201-15829B

Robust Summaries and Dossier for CAS No. 23500-79-0

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Existing Chemical	:	ID: 23500-79-0
CAS No.	:	23500-79-0
Product name	:	A-1846
EINECS Name	:	6-tert-butyl-3-(chloromethyl-2,4-xylenol
Molecular Formula	:	C13H19ClO

Producer related part

Company	:	Cytec Industries Inc.
Creation date	:	06.05.2003

Substance related part

Company	:	Cytec Industries Inc.
Creation date	:	06.05.2003
Status	:	
Memo	:	
Printing date	:	29.05.2003
Revision date	:	18.02.2005
Date of last update	:	28.02.2005
Number of pages	:	60
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type	:	organic
Physical status	:	solid
Purity	:	> 98.0 % w/w
Source	:	Cytec Industries Inc.
Reliability	:	(2) valid with restrictions. Methodological information not available.

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2,4-dimethyl-3-(chloromethyl)-6-tert-butylphenol

3-(chloromethyl)-6-(1,1-dimethylethyl)-2,4-dimethylphenol

4-tert-butyl-3-hydroxy-2,6-dimethylbenzyl chloride

6-tert-butyl-2,4-dimethyl-3-chloromethyl phenol

6-tert-butyl-3-chloromethyl-2,4-dimethylphenol

phenol, 3-(chloromethyl)-6-(1,1-dimethylethyl)-2,4-dimethyl-

phenol, 6-tert-butyl-3-chloromethyl-2,4-xylenol

1.3 IMPURITIES

Impurities	:	< 2.0% unidentified impurities
Source	:	Cytec Industries Inc. (personal communication)

Reliability : (2) valid with restrictions. Methodological information not provided.

1.4 ADDITIVES

Remark : A-1846 commercial grade is often stored in liquid form, dissolved in 11-13% methyl isobutyl ketone solvent.
Source : Cytec Industries Inc.

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : industrial
Category : Chemical industry: used in synthesis

Reliability : (1) valid without restriction
11.05.2003

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Exposure Limits : Internal Cytec PEL: 0.10 ppm; STEL: 0.24 ppm

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 : = 45 °C
Sublimation :
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The melting point of 45 degrees C is for the pure crystallized substance. The product is usually isolated with 11-13% methyl isobutyl ketone (CAS No. 108-10-1) added to store and handle the product in liquid form (Cytec Industries, Inc. material safety data sheet dated 10/30/00 for A-1846).
Reliability : (2) valid with restrictions
Methodology was not documented.
Flag : Critical study for SIDS endpoint
11.05.2003 (4)

Value : = -32 °C
Sublimation :
Method : other
Year :
GLP : no data
Test substance : other TS: commercially stored form containing 11-13% methyl isobutyl ketone solvent

Reliability : (2) valid with restrictions
Experimental details were not provided.
22.05.2003 (3)

2.2 BOILING POINT

Value : ca. 320 °C at 1013 hPa
Decomposition :
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : EPIWIN Mpbpwin (v1.40) was used to estimate boiling point by the adapted Stein and Brown Method. Inputs to the model were CAS No. 23500-79-0 and the measured melting point (45 degrees C).
Reliability : (2) valid with restrictions
Data were obtained by modeling.
Flag : Critical study for SIDS endpoint
11.05.2003 (11)

Value : = 156 °C at
Decomposition :
Method : other
Year :
GLP : no data
Test substance : other TS: commercially stored form containing 11-13% methyl isobutyl ketone solvent.

Reliability : (2) valid with restrictions
Experimental details were not provided.

22.05.2003 (3)

2.3 DENSITY

Type	:	relative density
Value	:	ca. 1.044 g/cm³ at 20 °C
Method	:	other
Year	:	
GLP	:	no
Test substance	:	other TS: commercially stored form containing 11-13% methyl isobutyl ketone solvent
Reliability	:	(2) valid with restrictions Experimental details were not provided.

11.05.2003 (3)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value	:	= .00042 hPa at 25 °C
Decomposition	:	no
Method	:	OECD Guide-line 104 "Vapour Pressure Curve"
Year	:	2004
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	No change in appearance of the test material was observed under the test conditions.
Test condition	:	The vapor pressure was determined using a vapor pressure balance system with measurements being made at several temperatures and linear regression analysis used to calculate the vapor pressure at 25 degrees C. Method 104 of the OECD Guidelines of July 27, 1995 was employed in accordance with OPPS 830.7950 US EPA Product Properties Guidelines. The temperature of the sample was controlled electronically with mass and temperature readings recorded automatically into a computer file. The test system consisted of a chamber separated into a lower and upper chamber by a shutter with an orifice. The test sample was placed into the lower test chamber, and the vapor pressure balance pan was situated in the upper chamber. At a given temperature, the system was evacuated and the shutter above the test sample in the lower chamber opened causing the escaping vapor jet to be directed at the scale pan in the upper chamber. The difference in mass readings with the orifice covered and uncovered is proportional to the vapor pressure at a given temperature. A sequence of runs was started after a sample of test material had been under vacuum for approximately 3 minutes. Temperature and pressure readings were taken between 25 and 35 degrees C with a one hour dwell time at 25 deg C between runs. The following equation provides the relationship of vapor pressure to the observed mass difference: $Vp = \partial m.g / A$, where Vp is vapor pressure in Pa; ∂m = mass difference in kg; g = acceleration due to gravity (9.813 m/sec^2) and A = area of the orifice

(7.06858 E-6 meter squared).

Vapor pressure is related to temperature by the following equation:

Log10 [Vp(Pa)] = {slope/(temperature (K)} + intercept.

A plot of Log10 Vp(Pa) versus reciprocal temperature (K) provides a straight line graph.

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

Nine separate runs were made and the mean vapor pressure value was calculated.

Test substance : The lot number of the test substance was CT-800-03. The test material was A-1846. It contained > 98% 6-tert-Butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities.

Reliability : (1) valid without restriction
Well-conducted guideline study following GLP.

Flag : Critical study for SIDS endpoint

27.09.2004 (23)

Value : ca. 12 hPa at 20 degrees C
Method ; other (measured)
GLP : no
Test substance : other: A-1846 dissolved in 11-13% methylisobutylketone
Remark : The vapor pressure corresponds to the solvent methyl isobutyl ketone
Reliability : (2) valid with restrictions
Experimental details were not provided.

14.05.2003 (3)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = 5.32 at 20 °C
pH value :
Method : other (calculated)
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : EPIWIN Kowwin (v1.66) was used to estimate the log Kow (Pow). The CAS No. (23500-79-0) was inputted into the model.

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

Flag : Critical study for SIDS endpoint

11.05.2003 (10)

Partition coefficient : octanol-water
Log pow : = 3.9 at °C
pH value :
Method : other (measured)
Year :
GLP : no data
Test substance : other TS: commercially stored form containing 11-13% methyl isobutyl ketone solvent

Reliability : (2) valid with restrictions
Experimental details were not provided.

22.05.2003 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	water
Value	:	= 3.46 mg/l at 20 °C
pH | **value**	:	= 1.8 - 1.9
pH | **concentration**	:	at °C
Stable	:	no
Deg. product	:	
Method	:	OECD Guide-line 105
Year	:	2004
GLP	:	yes
Test substance	:	as prescribed by 1.1 -1.4
Result	:	The preliminary estimate of water solubility was less than 0.0001 g/l.

In the definitive test, the concentrations of test material were <=1.9 x 10-5 g/l for Sample No. 1; 1.5 x 10-5 g/l for Sample No. 2, and none detected for sample No. 3. The pHs were 1.9, 1.8 and 1.8 (respectively) for Samples 1, 2, and 3. Samples Nos. 1, 2, and 3 were shaken at 30 degrees C for 24, 48, and 72 hours (respectively). The equilibration time (at 20 degrees C) was the same (24 hours) for all three samples.

The overall limit value was <=1.9 x 10-5 g/l.

The short-term assessment sample concentration was 4.04 x 10-4 g/l of solution and the pH was 2.2.

The linearity of the HPLC detector response with respect to concentration was assessed over the nominal concentration range of 0 to 100 mg/l, which was satisfactory with a correlation coefficient of 1000 being obtained. Recovery of the sampling procedure was assessed and found to be adequate (mean recovery was 95.6%).

During the definitive test, Samples 2A and Sample 3A were lost through accidents prior to analysis. This was concluded by the author not to impact significantly on the study, since negligible test material was found in the remaining solutions and the overall result was superseded by the short-term assessment flask.

The information obtained in the hydrolysis study run in conjunction with this study (O'Conner BJ and Mullee DM, Determination of Water Solubility and Hydrolysis as a Function of pH, Project Number 971/236, SafePharm Laboratories. 2004.) and data relating to the pH of the test material in water indicated that significant hydrolysis occurred during the course of the water solubility test. This also was evident from the significant degradation products observed in the chromatography. The pH of the sample solutions suggests that hydrolysis of the test material may be producing a strong acid such as hydrochloric acid. A possible hydrolysis pathway is the substitution of the chlorine on the benzyl chloride function with a hydroxyl group to generate a benzyl alcohol group and hydrochloric acid. This hypothesis is supported by the rapid acceleration of hydrolysis observed with the pH 9 sample.

The authors concluded that the instability of the test material to hydrolysis led to an underestimation of the potential water solubility. The authors therefore ran the WSKOW program, version 1.40, which estimated the water solubility to be 3.46 mg/l.

Test condition : The determination was carried out using the flask method (Method 105 of the OECD Guidelines of July 27, 1995 and Method 830.7840 of the OPPTS Guidelines).

A preliminary test was run by diluting an aliquot of the test material with glass double-distilled water. After shaking at 30 degrees C for three hours and standing at 20 degrees C for 3 hours, the solution was centrifuged at 2500 rpm for 10 minutes and then centrifuged further at 6000 rpm for 10 minutes. The solution was then sampled (excluding any excess undissolved test material), diluted and analyzed.

In the definitive test based on the preliminary result above, three samples of test material (Samples No. 1, 2, and 3) were prepared in glass, double-distilled water in three separate flasks. The flasks were shaken at approximately 30 degrees C for 24, 48, and 72 hours (respectively). After standing at 20 degrees for a period of not less than 24 hours, the contents of the flasks were filtered through glass wool to remove the majority of the remaining solids. The remaining solutions were then centrifuged at 10,000 rpm for 30 minutes and sampled, excluding most of the undissolved test material. The pH of each solution was measured.

An additional sample was prepared to allow the assessment of the short-term solubility of the test material in water due to observed instability to hydrolysis (see summary of hydrolysis study conducted by the same laboratory and same project number). An aliquot of test material was diluted to 400 ml with glass, double-distilled water. After shaking at 30 degrees C for 1 hour and standing at 20 degrees C for 1 hour, the solution was filtered through glass wool to remove a majority of the excess solids. The remaining solution was then centrifuged at 10,000 rpm for 30 minutes sampled (excluding excess, undissolved test material) and analyzed for pH.

The concentration of test material in the sample solutions was determined by high performance liquid chromatography (HPLC).

Duplicate aliquots (150 ml, A and B) were extracted from each of the three samples with three portions of dichloromethane. Each extract was filtered through anhydrous sodium sulphate. The combined extracts were then evaporated to dryness and the residue re-dissolved in 5 ml of acetonitrile for analysis.

Duplicate standard solutions of test material were prepared in acetonitrile at a nominal concentration of 15 mg/l.

The mean peak area of each standard was corrected to a nominal concentration of 15 mg/l and the mean value taken. The sample solution concentration (g/l) was calculated using the following equation:

C(spl) = [P(spl/P(std)] x C(std) x D x 0.001 , where:

C(spl) = sample concentration (g/l)
P(spl) = mean peak area of sample solution
P(std) = mean peak area of standard solution, corrected to nominal standard concentration
C(std) = nominal standard concentration (15 g/l)
D = sample dilution factor (0.033).

Test substance : The lot number of the test substance was CT-800-03. The test material was A-1846. It contained > 98% 6-tert-Butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities.

Reliability : (2) valid with restrictions
Well-conducted guideline study following GLP. However, study was unable to accurately measure water solubility, because the test material hydrolyzes rapidly in the presence of water. Therefore the study underestimates the true solubility of the test substance. This study provides useful supporting data regarding a lower limit of solubility and further evidence that rapid hydrolysis occurs in water.

Flag : Critical Study for SIDS endpoint
28.09.2004 (15)

Solubility in : water
Value : = 10.19 mg/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : slightly soluble (0.1-100 mg/L)
Stable : yes
Deg. product :
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : EPIWIN Wskow (v1.40) was used to estimate the water solubility. Inputs to the model are CAS No. 23500-79-0 and the measured melting point (45 degrees C).
Reliability : (2) valid with restrictions
Data were obtained by modeling.
14.05.2003 (12)

Solubility in : organic solvents
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : of very high solubility
Stable : yes
Deg. product :
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The pure crystalline substance can be liquified and dissolved into one liquid phase by adding 11-13% by weight methyl isobutyl ketone.
Reliability : (2) valid with restrictions
Experimental details were not provided.
22.05.2003 (3)

Solubility in : water
Value : = 500 mg/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa :
Description :
Stable :
Deg. product :
Method : other
Year :
GLP : no data
Test substance : other TS: commercially stored form containing 11-13% methyl isobutyl ketone solvent

Reliability : (2) valid with restrictions
Experimental details were not provided.

22.05.2003 (3)

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	:	= .0000000000143539 cm³/(molecule*sec)
Degradation	:	= 50 % after 8.9 hour(s)
Deg. product	:	
Method	:	other (calculated)
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN Aop Program (v1.90) was used to calculate the hydroxyl radical rate constant and half life. A CAS No. of 23500-79-0 was inputted.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

14.05.2003 (7)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	< 9.8 minute(s) at 25 °C
t1/2 pH7	:	< 6.4 minute(s) at 25 °C
t1/2 pH9	:	spontaneous at 25 °C
t1/2 pH 1.2	:	spontaneous at 37 °C
Deg. product	:	
Method	:	OECD Guide-line 111 "Hydrolysis as a Function of pH"
Year	:	2004
GLP	:	yes
Test substance	:	as prescribed by 1.1 – 1.4
Result	:	The minimum time period between sampling time points of 30 minutes was a constraint of the HPLC analysis run time. Testing at pHs 9 and 1.2 showed spontaneous hydrolysis at 25 degrees C and 37 degrees C respectively, with no parent test material remaining (even on the analysis of the initial samples).
Test condition	:	The test was carried out using Method 111 of the OECD Guidelines and Method 835.2110 of the OPPTS Guidelines. Buffer solutions were prepared of potassium hydrogen phthalate (for pH = 4); disodium hydrogen orthophosphate (anhydrous), potassium dihydrogen orthophosphate and sodium chloride (for pH = 7); and disodium tetraborate and sodium chloride (for pH = 9). The buffer solutions were sterilized by passing through a 0.2 micron membrane filter. In addition, these solutions were subjected to ultrasonication and degassing with nitrogen to minimize dissolved oxygen content. Sample solutions were prepared in stoppered glass flasks at a nominal concentration of 1.25 x 10-3 g/l in the three buffer solutions. A 1% co-solvent of acetonitrile was used to aid solubility. The buffer solutions were pre-equilibrated to the test temperature prior to sample solution preparation. The solutions were shielded from light while being maintained at the test temperature.

