

201-15741B

Revised Robust Summaries for

**Propanoic acid, 2-hydroxy-, compd. with 3-[2-(dimethylamino)ethyl]
1-(2-ethylhexyl) (4-methyl-1,3-phenylene)bis[carbamate] (1:1)**

CAS No. 68227-46-3

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Existing Chemical ID: 68227-46-3
CAS No. 68227-46-3

Producer Related Part
Company: PPG Industries, Inc
Creation date: 09-JAN-2003

Substance Related Part
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1.0.1 Applicant and Company Information

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Country: United States
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09-JAN-2003

1.1.0 Substance Identification

1.1.1 General Substance Information

1.1.2 Spectra

1.2 Synonyms and Tradenames

1.3 Impurities

1.4 Additives

1.7 Use Pattern

2.1 Melting Point

2.2 Boiling Point

2.3 Density

2.4 Vapour Pressure

Value: = .00000003492 hPa at 25 °C

Method: other (calculated)

GLP: no

Remark: The vapor pressure was estimated using the EPIWIN/MPBPWIN Program. The vapor pressure calculation was done by the modified Grain method.

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

10-JAN-2003

2.5 Partition Coefficient

Partition Coeff.: octanol-water

log Pow: = 4.38

Method: other (calculated)

Year: 2003

GLP: no

Remark: The Log Kow was calculated using the EPIWIN/WSKow Program.

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

09-JAN-2003

2.6.1 Solubility in different media

Solubility in: Water
Value: = 281.383 g/l at 20 °C
pH value: = 4.1
Descr.: very soluble (> 10000 mg/L)

Method: Directive 92/69/EEC, A.6
Year: 2004
GLP: yes
Test substance: other TS
Deg. product: not measured

Test condition: Triplicate saturated solutions of test material in Milli RO water was prepared for each successive time point (24h, 48h, and 72h). Samples were incubated at 30 ± 0.2 °C. During this incubation period, the samples were continuously shaken (ca 150 r.p.m.). On completion of the incubation period, the samples were removed and allowed to equilibrate in a water bath to 20 ± 0.2 °C for 24 hours. On completion of the equilibration period, the pH of each sample was recorded. The samples were then centrifuged (3000 r.p.m.; 5 min.). An aliquot of each supernatant was then diluted 250000 fold with 0.1% phosphoric acid in Milli Q water/acetonitrile (9:1, v/v) and analyzed, in duplicate.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.

Reliability: (1) valid without restriction

07-DEC-2004

(1)

2.6.2 Surface Tension

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2.7 Flash Point

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2.8 Auto Flammability

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2.9 Flammability

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2.10 Explosive Properties

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2.11 Oxidizing Properties

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2.12 Dissociation Constant

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2.13 Viscosity

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2.14 Additional Remarks

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3.1.1 Photodegradation

Type: air
Light source: other
DIRECT PHOTOLYSIS
Half-life t1/2: = 1.1 hour(s)

Method: other (calculated)
Year: 2003
GLP: no

Method: The half-life was calculated using the EPIWIN/AOPWIN Program. The hydroxyl radical rate constant was calculated to be 113.7966 E-12 cm³/molecule-sec.
Reliability: (2) valid with restrictions
Data were obtained by modeling.

10-JAN-2003

3.1.2 Stability in Water

Type: abiotic

Method: other (calculated)
Year: 2003
GLP: no

Method: Aqueous base/acid catalyzed hydrolysis was calculated using the EPIWIN/HYDROWIN Program. Total Kb for pH >8 at 25 °C was calculated to be 1.343E+1 L/mol-sec with a half-life calculated to be 14.339 hours.

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

09-JAN-2003

3.1.3 Stability in Soil

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3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
Media: water - air
Year: 2003
Air: .0032 % (**Fugacity Model Level I**)
Water: 14.4 % (**Fugacity Model Level I**)
Soil: 79.1 % (**Fugacity Model Level I**)

Method: The EPIWIN Program was used to conduct Level III fugacity modeling. A mass amount of 6.52% is estimated for sediment using the same model.

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

09-JAN-2003

3.3.2 Distribution

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, domestic
Concentration: 20 mg/l related to DOC (Dissolved Organic Carbon)
Contact time: 28 day(s)
Degradation: = 10.6 % after 28 day(s)
Result: under test conditions no biodegradation was observed
Deg. product: not measured

Method: OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year: 2004
GLP: yes
Test substance: other TS

Remark: A preliminary test was conducted to assess the inhibitory effects of test material to the microbial inoculum at the proposed test concentration. This test was conducted using a total of 4 bioreactors, one each for control, test item, reference item and toxicity control. Both the test and reference items were introduced to the system at 20 mg DOC/L. Traps were removed and titrated on Days 2, 4, and 5 for the control and reference and on Day 5 for the test and toxicity control. In the preliminary study, the test material was not considered to be inhibitory to the microbial inoculum under the test condition as 35% biodegradation was observed in the toxicity control bioreactor on Day 5.

