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**Substance:** 1,3,4-Thiadiazole, 2,5-bis(tert-nonyldithio)

**Summary Prepared by:** Petroleum Additives Panel
Health, Environmental and Regulatory Task Group

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# 1. GENERAL INFORMATION

## 1.1 Physico-chemical Data

### 1.1.1 Octanol Water Partition Coefficient

**Robust Summary 7-LogKow-1**

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|---|---|
| CAS No. | 89347-09-1 |
| Test Substance Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio); |
| Test Type | Octanol/Water Partition Coefficient |
| Method/Guideline | OECD Test Guideline 107 |
| GLP (Y/N) | Yes |
| Year | 1990 |
| Test Substance Stock Solution | A test substance stock solution was prepared as a mixture of radiolabeled and unlabeled test material. This mixture was diluted in 10 mL of methanol. The mean specific activity was 976 dpm/ug. |
| Remarks for Test Conditions | Water and n-octanol were added to each test vessel. Each sample was then spiked with 10 ul of test material stock solution. Test conditions were as follows:<br><br>20 mL distilled water and 1 mL of n-octanol (repeated)<br>20 mL distilled water and 2 mL of n-octanol<br>20 mL distilled water and 4 mL of n-octanol<br><br>The test temperature was 21$^{o}$C. Samples were shaken for five minutes and centrifuged for fifteen minutes at 4000 rpm to separate the phases. Each phase was sampled and the extract and stock solution were analyzed using liquid scintillation counting. Analytical data in units of radioactivity were converted to mass concentration units by using the specific activity of the test compound. The octanol/water ratio was calculated for each of three test conditions and expressed as a logarithm. |
| Results | <table><tr><th>Test Condition</th><th>Mean Log $P_{ow}$</th></tr><tr><td>20 mL distilled water and 1 mL of n-octanol</td><td>1.94 $\pm$ 0.27</td></tr><tr><td>20 mL distilled water and 1 mL of n-octanol</td><td>1.72$\pm$ 0.15*</td></tr><tr><td>20 mL distilled water and 2 mL of n-octanol</td><td>2.94$\pm$ 0.05</td></tr><tr><td>20 mL distilled water and 4 mL of n-octanol</td><td>2.77$\pm$ 0.24</td></tr></table> *Test condition repeat |
| Conclusion | The octanol/water partition coefficient of the test material was determined to range from 1.72 to 2.94 at 21°C under the stated test conditions. |
| Data Quality | Reliable without restriction |
| References | Confidential business information |
| Other | November 27, 2002 |

## 2. ENVIRONMENTAL FATE

### 2.1 Biodegradation

**Robust Summary 7-Biodeg-1**

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|---|---|
| *Test Substance* | |
| CAS # | 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| **Method** | |
| Method/Guideline Followed | OECD 301C, Ready Biodegradability, Modified MITI Test |
| Test Type (aerobic/anaerobic) | Aerobic |
| GLP (Y/N) | Y |
| Year (study performed) | 1989 |
| Contact time (units) | 28 days |
| Preparation of Activated Sludge | Sludge was sampled from 10 sites including 5 sewage treatment plants and 5 sites from rivers, lakes and the sea. The filtrate of the supernatant of an activated sludge in actual use was mixed with an equal volume of the filtrate of the supernatant of the newly collected sludge and the mixture was cultivated at pH 6-8 under sufficient aeration. Thirty minutes after ceasing the aeration of the sludge mixture, supernatant equal to 1/3 of the total volume was removed. An equal volume of 0.1% synthetic sewage was added to the remaining portion and the mixture was aerated again. This procedure was repeated once daily. Culturing was carried out at 25±°C. |
| Mineral Medium | Prepared as outlined in the OECD guideline. |
| Preparation of Test Solution Cultures | Culture 1: 300 mL purified water and 30 mg of test material (abiotic control)<br>Cultures 2, 3, 4: 300 mL mineral medium and 30 mg of test material<br>Culture 5: 300 mL mineral medium and 30 mg of aniline (positive control)<br>Culture 6: 300 mL mineral medium (blank)<br><br>30 mg/L (suspended solids) of activated sludge was added to test cultures 2, 3 and 4. The test apparatus was then assembled ensuring that it was airtight; oxygen uptake was then measured under conditions of darkness. Carbon dioxide was absorbed with soda lime No. 1. Magnetic stirrers stirred solutions. Oxygen uptake was recorded from all cultures continuously for 28 days. |
| Temperature of incubation: | 24-26 °C |
| Analytical method: | Analysis of test substance by high performance liquid chromatography (HPLC). Measurement of biochemical oxygen demand (BOD) by closed system oxygen consumption measuring apparatus. |
| Study termination: | At 28 days, the pH of the content of each test culture and the concentration (HPLC) of the test material were determined. |