Sample solutions at pHs of 4, 7, and 9 were maintained at 0.0 +/- 0.5 degrees C for a maximum period of 60 minutes. In addition, a sample solution was prepared at pH = 1.2 using a buffer solution of hydrochloric acid and potassium chloride. This solution was maintained at 37.0 +/- 0.5 degrees C for 30 minutes.

Aliquots of the sample solutions were taken from the flask at various times and the pH of each solution recorded.

The concentration of material in each sample solution was determined by high performance liquid chromatography (HPLC). An aliquot of each buffer solution and an aliquot of acetonitrile were analyzed to act as sample blanks and a standard blank, respectively. Duplicate standard solutions of test material were prepared in acetonitrile at a nominal concentration of 1.25 mg/l.

Regression data for the common logarithm of the concentration (g/l) versus time (hours) was generated for pHs of 4 and 7 at 0.0 +/- 0.5 degrees C, and the rate constant and half-life calculated using the following equations.

Rate constant (hours): K(obs) = - slope x 2.303

Half-life (hours): t(1/2) = 0.693/k(obs)

The mean peak area of each standard was corrected to a nominal concentration of 1.25 and the mean value taken. The concentration of the sample solutions (g/l) was calculated using the following equation:

C(spl) = [P(spl)/P(std)] x C(std) x 1/1000

where C(spl) = sample concentration (g/l); P(spl) = mean peak area of sample solution; P(std) = mean peak area of standard solution, corrected to nominal standard concentration; and C(std) = nominal standard concentration (1.25 mg/l).

The linearity of the detector response with respect to concentration was assessed over the nominal concentration range of 0 to 2.50 mg/l, and found to be satisfactory with a correlation coefficient of 1.000 being obtained.

Test substance : The lot number of the test substance was CT-800-03. The test material was A-1846. It contained > 98% 6-tert-Butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities.

Reliability : (1) valid without restriction
Well-conducted guideline study following GLP.

Flag : Critical study for SIDS endpoint

28.09.2004 (15)

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C

Remark : EPIWIN Hydrowin(v1.67) cannot estimate a rate constant for molecules other than esters, carbamates, epoxides, halomethanes and specific alkyl halides.

14.05.2003

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	:	.283 % (Fugacity Model Level III)
Water	:	10.4 % (Fugacity Model Level III)
Soil	:	% (Fugacity Model Level I)
Biota	:	52.6 % (Fugacity Model Level II/III)
Soil	:	36.7 % (Fugacity Model Level II/III)
Method	:	other
Year	:	2004
Remark	:	Inputs into the EPIWIN Program were measured melting point (45 degrees C), measured vapor pressure (.000315 mm Hg), and the CAS No. 23500-79-0. Default emission rates of 1000 kg/hr to air, water and soil were used.
Result	:	Half-lives in various media based on EPIWIN Biowin and EPIWIN Aop are air = 17.88 hours, water = 1440 hours, soil =1440 hours, and sediment = 5760 hours. Biowin ultimate is estimated to be months. The EPIWIN Pckoc Program (v1.66) estimates the soil/sediment partition constant to be 1.705 E+4. EPIWIN Henry (v3.10) was used to calculate a Henry's Law Constant of 6.85 E-7 atm-m3/mole at 25 degrees C.
Reliability	:	(2) valid with restrictions Data were obtained by modeling.
Flag	:	Critical study for SIDS endpoint

22.05.2003 (9)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	activated sludge, domestic
Contact time	:	29 day(s)
Degradation	:	= 7 (±) % after 28 day(s)
Result	:	other: not readily biodegradable
Kinetic of test subst.	:	3 day(s) = 0 % 10 day(s) = 0 % 16 day(s) = 0 % 24 day(s) = 5 % 29 day(s) = 10 %
Control substance	:	Benzoic acid, sodium salt
Kinetic	:	14 day(s) = 76 % 29 day(s) = 83 %

Deg. product : not measured
Method : OECD Guideline 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year : 2004
GLP : yes
Test substance : as prescribed by 1.1 – 1.4

Remark : An initial test performed with test material at a concentration of 10 mg C/l showed inhibition of CO2 evolution after 14 days. Following the recommendation of the OECD Guideline, it was considered appropriate to reduce the concentration to 5 mg C/l in order to minimize toxicity to the microorganisms.

The IC/TC ratio of the test material suspension in the mineral medium at the start of the test exceeded the recommended level of < 5%. This was considered to be due to the low TC concentration in the medium. This was considered to have no adverse effect on the study, given that the CO2 evolution in the control vessels satisfied the validation criterion given in the OECD TEST Guidelines.

Result : The test material attained 7% degradation after 28 days. The results of the inorganic carbon analysis of samples from the first absorber vessels on Day 29 showed an increase in all replicate vessels (with the exception of the second replicate for the standard material and the first replicate of the test material). The increases in inorganic carbon, coupled with the decrease in inorganic carbon in the first replicate of the test material were considered to be due to CO2 present in the solution being driven off by the addition of HCl on Day 28 and resulted in an increase in degradation of the test material from 7% on Day 28 to 10% on Day 29.

All criteria for validity were met, with the exception of the IC/TC ratio (see Remark above). The total CO2 evolution in the controls on Day 28 was 31.34 mg/l, the difference between the values for CO2 production at the end of the test for the replicate vessels was <20%, the toxicity control attained 60% degradation after 28 days, and sodium benzoate degradation was > = 60% by 14 days. Inorganic carbon analysis of the samples from the second absorber vessels on Day 29 confirmed that no significant carry-over of CO2 into the second absorber vessels occurred.

Observations made throughout the test indicated that the control, test, toxicity and positive control vessels contained light brown dispersions. A few particles of test material were visible in the test and toxicity control vessels. No undissolved material was visible in vessels containing the standard. The pH measured in the test vessels on Day 28 ranged from 7.4 - 7.6.

Test condition : Test material: The test material (21.9 mg) was dispersed in approximately 200 ml of culture medium prepared according to the OECD guideline and sonicated for approximately 30 minutes. The volume was adjusted to 3 liters, to give a 7.3 mg/l stock solution (equivalent to 5 mg carbon/l).

Standard/positive control: An initial stock solution of 1000 mg/l sodium benzoate was prepared by dissolving the standard material directly in culture medium and sonicating the solution for approximately 5 minutes. Additional medium (51.4 ml) was added to the vessel to give a final test concentration of 17.1 mg/l, equivalent to 10 mg carbon/l. The flask containing the test material was inverted several times to ensure homogeneity of the solution.

Toxicity control: The test material (21.9 mg) was dispersed in approximately 200 ml of culture medium prepared according to the OECD guideline and sonicated for approximately 30 minutes prior to dispersal in inoculated culture medium. An aliquot (51.4 ml) of the sodium benzoate stock solution also was added to the vessel. The volume was adjusted to 3

liters to give a concentration of 7.3 mg test material/l plus 17.1 mg sodium benzoate/l, equivalent to a total of 15 mg carbon/l.

Bacteria: A mixed population of activated sewage sludge microorganisms was obtained on March 15, 2004 from the aeration stage of the Severn Trent Water Plc sewage treatment plant at Loughborough, Leicestershire, UK, which treats predominantly domestic sewage. The sample was used on the day of collection and maintained under continuous aeration in the laboratory at 21 degrees C. The suspended solids level (3.4 g/l) was determined by filtering a 100 ml sample of the sludge by suction through a pre-weighed GF/A filter paper, drying the paper in an oven (105 degrees C for at least 1 hour), weighing the paper after cooling, and repeating the procedure until a constant weight was obtained.

Test conduct: Approximately 24 hours prior to testing, seven 5 liter glass culture vessels were filled with 2400 ml of culture medium and 26.5 ml of inoculum (for a total of 30 mg suspended solids/l) and aerated overnight. On Day 0, the test or standard material (for final concentrations of 5 or 10 mg carbon/l, respectively) were added to duplicate cultures. The test material plus the standard (for a final concentration of 15 mg carbon/l) were added to one vessel to act as a toxicity control. Two vessels left untreated served as inoculum controls. The culture vessels were sealed and CO2-free air was bubbled through the solution at a rate of approximately 40 ml/min. The CO2-free air was produced by passing compressed air through a glass column containing self-indicating soda lime granules. The solutions were stirred continuously with a magnetic stirrer and maintained in the dark at 21 degrees C.

The CO2 produced was collected in two 500 ml Dreschel bottles containing 350 ml of 0.05 M NaOH. The CO2 absorbing solutions were prepared using purified de-gassed water. Samples (2 ml) were taken from the first CO2 absorber vessel on Days 0, 1, 2, 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 27, 28 and 29. The second absorber vessel was sampled on Days 0 and 29. The pH of the solutions was measured on Day 28. After pH was measured, 1 ml of concentrated HCl was added to each vessel to drive off inorganic carbonates. The vessels were resealed, aerated overnight, and sampled on Day 29. All samples (with the exception of samples taken on Days 12 and 18) were analyzed immediately for CO2 (in triplicate) using a TOC analyzer. Samples (40 or 300 microliters) were injected into the inorganic carbon channel of the TOC analyzer. The instrument was calibrated using standard solutions of sodium carbonate. The samples taken on Days 12 and 18 were stored at -20 degrees C and were not analyzed.

Samples (20 ml) were removed for the test material and toxicity control vessels on Day 0 prior to the addition of the test material in order to calculate the Inorganic Carbon/Total Carbon (IC/TC) ratio in the test media. On days 0 and 28, samples (20 ml) were removed from the control and standard material vessels. All samples were filtered through 0.45 micron filters prior to DOC analysis. The samples (13 or 27 microliters) were injected into the total carbon and inorganic channels of the TOC analyzer in triplicate. The analysis was carried out at 680 degrees C using a platinum-based catalyst and zero grade air as the carrier gas. The instrument was calibrated using standard solutions of potassium hydrogen phthalate and sodium carbonate.

Calculation of carbon content: The total organic carbon content (TOC) of a 5 mg C/l solution of test material was 15 mg (21.9 mg x 68.86% carbon in the test material. The total organic carbon content (TOC) of a 10 mg C/l solution of the standard material was 30 mg (51.4 mg x 58.34 % carbon in sodium benzoate). The percentage degradation (or the percentage of the theoretical amount of CO2 produced) was calculated by subtracting the

amounts of inorganic carbon in the inoculum control from the test (or positive control) samples, dividing the result by the TOC, and multiplying the value by 100%. The percentage degradation from the results of the DOC analysis was calculated by subtracting the DOC values of the inoculum controls from the values of test or positive controls for Days 28 and Days 0, dividing the result on Day 28 by that of Day 0, subtracting it from 1, and multiplying it by 100%. The total CO2 evolution in the control vessels at the end of the test was equal to the mg inorganic carbon in the control x (100 divided by the %C of CO2) x (1/test volume).

Test for validity: The test was considered valid if the degradation of the standard was >= 60% by Day 14, the difference of the extremes of replicated values for CO2 production at the end of the test was < 20%, the total CO2 evolution in the control vessels at the end of the test did not exceed 40 mg/l, and the IC content of the test material at the beginning of the test was < 5% of the TC. The test material was considered to be readily biodegradable if degradation was >= 60% by 28 days. The toxicity control was considered non-inhibitory if degradation was >= 25% by Day 14.

Test substance : The test material was A-1846. It contained > 98% 6-tert-Butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities.

Reliability : (1) valid without restriction
OECD Guideline study.

Flag : Critical study for SIDS endpoint

27.04.2004 (19)

Type : aerobic
Inoculum :
Deg. product :
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : EPIWIN Biowin (v4.00) was used to predict biodegradability. A CAS No. of 23500-79-0 was inputted into the model.

Result : Linear Model Prediction: biodegrades fast (probability of 0.5694)
Non-linear Model Prediction: does not biodegrade fast (probability of 0.1513)
Ultimate Biodegradation Timeframe: months (value = 2.2195)
Primary Biodegradation Timeframe: weeks (value = 3.1692)
MITI Linear Model Prediction: does not biodegrade fast (value = 0.272)
MITI Non-Linear Model Prediction: does not biodegrade fast (value = 0.0430)

Reliability : (2) valid with restrictions
Data were obtained by modeling. All models except for the linear model are considered valid, since they are in agreement with experimental results.

14.05.2003 (13)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Elimination :
Method : other

Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The CAS No. (23500-79-0) was inputted into the EPIWIN BCF Program (v2.14) model.
Result	:	The log bioconcentration factor (log BCF) is calculated to be 2.945.
Reliability	:	(2) valid with restrictions Data were obtained by modeling.

22.05.2003 (9)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Oncorhynchus mykiss
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 8.2
LC50	:	= 11
Limit test	:	no
Analytical monitoring	:	yes
Method	:	OECD Test Guideline 203
Year	:	2004
GLP	:	yes
Test substance	:	other TS

Remark : Preliminary work showed that the half life of A-1846 in water was less than 1 hour at ambient temperature. Therefore, it was considered appropriate to assess the toxicity of the material based on the degradation product. Results of a preliminary study showed that the concentration of degradation product in a solution prepared from a saturated solution of A-1846 was 45 mg degradation product/l.

Concentrations of 0.45, 4.5 and 45 mg degradation product/l were tested in a preliminary range finding study. No mortality was noted at 0.45 and 4.5 mg degradation product /l. Mortalities were observed at 45 mg degradation product/l.

The temperature during the acclimation (14.1 -14.7 degrees C) and study period (13.6 – 14.4 degrees C) was in excess of the specified range of 12 +/- 2 degrees C. This was considered to have no effect on the results.

Result : Nominal/measured concentrations: Test solutions were clear and colorless throughout the duration of the test. Chemical analysis of the stock solution (also the highest concentration tested) showed a concentration of 48.4 mg degradation product/l. However, analysis of the saturated solution at 24, 48 and 72 hours showed a mean measured concentration of 62.3, 73.7, and 80.4 mg degradation product/l. This was not due to a problem with chromatography, since the standard response was constant.

As a consequence of the high concentrations, some of the test concentrations prepared by dilution of the saturated solution were in excess of the accepted 120% of nominal from 24 hours to the end of the test. Given that the measured concentrations of the degradation material increased during the test, the actual concentrations tested were based on the time-weighted mean measured concentrations. The time weighted concentrations calculated for the nominal concentrations of 4.5, 8.0, 14, 25 and 45 mg degradation product/l were 8.2, 10, 19, 28 and 47 mg/l, respectively.

Recovery/stability: The recovery analysis showed that measured concentrations of the degradation product ranged from 93-118% of fortification. The stability analysis showed the degradation product to be stable in the test medium over a 48-hour period.