Result: The test material attained 10% degradation after 28 days and failed to achieve a transition from 10% to 60% degradation in a 10 day window. Therefore, it can not be considered as readily biodegradable. The standard material, sodium benzoate, was readily biodegradable as 62% degradation was achieved on Day 6. The test item is not considered to be inhibitory to the microbial inoculum as >25% biodegradation (37%) was observed in the toxicity control on Day 14. The test material exhibited only negligible abiotic degradation (2%) by the end of the test.

Test condition: The test material was exposed to sewage treatment micro-organisms with culture medium in sealed vessels in the dark at a temperature of 20 °C for 28 days. A total of 7 bioreactors were used; 2 each for test material and control and one each for the toxicity control, abiotic control, and reference item. The test material was introduced to the test system at an addition rate of 20 mg DOC/L which required 62.5 mg of test material per 2 liter bioreactor. The reference item, sodium benzoate was also introduced to the test system

at 20 mg DOC/L which required 69 ml of a 1 g/L stock solution per 2 liter bioreactor. The test material was dissolved directly in culture medium. A toxicity control, containing the same concentrations of test material and sodium benzoate as in the test and reference bioreactor was prepared in order to assess any toxic effect of the test material on the sewage treatment micro-organisms used in the study. In addition, a control consisting of inoculated culture medium was included in the study. To prepare bioreactors, 1600 ml of mineral medium, 20 ml of microbial inoculum, and appropriate weight of test or reference material were added to the appropriate bioreactors and mineral medium was added to make up to a final volume of 2000 ml. The abiotic control was prepared in an identical manner to the other bioreactors, without the addition of microbial inoculum. A sterilizing agent (37% formalin) was also added to the abiotic control bioreactor to ensure any degradation exhibited was not attributable to a microbial population.

Each bioreactor was connected to 3 traps, each containing 100 ml of 0.0125M Ba(OH)₂. At trap collection the trap closest to the bioreactor was taken for titration and the 2 remaining traps were moved one place closer to the bioreactor. A new trap was then placed third in line. Trap changes were conducted on days 2, 4, 6, 9, 12, 14, 19, 21, 24, and 29. Each sample trap was then titrated with a few drops of phenolphthalein indicator solution against 0.05M HCL. The pH was determined in each reactor on Days 0, 28, and 29.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.

Reliability: (1) valid without restriction

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(2)

3.7 Bioaccumulation

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3.8 Additional Remarks

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AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: = 1.72 measured/nominal
EC50: = 4.2 measured/nominal

Method: OECD Guide-line 202
Year: 2004
GLP: yes
Test substance: other TS

Remark: Concentrations for the test were selected based on the results of range finding test. A range finding test was conducted over 48 hour at 0.1, 1.0, 10, and 100 mg/l with an untreated control. Duplicate vessels, each containing 5 Daphnia were prepared. After 48 hour, all Daphnia were immobile at the 10 and 100 mg/l concentrations.

Result: Mean measured concentrations for the exposure period were 0.36, 0.79, 1.72, 3.77, and 8.88 mg/l for the 0.46, 1.0, 2.14, 4.56, and 9.97 mg/l concentration levels, which were between 78 and 89% of nominal. Results from 48 hour samples showed that the concentrations were stable over the test period. Water quality parameters remained within acceptable limits over the test: pH 7.6-7.7, temperature 19.6-21.0°C, conductivity 652-687 uS/cm and dissolved oxygen 80.7-93.2% air saturation value. Water hardness was determined in a sample of the medium used to prepare the test solutions at initiation of the test as 270 mg CaCO₃/L.

After 24 hour, 20% (4 daphnia) were immobilized at 8.88 mg/l concentration. After 48 hour, 20 Daphnia (100%) were immobile at the 8.88 mg/l concentration and 4 Daphnia (20%) were immobile at 3.77 mg/l concentration. At the lowest concentration tested, 0.36 mg/l, there were 2 Daphnia immobile after 48 hour, however, this is not regarded as an effect of the test material as this is within the permitted effect levels for controls and it does not follow a standard concentration curve. The 24 hour EC₅₀ was >8.88 mg/l, the highest concentration tested. The 48 hour EC₅₀ was 4.2 mg/l.

Test condition: A test was conducted with twenty Daphnia at each treatment group and control, as 4 replicates of 5, at 0.64, 1.4, 3.0, 6.4, and 14 mg/l total test material with an untreated control. These concentrations were equivalent to 0.46, 1.0, 2.14, 4.56, and 9.97 mg/l of test material (corrected for 71.2% purity). Test solutions were prepared by parallel

dilution of a 100 mg/l stock solution. The stock solution was prepared by adding a weighed amount of test material to ElenDt M4 Daphnia medium and bringing to volume in a 250 ml metric flask. This was then placed in an ultrasonic bath for approximately 10 minutes to aid dissolution. Four vessels were prepared for each treatment group and control, each containing 100 ml of media. Five Daphnia were added to each vessel. Water quality parameters (pH, temperature, conductivity and dissolved oxygen concentration) were measured in one vessel for each treatment group at 24 intervals during the test. Test vessels were observed for any immobile Daphnia at 24 and 48 hour after initiation. Test vessels were maintained within a temperature controlled laboratory. A light cycle of 16 hour light and 8 h dark was in operation throughout the test. Samples were taken from freshly prepared test material and control media for chemical analysis at test initiation and from pooled samples from all replicates at 48 hour of the study. Statistical analysis of the observed immobilization data at each time point compared to the nominal concentration was conducted.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.
Reliability: (1) valid without restriction
 09-DEC-2004 (3)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Selenastrum capricornutum (Algae)
Endpoint: biomass
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: < .02 measured/nominal
EC10: measured/nominal
EC50: = .07

Method: EPA OPPTS 850.5400
Year: 2004
GLP: yes
Test substance: other TS

Remark: Test solution concentrations were selected based on the range finding test. In the range finding test, growth was inhibited by 16.6, 5.2, 16.3, and 99.3% at 0.001, 0.01, 0.1, and 1.0 mg/l respectively as indicated by cell numbers when compared with control cultures.