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| Method of calculating biodegradation values: | **Degree of degradation (%)(BOD) = [(biochemical oxygen demand of sludge plus test material) - (biochemical oxygen demand of control blank)] / (theoretical oxygen demand required when the test substance is completely oxidized) x 100**<br><br>Degree of degradation (%) (HPLC) = (Amount of residual test material in water) – (Amount of residual test material in sludge) / (Amount of residual test material in water) x 100 |
| | |
| *Results* | The mean biodegradation of the test substance was 2% by the BOD method and 5% by HPLC determination at 28 days. The degree of degradation of aniline (positive control) calculated by the BOD method was 74% at day 7 and 80% at day 14. The test material was not considered biodegradable under the study conditions. |
| *Conclusions* | The test substance was not readily biodegradable. |
| *Data Quality* | (1) Reliable without restriction |
| *References* | Confidential business information |
| *Other* | Updated: 11/27/2002 |

# 3. AQUATIC TOXICITY

## 3.1 Acute Toxicity to Fish

**Robust Summary 7-Fish Tox -1**

| ***Test Substance*** | |
|---|---|
| CAS # | 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| **Method** | |
| Method/Guideline followed | Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, EPA-660/3-75-009, April 1975 p. 61. |
| Test Type | Acute Toxicity to Fish (Static Test Method) |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1985 |
| Species/Strain | Fathead Minnows (Pimephales promelas) |
| Fish Number | 10/concentration |
| Fish Size | Average length 24 mm; Average weight 0.2 g |
| Analytical Monitoring | No |
| Nominal Test Substance Concentration Levels | 0 (control), 0 (solvent control), 100, 180, 320, 560 and 1000 mg/l |
| Test Concentration Preparation | Test solutions were prepared separately for each replicate test concentration by adding an appropriate aliquot (by weight) of test material directly to the test chambers. Prior to the addition of the test material 1.5 mL of dimethylformamide was added to each sample weight to increase dispersion of the test material in the dilution water. The solvent control also received 1.5 mL of dimethylformamide. The solutions were stirred vigorously prior to use. |
| Exposure Period | 96 hours |
| Exposure Conditions | Static test conditions. |
| Vehicle | None |
| Statistical Analysis | None required based on the results. |
| Dose Rangefinding Study | Yes |
| Test Chambers | 5-liter glass aquaria containing 15 liters of test solution |
| Diluent Water | Soft reconstituted water |
| Diluent Water Chemistry | Hardness 40-45 mg/l as $CaCO_3$<br>Alkalinity 30-35 mg/l as $CaCO_3$<br>Conductivity 130 umhos/cm<br>Dissolved Oxygen: 9.2 mg/L<br>PH: 7.2-7.6 |
| Photoperiod | 16 hours of light, 8 hours of dark |
| Temperature Range | 21-23 $^{O}C$ |
| Positive Control | Antimycin A |
| Remarks field for test | All organisms were observed for mortality and the number of individuals |

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| conditions | exhibiting clinical signs of toxicity or abnormal behavior at 2, 24, 48, 72, and 96 hours after initiation of test material exposure. A separate group of fish was exposed to Antimycin A as a positive control. |
| ***Results*** | After the preparation of the test material in the test chambers, an oily surface film and large yellow oily droplets were observed on the bottom of all test chambers. The amount of surface film and the size of the droplets increased with test material concentration. These observations were unchanged over the 96-hour duration of the study.<br><br>No mortality or unusual observations were observed at any test concentration. The positive controls confirmed the LD50 of Antimycin A.<br><br>Dissolved oxygen concentrations ranged from 7.1 to 9.5 mg/l during the study. These values represented 82-108% saturation at 23 and 22$^{o}$C respectively, and were considered adequate for testing. The pH values ranged from 7.1 to 7.8.<br><br>The 24, 48, 72 and 96-hour LC50s were each greater than 1000 mg/L (Nominal concentration). The 96 hour no observed effect level was 1000 mg/L. |
| ***Conclusions*** | Under the conditions of this study the 24, 48, 72 and 96-hour LC50s were each greater than 1000 mg/L (Nominal concentration). The 96 hour no observed effect level was 1000 mg/L. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of exposure concentration and due to the presence of test material on the surface and at the bottom of each test chamber. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 11/13/2002 |
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## 3.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

### Robust Summary 7 – Daph – 1

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|---|---|
| Test Substance | |
| CAS # | 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| Method | |
| Method/Guideline followed | OECD Guideline for Testing of Chemicals #202 Daphnia sp. Acute Immobilization Test and Reproduction Test (1984). |
| Test Type | Static acute toxicity test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 2006 |
| Species/Strain | Daphnia magna (Cladoceran)<br>Juveniles were obtained from an in-house colony. |
| Number of Daphnia | Definitive Study 20/concentration (5/replicate) |
| Analytical Monitoring | No |
| Exposure Period (unit) | 48 hours |
| Nominal Test Substance Concentration Levels | 0, 0.063, 0.13, 0.25, 0.50 and 1.0 mg/L |
| Test Concentration Preparation | A primary stock solution was prepared at a nominal concentration of 1.0 mg/L, the highest concentration tested, by mixing 15.0 mg of test material into 15 L of dilution water (UV sterilized well water). The stock solution was stirred for approximately 48 hours. Aliquots of the stock were proportionally diluted with dilution water to prepare 1 L of test solution at nominal concentrations of 0.063, 0.13, 0.25 and 0.50 mg/L. |
| Statistical methods | Since there was less than 50% mortality/immobility, the no-mortality/immobility concentration and the no-observed-effect concentration were determined by visual interpretation of the mortality, immobility and observation data. |
| Test chambers | Test chambers were 250-mL glass beakers containing 225 mL of test water. The depth of the test water in a representative chamber was 7.0 cm. |
| Diluent Water | Fresh aerated well water |
| Diluent Water Chemistry During 48 Hour Exposure Period. | Dissolved Oxygen: 8.5-8.8 mg/L<br>pH: 8.2-8.6<br>Specific Conductance: 300 µmhos/cm<br>Hardness: 132 mg/L as $CaCO_3$<br>Alkalinity: 176 mg/L as $CaCO_3$ |
| Photoperiod | 16 hours of light, 8 hours of dark |
| Temperature Range | 19.4-20.5$^{O}$C during exposure period |
| Remarks | Observations were made periodically to determine the numbers of dead and immobile organisms. Immobility was defined as a lack of movement by the organism except for minor activity of the appendages. The numbers of individuals exhibiting signs of toxicity or abnormal behavior also were evaluated. Observations were made approximately 5, 24 and 48 hours after test initiation. |