Element value: The LC50 (96 hr) with respective 95% confidence limits was 11 (10-12) mg degradation product/l The no observed effect concentration (NOEC) was based on the absence of toxicity at 8.2 mg degradation product/l. The highest concentration resulting in 0% mortality was 8.2 mg degradation product/l and the lowest concentration resulting 100% mortality was 19 mg degradation product/l.

Biological observations: Loss of equilibrium was noted at 10, 19 and 28 mg degradation product/l. After approximately 1 hour of exposure, all fish exposed to 45 mg degradation product/l nominal concentration were moribund. After approximately 27 hours of exposure, all 18 of the remaining fish exposed to 19 mg degradation product/l nominal concentration were moribund. All of these fish were killed and classified as mortalities for the following observational time point. Mortality data are listed in the following table.

	Cumulative Mortality (out of 20)					
Concentration	Time (hours) 3	6	24	48	72	96
Control	0	0	0	0	0	0
8.2	0	0	0	0	0	0
10	0	0	0	1	7	10
19	0	0	2	20	20	20
28	17	20	20	20	20	20
47	20	20	20	20	20	20

Observations: Test solutions were clear and colorless throughout the duration of the test.

Physico-chemical measurements: The temperature ranged from 13.6 – 14.4 degrees C throughout the test. The dissolved oxygen concentrations in the test water ranged from 9.3- 9.7 mg/l (90-94% of saturation) in fresh solutions and 8.1 – 9.1 mg/l (79-88% of saturation) in solutions that were 24 hours old. pH values ranged from 7.2 - 7.9. The pH of the solution decreased slightly with increasing concentration of test material.

Test condition : Test organisms: The juvenile rainbow trout used in the study were obtained from Brow Well Fisheries (location was not noted). They were maintained in a glass fiber tank with a “single pass” water renewal system. Fish were acclimated to test conditions from April 26 to May 10, 2004. The rate of mortality in the 7 days prior to the test was < 1%. All fish were on a 16 hour daylight/8 hour dark photoperiod, with 20 minute dawn and dusk transition periods. Fish received commercial trout pelleted food daily until 48 hours prior to testing. The mean weight and length of the fish at the end of the test were 1.01 +/- 0.20 (sd) g and 4.1 +/- 0.1 cm, respectively. Maximum loading was 0.1 g fish/liter of solution. The temperature of the acclimation water was 14.1 – 14.7 degrees C.

Test water: The water used for both the range-finding and definitive tests was the same as that used to maintain the stock fish. Laboratory tap water was dechlorinated by passage through an activated carbon filter and partly softened, resulting in water with a total hardness of approximately 100 mg/l as CaCO3. The dechlorinated, softened water was passed through a series of computer-controlled plate heat exchangers to achieve the required temperature. At the start of the study, the water had a particulate matter concentration of 0.1 mg/l, total water hardness of 144 mg/l as CaCO3, alkalinity of 90 mg/l as CaCO3, Chemical Oxygen Demand of 1.67 mg O2/l, conductivity of 461 microS/cm and Total Organic Carbon content of 1.2 mg C/l. The dissolved oxygen concentration in the water at the start of the test was 9.4 mg/l.

Test material: A saturated solution was prepared by stirring (1500 rpm) an excess (100 mg/l) of A-1846 in culture medium at 21 degrees C for 24, 48, 72 and 96 hours. After 24 and 48 hours, samples were taken and analyzed after centrifugation at 10000 and 40000g and after filtration through a preconditioned 2 micron filter to determine the amount of material in solution. The samples taken at 72 and 96 hours were taken and analyzed after filtration, since results for the 24 and 48-hour samples showed no difference between centrifugation and filtration. The samples taken at 24, 48, 72 and 96 hours contained 48, 59-60, 42 and 45 mg degradation

product/l, respectively.

Twenty two and a half liters of saturated solution was prepared by stirring an excess (100 mg/l) of A-1846 in culture medium for 24 hours at 1500 rpm and then removing any undissolved test material by filtration through a preconditioned 2 micron filter to give a saturated solution of the degradation product with a nominal test concentration of 45 mg/l. Aliquots (2.0, 3.56, 6.22 and 11.11 liters) of this saturated solution were each added to dechlorinated tap water and the volume adjusted to 20 liters to give test concentrations of 4.5, 8.0, 14 and 25 mg degradation product/l. The 45 mg/l solution also was tested. Each solution was inverted several times to ensure adequate mixing and homogeneity. A control containing medium only also was tested.

Test conduct: Approximately 20 liters of test medium were added to glass vessels (volume not stated). Ten fish were placed in each duplicate test and control vessels at random. The vessels were then covered to reduce evaporation and maintained at 13.6 – 14.4 degrees C under a photoperiod of 16 hours light, 8 hours dark, with 20 minute dawn/dusk transition periods. The fish did not receive food during the test and the vessels were not aerated. A semi-static design was employed, which involved daily renewal of the test medium. The study was terminated at 96 hours.

Water temperature, pH and dissolved oxygen concentrations were recorded daily. Any mortalities or symptoms of toxicity were recorded at 3, 6, 24, 48, 72 and 96 hours. Animals classified as dead had no respiratory movement and no response to physical stimulation. The concentration and stability of the degradation product in the test medium were verified by chemical analysis of fresh medium prepared at 0, 24, 48 and 72 hours, old medium prior to renewal at 24, 48, and 72 hours, and medium present a 96 hours. Each “old” solution was 24 hours old. Duplicate samples were taken at each at each time and stored frozen (approximately -20 degrees C) prior to analysis by high performance liquid chromatography (HPLC), using an CS-SIL-HS C18 column (5 micron, 250 x 4.6 mm internal diameter), ambient temperature, a UV/Vis detector wavelength of 210 nm, and an injection volume of 20 microliters. The eluant was a gradient of 0.1% v/v phosphoric acid in water:acetonitrile (50:50 at 0 minutes, to 10:90 at 15 minutes). The flow rate was 1 ml/min. A range of standard solutions from 0.12 to 59 mg/l was analyzed. Linearity was confirmed from 0 to 59 mg/l. Test medium was spiked with known concentrations of test material to determine stability and recovery.

The LC50 values and associated confidence limits at 3, 24, 48, 72 and 96 hours were calculated by the trimmed Spearman-Karber method, using the ToxCalc computer software package. The LC50 value at 6 hours was calculated using the geometric mean method. If there were no mortalities between 0 and 100%, then the geometric mean of the highest concentration showing no lethality and the lowest test concentration showing 100% lethality was calculated, and the 95% confidence limits were considered to be the concentrations resulting in 0 and 100% mortality.

Test substance : The test material was A-1846. It contained > 98% 6-tert-Butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities. The test material (A-1846) was not stable in the medium used in the test. The material actually used was the degradation product.

Reliability : (2) valid with restrictions
Guideline Study. Results were based on time-weighted concentrations, since the concentration of degradation product in the saturated stock solution used to prepare new test solutions every 24 hours increased over time.

Flag : Critical study for SIDS endpoint

07.08.2003 (18)

Type	:	other
Species	:	
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	
Analytical monitoring	:	no
Method	:	other
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN ECOSAR (v0.99) was used to obtain the calculated LC50 value. Inputs to the model are CAS Number 23500-79-0 and the measured melting point (45 degrees C).
Result	:	The LC50 values were 0.3 or 0.118 mg/l, depending on whether the molecule was classified as a phenol or benzyl halide (respectively).
Reliability	:	(3) invalid Values are not in agreement with experimental findings.

14.05.2003 (8)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 3.2
EC50	:	= 19
Limit Test	:	no
Analytical monitoring	:	yes
Method	:	OECD Test Guideline 202
Year	:	2004
GLP	:	Yes
Test substance	:	other TS
Remark	:	Preliminary work showed that the half life of A-1846 in water was less than 1 hour at ambient temperature. Therefore, it was considered appropriate to assess the toxicity of the material based on the degradation product. Concentrations of 0.45, 4.5 and 45 mg degradation product/l were tested in a preliminary range finding study. Concentrations of 0.45 and 4.5 mg/l had no effect on daphnia, and immobilization was observed at 45 mg/l.
Result	:	Nominal/measured concentrations: Test solutions were clear and colorless throughout the duration of the test. Chemical analysis of the saturated solution showed that the concentration of degradation product was 51.3 mg/l rather than 45 mg/l (as indicated in a preliminary test for solubility). Therefore, the measured concentrations (0.56, 1.0, 1.8, 3.2, 5.8, 10, 18, 30 and 51 mg degradation product/l) were higher than their respective nominal concentrations (0.45, 0.81, 1.4, 2.5, 4.5, 8.1, 14, 25 and 45 mg degradation product/l). Given these differences, the results were based on measured values. Recovery/stability: The recovery analysis showed that measured concentrations of the degradation product ranged from 90-101% of fortification. The stability analysis showed the degradation product to be stable in the test medium over a 48-hour period. Element value: The EC50 (24 hr) and EC50 (48 hr), with respective 95% confidence limits were 34 (28-43) mg degradation product/l and 19 (14-25)

mg degradation product/l. The no observable effect concentrations (based on 0 immobilization) for 24 and 48 hours were 10 and 3.2 mg degradation product/l, respectively.

Biological observations: The rate of mortality in the controls was 0 % at both time points. None of the organisms exposed to concentrations < = 3.2 mg degradation product/l were immobilized. Five out of 20 exposed to 5.8 or 10 mg degradation product/l (25%) were immobilized by 48 hours. At these concentrations, no immobilization was noted at 24 hours. A total of 3 (15%) and 9 (45%) of organisms exposed to 18 mg degradation product/l were immobilized by 24 and 48 hours, respectively. A total of 7 (35%) and 12 (60%) of organisms exposed to 30 mg degradation product/l were immobilized by 24 and 48 hours, respectively. A total of 16 (80%) and 18 (90%) of organisms exposed to 51 mg degradation product/l were immobilized by 24 and 48 hours, respectively.

Physico-chemical measurements: The temperature ranged from 20.3 - 21 degrees C at the beginning of the test and 21.6 - 21.7 degrees C at the end of the test. The dissolved oxygen concentrations in the test water ranged from 8.7- 9.0 mg/l (98-101 % of saturation) at the beginning of the test and 8.3 - 8.9 mg/l (95-102% of saturation) at the end of the test. pH values ranged from 7.5 - 7.8 at the beginning of the test and pH in all vessels at the end of the test was 8.3.

Test condition : Test organisms: The test was carried out using first instar Daphnia magna derived from in-house laboratory cultures. Adult Daphnia were maintained in polypropylene vessels containing approximately 2 liters of dechlorinated tap water at 21 degrees C. The lighting cycle was 16 hours light, 8 hours dark, with 20 minute dawn/dusk transition periods. Each culture was fed daily with a suspension of Chorella sp. algae. Culture conditions ensured that reproduction was by parthenogenesis. Gravid adults were isolated the day before initiation of the test, such that the young daphnids produced overnight were < 24 hours old. These young were removed from the cultures and used for testing. The diet and diluent water did not contain any contaminant that could affect the outcome of the study.

Growth/test medium: Dechlorinated tap water was the growth and test medium. Laboratory tap water was dechlorinated by passage through an activated carbon filter and partly softened, resulting in water with a total hardness of approximately 100 mg/l as $CaCO_3$. The dechlorinated, softened water was passed through a series of computer-controlled plate heat exchangers to achieve the required temperature. At the start of the study, the water had a particulate matter concentration of 0.2 mg/l, total water hardness of 146 mg/l as $CaCO_3$, alkalinity of 114 mg/l as $CaCO_3$, conductivity of 490 microS/cm and Total Organic Carbon content of 1.0 mg C/l.

Test material: A saturated solution was prepared by stirring (1500 rpm) an excess (100 mg/l) of A-1846 in culture medium at 21 degrees C for 24, 48, 72 and 96 hours. After 24 and 48 hours, samples were taken and analyzed after centrifugation at 10000g and 40000g and after filtration through a preconditioned 2 micron filter to determine the amount of material in solution. The samples taken at 72 and 96 hours were taken and analyzed after filtration since results for the 24 and 48 hour samples showed no difference between centrifugation and filtration.

A saturated solution was prepared by stirring an excess (100 mg/l) of A-1846 in culture medium at 21 degrees C for 24 hours and then removing any undissolved test material by filtration through a preconditioned 2 micron filter to give a saturated solution of the degradation product. Based on a preliminary study conducted in rainbow trout, this solution had a nominal concentration of 45 mg/l degradation product. Aliquots (10, 18, 32, 56, 100, 180, 320 and 560 ml) of this saturated solution were each added to

dechlorinated tap water and the volume adjusted to 1 liter to give test concentrations of 0.45, 0.81, 1.4, 2.5, 4.5, 8.1, 14 and 25 mg degradation product/l. Each solution was inverted several times to ensure adequate mixing and homogeneity. A control containing medium only also was tested.

The concentration and stability of the degradation product in the test medium were verified by chemical analysis at 0 and 48 hours. Duplicate samples were taken at each at each time and stored frozen (approximately -20 degrees C) prior to analysis by high performance liquid chromatography (HPLC), using an CS-SIL-HS C18 column (5 micron, 250 x 4.6 mm internal diameter), ambient temperature, a UV/Vis detector wavelength of 210 nm, and an injection volume of 20 microliters. The eluant was a gradient of 0.1% v/v phosphoric acid in water:acetonitrile (50:50 at 0 minutes, to 10:90 at 15 minutes). The flow rate was 1 ml/min. A range of standard solutions from 0.12 to 59 mg/l was analyzed. Linearity was confirmed from 0 to 59 mg/l. Test medium was spiked with known concentrations of test material to determine stability and recovery.

Test conduct: Approximately 200 ml of test medium was added to 250 ml glass jars. Ten daphnids were placed in each duplicate test and control vessels at random. The vessels were then covered to reduce evaporation and maintained at 21 degrees C under a photoperiod of 16 hours light, 8 hours dark, with 20 minute dawn/dusk transition periods. The daphnids did not receive food during the test and the vessels were not aerated. Solutions were not renewed during the test.

Water temperature was recorded daily. Dissolved oxygen concentrations and pH were recorded at the beginning and end of the test.

Immobilization or adverse reactions were recorded at 24 and 48 hours. Daphnia were considered to be immobilized if they were unable to swim for approximately 15 seconds after gentle agitation. The EC50 values and associated confidence limits at 24 and 48 hours were calculated by the maximum-likelihood probit method, using the ToxCalc computer software package.

Test substance : The test material was A-1846. It contained > 98% 6-tert-Butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities. The test material (A-1846) was not stable in the medium used in the test. The material used actually was the degradation product.

Reliability : (1) valid without restriction
Guideline Study

Flag : Critical study for SIDS endpoint

26.05.2003 (17)

Type : other
Species : Daphnia sp. (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : no
Method : other
Year : 2003
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Remark : EPIWIN ECOSAR (v0.99) was used to obtain the calculated LC50 value. Inputs to the model are CAS Number 23500-79-0 and the measured melting point (45 degrees C).