Result: Test material analyses showed that the mean measured concentrations at the initiation of the exposure phase were 0.03, 0.06, 0.14, 0.32 and 0.67 mg/l, which is the 100 to 103% of nominal values. After 96 hour exposure, the measured test concentrations were 0.01, 0.02, 0.09, 0.22, and 0.56 mg/l, which corresponded to 33, 33, 64, 71, and 84% of nominal values. Since measured concentrations of test material were clearly reduced compared to nominal, the results of the study

are based on the geometric mean of the initial and 96 hour analytical concentrations. These were 0.02, 0.03, 0.11, 0.27, and 0.61 mg/l. Samples from satellite vessels prepared at the lowest and highest concentrations without algae indicated that the test material may be absorbing/adsorbing to the biomass of algal cells. The 96 hour EC₅₀ value of test material for area under the growth curve (AUC) was calculated to be 0.07 mg/l. The No Observed Effect Concentration (NOEC) at 96 hour based on AUC was < 0.02 mg/l. The 96 hour EC₅₀ value based on growth rate was calculated to be 0.16 mg/l. The NOEC at 96 hour based on growth rate was 0.11 mg/l. The effect of the test material to algal cells was phytostatic and not phytotoxic, as no significant differences were found in growth rate after 9 days in fresh untreated medium. However, the cells in the two test material treatments were both noted to have formed clumps visible to the naked eye, compared to the controls where growth was normal.

Test condition: The test was conducted over 96 hour (exposure phase) and included a re-inoculation phase of 9 days. The test was conducted with nominal test material concentrations of 0.04, 0.09, 0.2, 0.43, and 0.94 mg/l total test material; these concentrations were equivalent to 0.03, 0.06, 0.14, 0.31, and 0.67 mg/l (corrected for 71.2% test material purity). An untreated control was also tested. A 100 mg/l test material solution was prepared by adding a weighed amount of test material to a flask and bringing to volume with ISO freshwater algal medium. This solution was sonicated for approximately 10 minutes to aid dissolution and used to prepare the test solutions. Flasks were prepared in triplicate for each treatment group with six flasks for the controls. Each flask contained 100 mL of media and was inoculated with *Pseudokirchneriella subcapitata*. The initial cell concentration in the test cultures was approximately 1×10^4 cells/mL. After 96 hour of exposure, test flasks from the 0.67 and 0.31 mg/l treatment groups were inoculated into fresh medium at the rate of 1:19 (exposure medium/fresh medium) and incubated for 9 days with the re-inoculated controls. The cultures and test vessels were maintained with an illuminated cooled orbital shaking incubator at a temperature range of 22-26°C. The algae was provided with continuous uniform illumination obtained from white fluorescent lamps. Light intensity on the shaker table was checked at 3 different places prior to the test and ranged from 4000 to 4300 lux. Algal cell concentrations were determined in each flask at 24, 48, 72, and 96 hour during the exposure phase and on Day 9 of the re-inoculation phase. Samples for analysis were taken from all concentrations and the controls at the start of the study and after 96 hour. Analyses were conducted for all collected samples. The pH of cultures were recorded at the beginning and end of the test.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.

Reliability: (1) valid without restriction

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4.4 Toxicity to Microorganisms e.g. Bacteria

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Endpoint: reproduction rate
Exposure period: 21 day(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: = .365 measured/nominal
EC50 Day 7 : = .69 measured/nominal

Method: OECD Guide-line 211
Year: 2004
GLP: yes
Test substance: other TS

Result: The corresponding time weighted mean measured concentrations were 0.015, 0.083, 0.365, 1.369 and 4.653 mg/l, which is equivalent to 30.0, 52.9, 73.3, 87.4 and 93.4 % of the nominal concentrations (corrected for purity). All results are based on time weighted means. A complete immobilization was observed in the 1.369 and 4.653 mg/l groups and the data on reproduction is not available for these groups. No decrease in reproduction was noted for all surviving treatment groups. The Day 7 EC₅₀ to Daphnia reproduction was estimated as 0.69 mg/l. As all parental daphnia were dead at the two higher concentrations and no reproduction effect was noted at 0.365 mg/l, the EC₅₀ for these time points was estimated to be between 0.365 mg/l to 0.69 mg/l (between the NOEC and Day 7 EC₅₀).

The Day 21 No Observed Effect Concentration (NOEC) to Daphnia reproduction was 0.365 mg/l, under the conditions of the test.