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| | Temperature was maintained at 19.4-20.5$^{O}$C throughout the test. No treatment related differences were observed in oxygen concentration or pH during the study. The solutions appeared clear and colorless at test initiation and termination.<br><br>Cumulative mortality/immobilization data was as follows:<br><br>Cumulative Immobilization/Mortality (%)<br>Concentration (mg/L ) \| Number of Daphnia \| 4 Hours \| 24 Hours \| 48 Hours<br>0 \| 20/intrerval \| 0 \| 0 \| 0<br>0.063 \| 20/intrerval \| 0 \| 0 \| 0<br>0.13 \| 20/intrerval \| 0 \| 0 \| 5<br>0.25 \| 20/intrerval \| 0 \| 0 \| 0<br>0.50 \| 20/intrerval \| 0 \| 0 \| 0<br>1.0 \| 20/intrerval \| 0 \| 0 \| 10<br><br>While there was no visible layer of test substance at the water surface, observations of daphnia trapped at the water surface were noted in each treatment group, increasing in number with increasing concentration. A small number of lethargic daphnia also were noted in the treatment groups during the study.<br><br>The No Observed Effect Concentration was <0.063 mg/L. The 48-hour EC50 value was >1.0 mg/L, the highest concentration tested. The no mortality/immobility concentration was 0.063 mg/L |
| Conclusions | The No Observed Effect Concentration was <0.063 mg/L. The 48-hour EC50 value was >1.0 mg/L, the highest concentration tested. The no mortality/immobility concentration was 0.063 mg/L |
| Data Quality | Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical confirmation of exposure concentrations. |
| References | Palmer S & Krueger H. A 48-hour Static Acute Toxicity Test with the Cladoceran (*Daphnia Magna)*. Wildlife International Project No.: 264A-110. November 16, 2006. |
| Other | Updated: 12/15/2006 |

## 3.3 Toxicity to Aquatic Plants (e.g. Algae)

**Robust Summary 7 – ALG – 1**

| *Test Substance* | |
|---|---|
| CAS # | 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| Method | |
| Method/Guideline followed | OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984) and OECD 2004 proposal for updating Guideline #201. |
| Test Type | Static acute toxicity test |
| GLP (Y/N) | Y |
| Year (Study Performed) | 2006 |
| Species/Strain | Freshwater algae, *Pseudokirchneriella subcapitata* (UTCC 37) |
| Element basis (# of cells/mL) | Approximately 1.0 x $10^6$ cells/mL, used to inoculate medium for an initial cell density of $10^4$ cells/mL. |
| Exposure period/duration | 72 hours |
| Range find test | No |
| Analytical monitoring | Not performed |
| Statistical methods | Area under the growth curve and growth rates were analyzed statistically by non-linear regression versus concentration or linear interpolation. 72-hour data evaluated for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests. |
| Remarks field for test conditions (fill as applicable) | Test Species: Cultures obtained from the Culture Collection of University of Toronto.<br><br>Loading Concentration: 0.063, 0.13, 0.25, 1.50, 1.0 mg/L<br><br>Test Concentration Preparation: A primary stock solution was prepared by dissolving 0.0100 g of test article in freshwater algal medium at a nominal concentration of 10 mg/L. The stock was mixed on a magnetic stir plate for approximately two days and appeared as a cloudy white solution. A secondary stock was prepared by diluting 100 mL of the primary stock to 1000 mL with algal medium for a nominal concentration of 1.0 mg/L, the highest test concentration. The secondary stock was proportionally diluted with freshwater algal medium to prepare the four additional test concentrations. Test concentrations were not corrected for percent active ingredient of the test article. All test solutions were clear and colorless.<br><br>Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Six 100-mL replicates were used in the Control and three replicates were used per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were plugged with foam stoppers. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day) for 72 hours. Cell densities were determined using an electronic particle counter at |