Result : The LC50 values were 0.535 or 0.118 mg/l, depending on whether the molecule was classified as a phenol or benzyl halide (respectively).

Reliability : (3) invalid

Values are not in agreement with experimental findings.

14.05.2003 (8)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Pseudokirchneriella subcapitata (Algae)
Endpoint	:	other: growth rate
Exposure period	:	72 hour(s)
Unit	:	mg/l
NOEC	:	5.1
EC50	:	6.6
Limit test	:	no
Analytical monitoring	:	yes
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	2004
GLP	:	yes
Test substance	:	other TS

Remark : Preliminary work showed that the half life of A-1846 in water was less than 1 hour at ambient temperature. Therefore, it was considered appropriate to assess the toxicity of the material based on the degradation product.

Concentrations of 0.45, 4.5 and 45 mg degradation product/l were tested in a preliminary study. Concentrations of 0.45 and 4.5 mg/l had no effect on growth and growth inhibition was observed at 45 mg/l.

Result : Nominal/measured concentrations: Chemical analysis of the saturated solution showed that the concentration of degradation product was 67.6 mg/l rather than 45 mg/l (as indicated in a preliminary test for solubility). Therefore, the measured concentrations (5.1, 10.4, 21, 39, and 70 mg degradation product/l) were higher than their respective nominal concentrations (2.89, 5.75, 11.5, 23 and 45 mg degradation product/l). Given these differences, the results were based on measured values.

Element value: The EbC50 (72 hr) and EbC50 (96 hr) were 6.6 and 6.4 mg degradation product/l, respectively. The ErC50 (0-96 hr) value with 95% confidence limits was 17 (15-20) mg degradation product/l, respectively.

There was no significant effect of 5.1 mg degradation product/l on area under the growth curve. All other test concentrations had a significant effect. Therefore the no effect concentration (NOEC) was 5.1 mg degradation product/l.

The NOEC for the positive control was 0.025 mg/l. The EbC50 (72 hr) and EbC50 (96 hr) for the positive control were 0.087 and 0.11 mg/l. The ErC50 (0-72 hr) was 0.21 mg/l for the positive control.

Cell density: The cell density of control cultures increased by a factor of 42 by 72 hours and 138 after 96 hours. No abnormalities were detected in any of the cultures exposed to concentrations < = 11.5 mg degradation product/l upon microscopic evaluation at 96 hours. Few intact cells were present in cultures exposed to 23 or 45 mg degradation product/l.

Regrowth occurred in the control and 2.89 mg degradation product/l test cultures after 72 hours, in the 5.75 mg degradation product/l culture after 96 hours, in the 11.5 mg degradation product/l culture after 120 hours, and in the 23 and 45 mg degradation product/l cultures after 144 hours. The results indicate that the material was algistatic.

Observations: All control and test cultures were clear and colorless at the start of the test. At 96 hours, all control and 2.89 mg degradation product/l

cultures appeared to be green dispersions. The 5.75 mg degradation product/l cultures were pale green, and cultures exposed to higher concentrations were clear, colorless solutions.

Physico-chemical measurements: Temperature was maintained at 24 +/- 1 degrees C throughout the test. The pH values of controls increased from pH 7.5 at 0 hours to 7.7 at 96 hours. The pH deviation was within the limits mentioned in the guideline (< 1.5 units).

Test condition : Test organisms: The test was carried out using Pseudokirchneriella subcapitata strain CCAP 278/4, obtained from the Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria. Cultures were maintained in the laboratory at 21 +/- 1 degrees C under continuous illumination (approx. 7000 lux) and aeration. Pre-culture conditions produced an algal suspension of 3.8 x 10E6 cells per ml in log phase growth. The suspension was diluted to a density of 1.78 x 10E6 cells/ml prior to use. Inoculation of 500 ml of test medium (see below) with 2.5 ml of this algal suspension produced an initial cell density of 1 x 10E4 cells/ml.

Growth/test medium: The culture medium was prepared according to the guideline using reverse osmosis purified deionized water. The pH was adjusted to 7.5 +/- 0.1 with 0.1 N NaOH or HCl. The medium was sterilized by 0.2 micron membrane filtration.

Test material: A saturated solution was prepared by stirring an excess (100 mg/l) of A-1846 in culture medium at 21 degrees C for 24 hours and then removing any undissolved test material by filtration through a preconditioned 2 micron filter to give a saturated solution of the degradation product. Based on a preliminary study conducted in rainbow trout, this solution had a nominal concentration of 45 mg/l degradation product. A series of dilutions was made from this saturated solution to give further stock solutions of 23, 11.5, 5.75 and 2.89 mg degradation product/l. An aliquot (500 ml) of each of the stock solutions was separately inoculated with algal suspension (2.5 ml) to give the required test concentrations of 2.89, 5.75, 11.5, 23 and 45 mg degradation product/l. Each solution was inverted several times to ensure adequate mixing and homogeneity.

The concentration and stability of the degradation product in the test medium were verified by chemical analysis at 0 and 96 hours. Duplicate samples were taken at each at each time and stored frozen (approximately -20 degrees C) prior to analysis by high performance liquid chromatography (HPLC), using an CS-SIL-HS C18 column (5 micron, 250 x 4.6 mm internal diameter), ambient temperature, a UV/Vis detector wavelength of 210 nm, and an injection volume of 20 microliters. The eluant was a gradient of 0.1% v/v phosphoric acid in water:acetonitrile (50:50 at 0 minutes, to 10:90 at 15 minutes). The flow rate was 1 ml/min. A range of standard solutions from 0.12 to 59 mg/l was analyzed. Linearity was confirmed from 0 to 59 mg/l. Test medium was spiked with known concentrations of test material to determine stability. Additional, spiked samples were analyzed following the addition of algae to determine if the presence of algae affected recovery of the test material.

Positive control: A positive control conducted every 6 months used zinc chloride as the reference material. Reference material (160 mg) was dissolved in culture medium and the volume adjusted to 1 liter to give a 160 mg/l stock solution. A series of dilutions were made to give 16, 1.6, 0.80, 0.40, 0.20, 0.10 and 0.050 mg/k stock solutions. An aliquot (250 ml) of each of the stock solutions except the 16 and 1.6 mg/l stock solutions was separately mixed with algal suspension (250 ml) to give the required test concentrations of 0.025, 0.050, 0.10, 0.20 and 0.40 mg/l.

Test conduct: Each concentration of test material and positive control was

tested in triplicate. Three negative control flasks containing algae but no test material also were prepared. Tests were conducted in 250 ml glass, conical flasks. Each flask contained 100 ml of the solutions prepared as described above. The flasks were plugged with polyurethane foam bungs and incubated at 24 +/- 1 degrees C under continuous illumination (approximately 7,000 lux) and constantly shaken at approximately 150 rpm for 72 hours. Samples were taken at 0, 24, 48, 72 and 96 hours and the cell densities determined using a Coulter® Multisizer Particle Counter.

The pH of each flask was determined at the beginning of the test and at 96 hours. The temperature was recorded hourly. Water samples were taken from the control and each test flask at 0 and 96 hours and replicates were pooled. Duplicate samples were taken on each occasion and stored at approximately -20 degrees C for further analysis (if necessary).

Statistical analyses: The areas under the curve for the control and test algae were calculated and the percentages of inhibition of growth at each concentration were compared by comparing the area under the test curve with that of the control curve. The percentage inhibition values were plotted against the test concentration and a line was fitted using the Xlfit 3 software package. The EC50 values with respect to the area under the growth curve (EbC50) for 0-72 and 0-96 hours were determined from the equation for the fitted line.

The maximum growth rate for each culture was calculated from the straight section of the growth curve. The percentage inhibition values for growth rate for each concentration were calculated and plotted against the test concentration and a line was fitted using the Xlfit 3 software package. The EC50 value with respect to the growth rate (ErC50) for 0-96 hours was determined from the equation for the fitted line.

The area under the curve and growth rate data at 96 hours for control and treated algae were analyzed using one way analysis of variance incorporating Bartlett's test for homogeneity of variance. Dunnett's multiple comparison procedure was used to compare data for treated algae to control. All statistical analyses were performed using the SAS computer software package. The critical level of significance was $p < 0.05$.

The test was considered to be valid if the cell concentration of the control cultures increased by a factor of at least 16 after 72 hours and 100 after 96 hours.

Regrowth experiment: A regrowth experiment was performed after 96 hours of exposure to test material to determine if the test material was algicidal or algistatic. Aliquots (0.5 ml) were removed from each replicate culture and the replicates pooled for each concentration. Fresh, sterile culture medium (100 ml) was added to ensure that the test concentrations were reduced to below those which caused inhibition of growth. The subcultures were incubated at 24 +/- 1 degrees C for 168 hours.

Test substance : The test material was A-1846. It contained > 98% 6-tert-Butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities. The test material (A-1846) was not stable in the medium used in the test. The material used actually was the degradation product.

Reliability : (1) valid without restriction
OECD Guideline study.

Flag : Critical study for SIDS endpoint.

19.04.2004 (16)

Species : other algae: green

Endpoint :

Exposure period : 96 hour(s)

Unit	:	mg/l
Limit test	:	
Analytical monitoring	:	no
Method	:	other
Year	:	2003
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	EPIWIN ECOSAR (v0.99) was used to obtain the calculated LC50 value. Inputs to the model are CAS No. 23500-79-0 and the measured melting point (45 degrees C).
Result	:	The LC50 values were 0.13 or 0.118 mg/l, depending on whether the molecule was classified as a phenol or benzyl halide (respectively).
Reliability	:	(3) invalid Values are not in agreement with experimental findings.

14.05.2003 (8)

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 : = 7.71 ml/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 10
Vehicle :
Doses : 5 and 10 ml/kg
Method : other
Year : 1979
GLP : no data

Remark : Based on a specific gravity of 1.044 at 20 degrees C, the LD50 dose in g/kg is 8.04.

Result : Four animals treated with 10 ml/kg died; 2 between 6-24 hours of dosing and the others on days 2 or 3 after dosing. None of the animals exposed to 5 ml/kg died. The LD50 value calculated was 7.71 (5.71 to 10.4) ml/kg.

All animals had a sluggish, unsteady gait after 1 hour of treatment. All animals treated with 5.0 ml/kg recovered within 2 days. Two animals treated with 10 ml/kg were prostrate 1 day after treatment. One of these animals recovered on day 2 (the other died on either day 2 or 3; see above). Survivors gained an average of 75 g over the course of the study.

Animals that died exhibited distended, gas-filled and injected stomachs, with glandular portions mottled pink and yellow; red kidney medullae; distended, liquid, blood-filled and injected intestines that were yellow and red in areas; and red adrenals. Survivors exhibited stomachs adhered to abdominal walls and livers at necropsy.

Test condition : Two groups of 5 nonfasted, male Hilltop-Wistar rats (90-120 g) were given 5.0 or 10.0 ml/kg test material by the oral route (presumably by gavage) as received. The length of the observation period after dosing is not clear (from 3-14 days); however, it probably was the standard 14 days since animals almost doubled their weight over the course of the experiment. All animals that died were subjected to gross necropsy. It is not clear when animals were terminated (from 3-14 days); however, they were necropsied at that time. The method of calculating the LD50 value was not listed.

Test substance : The test material was 80.5% pure. It is assumed that the material was the commercially isolated material that contains 11-13% methyl isobutyl ketone. It also contained 5.5% 6-t-butyl-2,4-dimethyl phenol. Other impurities were not identified.

Reliability : (2) valid with restrictions
The purity of the test material was not high. It is not known if doses were corrected for purity. The method of calculating the LD50 value was not listed. Only one sex was tested.

14.05.2003 (1)

Type : LD50
Value : > 2000 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male/female

Number of animals	:	6
Vehicle	:	other: dried arachis oil BP
Doses	:	2000 mg/kg
Method	:	OECD Guide-line 423
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	None of the animals died. Hunched posture and diarrhea were noted after treatment in all animals and lethargy and pilo-erection were found in males. Animals recovered 2-4 days after dosing. All animals gained a normal amount of weight over the observation period. No abnormalities were found at necropsy.
Test condition	:	The test material was freshly prepared as a suspension at 200 mg/ml in dried arachis oil BP. Homogeneity was assured by used of a Silverson homogenizer and vortex mixer. Animals [Sprague-Dawley Crl: CD(SD)IGSBR] were acclimated for at least 5 days before treatment. At the start of the study the males and females weighed 202 to 226 g and 216 to 230 g, respectively, and were approximately 8 weeks of age. Animals were allowed free access to food and water with the exception of an overnight fast immediately before dosing and for approximately 3-4 hours after dosing. The diet, drinking water and bedding were routinely analyzed for contaminants that could affect the outcome. Animals were housed in groups of 3/sex in rooms maintained at 19-25 degrees C, 30-70% relative humidity, and under a 12-hr light/dark cycle. All animals (3 per sex) were dosed with 2000 mg/kg at a volume of 10 ml/kg by gavage. The volume administered was calculated according to the fasted body weight at the time of dosing (females 216 - 230 g and males 202 - 226 g). Females were dosed prior to males. Sufficient time was allowed between dosing to confirm the survival of previously dosed animals. The animals were observed for death or overt signs of toxicity 0.5, 1, 2, and 4 hours after dosing and then daily for 14 days. Animals were weighed 7 and 14 days after treatment. Animals were killed by cervical dislocation 14 days after treatment. All animals were subjected to gross necropsies. External and internal (major organs) examinations were conducted. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.
Test substance	:	The material was described as "purified A-1846". The purity of the test substance was listed as >99% 6-tert-butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and <1% 6-tert-butyl-2,4-xylenol.
Reliability	:	(1) valid with restrictions
Flag	:	Critical study for SIDS endpoint

07.05.2003 (5)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	> 2000 ppm
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	6
Vehicle	:	
Doses	:	
Exposure time	:	4 hour(s)

Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : All 6 rats survived a 4-hour exposure to 2000 ppm (8.36 mg/l).
Reliability : (4) not assignable
There are not enough details to assign a reliability rating.

07.05.2003 (3)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : = 9.98 ml/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male
Number of animals : 14
Vehicle :
Doses : 0.8, 3.2, 6.4, 10.0 ml/kg
Method : other
Year : 1979
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Based on a specific gravity of 1.044 at 20 degrees C, the LD50 dose in g/kg is 10.4.
Result : One animal treated with 3.2 ml/kg died on day 3. Two animals treated with 10.0 ml/kg died (one on day 3 and another on day 5). The LD50 value was 9.98 (4.41 to 22.6) ml/kg.