Water quality parameters remained within the following range over the duration of the test: pH 7.26-7.92; temperature 19.2-22.3°C and dissolved oxygen 75.0-98.4 % ASV (air saturation value).

Test condition: A reproduction test was conducted at 0.07, 0.22, 0.7, 2.2 and 7.0 mg/l test material, with an untreated control. These concentrations are equivalent to 0.050, 0.157, 0.498, 1.566 and 4.984 mg/l, corrected for test item purity (71.2%). Ten Daphnia were exposed at each test concentration, with 20 for the untreated control. The Daphnia were <24 h old at test initiation and were housed singly for the duration of the test. Daphnia cultures were maintained in a synthetic medium

(Elendt M4). All testing was conducted using this medium. Samples were taken for chemical analysis at the beginning and end of 3 replacement periods over the duration of the test, on Days 0 and 3; 7 and 10; and 19 and 21 (for fresh and expired solutions respectively). Daphnia were fed on a diet of concentrated green algae (*Chlorella vulgaris*) for both culturing and testing. During the reproduction test Daphnia were fed daily at a rate of ca 0.1 0.2 mg C/parent Daphnia/day. Each vessel contained ca 60 mL of test or control media, as appropriate. The test was conducted under semi-static conditions, with media replacement conducted three times weekly (Monday, Wednesday and Friday).

All vessels were observed daily throughout the duration of the test for adult Daphnia health and neonate production. Neonates were discarded after counting. Males, where present, were discarded and discounted from the results. Daphnia were recorded as immobile if no movement was observed within 15 s following gentle agitation of the test vessel. Water quality parameters were measured at media change in a single replicate for each treatment group, prior to addition of the Daphnia, and in expired media for all vessels. Test vessels were maintained within a temperature-controlled laboratory with the aim of achieving a temperature in the range 18-22°C (maximum range of 2°C per vessel). A light cycle of 16 h light and 8 h dark was in operation throughout the test. Test vessels were not aerated during the test.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.
Reliability: (1) valid without restriction
09-DEC-2004 (5)

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

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4.6.2 Toxicity to Terrestrial Plants

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4.6.3 Toxicity to Soil Dwelling Organisms

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4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

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4.7 Biological Effects Monitoring

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4. Ecotoxicity

date: 09-DEC-2004
Substance ID: 68227-46-3

4.8 Biotransformation and Kinetics

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4.9 Additional Remarks

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5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: Sprague-Dawley
Sex: female
No. of Animals: 15
Vehicle: water
Doses: 175, 550 or 2000 mg/kg
Value: = 2000 mg/kg bw

Method: OECD Guide-line 425
Year: 2004
GLP: yes
Test substance: other TS

Remark: Prior to initiation of this acute oral study, the Neutral Red Uptake Bioassay was conducted to estimate a starting dose level. The Neutral Red Uptake Bioassay is used to quantitatively measure the toxicity of a test material to Balb/c 3T3 cultures by comparing the neutral red dye (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) uptake of test material treated Balb/c 3T3 cultures. The estimated LD₅₀ of the test material was determined to be 489 mg/kg. Based on this result, the starting dose level for acute oral toxicity study was set as 175 mg/kg.

Result: Mortality was noted as follows:

<u>Test sequence</u>	<u>Dose level (mg/kg)</u>	<u>Day of Death</u>
1	175	15 (Terminal Sacrifice)
2	550	15 (Terminal Sacrifice)
3	2000	15 (Terminal Sacrifice)
4	2000	3 (Found dead)
5	550	15 (Terminal Sacrifice)
6	2000	15 (Terminal Sacrifice)
7	2000	2 (Humanely Sacrificed)
8	550	15 (Terminal Sacrifice)
9	2000	15 (Terminal Sacrifice)
10	2000	2 (Humanely Sacrificed)
11	550	15 (Terminal Sacrifice)
12	2000	15 (Terminal Sacrifice)
13	2000	2 (Found dead)
14	550	15 (Terminal Sacrifice)
15	2000	2 (Found dead)

No adverse clinical signs were noted at 175 mg/kg. At 550 mg/kg, clinical signs were noted in 3 out of 5 animals from approximately 2 hour after dosing to Day 2. These included piloerection, staggering and/or noisy breathing. At 2000 mg/kg, clinical signs were noted from approximately 30 minutes after dosing up to the end of the observation period (Day 15).

The majority of clinical signs were noted from 2 hour after dosing up to Day 2 when 4 out of 5 premature deaths occurred. Predominant clinical signs included piloerection, cold to touch, labored breathing, staggering, subdued behavior, tremors and hunched posture. At 2000 mg/kg, 2 out of the 4 surviving animals had lost up to 25% of their Day 1 body weight by Day 15. No effects on body weight were observed for the other 2 animals receiving 2000 mg/kg or for animals receiving 175 or 550 mg/kg. At necropsy, in animals receiving 2000 mg/kg, one animal, which was humanely killed on Day 2, was noted to have its stomach distended by food, liquid and a small amount of gas and one animal surviving to the end of the observation period, had red staining on the skin around the muzzle. No other necropsy findings were observed in any other animals.