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| | 0, 24, 48 and 72 hours. pH was determined at 0 and 72 hours.<br>**Light: Continuous illumination approximately 7000 lux.**<br>Test temperature: 23.7-24.6 $^{o}$ C.<br>**Culture Media: As specified in the guideline.**<br>Method of calculating mean measured concentrations: Not applicable.<br>Exposure period: 72 hours |
| *Results* | After 72 hours of exposure, there were no apparent treatment-related effects upon growth at any concentration tested. There were no statistically significant differences between negative control growth and the growth in any treatment group.<br><br>The 72-hour $E_bC_{50}$, the concentration that reduced the area under the growth curve by 50%, was >1.0 mg/L.<br><br>The 72-hour $E_rC_{50,}$ the concentration that reduced growth rate by 50%, was >1.0 mg/L.<br><br>The 72-hour No Observed Effect Concentrations (NOEC) for area under the growth curve and growth rate were 1.0 mg/L (based on statistical significance).<br><br>The 72-hour No Observed Effect Concentrations (NOEC) for area under the growth curve based on calculated EC10/EC20 were 0.63/0.92 mg/L<br><br>The 72-hour No Observed Effect Concentrations (NOEC) for growth rate based on calculated EC10/EC20 were .1.0/>1.0 mg/L<br><br>The cell concentrations of the control cultures increased by a factor >16 during the study meeting the guideline requirement of at least a factor of 16 after 72 hours.<br><br>No abnormalities were observed in any of the control or treated cultures after 72 hours of exposure.. Control culture pH increased from 7.7 at 0 hour to 8.4 at 72 hours. |
| *Conclusions* | Both the growth and the growth rate of *Pseudokirchneriella subcapitata* were unaffected by the presence of the test material over the 72-hour exposure period. The 72-hour $E_bC_{50}$, the concentration that reduced the area under the growth curve by 50%, was >1.0 mg/L. The 72-hour $E_rC_{50,}$ the concentration that reduced growth rate by 50%, was >1.0 mg/L. The 72-hour No Observed Effect Concentrations (NOEC) for area under the growth curve and growth rate were 1.0 mg/L (based on statistical significance). |
| *Data Quality* | Reliable with restriction. Restriction due to the lack of analytical confirmation of exposure concentrations. |
| *References* | Desjardins D & Krueger H. (2006). A 72-Hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata)*<br>Wildlife International Project No.: 264A-111A |
| *Other* | Updated: November 13, 2006 |

# 4. MAMMALIAN TOXICITY

## 4.1 Acute Toxicity

### 4.1.1 Acute Oral Toxicity

**Robust Summary 7-Acute Oral -1**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| **Method** | |
| Method/Guideline followed | OECD Guideline 401 |
| Test Type | Acute oral toxicity |
| GLP (Y/N) | Not specified |
| Year (Study Performed) | 1981 |
| Species/Strain | Rats/Sprague-Dawley |
| Sex | Male/Female |
| No. of animals/dose | 5/sex/group and 2/sex/group |
| Vehicle | Corn oil |
| Route of administration | Oral (intragastric) |
| Dose level | 10,000 (5 /sex/group) and 5000 (2 /sex/group) mg/kg |
| Dose volume | 15 mL/kg |
| Control group | No |
| Chemical analysis of dosing solution | No |
| Remarks field for test conditions | A single dose of the test material/vehicle mixture was administered intragastrically to fasted (18 hours) male and female rats at each dose level. A control group was not included. The animals were observed 1, 3 and 4 hours after dosing and at least once/day thereafter for 14 days. Individual weights were recorded on the day of dosing. All animals were euthanized, and gross necropsies were performed, at the conclusion of the observation period. |
| ***Results*** | LD50 > 10 g/kg (males and females) |
| Remarks | There were no deaths during the study. Decreased motor activity was observed in nine of ten high dose animals within 24 hours of test material administration at 10,000 mg/kg. Within this same time period diarrhea was observed in 2 of 5 high dose females and in 1 of 2 low dose males. One male rat dosed at 10,000 mg/kg was observed to have a spleen with dark red edges. There were no other necropsy findings of note. |

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| ***Conclusions*** | The test article, when administered to Sprague Dawley rats had an acute oral LD50 of > 10 g/kg. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). Restriction due to the lack of individual animal necropsy data in the final report. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 11/25/2002 |
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### 4.1.2 Acute Inhalation Toxicity

**Robust Summary 7-Acute Inhalation-1**

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|---|---|
| ***Test Substance*** | |
| CAS # | CAS# 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| **Method** | |
| Method/Guideline followed | OECD Guideline 403 |
| Test Type | Acute Inhalation toxicity (Limit Test) |
| GLP (Y/N) | Not specified |
| Year (Study Performed) | 1981 |
| Species/Strain | Rats/Sprague-Dawley |
| Sex | Male and female |
| No. of animals/sex | 5 |
| Vehicle | None |
| Route of administration | Vapor inhalation (single 4 hour whole body exposure) |
| Dose level | 2.75 mg/L (Nominal Concentration) |
| Vehicle control group | No |
| Chamber analysis | No |
| Remarks field for test conditions | The above referenced guideline calls for the analytical confirmation of dose concentration. During this study the determination of chamber concentration was calculated as a nominal concentration based on test material usage and rate of airflow during exposure. The guideline also calls for an evaluation of animal body weights. Animals were weighed prior to exposure only.<br><br>One group of five rats/sex was exposed for 4 hours to the test material as a vapor generated by bubbling dry air at 5/liters/minute through 1 liter of test material heated to 94$^{o}$C. The vapor was delivered undiluted into a 37-liter Plexiglas exposure chamber. The nominal concentration of the test material in the atmosphere was 2.75 mg/L. Food and water were available ad libitum except during exposure. Animal observations for toxicological signs and mortality were recorded periodically during exposure and at least once daily during the 14-day observation period. Individual body weights were recorded on Day1 (immediately prior to exposure). Animals were sacrificed and subjected to a complete gross necropsy following the 14-day observation period. |
| ***Results*** | LC50 > 2.75 mg/L nominal concentration |
| Remarks | All animals survived the exposure and observation periods. A clear nasal discharge, red encrustation around the nose and eyes and salivation were observed in four of ten animals during the exposure period. One male animal exhibited diarrhea immediately following exposure and on the following day. No other significant physical |