No clinical signs of systemic toxicity were noted. Surviving animals in all groups treated with 3.2 to 10 ml/kg lost weight over the course of the study. Erythema, edema and ecchymosis and areas of necrosis were noted at the test site in several animals over the course of the study (times at which these observations were recorded were not noted). Scabs were noted in an undetermined number of animals at 14 days. Gross necropsies of survivors were normal. The animals that died exhibited red kidneys.
Test condition : Skin of the 14 animals (avg wt. 2246 - 2543 mg/kg) was clipped prior to treatment. Undiluted test material was applied at 0.8 ml/kg to two rabbits and at 3.2, 6.4 and 10.0 ml/kg to 3 groups of 4 animals each. The application site was then covered for 24 hours. Animals were observed for 14 days and weighed prior to euthanization and necropsy. Animals that died also were subjected to necropsy.
Test substance : The test material was 80.5% pure. It is assumed that the material was the commercially isolated material that contains 11-13% methyl isobutyl ketone. It also contained 5.5% 6-t-butyl-2,4-dimethyl phenol. Other impurities were not identified.
Reliability : (2) valid with restrictions
The purity of the test material was not high. It is not known if doses were corrected for purity. The method of calculating the LD50 value was not listed. Only one sex was tested.

14.05.2003 (1)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Semiocclusive
Exposure time	:	
Number of animals	:	6
Vehicle	:	
PDII	:	7
Result	:	highly irritating
Classification	:	
Method	:	Draize Test
Year	:	1979
GLP	:	no data
Test substance	:	other TS
Result	:	The mean values for erythema at intact sites were 2.17 and 4.00 after 24 or 72 hours, respectively. For abraded sites, these scores were 2.50 and 4.00, respectively. The mean values for edema at intact sites were 3.50 and 4.00 after 24 or 72 hours, respectively. At abraded sites, these scores were 3.83 and 4.00, respectively. The primary irritation score was 7.00. The structure of the tissue at the site of contact was destroyed or changed irreversibly in 24 hours or less.
Test condition	:	Test material (0.5 ml) was applied under a covered patch to both intact and abraded sites (the number of sites was not stated). The contact time was not stated. Skin was evaluated 24, and 72 hours after application for erythema and edema. The study was conducted according to an "FHSA procedure" and reactions were scored according to the method of Draize et al., J Pharmacol Exper Ther 82:377, 1944. The maximum possible value for a skin reaction (excluding necrosis) was 4. The primary irritation score was the sum of the mean values divided by 4.
Test substance	:	The test material was 80.5% pure. It is assumed that the material was the commercially isolated material that contains 11-13% methyl isobutyl ketone. It also contained 5.5% 6-t-butyl-2,4-dimethyl phenol. Other impurities were not identified.
Reliability	:	(1) valid without restriction The study was conducted according to an established method.

14.05.2003 (1)

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	no data
Exposure time	:	4 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	
Classification	:	
Method	:	other: DOT test
Year	:	1980
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	None of the 6 animals had necrosis. Therefore, the test material was not corrosive. No irritation scores were given.
Test substance	:	The test material was 80.5% pure. It also contained 5.5% 6-t-butyl-2,4-dimethyl phenol. Other impurities were not identified.
Reliability	:	(4) not assignable There are not enough details to assign a reliability rating.

14.05.2003 (21)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	
Result	:	highly irritating
Classification	:	irritating
Method	:	Draize Test
Year	:	1979
GLP	:	no data
Test substance	:	other TS
Result	:	The average corneal, iridial and conjunctival scores at 24 hours were 28.3, 4.2 and 13.7, respectively. At 48 hours, these values were 21.7, 4.2 and 13.3, respectively. At 72 hours, they were 18.3, 0.8 and 11.7, respectively. At any of the readings made at 24, 48 and 72 hours there was: 1) discernable opacity or ulceration of the cornea other than a slight dulling of the normal luster, 2) inflammation of the iris other than slight deepening of the folds, or slight circumcorneal injection, 3) a diffuse, deep-crimson red appearance of the conjunctivae, with individual vessels not easily discernable, and 4) an obvious swelling of the conjunctivae excluding cornea and iris, with partial eversion of the lids. There also was tissue destruction or irreversible change of tissue in 24 hours or less.
Test condition	:	Test material (0.1 ml) was applied to eyes of 6 rabbits. Eyes were evaluated 24, 48 and 72 hours later. They do not appear to have been washed. The test was conducted according to an "FHSA procedure" and eyes were scored according to the method of Draize et al. (J Pharmacol Exper Ther 82:377, 1944). The maximum possible scores for eye irritation reactions (excluding necrosis) are cornea: 80; iris: 10; and conjunctivae: 20.
Test substance	:	It is assumed that the material was the commercially isolated material that contains 11-13% methyl isobutyl ketone.
Reliability	:	(1) valid without restriction The study was conducted according to an established method.

14.05.2003 (1)

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	:	sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	34 days (males), 43-57 days (females)
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	15, 50 and 200 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	< 15 mg/kg bw
LOAEL	:	= 15 mg/kg bw
Method	:	OECD combined study TG422

Year : 2004
GLP : yes
Test substance : as prescribed by 1.1 – 1.4

Remark : Doses used in this study were based on the results of a preliminary study in which 250, 500 and 1,000 mg/kg/day test material was given by gavage at approximately the same time each day for 14 days. Mortality and clinical signs of toxicity occurred at 500 and 1,000 mg/kg/day. Test substance-related reductions in body weight gain and weight loss occurred at 250, 500, and 1000 mg/kg/day. Test substance-related gross lesions in the liver and stomach were observed in animals in these groups. Based on the results, dosages of 0, 15, 50, and 200 mg/kg/day were selected for the current study. The 50 mg/kg/day level was expected to produce no or minimal toxic effects. The 200 mg/kg/day level was expected to produce some systemic toxicity but no mortality. The 15 mg/kg/day level was expected to be the no observed-adverse-effect level (NOAEL).

The methods and results listed in this summary are for the repeated dose toxicity endpoints of the OECD 422 study. Methods and results for the reproductive and developmental toxicity endpoints are listed in their respective sections. The male and female rats utilized for pathology (gross pathology, organ weights, and histopathology) were also used as the P1 sires and dams in the Reproductive/Developmental Toxicity Screening Test (see Sections 5.8.1 and 5.8.2).

The reproductive and developmental methodologies and results are discussed in Sections 5.8.1 and 5.8.2, respectively.

Result : Test material stability and homogeneity: Results from the analysis of the test formulation samples indicated that the test substance was uniformly mixed, at the expected concentrations, stable in the vehicle for 5 hours at room temperature, and stable after 10 days of refrigeration at all dose levels. The stability of the test substance over the course of the study was confirmed by analyses conducted near the beginning and end of the study. No evidence of instability, such as a change in color or physical state, was observed.

Mortality and time to death: One male and 8 females died before their scheduled termination. The male death (200 mg/kg/day) and 1 female death (200 mg/kg/day) were dosing accidents. The cause of death was determined by gross findings (tracheal fluid) and microscopic observations (tracheal exudates and hemorrhage). The incidence of unscheduled female deaths (found dead, euthanized in extremis [i.e., unscheduled termination], and accidentally killed) was dose-related and included 0/10, 1/10, 2/10, and 5/10 females in the 0, 15, 50, and 200 mg/kg/day dose groups, respectively. The causes of death were, based on gross and microscopic pathology, recorded as dystocia (see Section 5.8.1). The incidence of dystocia-related deaths was 0/10, 1/10, 2/10, and 3/10 in the 0, 15, 50, and 200 mg/kg/day dose groups, respectively. The 200 mg/kg/day female with an “undetermined” cause of death was found dead on day 16 with severe autolysis. This death was interpreted to be most likely test substance related.

Body weight: There were test substance-related effects on body weight and body weight gain in males receiving 200 mg/kg/day. Body weight gain was 45% lower than control for the entire dosing period (days 1-34) as well as for the premating period (65% lower than control for days 1-15) at this dose level. Although weekly mean body weight gain was generally lower throughout dosing, it was most marked during the first week (97% lower than control for days 1-8). In addition, weekly mean body weights were 8%-10% lower than control throughout the study and were consistent with the reduced weight gain at this dose level. There were no test substance-related effects on body weights and body weight gains in males exposed to

15 or 50 mg/kg/day.

There were reductions in body weight gain in females during premating at 200 mg/kg/day and during gestation at all dose levels; these effects were considered test substance-related despite lack of statistical significance. The lower mean weight gain during premating (days 1-15) at 200 mg/kg/day was due to one animal (Animal Number 792) that displayed marked weight loss during this period and was subsequently found dead on test day 16. Maternal body weight gain for the entire gestation period (days 0- 21) was 16%, 16%, and 27% lower than control at 15, 50, and 200 mg/kg/day, respectively, as a result of a few animals in each group displaying low weight gain. There were no test substance-related effects on maternal body weight or weight gain during lactation days 0-4 at any dose level.

Food/water consumption: There were test substance-related effects on food consumption and food efficiency in males treated with 200 mg/kg/day. Food consumption was 17% lower than control for the premating period (days 1-15) at this dose level, as a result of lower food consumption during the first week of dosing (29% lower than control for days 1-8). The lower food efficiency during premating (days 1-8 and 1-15) at 200 mg/kg/day is consistent with the reduced weight gain and food consumption at this dose level.

There were reductions in food consumption and food efficiency during premating in females treated with 200 mg/kg/day and on food consumption during gestation in females treated with 50 or 200 mg/kg/day. These effects were considered test substance-related despite lack of statistical significance. The lower mean food consumption during premating (days 1-15) at 200 mg/kg/day was mainly due to one animal (Animal Number 792) that displayed marked weight loss and reduced food consumption during this period and was subsequently found dead on test day 16. The lower food efficiency during premating (days 1-8 and 1-15) at 200 mg/kg/day is consistent with the reduced weight gain and food consumption at this dose level. Maternal food consumption for the entire gestation period (days 0-21) was 10%-12% lower than control at 50 and 200 mg/kg/day, as a result of weekly food consumption that was 8%-15% lower than the mean weekly values for the control group. There were no test substance-related effects on food consumption or food efficiency during lactation days 0-4 at any dose level.

Description, severity, time of onset and duration of clinical signs: There were test substance-related clinical observations in males at 200 mg/kg/day, which consisted of lung noise (3/10 animals), diarrhea (3/10 animals), hunched over posture (2/10 animals) and stained fur (6/10 animals). In addition to displaying these signs, one male animal also had irregular respiration and dehydration. There were no substance-related clinical observations in males at 15 or 50 mg/kg/day.

Substance-related clinical observations occurred during premating in high dose females. These consisted of stained fur (4/10 animals), and diarrhea and hunched over posture (1/10 animals). Other clinical signs of toxicity observed during premating occurred in 1 high dose female immediately prior to death on test day 16, and included irregular respiration, stained/wet fur, diarrhea, and dehydration. During premating (days 1-15) this animal also lost 44 grams of body weight and had low food consumption (12.5 grams/day). There were test substance-related clinical observations in females during gestation at all dose levels; these signs were associated with dystocia in one animal at each level and included dehydration, diarrhea, immobility/lethargy, pallor, ptosis and stained/wet fur. Other test substance-related clinical observations were observed in a few females during gestation and lactation at 200 mg/kg/day and included irregular respiration and lung noise.

Hematological findings, incidence and severity: There were no adverse changes in hematologic parameters in males or females. The decrease in platelets in males dosed with 200 mg/kg/day (mean was 79% of control group mean; statistically significant) was not considered to be adverse due to the small magnitude of change. Numerical platelet counts were only available for 2 of the rats in this group (Animal Numbers 753 and 765). The platelet counts in these 2 males were lower than those of any other male on this study. The 2 other males (Animal Numbers 754 and 775) had inaccurate and low platelet counts due to platelet clumps. Historical platelet counts from approximate age-matched controls range from 925-1447 x10E3/uL (mean of 1149 x10E3/uL). The platelet count of 1 of the 2 males dosed with 200 mg/kg/day was minimally below the historical range for individual values, while the other one was just inside the range.

Clinical biochemistry findings, incidence and severity: There were no adverse changes in clinical chemistry parameters in male or female rats. Cholesterol concentration was minimally increased in some males dosed with 15, 50, or 200 mg/kg/day (not statistically significant at any dose, and not dose-related across the treatment groups). Cholesterol concentrations of individual males generally fell in the upper part of the range of control values. In addition, values for three males in each treated group were above the highest individual male in the concurrent control group. Therefore, increased cholesterol concentrations in some males of all three treated groups were considered to be treatment related. Cholesterol concentration was also minimally increased in some females dosed with 15 or 50 mg/kg/day, and in all females dosed with 200 mg/kg/day (statistically significant only at 200 mg/kg/day). Cholesterol concentrations of most of the rats treated with test substance were greater than the highest individual female in the concurrent control group. Therefore, increased cholesterol concentrations in most females of all 3 treated groups were considered to be treatment-related. Due to the minimal degree of change, all changes in cholesterol were considered to be non-adverse.

Gross pathology, incidence and severity: There were no test substance-related gross observations in this study. All gross observations at necropsy were interpreted to be either naturally occurring background lesions that are typical of rats of this age and stock or gross lesions associated with dosing accidents or dystocia (see Section 3.8.1).

Organ weight changes: A test substance-related increase in mean liver weight parameters was observed in male and female rats given 50 mg/kg/day or more of the test material. In males, mean relative (liver/body) liver weights were increased 7%, 16%, and 45% in rats given 15, 50, and 200 mg/kg/day, respectively (as compared to control values). In females, mean relative liver weights were increased 3%, 11%, and 21% in rats given 15, 50, and 200 mg/kg/day, respectively (as compared to control values). The increased values were statistically significant in both sexes at 200 mg/kg/day (absolute and relative weights) and in males at 50 mg/kg/day (relative weight). Increased liver weight parameters were considered test substance-related in males and females given 50 and 200 mg/kg/day. The liver weight effect correlated with hepatocellular hypertrophy observed microscopically in males given 50 mg/kg/day and females given 200 mg/kg/day (see microscopic findings). All other differences in organ weight values were considered unrelated to test substance administration.

Histopathology incidence and severity: Test substance-related microscopic findings were present in the liver and thyroid of male and female rats. Hypertrophy of centrilobular hepatocytes (graded as minimal) was observed in the livers of 6/10 males and 10/10 females given 200 mg/kg/day and 3/10 males given 50 mg/kg/day of the test substance. Microscopically, hepatocellular hypertrophy was characterized by an increased amount of

finely granular eosinophilic cytoplasm within centrilobular hepatocytes. There was no histomorphologic evidence of hepatocellular damage, and hepatic serum enzyme levels were not elevated. Thus, hepatocellular hypertrophy (and the associated increase in liver weights) was considered a test substance-related pharmacological response to the metabolism of the material and not adverse.

Minimal hypertrophy of thyroid follicular cells was present in 4/6 males and 1/9 females given 200 mg/kg/day of the test material. Although the incidence of minimal hypertrophy observed in the high-dose females (1/9) was within the range of normal, it was considered to be most likely treatment-related, since there was no hypertrophy observed in any other group in this study and the effect in males was unequivocal. Follicular cell hypertrophy was characterized by an increase in the height of the columnar epithelium of the thyroid follicles. Follicles were decreased in size, irregular in shape, and contained decreased amounts of normal pink colloid.