Under the conditions of the study, the median lethal oral dose (LD₅₀) in Sprague-Dawley rats is estimated to be equal to 2000 mg/kg.

Test condition: The acute oral toxicity was evaluated after a single gavage administration to rats. Based on the result from a in-vitro cytotoxicity test indicating a LD₅₀ of 489 mg/kg, the starting dose level was set as 175 mg/kg. Animals were treated at least 2 days apart, subsequent dose levels were selected based on the survival of the previously dosed animal. The test material was administered using water for irrigation as the vehicle, at a constant dose volume of 10 mL/kg. The animals were deprived of food overnight before dosing. Food was returned 3-4 hour after dosing. Animals were observed daily for signs of reaction to treatment for up to 14 days after dosing. Body weights were recorded weekly. Animals were subject to a necropsy examination either at premature death or on Day 15. Animals were killed by exposure to carbon dioxide and exsanguinated.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.

Reliability: (1) valid without restriction

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5.1.2 Acute Inhalation Toxicity

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5.1.3 Acute Dermal Toxicity

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5.1.4 Acute Toxicity, other Routes

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5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

5.2.2 Eye Irritation

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: 28 days
Frequency of treatment: once per day
Post exposure period: No
Doses: 2, 7.5, and 30 mg/kg/day
Control Group: yes, concurrent vehicle
NOAEL: = 2 mg/kg bw

Method: OECD Guide-line 407 "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-d Study"
Year: 2004
GLP: yes
Test substance: other TS

Remark: Dose levels were set after evaluation of a preliminary study (One Week Oral (Gavage) Dose Range Finding Study in Rats). Dose level selection took into account the maximum tolerated dose in the test model and other factors such as anticipated human exposure. In One Week Oral (Gavage) Dose Range Finding Study in Rats, dosing Sprague-Dawley rats at dose levels up to 1000 mg/kg/day resulted in body weight reduction and correlating food consumption performance in animals treated with as low as 30 mg/kg/day when compared to Controls.

Result: Analyzed concentrations were found to be within ±10% of the nominal concentration, indicating acceptable accuracy of formulation. The low coefficient of variation (2% or less) was indicative of satisfactory homogeneity. There was one premature decedent from the 2 mg/kg/day group during the study. Retrobulbar haemorrhage secondary to orbital venipuncture was considered to be the cause of death and was not considered to be related to treatment with test material.

Daily oral dosing with the test material for 4 consecutive weeks resulted in an overall reduction in body weight, body weight gain and food consumption performance in both sexes treated at 7.5 mg/kg/day. Decreased body weight gain and food consumption were also observed up to Day 7 of treatment in both sexes dosed at 30 mg/kg/day. However from Day 10 of treatment onwards, group mean body weight and food consumption

in animals treated at 30 mg/kg/day were comparable with Controls. Isolated incidences of excess salivation were noted throughout the study in a number of animals treated at 30 mg/kg/day. There were no treatment related effects in neurotoxicity clinical observations, motor activity, functional observations, water consumption, haematology and clinical chemistry evaluations, urinalysis and organ weights.

Tubular atrophy was found in the testes of 3 animals given 30 mg/kg/day. In Animal 20 the tubular atrophy was unilateral and was accompanied by unilateral agenesis of the epididymis. As a spontaneously occurring finding, unilateral agenesis of the epididymis would be expected to result in tubular atrophy of the testis on the same side. In the 2 other High dose group animals with bilateral tubular atrophy in the testes, sloughing of spermatogenic cells was found in the epididymides. There were no findings in testes and epididymides of animals treated at 7.5 mg/kg/day. Spermatid retention in the testes and sloughing of spermatogenic cells in the epididymides were observed one male treated at 2mg/kg/day. Marked tubular atrophy in the testes and oligospermia in the epididymis was observed in another male treated at 2 mg/kg/day. Available organ weight data confirm histology data.

However, there were no histological findings seen in the testes and epididymides of animals treated at 2 or 7.5 mg/kg/day in a subsequent Reproduction/Developmental Toxicity Screening Test in Rats (Inveresk Study Report 24474) which dosed a similar strain/age of animals used in this study. The findings in testes and epididymides in animals given 2 mg/kg/day may not be related to administration of the test material.

Test condition: Three groups of 5 male and 5 female Sprague-Dawley rats were dosed daily for 4 consecutive weeks by gavage at levels of 2, 7.5 and 30 mg/kg/day. A further group of 5 male and 5 female Sprague-Dawley rats received vehicle (water for irrigation) only and acted as a Control group.

The animals were monitored daily for any signs of ill health or reaction to treatment. Detailed functional observations were performed once during pretrial and during Week 4 of treatment. Additional detailed functional observations were also conducted during pretrial and once weekly up until Week 4. Body weights were recorded once during Pretrial then daily throughout dosing (twice weekly data reported). Food consumption was recorded twice weekly during pretrial and throughout treatment. Water consumption was assessed visually on a weekly basis. Ophthalmoscopic assessments were undertaken on all animals during pretrial and on all Control and High dose animals during Week 4. Urine and blood samples were both collected for laboratory investigations during Week 4. After 4 weeks of treatment, all animals were killed and necropsied. All animals were given a detailed post mortem

examination with major organs being weighed and/or placed in fixative. Tissues from all Control and High dose animals and testes and epididymides from all male animals were examined histologically.

Prior to study commencement, trial formulations of 0.1-200 mg/ml test material were investigated for stability, concentration and homogeneity. Formulations were analyzed on 2 occasions during the study treatment period, on Day 1 and during Week 4. On each occasion, triplicate samples were withdrawn from each formulation (including Control). These samples were analyzed to provide data on concentration and homogeneity.

Body weight, organ weight, food consumption, haematology, clinical chemistry, selected urinalysis data and selected neurotoxicity data were statistically analyzed. Histological incidence data were analyzed using Fisher's Exact Probability Test.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.

Reliability: (1) valid without restriction

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5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA1535, TA98, TA1537, TA100 and Escherichia coli WP2uvrA
Concentration: 1 to 333 ug per plate
Cytotoxic Concentration: 333 µg/plate for in the presence of S9, 50 µg/plate for in the absence of S9
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 2004
GLP: yes
Test substance: other TS

Result: The test material did not induce mutagenic activity in any of the 5 bacterial strains used, in either activation condition. Toxicity, observed as a thinning of the background lawn of microcolonies occurred. In the first assay, toxicity was observed at 333 µg/plate with all four Salmonella strains in the presence of S9 and with all 5 strains in the absence of S9 mix. In the second assay, toxicity was observed at 333 µg/plate with all four Salmonella strains in the presence of S9 mix and at 50 and 167 µg/plate with all Salmonella strains and at 167 µg/plate with E. coli in the absence of S9 mix. Toxicity was also observed as a reduction in the number of revertant colonies. A reduction in colony numbers was observed in TA98, TA100 and TA1535 (second assay only) at 333

Test condition: $\mu\text{g}/\text{plate}$ in the presence of S9 mix. In the absence of S9 mix, a reduction was observed with TA98 and E coli at 167 $\mu\text{g}/\text{plate}$. The test substance was tested for mutagenicity activity in Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 and Escherichia coli WP2uvrA. Two independent tests (one direct plate and one pre-incubation) were conducted on agar plates in the presence and absence of an S-9 mix. The solvent used was dimethylsulphoxide. The metabolic activation system, S-9 mix was prepared from male Fischer rats that had been induced by an injection of Aroclor 1254. For S. typhimurium strains, sterile 1.0 mM L-histidine.HCl/1.0 mM biotin solution was added, 5 ml per 100 ml of soft agar. For E. coli WP2uvrA, 1.0 ml of 1.35 mM L-tryptophan was added per 100 ml agar. These agars were thoroughly mixed prior to use and maintained in a water bath at 45°C. For the direct plate assay, volumes of soft agar (2 ml) were dispensed into small sterile tubes. 0.5 ml of S9 mix or 0.05 M phosphate buffer, pH 7.4, 0.1 ml of bacteria, and 0.1 ml of solvent or test solution were added to the tube and mixed and poured onto minimal medium plates that contained 20 ml of 1.5% purified agar, in Vogel-Bonner Medium E with 2% glucose. When the soft agar had set, the plates were inverted and incubated 37°C for 2 or 3 days. For the pre-incubation assay, volumes of S9 mix or 0.05 M phosphate buffer, pH 7.4 (0.5 ml) were dispensed into sterile tube. This was followed by 0.1 ml of bacteria and 0.1 ml of solvent or test solution. The tubes placed in a shaking incubator at 37°C for 20 minutes. And then, 2 ml of soft agar was added to each tube. The tube contents were mixed and poured onto agar plates. When the soft agar had set, the plates were inverted and incubated at 37°C for 2 or 3 days. The dose levels for the first mutation assay both in the presence and absence of S9 mix and second mutation assay in the presence of S9 mix were 1, 3.3, 10, 33.3, 100, and 333 $\mu\text{g}/\text{plate}$. The dose levels for the second mutation assay in the absence of S9 mix were 0.5, 1.7, 5, 17, 50, and 167 $\mu\text{g}/\text{plate}$. Triplicate plates were prepared for each bacterial strain and dose level. The positive controls in the absence of metabolic activation were N-ethyl-N-nitrosoguanidine (E. coli), sodium azide (TA 100 and TA 1535), 9-aminoacridine (TA 1537), and 2-Nitrofluorene (TA 98). The positive control for in the presence of metabolic activation with all strains was 2-aminoanthracene. The test material was considered a mutagen if the increase was twofold (1.5 fold for TA100) and if a dose related and reproducible increase in the number of revertants over background.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.

Reliability: (1) valid without restriction

09-DEC-2004 (8)

Type: Chromosomal aberration test
System of testing: Chinese hamster ovary cells
Concentration: 5 - 80 µg/ml
Cytotoxic Concentration: 10 µg/ml (without S9) and 22.5 µg/ml (with S9)
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 473
Year: 2004
GLP: yes
Test substance: other TS

Result: The test material was toxic to Chinese hamster ovary cells in vitro in both the presence and absence of S9 mix. It was tested to 40 µg/ml in the presence of S9 mix and to 80 µg/ml in the absence of S9 mix (Test 1). Toxicity was noted at 30-40 µg/ml in the presence of S9 mix and at 20-80 µg/ml in the absence of S9 mix. In Test 2, the test material was tested to 30 µg/ml in the presence of S9 mix and to 25 µg/ml in the absence of S9 mix. Toxicity was noted in cultures treated with 22.5-30 µg/ml (presence of S9 mix) and in cultures treated with 10-25 µg/ml (absence of S9 mix). There was no evidence that the test material induced structural chromosomal aberrations in either the presence or absence of S9 mix.