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| | observations were recorded. Three animals had spongy lungs and/or brown foci through all lung lobes. Chamber oxygen concentration during exposure was 19.5%. |
| ***Conclusions*** | Following 4-hour whole body exposure to the test material vapor the LC50 in male and female Sprague Dawley rats was > 2.75 mg/L nominal concentration. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). Restriction due to the lack of analytical characterization of exposure concentration and due to the lack of individual animal necropsy data in the final report. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 11/25/2002 |
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### 4.1.3 Acute Dermal Toxicity

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|---|---|
| ***Test Substance*** | |
| CAS # | CAS# 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| **Method** | |
| Method/Guideline followed | OECD Guideline 402 |
| Test Type | Acute dermal toxicity (Limit Test) |
| GLP (Y/N) | Not specified |
| Year (Study Performed) | 1981 |
| Species/Strain | Rabbits/New Zealand White |
| Sex | Male and female |
| No. of animals/sex/group | 5 |
| Vehicle | None |
| Route of administration | Dermal |
| Dose level | 2 g/kg |
| Control group included | No |
| Remarks field for test conditions | This study deviates from the above referenced guideline in that the dosing site was abraded prior to treatment. This was not considered a significant deviation from the guideline that would adversely affect the study results.<br><br>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Immediately prior to dosing the skin was abraded. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and five female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze pad and elastic film. The application site was washed clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs daily for 14 days after treatment. Individual body weights were recorded on the day of dosing. Gross necropsies were performed on all animals on Day 14. |
| ***Results*** | LD50 > 2.0 g/kg (males and females) |
| Remarks | No mortality was observed. One male rabbit had diarrhea on days 2, 3 and 4, as did one female rabbit on day 3. This female also exhibited this finding immediately prior to dosing. All animals were unremarkable from day 5 through study termination. No gross necropsy effects were evident. |

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| ***Conclusions*** | The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 2.0 g/kg. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code). Restriction due to the lack of individual animal necropsy data in the final report. |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 11/25/2002 |
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## 4.2 Genetic Toxicity

**Robust Summary 7-Gentox-1**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| **Method** | |
| Method/Guideline followed | OECD Guideline 473 |
| Test Type | *In Vitro* Chromosomal Aberration Assay in CHO Cells |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1989 |
| Test System | Chinese hamster V79 Cells |
| Culture Preparation and Maintenance | Cells were thawed and cultured at 37$^{o}$C, in 4.5% $CO_2$ in air in plastic flasks. Seeding is performed at 5 x $10^5$ cells/flask in 15 mL MEM medium containing 10% fetal bovine serum. |
| Exposure Method | Dilution |
| Test Substance Doses/concentration levels | 50 uL samples of concentrations of 1.0, 10, 20 ug/mL were evaluated with and without metabolic activation. |
| Metabolic Activation | With and without S9 fraction mix of livers of Aroclor 1254 pretreated Wistar rats. |
| Vehicle | Ethanol (final concentration did not exceed 1% v/v). |
| Positive Control concentration levels by activation status | Ethylmethanesulfonate, 0.72 mg/mL without activation<br>Cyclophosphamide, 1.4 ug/mL with activation |
| Statistical Analysis | Statistical analysis of the data was not performed. Test data were consistent with control data. |
| Test Substance Solubility | Test substance solubility in the vehicle was determined. |
| Dose rangefinding study | Test substance and vehicle control tested in duplicate cultures each with and without activation. Test substance tested at concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10, 15, 20 ug/ml without activation. Test substance tested at concentrations of 0.5, 1.0, 5.0, 10, 15, 20 ug/ml with activation. Cytotoxicity and mitotic indices were evaluated. |
| Remarks field for test conditions | A pretest dose range finding study was conducted at concentrations up to 20 ug/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. Cyclophosphamide (positive control) was tested with activation and ethylmethanesulfonate (positive control) was tested without activation. Prepared cultures were treated with test substance or control material and were incubated for 24 hours at 37$^{o}$C, in 4.5% $CO_2$ in air. Twenty-one and one half hours after the start of treatment the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.2 ug/mL. 2.5 hours later two slides were prepared for each group using Giemsa |

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| | stain. Two-slides/treatment group were evaluated. 200 metaphase cells (100 per culture) each containing 21-23 chromosomes per treatment group were scored. Chromosomes were counted for each cell. Chromosome aberrations, either chromosome or chromatid type were recorded. Gaps were excluded from the total aberration frequency. Mitotic index was determined. The percent of aberrant cells and the frequency of aberration (%) per treatment group were determined. In order for a test substance to be considered to have induced a positive response compared to vehicle control a statistically significant dose related increase in the number of aberrant cells or a significant and reproducible positive response for at least one of the test points were required. |
| ***Results*** | The test substance was not mutagenic in this assay with or without metabolic activation. |
| Remarks | In the prestudy toxicity evaluation, colony-forming ability, in the absence and presence of metabolic activation, at the 20 ug/mL test substance concentration was clearly reduced. Precipitate was evident at the higher concentrations.<br><br>In the main study the mitotic index was reduced after treatment with the highest dose level only in the absence of metabolic activation.<br><br>The test substance did not increase the frequency of cells with aberrations at any dose level, with or without metabolic activation. The aberration rates of the treated cells (0.0-2.5%) were in the range of the control values (0.0-3.5%). The positive control group had a higher percentage of aberrant cells than the vehicle control group with and without activation. |
| ***Conclusions*** | The test material was not genotoxic under the conditions of this study. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 11/26/2002 |
| | |

**Robust Summary 7-Gentox-2**

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| **Method** | |
| Method/Guideline followed | OECD Guideline 471 |
| Test Type | Bacterial Reverse Mutation Assay |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1989 |
| Test System | *Salmonella typhimurium* and *Escherichia Coli* |
| Strains Tested | *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia Coli* tester strain WP2*uvr*A |
| Exposure Method | Plate incorporation |
| Test Substance Doses/concentration levels | Initial and Confirmatory assays:<br>*Salmonella* + (S9): 0.0, 10, 100, 333.3, 1000, 5000 ug/plate<br>*Salmonella* - (S9): 0.0, 10, 100, 333.3, 1000, 5000 ug/plate<br>WP2*uvr*A + (S9): 0.0, 10, 100, 333.3, 1000, 5000 ug/plate<br>WP2*uvr*A - (S9): 0.0, 10, 100, 333.3, 1000, 5000 ug/plate |
| Metabolic Activation | With and without (S9 fraction-liver from Aroclor 1254 treated rats) |
| Vehicle | Ethanol |
| Tester strain, activation status, Positive Controls and concentration level | TA98 +S9 2-aminoanthracene 10.0 ug/plate<br>TA98 -S9 4-nitro-o-phenylene-diamine 50.0 ug/plate<br>TA100 +S9 2-aminoanthracene 10.0 ug/plate<br>TA100 -S9 sodium azide 10.0 ug/plate<br>TA1535 +S9 2-aminoanthracene 10.0 ug/plate<br>TA1535 -S9 sodium azide 10.0 ug/plate<br>TA1537 +S9 2-aminoanthracene 10.0 ug/plate<br>TA1537 -S9 4-nitro-o-phenylene-diamine 50.0 ug/plate<br>TA1538 +S9 2-aminoanthracene 10.0 ug/plate<br>TA1538 -S9 4-nitro-o-phenylene-diamine 50.0 ug/plate<br>WP2*uvr*A +S9 2-aminoanthracene 10.0 ug/plate<br>WP2*uvr*A –S9 methyl methane sulfonate 10.0 ug/plate |
| Vehicle Control | Ethanol |
| Dosing Solution Analysis | No |
| Statistical Analysis | Mean revertant colony count and standard deviation were determined for each dose point. |
| Dose Rangefinding Study | Conducted in triplicate using tester strains TA98, TA100 and WP2*uvr*A and doses of test material ranging from 1.0 to 5000.0 ug/plate, with and without metabolic activation. Cytotoxicity was evaluated. |
| S9 Optimization Study | No |
| Remarks field for test conditions | In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle |

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| | controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 100 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) or S9 buffer, and 2000ul of top agar. This was overlaid onto the surface of 20 ml minimal bottom agar in a petri dish. Plates were incubated for 72 hours at 37$^{o}$C in the dark. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate. An automatic colony counter was utilized. If precipitate was present then colonies were counted by hand.<br><br>The test article was considered positive if either a significant dose related increase in the number of revertants or a significant and reproducible increase for at least one concentration was induced. A significant response was considered as follows: TA100 - 2x increase in number of revertants; TA1535, 1537, 1538, 98 - 3x increase in number of revertants. In addition a dose dependent increase in the number of revertants was regarded as an indication of possibly existing mutagenic potential regardless whether the highest dose induced the described enhancement factor or not. |
| ***Results*** | The test substance was not genotoxic in this assay with or without metabolic activation. |
| Remarks | As a result of the dose range finding study dose levels of 10.0 to 5000 ug/plate were selected for the mutagenic assays.<br><br>The test material was not cytotoxic to any tester strain at up to the highest concentration tested with or without metabolic activation.<br><br>No significant and reproducible dose dependent increases in revertant colony numbers were obtained in any strain evaluated with or without metabolic activation. The test material was not considered genotoxic to any tester strain with or without metabolic activation.<br><br>The positive control for each respective test strain exhibited at least a 5-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response. |
| ***Conclusions*** | Under the conditions of this study, the test material was not mutagenic. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Unpublished confidential business information |
| ***Other*** | Updated: 11/26/2002 |
| | |

## 4.3 Repeated Dose Toxicity

### Robust Summary 7 – RepeatDose – 1

| ***Test Substance*** | |
|---|---|
| CAS # | CAS# 89347-09-1 |
| Chemical Name | 1,3,4-thiadiazole, 2,5-bis(tert-nonyldithio) |
| **Method** | |
| Method/Guideline followed | OECD 407 |
| Test Type | 28-day oral (gavage) toxicity study in rats |
| GLP (Y/N) | Y |
| Year (Study Performed) | 1989 |
| Species | Rat |
| Strain/Age | Wistar rat, KFM-Han., SPF-quality, approximately eight weeks old at receipt |
| Route of administration | Oral (gavage) |
| Duration of test | 28 days of treatment, 14 days of recovery |
| Doses/concentration levels | 0, 50, 200, 1000 mg/kg/day |
| Dose Volume | 10 mL/kg |
| Vehicle | Polyethylene glycol (PEG 400) |
| Sex | Males and females |
| Exposure period | Four week treatment duration |
| Frequency of treatment | 7 days/week |
| Number of animals/sex/group | Groups 1 and 4: 10/sex/group<br>Groups 2 and 3: 5/sex/group |
| Post exposure observation period | 14 days, Groups 1 and 4, 5/sex/group |
| Test Material Analysis | Yes (concentration, homogeneity and stability of gavage mixtures) |
| Statistical methods | Body weight, body weight gain, food consumption, clinical pathology data, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistical tests included Dunnett's test, Steel test, Fisher's Exact test, and an Analysis of Variance. |
| Remarks field for test conditions | The test material was prepared and administered daily. Mortality checks and clinical observations were performed once daily. Body weight and food consumption were measured once weekly. Hematology, clinical chemistry and urinalysis were evaluated at weeks 4 and 6 from fasted animals. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically from the control and high dose groups including the adrenals, heart, kidneys, liver, spleen and gross lesions. Target tissues (liver and kidney) identified in the high dose were examined in the low and mid dose and recovery groups. Deviations from the OECD 407 test guidelines included: |

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| | • A functional observational battery for neurotoxicity was not performed.<br>• Limited microscopic pathology was performed. |
| ***Results*** | |
| Remarks | All animals survived the duration of the study. Diarrhea was observed in all control and treated animals during the treatment period. This finding was not evident during recovery. Diarrhea was attributed to the use of polyethylene glycol as the vehicle.<br><br>Ophthalmoscopic examinations were unremarkable at the termination of dosing and recovery.<br><br>Statistically significant increases observed in the body weights of the high dose females throughout the study were considered treatment related. Terminal recovery body weights in the high dose females were increased 10%. No other treatment related effects were observed in the body weight or food consumption data of the test material treated animals.<br><br>The mean hematology data of the treated males and females were considered unremarkable following the four week treatment and two week recovery periods.<br><br>Differences from control values observed in the treated males at four weeks included: a 15% decrease from control in mean glucose levels in the high dose males; an 18% increase in mean total cholesterol in the mid and high dose males; an 110% increase in mean gamma-glutamyl transferase activity in the high dose males and 5% increases in high dose male mean albumin and protein levels. The high dose females exhibited a 26% increase in mean LDH values at 4 weeks, and increases in mean albumin (14%) and protein (11%) levels. Treated male and female recovery values were unremarkable.<br><br>Mean urine volumes were increased in the high dose males (55%) and females (32%) at 4 weeks but not following recovery.<br><br>The mean absolute and relative (to body weight) liver weights of the high dose males and females were increased compared to the controls following four weeks of treatment. Absolute (26%) and relative (13%) liver weights remained elevated in the high dose females following recovery. Terminal recovery body weights in the high dose females were increased 10%.<br><br>The mean absolute and relative (to body weight) kidney weights of the high dose males and females, mid dose males and low dose females were increased compared to the controls following four weeks of treatment. Relative kidney weights remained elevated in the high dose females (16%) following recovery. Terminal recovery body weights |

in the high dose females were increased 10%.

Mean absolute spleen weights were increased in the high dose males and mid and high dose females. Relative spleen weights were increased in the mid and high dose males and females. The increased relative spleen weights observed in the mid and high dose females did not exhibit a dose response. Spleen weights were unremarkable following recovery.

Absolute and relative adrenal, pituitary, testes and ovary weights were unremarkable in the treated males and females following both 4 weeks of treatment and 2 weeks of recovery.

% Change From Control – Absolute Organ Weights [a]

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| mg/kg/day | 50 | 200 | 1000 | 50 | 200 | 1000 |
| liver | +17 | +3 | +29* | +9 | +6 | +37** |
| kidney | +10 | +11 | +28* | +16 | +8 | +19** |
| spleen | +14 | +14 | +27 | +5 | +35** | +16 |

% Change From Control – Organ Weights/Body Weight [a]

| | | | | | | |
|---|---|---|---|---|---|---|
| liver | +17 | +9 | +34** | +12 | +6 | +36** |
| kidney | +6 | +19* | +33** | +20** | +8 | +18** |
| spleen | +12 | +21 | +30* | +8 | +34** | +15 |

[a] 4 week necropsy

* $p \leq 0.05$; ** $p \leq 0.01$

No treatment related macroscopic findings were evident following 4 weeks of treatment or two weeks of recovery.

Microscopic findings considered treatment related included slight hepatic parenchymal hypertrophy in some high dose males and females which was not evident after recovery and the presence of renal tubular inclusion bodies in increased numbers in the mid and high males and high dose females. The presence of inclusion bodies was reduced or absent following recovery. In addition slight renal tubular degeneration was seen in some high dose males at 4 weeks but not after recovery.

Analysis of gavage mixture confirmed that all dosing solutions were homogeneous, stable for their period of use and at acceptable concentrations.

The Study Director concluded that the no observed adverse effect level (NOAEL) was 50 mg/kg/day based on clinical pathology and microscopic changes observed following treatment at 200 and 1000 mg/kg/day.

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| ***Conclusions*** | The Study Director concluded that the NOAEL was 50 mg/kg/day. |
| ***Data Quality*** | Reliable without restriction (Klimisch Code) |
| ***References*** | Unpublished confidential business information<br>RCC Study No.: 226168 |
| ***Other*** | Revised 1/31/2006 |
| | |

## 4.4 Reproductive and Developmental Toxicity

**Robust Summary 7 – Repro/Devel – 1**

| ***Test Substance*** | |
|---|---|
| CAS # | 5-Ethyoxy- 3-trichlormethl-1,2,4- thiadiazole (structural analog of CAS# 89347-09-1) |
| Chemical Name | 5-Ethyoxy- 3-trichlormethl-1,2,4- thiadiazole (ETMT) |
| **Method** | |
| Test Type | A Two Litter Three Generation Reproductive Study in Rats |
| GLP (Y/N) | Not Specified |
| Year (Study Published) | 1980 |
| Species | Rat |
| Strain | Sprague-Dawley CD, 28 days of age at initiation of treatment |
| Route of administration | Orally by dietary admixture |
| Duration of test | Through weaning of the F3b generation |
| Dose levels | 0, 10, 80 and 640 ppm active ingredient |
| Vehicle control | Corn Oil (10 mL/6 kg of feed) |
| Sex | Males and Females |
| Frequency of treatment | Ad libitum, 7 days/week |
| Analytical confirmation of concentration. | Not Specified |
| Control and treatment groups | F0 generation: 25/sex/group |
| Post exposure recovery period | None |
| Mating ratio | One male to one female |
| Duration of mating period | Up to three weeks |
| Statistical methods | Not Specified |
| Dose range finding study | A prior three-month study was conducted. |

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| Remarks field for test conditions | Twenty-eight day old CD-rats were randomly separated into four groups, each containing 25 males and 25 females. These animals represented the F0 generation. One group was assigned to each of test concentrations or to the vehicle control group. The test article was administered via the diet. After 11 weeks exposure to treated or control diets 20 rats/sex/group were mated within groups to form the F1a generation, females being rotated to a different male for three consecutive weeks if necessary. The number of matings, pregnancies and litters, pups in the litter at 1, 4 and 21 days and weaning weight at 21 days were noted. Litters containing more than 10 pups were culled to 10 on day 4 of lactation. Indices evaluated included fertility (pregnancies/matings), gestation (litters cast/pregnancies), viability (live at day 4/live born) and lactation (weaned/live minus discards at day 4). Ten days after weaning of the last litter, the F0 rats were remated to produce the F1b generation.<br><br>Twenty-five male and female F1b rats from each group were continued on their parents control or treated diets and at approximately 105 days of age, 20/sex of each group were mated and the same procedures used with the F0 generation were continued through the production of the F2a and F2b generations. F2b animals continued on study through the production and weaning of the F3a and F3b generations. Histopathological examinations were conducted on 10 F3b offspring/sex from each study group at about 2 months of age. The examined tissues included the heart, liver, spleen, kidneys, testes, lung, bladder, stomach, small and large intestine, cecum, bone marrow, skeletal muscle, skin, brain, pituitary, thymus, thyroid, pancreas, adrenal, ovaries and gross lesions. |

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| ***Results*** | No significant differences were found between the control and test article treated animals in fertility, gestation, viability, or lactation indicies, or in mean numbers of offspring born and weaned/litter.<br><br>Weaning weights of pups on the 0, 10 and 80 ppm diets were comparable. Weaning weights at 640 ppm were significantly lower than control. Significantly lower body weights were also observed in parent rats at 640 ppm.<br><br>Histological studies on second litter off spring in the third generation showed no effects of exposure to the test article.<br><br>Based on the experimental conditions of this study, the No Observed Effect Level (NOEL) for parental toxicity was considered to be 80 ppm. The NOEL for toxic effects on reproductive performance was 640 ppm. The NOEL for pup development was 80 ppm. |
| ***Conclusions*** | Based on the experimental conditions of this study, the No Observed Effect Level (NOEL) for parental toxicity was considered to be 80 ppm. The NOEL for toxic effects on reproductive performance was 640 ppm. The NOEL for pup development was 80 ppm. |
| ***Data Quality*** | Reliable with restriction (Klimisch Code) Restriction due to the lack of published information on analytical confirmation of the dietary concentrations of the test article treated groups. |
| ***References*** | Toxicology and Applied Pharmacology 56, 164-170 (1980) |
| ***Other*** | Updated: 10/20/06 |