Functional observational battery (FOB): Test substance-related decreases in hindlimb grip strength (males only) and motor activity (males and females) occurred at 200 mg/kg/day. There were no effects at any dosage in P1 males or females on forelimb grip strength, hindlimb foot splay, rearing, or any of the other behavioral parameters evaluated in the FOB.

Test condition : Animals: On February 19, 2004, 45 male and 45 female Crl:CD®(SD)IGS BR rats, with an assigned birth date of December 29, 2003 were received from Charles River Laboratories, Inc., Raleigh, North Carolina for use on this study. The rats were approximately 52 days old upon arrival and weighed approximately 210.1-241.9 grams (males) and approximately 176.5-211.0 grams (females) the day after arrival. Male and female rats were nonsiblings; females were nulliparous. Upon arrival at the laboratory, the rats were quarantined for 6 days of the 13-day pretest period. They were observed daily for any clinically apparent signs of disease or injury and weighed once. On the basis of acceptable body weight gains and clinical observations, all surviving rats were released from quarantine on test day -8 (February 24, 2004) and randomly assigned to 4 treatment groups based on weight. The randomization resulted in a distribution in which the mean body weights for all groups within a sex were not statistically different ($p > 0.05$). Rats not assigned to a test group were euthanized and discarded without pathological evaluation (except for 2 males that were sent to necropsy for a gross examination and blood collection for serology).

The rats were approximately 65 days old at the start of treatment (March 3, 2004), with body weights ranging from 276.6–385.6 grams (males) and 216.0-248.5 grams (females). All animals were housed individually during non-mating or delivery/lactation periods in stainless steel, wire-mesh cages suspended above cageboards. During breeding, all rats were housed in pairs in similar cages. Females were housed individually in polycarbonate pans with bedding from day 20 of gestation through delivery and lactation. Animal rooms were maintained at an acceptable temperature of 18-26ºC (targeted at 22º-24ºC) and relative humidity of 30%-70% (targeted at 40%-60%). Animal rooms were artificially illuminated (fluorescent light) on a 12-hour light/dark cycle (approximately 0600-1800 hours). Occasional excursions outside the accepted ranges were minor and did not affect the study. All rats were fed pelleted PMI® Nutrition International, Certified Rodent LabDiet® 5002 ad libitum, except when fasted. Tap water from United Water Delaware was provided ad libitum. Water and feed were analyzed periodically and found to contain no contaminants that would affect the outcome of the study.

Test material: Test material was mixed with technical grade polyethylene glycol (PEG 400) at 1.88, 6.25 and 25 mg/ml to provide for administration of 15, 50 and 200 mg/kg bw/day. The formulations were adjusted for sample

purity. The dosing volume was 8 ml/kg. Formulations of the test substance in the vehicle were prepared daily and stored refrigerated until used. Once 10-day refrigerated storage stability was established, formulations were prepared once a week and stored refrigerated.

Samples from dosing formulations prepared March 3, 2004 containing A-1846 at concentrations of 1.88, 6.25 and 25.0 mg/ml were collected for homogeneity/concentration verification and 5- hour room temperature stability analysis. Samples from the same preparation of the dosing formulations were collected on March 12, 2004 after 10 days of refrigeration for concentration verification and 5-hour room temperature stability analysis. Whenever samples were collected, a sample of the vehicle was also collected and analyzed. Concentrations of A-1846 in dosing formulations were measured by high performance liquid chromatography (HPLC).

Study design: The animals were randomly allocated by weight to 4 groups of 10 animals per sex receiving either 0 (vehicle), 15, 50 or 200 mg/kg/day. All animals were dosed once daily by gavage for 14 days prior to cohabitation and during the cohabitation period (up to 2 weeks).

During breeding, each female was housed on a 1:1 basis with a randomly selected, nonsibling male of the same dosage level, in the male's cage. Animals were cohoused until evidence of copulation was observed (intravaginal copulation plug or sperm in a daily vaginal lavage sample) or until 2 weeks had elapsed. The day evidence of copulation was observed was designated as day 0 of gestation. A second pairing with a proven male of the same dose group was performed for 2 pairs each in the 50 and 200 mg/kg/day groups due to lack of evidence of copulation during the first pairing. Due to severe clinical signs of toxicity on test day 15, animal Number 792 was not cohoused and was subsequently found dead on test day 16. Male and female rats showing no evidence of copulation continued to be dosed after the end of the cohabitation period until termination. Females showing evidence of copulation were dosed throughout gestation. Pregnant females in the process of delivery or showing signs of delivery were not dosed. Females were dosed after delivering litters, until day 3 postpartum.

Parameters evaluated: Cage-site examinations to detect moribund or dead animals and abnormal behavior and/or appearance were performed on all animals at least once daily during quarantine and predosing and twice daily thereafter. All animals were examined daily at approximately the same time of day (± 2 hours) for acute/systemic toxicity and signs of delivery. Once during pretest (baseline), and weekly thereafter at approximately the same time of day (+ 2 hours), animals were individually handled and examined for abnormal behavior and appearance. Observations included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling, presence of clonic, tonic, stereotypical, or bizarre behavior.

All females were weighed once a week prior to mating and at termination. P1 females were weighed on days 0, 7, 14, and 21 of gestation and on days 0 and 4 of lactation. Females without evidence of copulation, those that copulated and did not deliver a litter, and males were weighed on a weekly schedule. Individual food consumption was determined weekly throughout the premating period, ending on test day 15. Food consumption was not measured during cohabitation for males and females or after cohabitation for males. Individual food consumption of pregnant females was recorded on gestation days 0, 7, 14, and 21, and for lactating females on lactation days 0 and 4. Food consumption was not measured for females without evidence of copulation or for those that did not deliver a

litter.

Functional observational battery (FOB) observations were performed once on 5 randomly selected male rats per group on day 29 (near the end of the dosing period), and once on 5 randomly selected female rats per group on day 14 (near the end of the premating period). The FOB included the following observations: home cage (acclimation, posture, convulsions/ tremors, palpebral (eyelid) closure, gait/coordination), handling (ease of removal from cage, lacrimation, exophthalmus, vocalizations, salivation, fur/skin appearance, piloerection, dehydration, emaciation, mucous membranes, muscle tone), open field (righting reflex, vocalizations, posture, palpebral closure, tremors, diarrhea, gait/coordination, polyuria, rate/ease of respiration, convulsions, muscle spasms/fasciculation) sensory (tail pinch, auditory response, pupillary constriction and foot splay), grip strength (hindlimb and forelimb. Motor activity (MA) measurement was conducted immediately following the FOB assessment.

A clinical pathology evaluation was conducted on the first 5 surviving rats/sex/group approximately 14 days after initiation of the study (hematology and clinical chemistry) and at termination (coagulation only). Only 4 males could be evaluated in the high-dose group on day 14 due to the death of 1 male on test day 3 and because the 5 last males in the group were undergoing neurobehavioral evaluation on that day.

The day before collection of samples for hematology and clinical chemistry, these animals were fasted after 3 p.m. (at least 15 hours). Blood samples for hematology (complete blood count, red blood cell count, red cell distribution width, hemoglobin, absolute reticulocyte count, hematocrit, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total and differential white blood cell count, prothrombin time, activated partial thromboplastin time) and clinical chemistry (aspartate aminotransferase, alanine aminotransferase, urea nitrogen, creatinine, cholesterol, glucose , total protein, albumin, total bile acids, sodium, potassium) measurements were collected from the orbital sinus of each animal while the animal was under carbon dioxide anesthesia. Blood samples for coagulation parameters were collected at termination (day 34 for males, and day 4 of lactation [days 43-57] for females) from the abdominal vena cava of each animal (non-fasted) while the animal was under carbon dioxide anesthesia. Additional blood collected from the vena cava was placed in a serum tube, processed to serum, and frozen at -80°C. Serum was discarded without analysis because further tests were not required to support experimental findings. All blood samples were evaluated for quality by visual examination prior to analysis. Results were maintained in the study records and reported only if the sample was analyzed.

Surviving male rats (up to 10/sex/group) were euthanized and necropsied on test day 34. Surviving female rats (up to 10/sex/group) were euthanized and necropsied on day 4 of lactation. The following tissues were collected from rats that were found dead or accidentally killed, euthanized in extremis, and the first 5 males and 5 females that survived to termination: liver, spleen, stomach, thymus, duodenum, mandibular lymph node, testes, jejunum, mesenteric lymph node, epididymides, ileum, bone marrow, prostate, cecum, seminal vesicles, colon, endocrine system, coagulating glands, rectum, thyroid gland, adrenal glands, kidneys, ovaries (with oviducts), urinary bladder, cervix, brain (3 sections) uterus, spinal cord (3 levels), vagina, lungs, sciatic nerve, trachea, heart, femur. Gross observations, suspected target organs (liver and stomach), and organs of the reproductive system were collected from all rats that survived to termination.

The testes and epididymides of all males terminated at the end of the study were weighed. The following tissues were weighed from the first 5 rats from each group:liver, kidneys, adrenal glands, thymus, spleen, brain, and heart. Relative organ weights (percent of final body weight; ratio to brain weight) were calculated. Final body weights, determined just prior to necropsy, were used in the assessment of organ weight changes. Organs from rats found dead, euthanized in extremis, or accidentally killed were not weighed. Organs were inadvertently weighed from an additional (i.e., 6th) 15 mg/kg/day rat (Animal Number 832) that survived to termination but was not one of the first 5 rats. These data were included in the weight data tables and were used in the calculation of the means.

Testes and epididymides were fixed in Bouin's solution. All other tissues were fixed in 10% neutral buffered formalin. Processed tissues were embedded in paraffin, sectioned approximately 5-6 microns thick, stained with hematoxylin and eosin (H&E), and examined microscopically by a veterinary pathologist. All collected tissues from the first 5 terminal rats/sex in the control and high-dose (200 mg/kg/day) groups were examined microscopically. All tissues from rats found dead, euthanized in extremis, or accidentally killed were similarly examined. Reproductive tissues and gross observations were examined from all males and females. Target organs that were collected from all rats (liver) were examined from all rats. Target organs that were not collected from all rats (thyroid) were examined for the rats from which they were collected (the first 5 terminal rats/sex/dose and all unscheduled deaths). Non-target organs that were initially suspected to be target organs based on the previous 2-week range-finding study (stomach) were examined from all control and high-dose males and females (10/sex/group).

Statistical evaluations: Body weight, body weight gain, food consumption, food efficiency, clinical pathology and organ weight data were analyzed by Levene's test for homogeneity and the Shapiro-Wilk test for normality. Parametric data were analyzed by the one-way analysis of variance and Dunnett's test, and nonparametric data were analyzed by the Kruskal-Wallis test and Dunn's test. Clinical observation and FOB data (with the exception of motor activity, grip strength, foot splay and rearing) were analyzed by the Cochran-Armitage test for trend. Motor activity, grip strength, foot splay and rearing were analyzed for normality and homogeneity as described above. Parametric data were analyzed by a repeated measured analysis of variance, followed by linear contrasts. Nonparametric data were analyzed by sequential application of the Jonckheere-Terpstra trend test, unless a normalizing, variance stabilizing transformation could be found and the repeated measures analysis of variance repeated. The critical level of significance was $p < 0.05$.

Test substance : A-1846 was stable over the course of the study. The average amount of active ingredient was 99.8% ± 2.0 in samples analyzed March 5, 2004 and April 30, 2004, respectively.

Reliability : (1) valid without restriction
OECD Guideline study

Flag : Critical study for SIDS endpoint.

01.11.2004 (14)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test

System of testing : S. typhimurium strains TA98, TA100, TA1535 and TA1537 and E. Coli WP2uvrA-

Test concentration : 5 to 1500 micrograms/plate for Salmonella strains; 50 to 5000 micrograms/plate for E. coli

Cytotoxic concentr. : 500 micrograms/plate (without S-9) and 1500 micrograms/plate (with S-9) for Salmonella strains; 5000 micrograms/plate for E. coli
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 471
Year : 2000
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : This study fills the mutagenicity endpoint.
Result : The preliminary experiment revealed that the test material was toxic at concentrations > = 500 and 1500 micrograms/plate in strain TA100 without and with S-9, respectively. Although the background lawn was reduced somewhat in WP2uvrA- exposed to 5000 micrograms/plate (without S-9), toxicity was not considered to be excessive. Therefore, 1500 and 5000 micrograms/plate were the upper limits of concentrations in the mutation study with Salmonella and E. coli, respectively.

In both experiments, there was no significant increase in the number of mutants in any strains incubated with any concentration of test material (with or without S-9). All of the positive controls induced at least a 3-fold increase in the number of revertants. No precipitate was observed on the plates in the presence or absence of S-9. Spontaneous mutation rates in negative controls were acceptable.
Test condition : The Salmonella strains were obtained from the University of California at Berkeley, and E coli strain WP2uvrA- was obtained from the British Biological Research Association. Bacteria were stored frozen until used. Prior to use, characterization checks were carried out to confirm the amino-acid requirement, presence of rfa, R factors, uvrA or uvrB mutation and the spontaneous reversion rate. Overnight sub-cultures were prepared in nutrient broth and incubated at 37 degrees C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

The test material was weighed and approximate half-log dilutions prepared in dried dimethyl sulfoxide. A preliminary study using 0.15 to 5000 micrograms/plate was used to determine the toxicity of the material. Based on the results, the mutation study with the 4 Salmonella strains was conducted with 5, 15, 50, 150, 500, and 1500 micrograms/plate, and the study with E. coli was performed with 50, 150, 500, 1500 and 5000 micrograms/plate.

Measured aliquots (0.1 ml) of each of the bacterial cultures were dispensed into sets of test tubes followed by 2.0 ml of molten, trace histidine supplemented top agar, 0.1 ml of the test material formulation, vehicle or positive control [2, 3 or 5 micrograms/plate N-ethyl-N'-nitro-N-nitirosoguanidine (ENNG) for E. coli WP2uvrA- and Salmonella strains TA100 and TA1535 without S-9, respectively; 80 micrograms/plate 9-aminoacridine (AA) for strain TA1537 without S-9; 0.2 micrograms/plate 4-nitroquinoline-1-oxide (4NQO) for strain TA98 without S-9; 1 or 10 micrograms/plate 2-aminoanthracene (2AA) for Salmonella strain TA100 and E. coli WP2uvrA- with S-9, respectively; 2 micrograms/plate 2AA for strains TA1535 and TA1537 with S-9; and 5 micrograms/plate benzo(a)pyrene (BP) for strain TA98 with S9], and either 0.5 ml S9-mix or phosphate buffer. S-9 was prepared in-house from the livers of male Sprague-Dawley rats that had been orally induced with phenobarbitone/ beta-naphthoflavone. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). The procedure was repeated in triplicate, for each bacterial strain and for each concentration of test material (with and without S-9 mix).

All plates were incubated at 37 degrees for approximately 48 hours and the frequency of revertant colonies assessed using a Domino colony counter.

A second experiment was performed using the same test conditions as in the first.