The test material induced slight increases in polyploidy in the absence of S9 mix, in the cultures harvested 48 h post treatment, at doses of 13.33 and 15 µg/ml with cell survival counts of approximately 24% and 10% respectively and sparse metaphase cells. Slight increases in polyploidy were also seen in the presence of S9 mix with 6 h treatment and 24 h harvest. In Test 1, a slight increase was noted at 25 µg/ml. Although no toxicity was observed at 25 µg/ml in Test 1, toxicity was noted at this concentration in Test 2. A slight increase in polyploidy was noted in Test 2 at 22.5 µg/ml where toxicity was observed. No increases in polyploidy were noted in the cultures treated with 25 µg/ml in Test 2.

It was concluded that test material did not induce structural aberrations when tested with Chinese hamster ovary cells in vitro. Slight increases in polyploidy were noted in the cultures in the absence of S9 mix, 22 h treatment, 48 h harvest and in the presence of S9 mix, 6 h treatment, 24 h harvest. These increases are not likely to be biologically relevant based on the small increases in the number of polyploid cells at or near toxic concentrations and no dose response curve being evident.

Test condition: Chromosomal aberrations assays were performed with duplicate, Chinese hamster ovary (CHO) cell cultures. This study was conducted incorporating 2 independent tests. Dimethylsulphoxide was the vehicle and cyclophosphamide and methyl methanesulphonate were the positive controls used in both tests. Both tests were conducted in the presence and absence of a post-mitochondrial supernatant fraction obtained

from the livers of adult, male rats treated with Aroclor 1254 (S9) and a NADPH-generating system. Dose levels used for test were 5, 10, 15, 20, 25, 30 and 40 µg/ml in the presence of S9 mix and 5, 10, 20, 30, 40, 60 and 80 µg/ml in the absence of S9 mix for the first experiment. In the second experiment, the dose levels selected were 10, 15, 20, 22.5, 25, 27.5 and 30 µg/ml in the presence of S9 mix and 5, 10, 11.67, 13.33, 15, 20 and 25 µg/ml in the absence of S9 mix. Cultures, established approximately 20 h before testing, were treated for 6 h in the presence or 22 h in the absence of S9 mix.

Treatment with the test item was as follows:

<u>S9 Mix</u>	<u>Growth Medium</u>	<u>S9 mix</u>	<u>Dosing Solution</u>	<u>Final volume</u>
with	4.5 ml	0.5 ml	50 µl	~5 ml
without	5 ml	-	50 µl	~5 ml

After treatment, cells were washed twice with serum free medium, then full growth medium was added. Colcemid was added to all cultures at a final concentration of 0.1 µg/ml and incubation continued for 2 hours. Cultures were harvested at 24 h (Test 1 and 2) or 48 h (Test 2, absence of S9 mix) post treatment and then fixed. For both experiments, 3 slides per culture were made. The slides were stained with Giemsa. Based on the toxicity (ie cell counts and slide/culture observations) and osmolality, 3-4 concentration levels were selected for assessment of chromosomal aberrations. From 2-3 slides per culture, up to 50 metaphase cells per slide, a total of 100 metaphase cells per culture, were examined where possible. The number of chromosomes in each metaphase cell and all abnormalities were recorded. As cultures harvested at both culture times were negative with regards to structural aberrations, a further assessment of polyploidy was made. For this assessment, approximately 300 metaphase cells were cursorily examined and deemed to be either diploid, polyploid or endoreduplicated.

For the clastogenicity, a negative response was recorded if responses from the test item treated cultures are within the 95% confidence limits for the historical negative control data. The response at a single dose was classified as significant if the percent of aberrant cells is consistently greater than the 99% confidence limits for the historical negative control data or greater than double the frequency of an elevated vehicle or untreated control culture if appropriate. A test was positive if the response in at least one acceptable dose level is significant by the criterion described above.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.

Reliability: (1) valid without restriction

09-DEC-2004

(9)

5. Toxicity

5.6 Genetic Toxicity 'in Vivo'

5.7 Carcinogenicity

5.8.1 Toxicity to Fertility

5.8.2 Developmental Toxicity/Teratogenicity

5.8.3 Toxicity to Reproduction, Other Studies

Type: other
In Vitro/in vivo: In vivo
Species: rat
Strain: Sprague-Dawley **Sex:** male/female
Route of administration: gavage
Exposure period: Males - 4 weeks, females - 2 weeks prior to mating
until day 4 of lactation
Frequency of treatment: once daily
Duration of test: Day 4 of lactation
Doses: 2, 7.5, and 50 mg/kg/day
Control Group: yes, concurrent vehicle
Result: NOEL - 2mg/kg

Method: other
Year: 2004

Method: The design of this Reproduction/Developmental Toxicity Screening study is based on OECD Guideline 421.

Result: The analyzed concentrations were within $\pm 10\%$ of the nominal concentration, indicating acceptable accuracy of formulation. Among males treated at 7.5 and 50 mg/kg/day, there was a reduction in weight gain during the first week of treatment. Weight gain thereafter was similar to Control. Food consumption at 50 mg/kg/day was reduced during the first week of treatment; thereafter, consumption at this level was similar to control. Among females treated at 7.5 and 50 mg/kg/day, there was a dose-related reduction in body weight gain over the first week of treatment. Over the second week of treatment, weight gain at 50 mg/kg/day was greater than Control, but at 7.5 mg/kg/day weight gain over the second week was also lower than Control, such that the overall weight gain to Day 14 of treatment and absolute body weight at Day 14 were significantly lower than Control. Food consumption at 50 mg/kg/day was reduced during the first week of treatment; consumption at 7.5 mg/kg/day was reduced during the first 2 weeks of treatment.

During gestation, mean weight gains and food consumption at 7.5 and 50 mg/kg/day were lower than Control; food consumption over Days 0-4 of lactation at 7.5 mg/kg/day was also lower than Control. These differences were not statistically significant. At 50 mg/kg/day, most animals showed piloerection and excessive salivation; both of these signs were observed intermittently. At 7.5 mg/kg/day, three animals showed piloerection.

At 50 mg/kg/day, 6/10 males had low testes weights; these testes had marked seminiferous epithelial degeneration and mild interstitial cell hyperplasia. There was mild to marked oligospermia and moderate to marked sloughing of spermatogenic cells in the epididymides of these animals; group mean epididymides weight at this level was lower than Control. At 50 mg/kg/day, 2/10 pairs failed to mate; both males had low testes weights.

The mean duration of gestation at 50 mg/kg/day was slightly increased. The mean number of implants at 50 mg/kg/day was lower than Control, and pup mortality markedly increased, such that surviving pups at this level were sacrificed for welfare considerations. At 7.5 mg/kg/day, reproductive effects were confined to a reduced number of implants, and therefore of pups born, and slight reduction in mean litter and pup weights.

The No Observed Effect Level (NOEL) for both sexes of adults, and for reproductive effects, was 2 mg/kg/day.

Test condition: Sprague-Dawley rats were randomized into 3 test groups and one negative Control group, each containing 10 males and 10 females. Males were treated once daily for 2 weeks prior to mating through until necropsy after 4 weeks of treatment. Females were treated once daily for 2 weeks prior to mating, then throughout mating, gestation and until ca Day 4 of lactation. Dose levels were 0, 2, 7.5 and 50 mg/kg/day. The animals were dosed once daily orally by gavage at a dose volume of 20 ml/kg. The animals were monitored for clinical signs of toxicity. Individual body weights were recorded one week prior to commencement of dosing, then daily to determine the correct dose volume to be administered. For both sexes, food consumption was recorded weekly from one week prior to commencement of treatment through until pairing for mating. For males, consumption was also recorded for a complete one-week period, after cohabitation with females. For mated females, consumption was recorded over Days 0-7, 7-14 and 14-20 of gestation, then Days 0-4 of lactation.

Animals were paired on a one male to one female basis, with both animals being in the same treatment group. Each female was transferred to the cage of its appropriate co-group male near the end of the work day, where it remained until mating had occurred. Vaginal lavages were taken daily early each morning from the day of pairing until mating had occurred, and the stage of oestrus observed in each lavage was recorded.

The day of detection of a copulatory plug in situ and/or sperm in the lavage was designated Day 0 of gestation. The day of birth of the litter (day on which first pups were born) was designated Day 0 of lactation. The females were allowed to litter normally. The duration of gestation in days was evaluated. The number of live and dead pups born in each litter was recorded as soon as possible after completion of parturition on Day 0 of lactation. The live pups were sexed, counted and examined for the presence of milk in the stomach and for any externally visible abnormalities again on Day 4 of lactation. These pups were weighed en masse, sexes separate, on Days 1 and 4 of lactation. All adults were submitted for necropsy, with epididymides and testes weighed. Histopathology was conducted on the epididymides and testes of all males, and on the ovaries of all the Control and High dose females.

Prior to study commencement, trial formulations of 0.1-200 mg/mL test material were investigated for stability, concentration and homogeneity. Formulations were analyzed on 2 occasions during the study treatment period, on Day 1 and during Week 4. On each occasion, triplicate samples were withdrawn from each formulation (including Control).

Body weight and food consumption of males, and of females prior to mating were analyzed by analysis of variance or the Kruskal-Wallis non-parametric analysis as appropriate. Organ weight data were analyzed by analysis of variance and analysis of covariance using the terminal body weight as the single covariate. Histology data were analyzed by Fisher's Exact Probability Test.

Test substance: Test material was specially prepared for toxicity testing and contained 71.2% in water.

Reliability: (1) valid without restriction (10)
09-DEC-2004

5.9 Specific Investigations

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5.10 Exposure Experience

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9. References

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