An experiment was considered valid if all vehicle and untreated controls had numbers of spontaneous revertants within historical ranges, the appropriate characteristics of each tester strain were confirmed, all tester strain cultures had approximately 1 to 9.9 x 10E9 bacteria/ml before treatment, positive controls induced at least a 2-fold increase in revertants, there was a minimum of 4 non-toxic test material doses, and there was no evidence of excessive contamination.

A test material was considered positive in the assay if the material induced a reproducible, dose-related and statistically (Dunnett's method of linear regression) significant increase in revertants in at least one strain.

Test substance : The material was described as "purified A-1846". The purity of the test substance was listed as >99% 6-tert-butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and <1% 6-tert-butyl-2,4-xylenol

Reliability : (1) valid with restrictions

Flag : Critical study for SIDS endpoint

07.05.2003 (22)

Type : Ames test
System of testing : S. typhimurium strains TA-98, TA-100, TA-1535, and TA-1537 and E. coli strain WP2uvrA-
Test concentration : 1000 micrograms/plate
Cytotoxic concentr. : 1000 micrograms/plate
Metabolic activation : with and without
Result : negative
Method : other
Year : 1978
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The results of the tests were negative. Large zones of inhibition were present on all plates in the disc test.

Test condition : Test material was dissolved in ethanol. A disc test was performed on all strains and a plate test was performed on strains TA-1535 and TA-1537. The material was tested with and without metabolic activation. Only one dose was tested (1000 mg/plate or disc). No other study details were given.

Test substance : Purity of the test material was 90.1%. Impurities were not listed. The concentration tested was based on 100% active material.

Reliability : (4) not assignable
There are not enough details to assign a reliability rating.

07.05.2003 (2)

Type : Chromosomal aberration test
System of testing : human lymphocytes
Test concentration : 17.5, 35, 70, 105, 140 and 280 micrograms/ml without S9 and 35, 70, 140, 210, 280 and 420 micrograms/ml with S9.
Cytotoxic concentr. : 140 micrograms/ml
Metabolic activation : with and without
Result : positive
Method : Other: OECD Test Guideline 473 and Method B10 of the EC Commission Directive 92/69/EC
Year : 2004
GLP : yes

Test substance : as prescribed by 1.1 – 1.4

Result : Preliminary test: Metaphase cells were present up to 283.38 and 566.75 micrograms/ml in the 4(20)-hour treatments in the absence and presence of S9, respectively. The maximum concentration with metaphases present in the 24-hour continuous exposure was 70.84 micrograms/ml. A precipitate of test material was observed in the parallel, blood-free cultures at concentrations > = 566.75 micrograms/ml in the 4(20)-hour pulse exposure groups and at concentrations >= 283.38 micrograms/ml in the continuous exposure group. Based on the results, a 4(20)-hour pulse design with maximum concentrations of 280 micrograms/ml (without S9) and 420 micrograms/ml (with S9) were chosen for the definitive study.

Definitive test: Scorable metaphases were present at concentrations up to 140 micrograms/ml in the absence of metabolic activation. In the presence of metabolic activation, the maximum concentration with scorable metaphases was 210 micrograms/ml. In the absence or presence of S9, the amount of mitotic inhibition observed in cultures treated with 140 micrograms/ml was 52% and 48%, respectively.

The frequencies of vehicle control cells with aberrations were similar to historical controls. The positive controls resulted in significant increases in the frequencies of cells with aberrations (34% for mitomycin C and 32% for cyclophosphamide). The test material caused a significant increase in the frequency of cells with aberrations at 105 (6% in treated vs. 0.5% in control) and 140 micrograms/ml (4.5% in treated vs. 0.5% in control) in the absence of S9 and 140 micrograms/ml (10.0% in treated vs. 0% in control) in the presence of S9. In both treatment groups, the predominant aberrations (other than gaps) were chromatid breaks and exchanges. The incidence of cells with polyploidy was 0% in all evaluated cultures.

Test condition : Cells: Whole blood was withdrawn from the peripheral circulation of a volunteer who had been previously screened for suitability. The volunteer had not been exposed to high levels of radiation or hazardous chemicals and had not knowingly recently suffered from a viral infection. The cell-cycle time for the lymphocytes from the donor used in the experiment was determined using bromodeoxyuridine incorporation to assess the number of first, second and third division metaphase cells and calculate the average generation time. The average generation time for the regular donors used in the laboratory was approximately 17 hours under typical experimental conditions.

Duplicate lymphocyte cultures for each test condition were established by mixing 0.65 – 0.75 ml heparinized whole blood, 0.1 ml Li-heparin and 0.1 ml phytohemagglutinin into 9.05 – 9.15 ml Eagle's Essential Medium with HEPES buffer (MEM) supplemented with L-glutamine, penicillin/ streptomycin, amphotericin B and 15% fetal calf serum and dispensing the mixture into sterile plastic flasks. Cells were grown at 37 degrees C with 5% CO2 in air.

Test materials: The test material was dissolved in dimethyl sulfoxide at a concentration of 10 mM and serially diluted so that addition of 0.1 ml of the diluted solution to the test cultures would result in the required concentrations. There was no significant change in pH when the test material was dosed into the media and the osmolality did not increase by more than 50 mOsm. The positive controls in the absence and presence of S9 were mitomycin C (final concentration of 0.4 micrograms/ml) and cyclophosphamide (final concentration 5 micrograms/ml), respectively. Cyclophosphamide was dissolved in dimethylsulfoxide before use.

Microsomal enzyme fraction: S9 was prepared from the livers of male, Sprague-Dawley rats weighing approximately 250 g. The rats had received three daily oral doses of a mixture of phenobarbitone (80 mg/kg) and beta-

naphthoflavone (100 mg/kg), prior to S9 preparation on the fourth day. The S9 was stored at -196 degrees C until use.

Preliminary toxicity test: A preliminary toxicity test was performed on cell cultures using a 4 hour exposure time with and without metabolic activation, followed by a 20-hour recovery period, and a continuous exposure of 24 hour without metabolic activation. The dose range of test material was 8.86 to 2267 micrograms/ml. Slides of each culture were prepared and examined for mitotic index. Mitotic index data were used to estimate concentrations used for the definitive study. Parallel flasks containing culture medium without whole blood were established to observe possible precipitation of test material at the beginning and end of the exposure period.

Definitive test: After approximately 48 hours of incubation, the cultures were transferred to tubes and cells were pelleted by centrifugation. Approximately 9 ml of the culture medium was removed, reserved, and replaced with the required volume of MEM (including serum) and 0.1 ml of the appropriate solution of vehicle control, test material (for final concentrations of 17.5, 35, 70, 105, 140 and 280 micrograms/ml without S9 and 35, 70, 140, 210, 280 and 420 micrograms/ml with S9) or positive control. One ml of 20% S9 mix was added to the cultures requiring metabolic activation. The total volume for each concentration was 10 ml.

After 4 hours of incubation at 37 degrees C, the cells were pelleted by centrifugation, washed with 8 ml of MEME culture medium, and resuspended in the reserved, original culture medium. The cells were then returned to the incubator for an additional 20 hours. Colcemid (0.1 micrograms/ml) was added to each culture two hours prior to harvest to arrest cells in metaphase.

At harvest, cells were sedimented by centrifugation, culture medium was drawn off and hypotonic KCl (0.075 M) was added to swell the cells. After approximately 14 minutes (including centrifugation), most of the hypotonic solution was drawn off and discarded. The cells were resuspended and then fixed by dropping the KCl cell suspension into fresh methanol:glacial acetic acid (3:1, v/v). The fixative was changed at least three times and the cells stored at 4 degrees C for at least 4 hours to ensure complete fixation. The lymphocytes were resuspended in several ml of fresh fixative, spun in a centrifuge, and resuspended in a small amount of fixative. Several drops of the suspension were dropped onto wet microscope slides and left to air dry. Dry slides were stained with 5% Giemsa for 5 minutes, rinsed, dried and coverslipped using mounting medium. Each slide was labeled and coded.

The slides were examined under a microscope for toxicity, extent of precipitation and quality of the metaphases. A total of 2000 lymphocyte cell nuclei were counted and the number of cells in metaphase recorded and expressed as the mitotic index. The first 100 consecutive, well-spread metaphases from each culture chosen for evaluation (70, 105 and 140 micrograms/ml without S9 and 35, 70 and 140 micrograms/ml with S9) were scored for the presence of chromosome aberrations. If approximately 50% of the cells had aberrations, counting was terminated at 50 cells. If the cell was diploid (44-48 chromosomes), any gaps, breaks or rearrangements were noted according to the International System to Chromosome Nomenclature (1985). Cells with aberrations were reviewed as necessary by a senior cytogeneticist prior to decoding.

Data evaluation: The frequencies of cells with aberrations excluding gaps and the frequency of polyploid cells in test cultures was compared to control using the Fisher's Exact test. The critical level of significance was $p < 0.01$.

Test substance : The test material was A-1846. It contained > 98% 6-tert-Butyl-3-

(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

(20)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : mouse
Sex : male
Strain : CD-1
Route of admin. : gavage
Exposure period : 24 or 48 hours
Doses : 250, 500 or 1000 mg/kg
Result : negative
Method : Other: OECD Test Guideline 474 and Method B12 of the EC Commission Directive 2000/32/EEEC
Year : 2005
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : This is the critical study for the chromosome aberrations endpoint.

A preliminary toxicity study was conducted in males and females gavaged with 1000 or 2000 mg/kg bw. Premature death, hunched posture, lethargy, ptosis, ataxia, prostration, decreased respiratory rate, laboured respiration, ataxia, tip-toe gait and distended abdomen were noted in the 2000 mg/kg bw group. Symptoms of toxicity in animals dosed with 1000 mg/kg bw were hunched posture, lethargy and ptosis. There was no marked difference in the toxicity of the material in males and females. Therefore, the micronucleus study was only conducted in males. Based on the results of the preliminary test, the highest concentration chosen for use in the micronucleus study was 1000 mg/kg bw.

Result : None of the animals died. Clinical signs observed in animals treated with concentrations ≥ 250 mg/kg were hunched posture, lethargy and ptosis.

The results of the study are shown in the table below. There was no evidence of a significant increase in the incidence of micronucleated PCE in animals dosed with the test material, as compared to vehicle controls. The positive control group showed a marked increase in the incidence of micronucleated cells, indicating that the study was valid. A significant decrease in the PCE/NCE ratio and clinical signs were observed in the 24 hour 1000 mg/kg bw group, indicating that systemic absorption and exposure to the bone marrow had occurred.

Group	Number of micronucleated PCE/2000 PCE (mean +/- SD)	PCE/NCE Ratio (mean +/- SD)
Vehicle control[a]	1.4 +/- 1.0	0.68 +/- 0.28
Vehicle control[b]	0.9 +/- 1.5	0.79 +/- 0.31
A-1846		
250 mg/kg[a]	0.9 +/- 0.9	0.75 +/- 0.26
500 mg/kg[a]	0.9 +/- 1.2	0.72 +/- 0.12
1000 mg/kg bw[a]	1.3 +/- 1.1	0.71 +/- 0.15
1000 mg/kg bw[b]	1.4 +/- 2.1	0.52 +/- 0.14*
Positive control[a]	32.0 +/- 12.1***	1.64 +/- 0.54

[a] 24 hours ; [b] 48 hours
* = $p < 0.05$, *** = $p < 0.001$

Test condition : Animals: Male Crl:CD-1(ICR)BR mice (5-8 weeks old, 20-39 g) were acclimated for a minimum of 7 days before being used in the test. They were housed in groups of up to seven in solid-floor polypropylene cages with woodflake bedding and allowed free access to drinking water and food (Certified Rat and Mouse Diet Code 5LF2, BCM, IPS Limited, London, UK). The temperature and relative humidity were set to fall between 19-25 degrees C and 30-70%, respectively. Any occasional deviations fro these limits were not considered to have affected the study. The rate of air exchange was approximately 15 changes/hr. A 12 hour light/dark cycle was maintained.

Study conduct: The mice were randomized into 3 treatment groups of 7 animals (250, 500 and 1000 mg/kg bw), an additional group of 7 high dose animals, one positive control group of 5 animals (50 mg/kg bw cyclophosphamide) and two negative control groups of 7 animals (Arachis oil). For each dose of test material, a solution was prepared by adding the material to Arachis oil. The test and control materials were administered by gavage in a volume of 10 ml/kg. One group of mice from each dose level was killed by cervical dislocation 24 hour after treatment and the additional group of high dose animals was killed after 48 hours. The two vehicle control groups were killed 24 hours after dosing and the positive control group was killed 24 hour after dosing. All animals were observed for signs of overt toxicity and death one hour after dosing, once daily (as applicable), and immediately prior to termination.

Immediately following termination, both femurs were removed from each mouse and aspirated with fetal calf serum. Bone marrow smears were prepared following centrifugation and resuspension. The smears were air dried, fixed in absolute methanol, stained in Giemsa, allowed to air dry, and coverslipped using mounting medium.

Slides from each animal were coded and blindly evaluated for the presence of micronuclei. Two thousand polychromatic erythrocytes (PCE) and one thousand normochromatic erythrocytes (NCE) from each slide were screened for micronuclei under a microscope (at unlisted magnification). The ratio of PCE/NCE was calculated and used as an index of toxicity of the test material.

Statistical analyses: All data were statistically analyzed using appropriate methods as described by the UKEMS Sub-committee on Guidelines for Mutagenicity Testing Report, Part III (1989). The data were analyzed following a square root transformation [of (x + 1)], using Student's t-test (two tailed). Any significant results were uncovered using a one-way analysis of variance (ANOVA).

A positive response occurred when a statistically significant, dose-dependent, toxicologically relevant increase in the number of micronucleated PCE was observed at either 24 or 48 hours, compared to the control group. The test was considered to be negative if these criteria were not fulfilled. A positive response to bone marrow toxicity was demonstrated when the dose group mean PCE/NCE ratio was significantly lower than the concurrent vehicle control group.

Test substance : The test material was A-1846. It contained > 98% 6-tert-Butyl-3-(chloromethyl)-2,4-xylenol (CAS# 23500-79-0) and < 2% of unidentified impurities.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
18.02.2005 (6)

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 : 34 days (males), 43-57 days (females)
Frequency of treatm. : daily
Premating exposure period
Male : 14 days
Female : 14 days
Duration of test : to lactation day 4
No. of generation studies : 1
Doses : 15, 50 and 200 mg/kg bw/day
Control group : yes, concurrent vehicle
NOAEL parental : <15 mg/kg bw
NOAEL F1 offspring : = 15 mg/kg bw
Method : OECD Guideline 422
Year : 2004
GLP : yes
Test substance : as prescribed by 1.1 – 1.4

Remark : The NOAELs for systemic and developmental toxicity are < 15 mg/kg bw/day and 15 mg/kg/day, respectively. The repeated dose and developmental toxicity components of this study are discussed in detail in Sections 5.4 and 5.8.2, respectively.

The male and female rats used as the P1 sires and dams were also utilized for pathology (see Section 5.4).

Result : The NOAEL for systemic toxicity was <15 mg/kg bw/day, due to the mortality and clinical signs associated with dystocia (difficult labor) and reduced body weight gain in females at all dose levels during gestation. Systemic effects noted at 50 and 200 mg/kg bw/day are described in detail in Section 5.4.

The NOAEL for reproductive effects was <15 mg/kg/day due to the occurrence of dystocia at all dose levels. However, there were no adverse reproductive effects seen in the males. All mating and fertility indices at any

dose level were within the normal range. Therefore, this material caused reproductive toxicity effects only in the female rats.

There was no embyrolethality or gross abnormalities of the pups noted in this study. Maternal toxicity was seen at all dose levels. The accompanying reduced pup weights at 50 and 200 mg/kg/day are therefore not considered to be clear evidence of an effect. This material is not classified as a developmental toxicant.

Test condition : Animals: On February 19, 2004, 45 male and 45 female Crl:CD®(SD)IGS BR rats, with an assigned birth date of December 29, 2003 were received from Charles River Laboratories, Inc., Raleigh, North Carolina for use on this study. The rats were approximately 52 days old upon arrival and weighed approximately 210.1-241.9 grams (males) and approximately 176.5-211.0 grams (females) the day after arrival. Male and female rats were nonsiblings; females were nulliparous. Upon arrival at the laboratory, the rats were quarantined for 6 days of the 13-day pretest period. They were observed daily for any clinically apparent signs of disease or injury and weighed once. On the basis of acceptable body weight gains and clinical observations, all surviving rats were released from quarantine on test day -8 (February 24, 2004) and randomly assigned to 4 treatment groups based on weight. The randomization resulted in a distribution in which the mean body weights for all groups within a sex were not statistically different ($p > 0.05$). Rats not assigned to a test group were euthanized and discarded without pathological evaluation (except for 2 males that were sent to necropsy for a gross examination and blood collection for serology).

The rats were approximately 65 days old at the start of treatment (March 3, 2004), with body weights ranging from 276.6–385.6 grams (males) and 216.0-248.5 grams (females). All animals were housed individually during non-mating or delivery/lactation periods in stainless steel, wire-mesh cages suspended above cageboards. During breeding, all rats were housed in pairs in similar cages. Females were housed individually in polycarbonate pans with bedding from day 20 of gestation through delivery and lactation. Animal rooms were maintained at an acceptable temperature of 18-26ºC (targeted at 22º-24ºC) and relative humidity of 30%-70% (targeted at 40%-60%). Animal rooms were artificially illuminated (fluorescent light) on a 12-hour light/dark cycle (approximately 0600-1800 hours). Occasional excursions outside the accepted ranges were minor and did not affect the study. All rats were fed pelleted PMI® Nutrition International, Certified Rodent LabDiet® 5002 ad libitum, except when fasted. Tap water from United Water Delaware was provided ad libitum. Water and feed were analyzed periodically and found to contain no contaminants that would affect the outcome of the study.

Test material: Test material was mixed with technical grade polyethylene glycol (PEG 400) at 1.88, 6.25 and 25 mg/ml to provide for administration of 15, 50 and 200 mg/kg bw/day. The formulations were adjusted for sample purity. The dosing volume was 8 ml/kg. Formulations of the test substance in the vehicle were prepared daily and stored refrigerated until used. Once 10-day refrigerated storage stability was established, formulations were prepared once a week and stored refrigerated.

Study design: The animals were randomly allocated by weight to 4 groups of 10 animals per sex receiving either 0 (vehicle), 15, 50 or 200 mg/kg/day. All animals were dosed once daily by gavage for 14 days prior to cohabitation and during the cohabitation period (up to 2 weeks).

During breeding, each female was housed on a 1:1 basis with a randomly selected, nonsibling male of the same dosage level, in the male's cage. Animals were cohoused until evidence of copulation was observed (intravaginal copulation plug or sperm in a daily vaginal lavage sample) or

until 2 weeks had elapsed. The day evidence of copulation was observed was designated as day 0 of gestation. A second pairing with a proven male of the same dose group was performed for 2 pairs each in the 50 and 200 mg/kg/day groups due to lack of evidence of copulation during the first pairing. Due to severe clinical signs of toxicity on test day 15, animal Number 792 was not cohoused and was subsequently found dead on test day 16. Male and female rats showing no evidence of copulation continued to be dosed after the end of the cohabitation period until termination. Females showing evidence of copulation were dosed throughout gestation. Pregnant females in the process of delivery or showing signs of delivery were not dosed. Females were dosed after delivering litters, until day 3 postpartum.

Parameters evaluated: Cage-site examinations to detect moribund or dead animals and abnormal behavior and/or appearance were performed on all animals at least once daily during quarantine and predosing and twice daily thereafter. All animals were examined daily at approximately the same time of day (± 2 hours) for acute/systemic toxicity and signs of delivery. Once during pretest (baseline), and weekly thereafter at approximately the same time of day (+ 2 hours), animals were individually handled and examined for abnormal behavior and appearance. Observations are described in Section 5.4

All females were weighed once a week prior to mating and at termination. P1 females were weighed on days 0, 7, 14, and 21 of gestation and on days 0 and 4 of lactation. Females without evidence of copulation, those that copulated and did not deliver a litter, and males were weighed on a weekly schedule. Food consumption was measured as described in Section 5.4.

The day when delivery was complete was designated day 0 postpartum. At each examination period (days 0 and 4 postpartum), offspring were individually handled and examined for abnormal behavior and appearance; any dead or abnormal pups were recorded. Dams that had no live pups remaining during lactation were euthanized. Live and dead pups in each litter were counted by sex as soon as possible after delivery was completed. Live pups in each litter were counted by sex, individually weighed and examined grossly. All pups were euthanized by decapitation.

Functional observational battery (FOB) observations were performed once on 5 randomly selected male rats per group on day 29 (near the end of the dosing period), and once on 5 randomly selected female rats per group on day 14 (near the end of the premating period) as described in Section 5.4.

A clinical pathology evaluation was conducted on the first 5 surviving rats/sex/group approximately 14 days after initiation of the study (hematology and clinical chemistry) and at termination (coagulation only). Only 4 males could be evaluated in the high-dose group on day 14 due to the death of 1 male on test day 3 and because the 5 last males in the group were undergoing neurobehavioral evaluation on that day.

The day before collection of samples for hematology and clinical chemistry, these animals were fasted after 3 p.m. (at least 15 hours). Blood samples for hematology and clinical chemistry measurements were collected and analyzed for parameters described in Section 5.4

Surviving male rats (up to 10/sex/group) were euthanized and necropsied on test day 34. Surviving female rats (up to 10/sex/group) were euthanized and necropsied on day 4 of lactation. Organs described in Section 5.4, including testes, seminal vesicles, ovaries (with oviducts), cervix, uterus, and vagina were collected. Selected organs, including the testes and epididymides (see Section 5.4) from the first 5 rats from each group were

weighed. Final body weights, determined just prior to necropsy, were used in the assessment of organ weight changes. Organs from rats found dead, euthanized in extremis, or accidentally killed were not weighed. Organs were inadvertently weighed from an additional (i.e., 6th) 15 mg/kg/day rat (Animal Number 832) that survived to termination but was not one of the first 5 rats. These data were included in the weight data tables and were used in the calculation of the means.

Testes and epididymides were fixed in Bouin's solution. All other tissues were fixed in 10% neutral buffered formalin. Processed tissues were embedded in paraffin, sectioned approximately 5-6 microns thick, stained with hematoxylin and eosin (H&E), and examined microscopically by a veterinary pathologist. All collected tissues from the first 5 terminal rats/sex in the control and high-dose (200 mg/kg/day) groups were examined microscopically. All tissues from rats found dead, euthanized in extremis, or accidentally killed were similarly examined. Reproductive tissues and gross observations were examined from all males and females. Additional organs that were examined are described in Section 5.4.

Statistical evaluations: Statistical analyses performed on repeated dose toxicity parameters are described in Section 5.4. Gestation length, numbers of implantation sites, corpora lutea counts, implantation efficiency, mean number of pups per litter, percent born alive, and 0-4 day viability data were analyzed by Levene's test for homogeneity and the Shapiro-Wilk test for normality. Parametric data were analyzed by the one-way analysis of variance and Dunnett's test, and nonparametric data were analyzed by the Kruskal-Wallis test and Dunn's test. The mating, fertility and gestation indices were analyzed by the Cochran-Armitage test for trend. Sex Ratio and Mean Pup Weight (covariates: litter size, sex ratio) were analyzed using the Exact Mann-Whitney test with a Bonferroni-Holm adjustment. Male and female parental data were evaluated separately. For litter parameters, the proportion of affected pups per litter or the litter mean were used as the experimental unit for statistical evaluation. The critical level of significance was $p < 0.05$.

Test substance : A-1846 was stable over the course of the study. The average amount of active ingredient was 99.8% ± 2.0 in samples analyzed March 5, 2004 and April 30, 2004, respectively.

Reliability : (1) valid without restriction
OECD Guideline study

Flag : Critical study for SIDS endpoint.

01.11.2004 (14)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	34 days (males), 43-57 days (females)
Frequency of treatm.	:	daily
Duration of test	:	14 days prior to mating to lactation day 4
Doses	:	15, 50 and 200 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	< 15 mg/kg bw
NOAEL teratogen.	:	= 200 mg/kg bw
Result	:	not a developmental toxicant
Method	:	other: OECD Guideline 422
Year	:	2004
GLP	:	yes
Test substance	:	as prescribed by 1.1 – 1.4

Remark : The NOAELs for systemic and reproductive toxicity were < 15 mg/kg bw/day. The repeated dose and fertility components of this study are discussed in detail in Sections 5.4 and 5.8.1.

The male and female rats used as the P1 sires and dams were also utilized for pathology (see Section 5.4).

Result : The NOAEL for systemic toxicity was <15 mg/kg bw/day, due to the mortality and clinical signs associated with dystocia (difficult labor) and reduced body weight gain in females at all dose levels during gestation. Systemic effects noted at 50 and 200 mg/kg bw/day are described in detail in Section 5.4.

The NOAEL for reproductive effects was <15 mg/kg/day due to the occurrence of dystocia at all dose levels. However, there were no adverse reproductive effects seen in the males. All mating and fertility indices at any dose level were within the normal range. Therefore, this material caused reproductive toxicity effects only in the female rats.

There was no embyrolethality or gross abnormalities of the pups noted in this study. Maternal toxicity was seen at all dose levels. The accompanying reduced pup weights at 50 and 200 mg/kg/day are therefore not considered to be clear evidence of an effect. This material is not classified as a developmental toxicant.

Test condition : Animals: On February 19, 2004, 45 male and 45 female Crl:CD®(SD)IGS BR rats, with an assigned birth date of December 29, 2003 were received from Charles River Laboratories, Inc., Raleigh, North Carolina for use on this study. The rats were approximately 52 days old upon arrival and weighed approximately 210.1-241.9 grams (males) and approximately 176.5-211.0 grams (females) the day after arrival. Male and female rats were nonsiblings; females were nulliparous. Upon arrival at the laboratory, the rats were quarantined for 6 days of the 13-day pretest period. They were observed daily for any clinically apparent signs of disease or injury and weighed once. On the basis of acceptable body weight gains and clinical observations, all surviving rats were released from quarantine on test day -8 (February 24, 2004) and randomly assigned to 4 treatment groups based on weight. The randomization resulted in a distribution in which the mean body weights for all groups within a sex were not statistically different ($p > 0.05$). Rats not assigned to a test group were euthanized and discarded without pathological evaluation (except for 2 males that were sent to necropsy for a gross examination and blood collection for serology).

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Reliability : (1) valid without restriction
OECD Guideline study

01.11.2004 (14)

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

(1) Brown DR (1979). Antioxidant A-1846 Range Finding Toxicity Tests: Single Oral Dose, Single Dermal Dose, Skin Irritation, Eye Irritation. Carnegie-Mellon University Institute of Research, Report No 42-556, for American Cyanamid Company, dated July 26 (unpublished study).

(2) Caterson C (1978). Mutagenicity test report (Ames Salmonella test) for A-1846. Report Number M78-170 from CRD Division, Plastics Additives Department, American Cyanamid (unpublished study).

(3) Cytec Industries, Inc. (2000). Material safety data sheet for A-1846, dated 10/30/2000.

(4) Cytec Industries, Inc. (2003). Communication.

(5) Driscoll R (2000). Purified A-1846 (CT-684-00). Acute oral toxicity in the rat - acute toxic class method. SafePharm Laboratories Limited Project Number 971/115 for Cytec Industries Inc., dated October 3 (unpublished study).

(6) Durward R. 2005. A-1846 (CT-800-03): Micronucleus test in the mouse, SafePharm Laboratories Project Number: 971/277, for Cytec Industries Inc., dated January 20, 2005

(7) EPIWIN Aop Program (v1.90).

(8) EPIWIN ECOSAR (v0.99).

(9) EPIWIN Fugacity Level III Program

(10) EPIWIN Kowwin (v1.66).

(11) EPIWIN Mpbpwin (v1.40).

(12) EPIWIN Wskow (v1.40).

(13) EPIWRSIN Biowin (v4.00).

(14) Mylchreest E (2004). A-1846: Combined Repeated Dose Toxicity Study With the Reproduction/Developmental Toxicity Screening Test in Rats. E.I. du Pont de Nemours and Company Haskell Laboratory for Health and Environmental Sciences, Laboratory Project ID DuPont-14109, dated October 19, 2004 (unpublished study).

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(17) SafePharm Laboratories (2004). A-1846 (CT-800-03): Acute toxicity to Daphnia Magna. SPL Project Number: 971/240, dated Aug 11, 2004 (unpublished study).

(18) SafePharm Laboratories (2004). A-1846 (CT-800-03): Acute toxicity to rainbow trout (Oncorhynchus mykiss). SPL Project Number: 971/239, dated July 27, 2004 (unpublished study).

(19) Safepharm Laboratories (2004). A-1846 (CT-800-03): Assessment of ready biodegradability; CO2 evolution test. SPL Project Number: 971/242, dated June 9, 2004 (unpublished study).

(20) Safepharm Laboratories (2004). A-1846 (CT-800-03): Chromosome aberration test in human lymphocytes in vitro. SPL Project Number: 971/238, dated September 7, 2004, (unpublished study).

(21) Shaffer CB (1980). Antioxidant A-1846 skin irritation test (D.O.T.). Carnegie-Mellon University Institute of Research Report No. 43-502 to American Cyanamid Company, dated January 31 (unpublished study).

(22) Thompson PW (2000). Purified A-1846 (CT-684-00). Reverse mutation assay "Ames Test" using Salmonella Typhimurium and Escherichia Coli. SafePharm Laboratories Limited Project Number 971/116 for Cytec Industries Inc., dated October 30 (unpublished study).

(23) Tremain SP (2004). Determination of Vapour Pressure, Project Number 971/237, SafePharm Laboratories (unpublished study).

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT