US EPA HPV Chemical Challenge Program

# ROBUST SUMMARIES FOR ETHANOL, 2-(HYDROXYMETHYLAMINO)- (CAS No. 34375-28-5)



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## INTRODUCTION

Troy Chemical Corporation (www.troycorp.com) has prepared the following robust summaries for Ethanol, 2-(hydroxymethylamino)-, otherwise known as 2-Hydroxyethyl aminomethylalcohol (Troysan 174; CAS# 34375-28-5), as part of its contribution to the U.S. Environmental Protection Agency's (EPA) High Production Volume (HPV) Challenge Program. The US HPV Challenge Program is directed at chemicals falling under the jurisdiction of the Toxic Substances Control Act (TSCA) and the initial list of HPV chemicals were identified from the 1990 TSCA Inventory Update. While Ethanol, 2-(hydroxymethylamino)- was on the HPV candidate list, Troy is currently not aware of any uses of this compound governed by TSCA. Troy's production of this compound is limited to pesticidal applications that are governed by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). Nonetheless, as the goal of the HPV Challenge Program is to ensure that the American public has access to basic information about the chemicals manufactured in the U.S., Troy has provided these robust summaries.

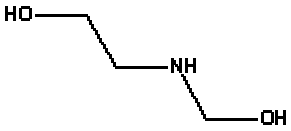
Background on Troysan 174 and FIFRA Registration

Troysan 174 is a water-soluble liquid bactericide recommended for concentrates and tankside application in all aqueous based metalworking fluids. Troysan 174 is a broad spectrum, non-metallic organic biocide designed for use in products that are subject to bacterial deterioration.

Molecular Formula: $C_3H_9NO_2$

Molecular Weight: 91.1096

Chemical Structure:




Troysan 174 (FIFRA registration #5383-11) helps buffer formulations, and protects machines, tools and work pieces from corrosion. Examples of products in which Troysan 174 can be used are latex paints, resin emulsions, adhesives, pigment dispersions, joint cements, cutting oils, and drilling additives.

Troysan 174 was the subject of a review by EPA's Office of Pesticides as part of the FIFRA Reregistration Eligibility Decision (RED) program (Case 3070). As such, many of the studies contained in this robust summary submission were reviewed by EPA and are discussed in the RED.

## 1.0 Melting Point

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)

### Method

| | |
|---|---|
| Method/guideline followed: | EPA 63-3 |
| GLP: | Yes |
| Year: (study performed): | 1990 |

### Test Conditions

Observation at 20°C

### Results

When examined at room temperature (20°C) the material exists as a liquid and therefore specific testing to determine melting point is not appropriate (see EPA Guidelines 63-3).

### Conclusions

Material exists as a liquid at room temperature (20°C).

### Reliability

Klimisch Code = 1

### References

Troy Chemical Corporation, Newark, NJ, “Product Chemistry – Troysan 174, (2[(Hydroxymethyl)amino] ethanol),” October 24, 1990.

## 2.0 Boiling Point

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
Purity 98.88%

### Method

| | |
|---|---|
| Method: | OECD 102 |
| GLP: | Yes |
| Year: (study performed): | 1990 |

### Test Conditions

The product was tested according to OECD Method 102 "Boiling Point/Boiling Range," the Siwoloboff method. Lot # 9010-6514 was tested in triplicate.

### Results

| | |
|---|---|
| Boiling point value in °C: | 110 °C |

### Conclusions

Results of triplicate testing were 115 °C, 110 °C, and 105 °C, with an average value of 110 °C.

### Reliability

This study was conducted in compliance with EPA GLP Standards (40CFR 160) (Study Author) according to EPA Guidelines 63-3.

### References

Troy Chemical Corporation, Newark, NJ, "Product Chemistry – Troysan 174, (2[(Hydroxymethyl)amino] ethanol)," October 24, 1990.

## 3.0 Vapor Pressure

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
Batch 9709-4882

### Method

| | |
|---|---|
| Method: | Calculated OPPTS 830.7950 |
| GLP: | Yes |
| Year: | 1997 |

### Test Conditions

The vapor pressure of Troysan 174 was determined using the static method presented in the guideline. An instrument constructed by Case Consulting Laboratories, Inc. was used. The zero-indicator and nitrogen pressures were measured using pressure transducers.

The test substance was places in a glass, round-bottomed flask and attached to the instrument. The test substance was degassed by immersing the flask in a dry ice/ethanol bath and applying a high vacuum. The test substance was warmed to room temperature under vacuum, cooled again in dry ice/ethanol and the limited headspace was evacuated.

The pressure produced by the test substance at a particular temperature was recorded. The temperature was raised by an arbitrary amount (~9°C), the system brought to thermodynamic equilibrium and the new pressure recorded. This cycle was repeated for a total of four data points.

The log of the pressure was plotted against the reciprocal of the absolute temperature. Vapor pressures at 20 and 25°C were calculated from this curve.

No statistics methods were applied.

### Results

| | |
|---|---|
| Vapor Pressure value: | VP (20.0°) = 1585 Pa |
| | VP (25.0°) = 2116 Pa |
| Decomposition: | No |

Measured Vapor Pressures

| | |
|---|---|
| 24.6°C | 2082 Pa |
| 30.8°C | 2904 Pa |
| 38.8°C | 4453 Pa |
| 46.9°C | 6785 Pa |

Calculated Vapor Pressures

| | |
|---|---|
| 20.0°C | 1585 Pa |
| 25.0°C | 2116 Pa |

**Conclusions**

VP (20.0°) = 1585 Pa
VP (25.0°) = 2116 Pa

**Reliability**

Klimisch Code = 1

The study was conducted according to OPPTS Guidelines 830.7950, and in accordance with GLPs. (Reviewer)

The study, "Physical and Chemical Characteristics of Troysan® 174: Viscosity and Vapor Pressure," reported herein was conducted in compliance with the Good Laboratory Practice Standards as set forth in Title 40 Part 160, of the Code of Federal Regulations of the United States of America and as specified in 54 Federal Register 34067 (August 17, 1989).

**Reference**

Case Consulting Laboratories, Inc. Whippany, NJ, "Physical and Chemical Characteristics of Troysan 174: Viscosity and Vapor Pressure," December 1997.

## 4.0 Water Solubility

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)

### Method

| | |
|---|---|
| Method: | OECD 105 |
| GLP: | Yes |
| Year: | 1990 |

### Test Conditions

OECD Guidelines for Testing of Chemicals, Section 1, No. 105, "Water Solubility," May 12, 1981. Preliminary solubility data indicated that the sample was miscible in two of the solvents to be tested: water and isopropanol. Therefore, the OECD method was modified for these solvents by equilibrating weight: weight (1:1) mixtures of sample and solvent at 25°C for 24 h, then analyzing the solutions to determine if the sample had been altered. Solubility was determined for technical grade Troysan 174 (Lot No. 89095340, 97.2% active ingredient) in water, octanol, and isopropanol. The study was monitored by supercritical fluid chromatography (SFC).

### Results

Troysan 174 was found to be completely miscible with water at 25.0 ± 0.5°C. Comparison of GC profiles for the sample with a standard indicated that the Troysan 174 had not been altered significantly. (Study author)

### Conclusions

Troysan 174 was found to be completely miscible with water at 25.0 ± 0.5°C.

### Reliability

Klimisch Code = 1

This study was conducted in compliance with OECD Guideline 105 and in accordance with the EPA GLP standards (40 CFR 160). (Study author)

## References

Midwest Research Institute, Kansas City, MO, “Product Chemistry for Troysan 174, Guidelines Series 63 Physical and Chemical Characteristics Solubility Study,” Laboratory Project ID 9555-F(02), October 19, 1990.

## 5.0 Stability in Water

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
Purity 98.7%

### Method

| | |
|---|---|
| Method/guideline followed: | EPA Pesticide Assessment Guidelines Subdivision N, Section 161-1 |
| Type (test type): | Aqueous Hydrolysis at pH 5, 7, 9 (25°C) |
| GLP: | Yes |
| Year: (study performed): | 1992 |

### Test Conditions

| | |
|---|---|
| Duration: | 30 days |
| Statistical Methods: | Mean and standard deviation were calculated, and a linear regressions analysis was performed. |

The aqueous hydrolysis of 2[(Hydroxymethyl)amino])ethanol, was followed for 30 days in buffered solutions at pH 5, 7 and 9. Since radioactively labeled material was not available for the study, the 95% grade compound was used and chemical analysis was used to identify the breakdown products. The initial amount of test material dissolved in the buffers was 250 ug/mL. The buffers were autoclaved after preparation and before the test substance was added. Efforts were made to maintain sterility. For the duration of the study, the solutions were maintained in darkness in a water bath adjusted to 25°C. The temperature of the bath was recorded once daily, with an average of 25°C, ranging from 24.9 to 25.1° C. The solutions were sampled for immediate chromatographic analysis at intervals of 0, 1, 3, 7, 14, 21 and 30 days. Samples from each time point were also placed in the freezer for further analysis. Liquid chromatographic analysis of the fresh samples using post-column detection allowed quantitation of primary amines.

Validation:
*Analysis of Amines by Post-Column Derivatization with o-Phthaladehyde.*
A validation study was undertaken, using buffer samples fortified with known amounts of Troysan 174 on the day of testing. Each set consisted of three replicate sample of Troysan 174 (at 0, 125 and 250 μg/mL) in each buffer (pH 5.0, 7.0 and 9.0). Fortification recovery data are presented in Appendix B. The average recovery for validation fortifications at 250 μg/mL averaged 96.4% at pH 5.0, 103.6% at pH 7.0 and 98.1% at pH 9.0.

*GC-MS Analysis of Troysan 174 Reference Substance.* After the last sampling interval (30 days), the reference standard of Troysan 174 used in the study was dissolved in acetonitrile and analyzed by gas chromatography-mass spectroscopy (See Method 147S09C in Appendix A). The results show a single peak with mass spectrum indicating that the original test substance was intact and had not deteriorated during the time of the study.

Analysis of the study samples indicates that Troysan 174-derived formaldehyde is strongly persistent in the buffered solutions (Table II, Figures 4-6). Recovery of Troysan-derived formaldehyde was not as quantitative over the entire length of the study (Table II), but the data suggest long half-lives for this degradation product.

**Results**

Breakdown products: Formaldehyde and ethanolamine

There was immediate, or nearly immediate hydrolysis of Troysan at pH 5.0, 7.0 and 9.0 at 25°C.

Troysan 174 dissolved in water and ethanolamine co-eluted in two dissimilar chromatographic systems (reversed-phase, ion-exchange HPLC) and gave similar fluorescence responses on a molar basis. The recovery of these two substances together after thirty days (calculated as a percentage of the initial, using Troysan 174 dissolved in water as the standard) averaged 91.5% at pH 5, 97.5% at pH 7 and 97.1% at pH 9. The buffered solutions were also assayed for formaldehyde using the selective colorimetric reaction with Chromotropic acid. This method showed that Troysan 174-derived formaldehyde could be detected in all study samples, beginning with the time zero samples. To check for rapid hydrolysis of Troysan 174 to release formaldehyde, samples of control buffer at pH 5.0, 7.0 and 9.0 from the original preparation were spiked with 250 ug/ml Troysan 174 (using a fresh solution of the reference standard) and immediately assayed for formaldehyde and amines, or were incubated with a cation-exchange resin for five minutes prior to these assays.

The results indicate that the same quantity of formaldehyde was assayable with or without prior removal of amines by ion-exchange. Chromatographic analysis of these solutions for primary amines showed that the treatment had removed between 55 and 95% of the amines assayed in the solutions not treated with ion-exchange resin. Gas chromatography–mass spectroscopy of the original reference standard of Troysan 174 confirms that this compound was present prior to preparation of aqueous solutions. Therefore, the study determined that Troysan

174 is subject to rapid hydrolysis in aqueous solution at each buffer pH included in the study – pH 5, 7 and 9.

## Conclusions

The hydrolysis of Troysan 174 to formaldehyde and ethanolamine was too rapid to be quantified.

## Reliability

Klimisch Code = 1

Study conducted in compliance with EPA GLP standards (40CFr 160 and according to EPA Pesticide Guidelines Subdivision N 161-1 (Study author)

## References

EPL Bio-Analytical Services, Inc. Harristown, IL, “Aqueous Hydrolysis of 2-(Hydroxymethyl) amino ethanol,” Laboratory Project Identification 147S09, November 2, 1992.

## 6.0 Acute Toxicity to Fish

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
97% pure

### Method

| | |
|---|---|
| Method/guideline followed: | EPA Pesticide Assessment Guidelines FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, EPA 540/9-82-024, Oct. 1982 and ASTM Standard E 729-88, Standard Practice for Conducting Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians |
| Test type: | 96 hour flow-through, LC50 |
| GLP: | Yes |
| Year *(study performed)*: | 1990 (Report finalized 1992) |
| Species/Strain/Supplier: | Rainbow Trout (*Oncorhynchus mykiss*), Fattig Fish Hatchery, Brady NE |
| Exposure period [Duration]: | 96 hours |

### Test Conditions

Test fish:

| | |
|---|---|
| Age: | Juveniles |
| Wet Weight: | Mean – 0.72 g, Range 0.39 to 1.0 g |
| Length: | Mean – 44 mm, Range 39 to 48 mm |
| Number of replicates: | 2 |
| Fish per replicate: | 10 |

Dilution water source: Fresh water was obtained from a well 45 meters deep that was located on the test laboratory site. The well water was characterized as medium hard water.

Exposure vessel type:

A continuous-flow diluter was used to deliver each concentration of the test substance and a negative (well water) control. A syringe pump (Harvard Apparatus, Inc., Model 22) was used to inject the test substance stock solution into a delivery line where the test substance was mixed with dilution water to form a concentrated solution.

The diluter was adjusted so that each test chamber received 6 volume additions of test water every 24 hours. The delivery pump was calibrated before the test, and the general operation of the diluter was checked visually at least two times per day during the test.

Test chambers were Teflon®-lined, 25-L polyethylene aquaria filled with 15 L of test water. The depth of the test water in each chamber was approximately 17 cm. The test chambers were randomly positioned in a temperature-controlled water bath designed to maintain a temperature of 12±1˚C. The water bath was enclosed in a plexiglass ventilation hood in order to minimize any potential for cross-contamination.

Water chemistry in test (D.O., pH) in the control and one concentration where effects were observed:

During the 14-day holding period immediately preceding the test, water temperatures ranged from 11.0 to 11.3°C. The pH of the water during the holding period ranged from 7.5 to 7.9, alkalinity was between 188 and 194 mg/L, as $CaCO_3$, and hardness ranged from 132 to 148 mg/L, as $CaCO_3$.

Temperature was measured in each test chamber at the beginning and end of the test using a calibrated hand-held thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the study was 12±1°C. Hardness and alkalinity measurements were made by titration in accordance with Standard Methods for the Examination of Water and Wastewater (3). Conductivity was measured using a Yellow Springs Instruments Model 33 Salinity-Conductivity-Temperature meter. The pH and dissolved oxygen content of the water in alternate replicates of each treatment and control group were measured at 24-hour intervals during the study. Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter.

Observations:

All organisms were observed to evaluate the numbers of mortalities and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior. Typical abnormalities that were evaluated included surfacing, lethargy, hyperexcitability, discoloration, erratic swimming, etc. Observations were made approximately 2, 24, 48, 72, and 96 hours after initiation of the test.

Test temperature range*:* 12° C ± 1
Method of calculating mean measured concentrations*:*
Water samples were collected from each replicate test chamber at test initiation, and at test termination to measure concentrations of the test substance.

Statistics:
The pattern of mortality in this study did not allow for calculating an LC50 value; therefore, an estimation of the LC50 value was made by a visual inspection of the mortality data.

**Remarks (Test Conditions)**

Rainbow trout were exposed to a geometric series of five test concentrations and a negative (well water) control. Two replicate test chambers were maintained in each treatment and control group, with 10 fish in each test chamber. Nominal test concentrations were based upon the results of acute range finding toxicity tests. Nominal test concentrations selected were 13.0, 21.6, 36.0, 60.0, and 100 mg of Troysan 174/L. Samples of test water were collected from each treatment and control group at test initiation and termination. The samples were shipped to EPL Bio-Analytical Services for analysis.

Delivery of the test substance was initiated approximately 4 hours prior to the introduction of the fish to the test water in order to achieve equilibrium of the test substance in the test chambers. The fish were impartially removed from holding tanks in groups of two and released into the test chambers at test initiation. Observations of mortality and other clinical signs of toxicity were made at 2, 24, 48, 72, and 96 hours. Cumulative percent mortality values in the treatment groups were used to calculate LC50 values at 24, 48, 72, and 96 hours. The no observed effect concentration was determined by visual examination of the mortality, and clinical observation data.

**Results**

Nominal concentrations (as mg/L): 0, 13.0, 21.6, 36.0, 60.0 and 100 mg/L
Element value: 96 hour LC50 = > 100 mg/L

NOEC = 100 mg/L

Daily observations of mortality and other signs of toxicity observed during the test are shown in Table 2. Rainbow trout in the negative control group appeared healthy and normal throughout the test. Similarly there was no mortality or signs of toxicity observed in the 13.0 mg/L treatment group. All fish in that group appeared healthy and normal throughout the test.

While there were no mortalities during the 96-hour exposure period at any of the test concentrations equal to or greater than 21.6 mg/L, small numbers of fish in the 21.6, 36.0, and 60.0 mg/L treatment groups exhibited discoloration or a slight loss of equilibrium. However, all fish in the 100 mg/L treatment group appeared healthy and normal throughout the test. Since the numbers of fish exhibiting normal clinical signs was small and not concentration-dependant, the signs were not considered to be treatment related.

Table 3
LC50 Values

Sponsor: Troy Chemical Company
Test Substance: Troysan 174
Test Organism: Rainbow Trout (Oncorhynchus mykiss)
Dilution Water: Well Water

| Time | LC50 (mg/L) | Lower 95% Confidence Limits | Upper 95% Confidence Limits | Statistical Method |
|---|---|---|---|---|
| 24 Hours | >100 | N/A | N/A | N/A |
| 48 Hours | >100 | N/A | N/A | N/A |
| 72 Hours | >100 | N/A | N/A | N/A |
| 96 Hours | >100 | N/A | N/A | N/A |

N/A = Not Applicable

**Conclusions**

The 96-hour LC50 value for rainbow trout exposed to Troysan 174 was >100 mg/L. The 96-hour no observed effect concentration was 100 mg/L, as determined by visual examination of the toxicity data.

**Reliability**

Klimisch Code = 1

The study was conducted according to EPA FIFRA and ASTM standard protocols as well as in compliance with GLPs. GLP compliance statements were provided by Wildlife International Inc. for the biological portion of the study, and EPL Bioanalytical Services Inc. for the analytical portion of the study.

## References

Wildlife International LTD, Easton, MD, "Troysan 174: A 96-Hour Flow-Through Toxicity Test with the Rainbow Trout (*Onchorhynchus mykiss)*, Amended Final Report," November 25, 1992.

## 7.0 Acute Toxicity to Aquatic Invertebrates

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
97% purity

### Method

| | |
|---|---|
| Method/guideline followed: | EPA Series 72 of Pesticide Assessment Guidelines, Subdivision E Hazard; ASTM Standard E 729-88 |
| Test type: | Flow-through |
| GLP: | Yes |
| Year: (study performed): | 1992 |
| Analytical Monitoring: | Yes |
| Species/Strain*:* | *Daphnia magna* |
| Exposure period [Duration]: | 48 hours |

Statistical methods:
The computer program of C.E. Stephan was used to calculate the EC50 and 95% confidence interval by probit analysis, the moving average method, or binomial probability with nonlinear interpolation. The binomial method was used to evaluate mortality at 24 and 48 hours. The No Observed Effect Concentration was determined by visually inspecting the mortality data.

### Test Conditions

| | |
|---|---|
| Test organism: | *Daphnia magna* |
| Organism source/supplier: | Wildlife international Ltd. Cultures |
| Age at study initiation: | < 24 hours |
| Control group: | Yes, dilution water |

Test organism pretreatment, breeding method:
The daphnids were cultured and held in water from the same source and at the same temperature as used during the test. Daphnids in culture showed no signs of disease or stress. Neonates were obtained for testing by transferring individual adult daphnids to dilution water 24 hours prior to test initiation. The progeny produced by three or more adults during this 24-hour period were used as test organisms. At test initiation, the juvenile daphnids were carefully collected from the cultures, and transferred to the test chambers. Daphnids in the cultures were fed a mixture of yeast, Cerophyll®, and trout chow, as well as a suspension of the freshwater green alga *Selenastrum capricornutum.* The adults were fed during the

24-hour isolation period prior to test initiation, but neonates were not fed during the test.
Test concentrations: 13.0, 21.6, 36.0, 60.0 and 100 mg/L

Stock solutions preparation (vehicle, solvent, concentrations) and stability:
A stock solution of the test substance was prepared at a concentration of 0.848 g/ml by dissolving Troysan 174 in deionized water. The stock was delivered to the diluter and mixed with well water to achieve the desired test concentrations.

Test temperature range*:* 20±1°C

Exposure vessel type (e.g., size, headspace, sealed, aeration, number per treatment):
The diluter was adjusted so that each test chamber received approximately 6 volume additions of test solution every 24 hours. The syringe pump was calibrated before initiation of the test and the general operation of the diluter was checked visually at least two times per day during the test.

Test compartments were constructed from glass cylinders, 5 cm in diameter and 10 cm in length. The depth of the test solution in each test compartment was approximately 6 cm; leaving 4 cm headspace. Test chambers were randomly positioned in a temperature controlled water bath to maintain a temperature of 20±1°C. The water bath was enclosed in a plexiglass ventilation hood in order to minimize potential cross contamination.

Dilution water source:
The water used for holding and testing was fresh water obtained from a well 45 meters deep located on the Wildlife International Ltd. Site. The well water was passed through a sand filter to remove particles greater than 25 um and pumped into a 37,800 L storage tank where the water was aerated with spray nozzles. Prior to delivery to the diluter system, the water again was filtered to remove microorganisms and particles.

Dilution water chemistry:

Appendix I
Conductivity, Hardness, Alkalinity, and pH of Well Water Measured During the 4-Week Period Immediately Preceding the Test

| | |
|---|---|
| Sponsor: | Troy Chemical Company |
| Test Substance: | Troysan 174 |
| Test Organism: | Cladoceran, Daphnia magna |
| Dilution Water: | Well Water |

| | Mean | Range |
|---|---|---|
| Conductivity (μmhos/cm) | 350 (n = 4) | 340-360 |
| Hardness (mg/L as $CaCO_3$) | 150 (n = 4) | 144-160 |
| Alkalinity (mg/L as $CaCO_3$) | 196 (n = 4) | 196-198 |
| pH | 7.9 (n = 4) | 7.6-8.2 |

Lighting (quality, intensity and periodicity):
Lighting used to illuminate the cultures and test chambers during holding and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (e.g., Chroma 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided at dawn and dusk to avoid sudden changes in light intensity. Light intensity during the study was approximately 20 to 70 footcandles at the surface of the water.

Water chemistry in test (D.O., pH) in the control and at least one concentration where effects were observed:

Table 1
Temperature, Dissolved Oxygen, and pH of Water in the Test Chambers

Sponsor: Troy Chemical Company
Test Substance: Troysan 174
Test Organism: Cladoceran, Daphnia magna
Dilution Water Well Water

| Nominal Concentration (mg/L) | Replicate | 0 Hours Temp (°C) | 0 Hours DO[1] (mg/L) | 0 Hours pH | 48 Hours Temp (°C) | 48 Hours DO (mg/L) | 48 Hours pH |
|---|---|---|---|---|---|---|---|
| Negative Control | A | 20.0[2] | 8.8 | 8.2 | 19.7 | -- | -- |
| | B | 19.9 | -- | -- | 19.6 | 9.4 | 8.2 |
| 13.0 | A | 19.9 | 9.0 | 8.4 | 19.6 | -- | -- |
| | B | 19.9 | -- | -- | 19.5 | 9.2 | 8.3 |
| 21.6 | A | 19.9 | 8.8 | 8.5 | 19.7 | -- | -- |
| | B | 19.9 | -- | -- | 19.6 | 9.0 | 8.4 |
| 36.0 | A | 19.9 | 8.8 | 8.7 | 19.6 | -- | -- |
| | B | 19.9 | -- | -- | 19.5 | 9.2 | 8.9 |
| 60.0 | A | 19.9 | 8.8 | 8.9 | 19.6 | -- | -- |
| | B | 19.9 | -- | -- | 19.5 | 9.2 | 9.0 |
| 100 | A | 19.9 | 8.9 | 9.1 | 19.6 | -- | -- |
| | B | 19.8 | -- | -- | 19.5 | 9.4 | 9.0 |

[1] A dissolved oxygen concentration of 5.4 mg/L represents 60% saturation at 20°C in fresh water.
[2] Temperature measured continuously in negative control replicate A ranged from approximately 19.7 to 20.3 C.

The 0-hour hardness, alkalinity, and conductivity values of the dilution water were 148 mg/L as $CaCO_3$, 198 mg/L as $CaCO_3$, and 330 µmhos/cm, respectively.

Element (unit) basis: mg/L (immobilization, mortality)
Test design: 2 replicates with 10 organisms each for each test concentration
Exposure period: 48 hours
Analytical monitoring:
Water samples were collected from each replicate test chamber at test initiation and termination to measure concentrations of the test substance. Samples were shipped to EPL Bio-Analytical Services for analysis.

Daphnids were exposed to a geometric series of five test concentrations and a negative (well water) control. Two replicate test chambers were maintained in each treatment and control group, with 10 daphnids in each test chamber. Nominal test concentrations were based upon the results of acute range finding toxicity tests. Nominal test concentrations selected were 13.0, 21.6, 36.0, 60.0 and 100 mg/L of Troysan 174. Samples of test water were collected from each

treatment and control group at the beginning of the test and at test termination. The samples were shipped to EPL Bio-Analytical Services for analysis.

Delivery of the test substance was initiated approximately 18.5 hours prior to the introduction of the daphnids to the test water in order to achieve equilibrium of the test substance in the test chambers. Daphnids were impartially removed from holding tanks in groups of two and released in to the exposure chambers at test initiation. Observations of mortality and other clinical signs of toxicity were made on the day of tests initiation and after approximately 24 and 28 hours. Cumulative percent mortality values in the treatment groups were used to calculate EC50 values at 24 and 48 hours. The no observed effect concentration was determined by visual examination of the mortality, immobilization, and clinical observation data such as surfacing lethargy, erratic swimming, and floating.

**Results**

Nominal concentrations (mg/L): 0, 13.0, 21.6, 36.0, 60.0 and 100 mg/L
Measured concentrations (mg/L):
24 hours: 0.3, 10.0, 18.2, 34.3, 60.5, 107.5 mg/L
48 hours: 0, 6.7, 9.9, 37.1, 48.1, 72.5 mg/L
EC50 values and statistical results:

Table 3
EC50 Values

Sponsor: Troy Chemical Company
Test Substance: Troysan 174
Test Organism: Cladoceran, Daphnia magna
Dilution Water: Well Water

| Time | EC50 (mg Troysan 174/L) | Lower 95% Confidence Limits | Upper 95% Confidence Limits | Statistical Method |
|---|---|---|---|---|
| 24 Hours | 54.8 | 36.0 | 100 | Binomial |
| 48 Hours | 27.9 | 21.6 | 36.0 | Binomial |

N/A = Not Applicable

Temperatures were within the 20±1°C range established for the test. Dissolved oxygen concentrations measurements exceeded 60% of saturation throughout the test.

Daily observations of mortality and other signs of toxicity were recorded. Daphnids in the negative control group appeared healthy and normal throughout the test. Similarly there was no mortality, immobilization or signs of toxicity

observed in the 13.0 mg/L treatment group. All daphnids in that group appeared healthy and normal throughout the test.

While there were no mortalities or other signs of toxicity during the first 24 hours of exposure in the 21.6 and 36.0 mg/l treatment groups, by 48 hours mortality in those two groups was 5% and 95% respectively. At 60.0 mg/L there was 65% mortality within the first 24 hours, and 100% mortality by 48 hours. At 100 mg/L the highest concentration tested, 100% mortality occurred within 24 hours of test initiation.

## Conclusions

The 48 hour EC50 value for daphnids exposed to Troysan 174 was 27.9 mg/L. The 95% confidence limits for the EC50 value were 21.6 and 36.0 mg/L. The 48hour No Observed Effect Concentration was 13.0 mg/L, as determined by visual observation of the toxicity data.

## Reliability

Klimisch Code = 1

The study is reliable without restriction. (Reviewer)
This study was certified as being conducted in compliance with GLPs as published by EPA in 40CFR Part 160 (August 1989) with the following items noted: water samples from the study were collected to verify test concentration and stability. The analytical portion of the study was also certified as being conducted according to GLPs published by EPA in 40CFR Part 160 by EPL Bioanalytical services Inc. The test substance was characterized by the sponsor. (Study author)

The study was conducted according to the procedures based on EPA Series 72 of Pesticide Assessment Guidelines, Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms and ASTM Standard E 729-88, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. (Study author)

## References

Wildlife International Ltd., Easton, MD, “Troysan 174: A 48 hour flow-through acute toxicity test with the Cladoceran (*Daphnia magna*)- Amended Final Report, FIFRA Subdivision E, Series 72-2.” November 25, 1992.

## 8.0 Acute Toxicity

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)

### Method

| | |
|---|---|
| Method/guideline followed: | Not reported |
| Type *(test type):* | Acute oral |
| GLP: | Yes |
| Year (study performed): | 1986 |
| Species/Strain: | Rat/ Sprague-Dawley |
| Sex: | Male and Female in first study, Female in second study |
| No. of animals per sex per dose: | 5 Male, 5 Female per dose |
| Vehicle: | Distilled water |
| Route of administration: | Oral, gavage |

The test material was administered orally in a single dose by means of gavage at a constant dose volume of 10 ml/kg.

The rats were observed frequently on the day of dosing and for 14 days following. They were weighed immediately prior to dosing, 7 days after dosing and at death or sacrifice at the end of the 14 day observation period.

At the end of the observation period and sacrifice by nitrogen asphyxiation, each animal was subjected to a gross post mortem examination.

The LD50 values were calculated based on the mortality pattern by probit analysis (Finney, 1971).

### Test Conditions

| | |
|---|---|
| Age: | 6 – 8 weeks |
| Doses: | 750, 1250, 2100 and 3500 mg/kg (for males)<br>2500, 3150, 4000 and 5000 mg/kg (for females in first study)<br>600, 1200, 1800 and 2400 mg/kg (for females in second study) |
| Post dose observation period: | 14 days |

### Results

Value:

LD50 (Males): 1620 mg/kg, 95% confidence limits 1069-2455 mg/kg
LD50 (Females): 1956 mg/kg, 95% confidence limits 1110-3093 mg/kg

Number of deaths at each dose level:

Mortality by dose and sex (first study):

| Dose Level ($mg.kg^{-1}$) | ♂ | ♀ |
|---|---|---|
| 750 | 0/5 | - |
| 1250 | 1/5 | - |
| 2100 | 4/5 | - |
| 2500 | - | 5/5 |
| 3150 | - | 5/5 |
| 3500 | 5/5 | - |
| 4000 | - | 5/5 |
| 5000 | - | 5/5 |

Mortality by dose (female only, second study):

| $mg.kg^{-1}$ | ♀ |
|---|---|
| 600 | 0/5 |
| 1200 | 0/5 |
| 1800 | 2/5 |
| 2400 | 4/5 |

Due to the unexpected mortality in the first study, it was decided to repeat the study using lower dose levels for females only.

Time of death: 4 hours to 1 day (first study)
1 day (second study)

Description, severity, time of onset and duration of clinical signs at each dose level:
Clinical signs noted at 0.5 hr to 4 days after dosing, including hyperkinesias, sedation, coma, prostration, piloerection, soiled coat, ataxia and haemodaccryorrhea.

Necropsy findings, included doses affected, severity and number of animals affected:
Premature decedents showed red stained fluid in the gut. No abnormalities were noted, at post mortem, in survivors sacrificed after the 14-day observation period, except for one male which showed a gas-filled gut and stomach.

Potential target organs: None identified

Body weight gains in the first study were generally lower than expected with one male rat having lost weight after 7 days, and another having lost weight at the end of the 14-day observation period.

Clinical signs in the second study were similar to those noted in the first study. Deaths occurred 1 day after dosing. No abnormalities were noted at post mortem in premature decedents or in animals sacrificed after the 14-day observation period. Body weights were within acceptable limits.

## Conclusions

LD50 (Males): 1620 mg/kg, 95% confidence limits 1069-2455 mg/kg; LD50
LD50 (Females): 1956 mg/kg, 95% confidence limits 1110-3093 mg/kg

## Reliability

Klimisch Code = 1

The study was performed in accordance with the principles of Good Laboratory Practice. While a specific method was not identified in the study report, there is sufficient detail to conclude that the methodology followed is comparable to EPA guidelines for the conduct of acute studies. (Reviewer)

## References

Inveresk Research International, Scotland, “Compound 174: Acute Toxicity Tests,” IRI Project No. 235629, November 1986.

## 9.0 Genetic Toxicity In Vivo

### Test Substance

Identity *(purity)*: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
74.3% Purity; Nuosept 91 (Huls America Inc.), Lot 074-002

### Method

| | |
|---|---|
| Method/guideline followed: | OECD Guideline 474 |
| Type: | Micronucleus assay |
| GLP: | Yes |
| Year: | 1995 |
| Species: | Mouse Strain Charles River CD1 |
| Sex: | Male and Female |
| Route of administration: | Oral, gavage |
| Doses/concentration levels: | 150, 300 and 600 mg/kg |

Statistical methods:
This study was designed to assess the potential induction of micronuclei by 2-(Hydroxymethyl)aminoethanol in bone marrow cells of mice.

Mice were treated with a single acute oral administration of the test substance by intragastric gavage at dosages of 150, 300 and 600 mg/kg. A preliminary toxicity test had previously shown 600 mg/kg to be approximately the maximum tolerated dosage.

Negative and positive control groups were dosed in an identical manner, orally by intragastric gavage. The negative control group received the vehicle, water. The positive control group was treated with mitomycin C at 12 mg/kg bodyweight.

Bone marrow smears were obtained from five male and five female animals in the negative control and test substance groups at each of 3 sampling times; these being 24, 48 or 72 hours after dosing. Bone marrow smears were obtained from the positive control group 24 hours after dosing. One smear from each animal was examined for the presence of micronuclei in 1000 polychromatic erythrocytes. The ratio of polychromatic to normochromatic erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. A record of the incidence of micronucleated normochromatic erythrocytes was also kept.

### Test Conditions

| | |
|---|---|
| No. of animals per dose: | 15 Male, 15 Female |
| Vehicle: | Water |
| Duration of test: | 72 hours |

| | |
|---|---|
| Frequency of treatment: | Single dose |
| Sampling times: | 24, 48 and 72 hours |
| Positive Control: | Mitomycin C, 12 mg/kg bodyweight |

The positive control produced large, highly significant increases in the frequency of micronucleated polychromatic erythrocytes.

| | |
|---|---|
| Negative Control: | Water |

Criteria for evaluating Results:

A positive response is normally indicated by a substantial, statistically significant increase ($P < 0.01$) in the incidence of micronucleated polychromatic erythrocytes compared to the incidence for the concurrent vehicle control group for at least one of the sampling times; individual and/or group mean values should exceed the laboratory historical control range (see Appendix 4). A negative result is indicated where individual and group mean incidences of micronucleated polychromatic erythrocytes for animals treated with the test substance are not significantly greater ($P > 0.01$) than incidences for the concurrent control group and where these values fall within the historical control range. An equivocal response is obtained when the results cannot be adequately classified using the criteria for a positive or negative response.

Bone marrow cell toxicity (or depression) is normally indicated by a substantial, statistically significant decrease ($P < 0.01$) in the ratio of polychromatic to normochromatic erythrocytes. This decrease would normally be evident at both the 48 and 72 hour sampling points, a decrease at the 24 hour time point is not necessarily expected because of the relatively long transition time of erythroid cells [late normoblast → polychromatic erythrocyte (approximately 6 hours) → normochromatic erythrocyte (approximately 30 hours)]. A very large decrease in this ratio would be indicative of a cytotoxic effect.

**Results**

| | |
|---|---|
| Genotoxic effects: | Negative |

Mortality at each dose level by sex:
Three male and five female animals died after treatment with the highest level of 2-(Hydroxymethyl)aminoethanol in the micronucleus test. At *post mortem* examination, none of these animals showed signs of misdosing. These animals were replaced by animals from the concurrently treated satellite group. Clinical signs for animals treated with 2-(Hydroxymethyl)aminoethanol are detailed in Appendix 3. No adverse clinical signs were obtained for the vehicle control or positive control treated animals over the duration of the test.

**Preliminary toxicity test and micronucleus test - Mortality data**

| Phase | Group | Treatment | Dosage (mg/kg) | Mortality ratio (No. of deaths) (No. dosed) | | |
|---|---|---|---|---|---|---|
| | | | | ♂ | ♀ | Combined |
| I | 1 | 2-(Hydroxymethyl)-aminoethanol | 450 | 0/2 | 0/2 | 0/4 |
| | 2 | | 600 | 0/2 | 0/2 | 0/4 |
| | 3 | | 800 | 0/2 | 2/2 | 2/4 |
| | 4 | | 1000 | 2/2 | 2/2 | 4/4 |
| II | 5 | 2-(Hydroxymethyl)-aminoethanol | 416.6 | 0/2 | 0/2 | 0/4 |
| | 6 | | 500 | 0/2 | 1/2 | 1/4 |
| | 7 | | 600 | 0/2 | 0/2 | 0/4 |
| | 8 | | 720 | 1/2 | 0/2 | 1/4 |
| Micronucleus test | 1 | Vehicle | - | 0/15 | 0/15 | 0/30 |
| | 2 | 2-(Hydroxymethyl)-aminoethanol | 150 | 0/15 | 0/15 | 0/30 |
| | 3 | | 300 | 0/15 | 0/15 | 0/30 |
| | 4 | | 600 | 3/20 | 5/20 | 8/40 |
| | 5 | Mitomycin C | 12 | 0/5 | 0/5 | 0/10 |

A slight but statistically significant increase (P< 0.01 using Kruskal-Wallis's test) in the frequency of micronucleated polychromatic erythrocytes was obtained at the 24 hour sampling time for animals treated at the intermediate level (300 mg/kg) of the test substance. This increase was small and was not dose related (P>0.01 using Jonckheere's test for trend) and was not apparent at the later sampling times. Slides from this group along with those from the concurrent vehicle control were re-examined by a second slide reader and, in this instance, no significant increase in the incidence of micronucleated polychromatic erythrocytes was recorded. In all cases the group mean and individual animal incidence of micronucleated polychromatic erythrocyte values obtained were well within the laboratory historical control range. The increase recorded in the original examination is therefore thought to be the result of chance variation and is not indicative of chromosome damage. At all other sampling times and does levels, mice treated with 2-(Hydroxymethyl)aminoethanol did not show any significant increase in the frequency of micronucleated polychromatic erythrocytes.

There was no significant decrease in the ratio of polychromatic to normochromatic erythrocytes after treatment of the animals with 2-(Hydroxymethyl)aminoethanol.

Ratio of polychromatic to normochromatic erthrocytes(p/a)

2-(Hydroxymethyl)aminoethanol failed to cause any significant decreases in the ratio of polychromatic to normochromatic erthrocytes [P>0.01 using Kruskal0Wallis' test] at any of the three dose levels or sampling times.

Mitomycin C did not cause any statistically significant decreases in the p/a ratio at the 24 hour sampling time [P>0.01 using Wilcoxon's sum of ranks test].

## Conclusions

Since the test substance did not cause any substantial or confirmable increase in the incidence of micronucleated polychromatic erythrocytes or any significant decrease in the p/n ration, it is concluded that 2-(Hydroxymethy) amino ethanol did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered orally in this in vivo test procedure. (Study author)

## Reliability

Klimisch Code = 1, Reliable without restriction

This study was conducted in compliance with the following Good Laboratory Practice standards: UK Compliance Programme, EC Council Directive 87/18, OECD No. 45 (1992), Japan Ministry of Agriculture, Forestry and Fisheries (No. 3850), US EPA (TSCA) 40 CFR Part 792, US FDA 21 CFR Part 58. (Study author.) This study was conducted to meet EPA FIFRA Series 84-1 (mutagenicity tests). A certificate of analysis for the test substance was provided in the study report. (Reviewer)

## References

Huntingdon Research Centre, Ltd., England, " 2-(Hydroxymethyl)aminoethanol Mouse Micronucleus test," HRC Study Report No. TCC5/941605, January 30, 1995.

## 10.0 Genetic Toxicity In Vitro

(A) Unscheduled DNA Synthesis

### Test Substance

Identity (*purity*): Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
100% Purity. Batch 8904-5016. A copy of the certificate of test substance receipt and the certificate of analysis from Troy Chemical Corporation are included in the study report.

### Method

| | |
|---|---|
| Method/guideline followed: | EPA Guideline 84-2(b) |
| Type: | Mammalian cell unscheduled DNA synthesis |
| System of testing: | Nonbacterial |
| GLP: | Yes |
| Year (study performed): | 1989 |

Species/Strain or cell type and or cell line: Rat/ Fischer 344/ adult hepatocytes

This study was conducted to assess the ability of the test compound to induce unscheduled DNA synthesis in primary cultures of adult rat hepatocytes, as measured by silver grain counts in photographic emulsion formed by radiation from [6 $^{3}$H]-thymidine taken up by the cells. Cultures were established with cells derived from the collagenase-perfused liver of a young adult male Fischer 344 rat. Eight doubling concentrations from 0.78125 to 100 ug/ml were tested in 2 independent experiments.

The cells were examined microscopically at approximately 1000x magnification under oil immersion using a Leitz Dialux 20L microscope.

Unscheduled DNA Synthesis was measured by counting nuclear grains and subtracting the average number of cytoplasmic grains in 3 nuclear sized areas adjacent to each nucleus (background count). This value was referred to as the net nuclear grain count. The data were recorded as the average net grain counts for 3 cultures.

### Test Conditions

| | |
|---|---|
| Test concentrations: | 0.78125, 1.5625, 3.125, 6.25, 12.5, 25.0, 50.0 and 100.0 ug/ml (in both the first and second assays) |

Positive Controls:
Michler's ketone (direct acting) at 2.0 and 8.0 ug/ml

2-Acetylamino fluorine (2-AAF) (indirect acting) at 0.5 and 2.0 ug/ml
Both direct and indirect acting positive controls demonstrated the sensitivity of the assays. (Study author)

Solvent: Dimethylsulphoxide (DMSO)

Vehicle controls were considered valid, having met the requirements as specified by the acceptance criteria. (Study author)

Criteria for evaluating results:
The cell cultures were scored, and the results assessed according to the criteria of Butterworth et al (2), in conjunction with historical data generated in-house.

**Results**

Cytotoxic concentration: 50 – 100 ug/ml
Genotoxic effects: Negative

In the first assay, results were obtained up to 25 µg/ml with no evidence of unscheduled DNA synthesis at any concentration tested. In the second assay, results were obtained up to 25 µg /ml with no evidence of unscheduled DNA synthesis at concentrations of 12.5 µg /ml and lower. Toxicity at concentrations of 50 and 100 µg /ml prevented assessment in both assays. At the concentration of 25.5 µg/ml there was, however, a slight increase in the mean net grains per nucleus, accompanied by an increase in the percentage of cells adjudged to be in repair. The increase obtained did not meet the criteria required for a positive response, was not similarly observed in the first experiment and was not associated with a dose-related trend at lower concentrations.

**Conclusions**

It was concluded that unscheduled DNA Synthesis had not been demonstrated by 2-[(Hydroxymethyl) amino] ethanol when tested in dimethylsulphoxide at concentrations extending into the toxic range. (Study author)

**Reliability**

Klimisch Code = 1

This study was conducted according to a protocol that follows EPA Guideline 84-2(b) and in compliance with the Good Laboratory Practice regulations as described by the EPA (40 CFR Parts 160 and 792). (Study author) The test substance is of sufficient purity and is well characterized. A copy of the certificate

of test substance receipt and the certificate of analysis from the supplier (Troy Chemical Corporation) are included in the study report. (Reviewer)

## References

Inveresk Research International, Ltd., Scotland, “2[(Hydroxymethyl) Amino] Ethanol: Assessment of Genotoxicity in an Unscheduled DNA Synthesis Assay Using Adult Rat Hepatocytic Primary Cultures,” IRI Project No. 740842, December 13, 1989.

(B) Bacterial Reverse Mutation Assay

**Test Substance**

Identity (purity): Troysan 174, (2[(Hydroxymethyl)amino] ethanol) 98.2 – 98.5% Purity. Batch Nos. 8904-5016 and 9011-6630. Copies of test substance receipts and supplier certificates of analysis are included in the study report.

**Method**

| | |
|---|---|
| Method/guideline followed: | EPA Pesticide Assessment Guidelines Subdivision F, Series 84-2 |
| Type: | Bacterial reverse mutation assay |
| System of testing: | Bacterial |
| GLP: | Yes |
| Year (study performed): | 1991 |
| Cell line (bacterial or non-bacterial): | Bacterial |
| *Salmonella typhimurium* strains: | TA 1535, TA 1537, TA 1538, TA 98, TA 100 |
| *Escherichia coli*: | WP2uvrA- (pKM101) |
| Metabolic activation: | Yes, S9 Mix |
| Chemical used for induction: | Polychlorinated biphenyl mixture (Aroclor 1254) |

Concentrations tested:

First experiment:

+S9 mix: 3 µg, 10 µg, 33 µg, 100 µg, 333 µg and 1000 µg per plate
-S9 mix: 1.7 µg, 5 µg, 16.7 µg, 50 µg, 167 µg and 500 µg per plate

Second experiment:

+S9 mix: 50 µg, 100 µg, 150 µg, 200 µg, 250 µg, 300 µg, 350 µg, 400 µg and 450 µg per plate
-S9 mix: 50 µg, 100 µg, 200 µg, 300 µg, 400 µg, 500 µg and 600 µg per plate

The tests were conducted on agar plates in the presence and absence of an Aroclor 1254 induced rat liver preparation and co-factors (S9 mix) required for mixed-function oxidase activity. The direct plate incorporation method was employed.

**Test Conditions**

Number of replicates: 3
Positive Controls: Positive control substances were 2-aminoanthracene (2-AAN), methyl methanesulphonate (MMS), sodium azide, 9-aminoacridine and 2-nitrofluorene. Concurrent positive controls demonstrated the sensitivity of the assay and the metabolizing activity of the S9 mix.

Solvent*:*
Dimethylsulphoxide (DMSO) (AnalaR grade) was used as the solvent for the positive control substances except sodium azide, which was dissolved in sterile, ultra-pure water.

Sterile ultra-pure water was used as the vehicle control.

Criteria for evaluating result:

A test was considered acceptable if for each strain:

i) the bacteria demonstrated their typical responses to crystal violet, ampicillin and u.v. light.

ii) at least 2 of the vehicle control plates were within the following ranges: TA 1535, 4-30; TA 1537, 1-20; TA 98, 10-60; TA 100, 60-200 and TA 1538, 5-35; E. coli WP2uvrA⁻ (pKM101), 10-100.

iii) on at least 2 of the positive control plates there were x 2 the mean vehicle control mutant numbers per plate, or in the case of TA 100, x 1.5 the mean vehicle control mutant numbers per plate.

If the mean colony count on the vehicle control plates was less than 10 then a value of 10 was assumed for assessment purposes. In such cases a minimum count of 20 was required on at least 2 of the positive control plates.

iv) no toxicity or contamination was observed in at least 4 dose levels.

v) in cases where a mutagenic response was observed, that no more than one dose level was discarded before the dose which gave the highest significant mean colony number.

Where these criteria were met, a significant mutagenic response was recorded if there was:

i) for S. typhimurium strains TA 1535, TA 1537, TA 1538 and TA 98, and for E. coli, at least a doubling of the mean concurrent vehicle control values at some concentration of

the test substances and, for S. typhimurium strain TA 100, a 1.5-fold increase over the control value. If the mean colony count on the vehicle control plates was less than 10 then a value of 10 was assumed for assessment purposes. In such cases a minimum count of 20 was required before a significant mutagenic response was identified.

ii) a dose related response, although at high dose levels this relationship could be inverted because of, for example, (1) toxicity to the bacteria generally, (2) specific toxicity to the mutants and (3) inhibition of foreign compound metabolising enzymes where mutagens require metabolic activation by the liver.

iii) a reproducible effect in independent tests.

**Results**

Cytotoxic concentration:

With metabolic activation:
In the presence of S9 mix, toxicity resulting in the complete killing of the background lawn of microcolonies was observed at 3333 ug and 10,000 ug per plate. Less severe toxicity was also observed at 1000 ug per plate. Toxicity to the bacteria was observed from 350 ug per plate in the presence of S9 mix.

Without metabolic activation:
In the absence of S9 mix, complete killing of the background lawn of microcolonies was observed at 1,000, 3,333 and 10,000 ug per plate.

Toxicity to the bacteria in the second experiment was similar to that observed in the first experiment. Toxicity to the bacteria was observed from 500 ug per plate in the absence of S9 mix.

Genotoxic effects: Positive, dose related response

With metabolic activation:
In the first experiment, mutagenic activity was observed in the presence of S9 mix in TA 1535 at 10, 33 and 333 ug per plate.

In the second experiment, the response in TA 1535 was not reproduced. TA 100 in the presence of S9 mix showed a dose related response.

Without metabolic activation:
No mutagenic activity was observed in the absence of S9 mix.

Precipitation concentration if applicable:
No precipitation occurred in any of the experiments.

The vehicle control values were within the normal ranges experienced in the laboratory and reported in the literature with the selected strains of *S. typhimurium.*

The results obtained in the positive control groups were generally within the normal ranges expected for each bacterial strain and activation condition.

All tests were acceptable according to the study criteria except:

Experiment 1: TA 1538 in the presence of S9 mix was contaminated.

Experiment 2: TA 1535 in the absence of S9 mix, where the positive controls were low; TA 1538 and TA 98 in the absence of S9 mix, where the vehicle controls were low.

Experiment 3: TA 98 in the absence of S9 mix, where the vehicle controls were low.

Experiment 4: TA 98 in the absence of S9 mix, where the vehicle and positive controls were low.

In the first experiment, mutagenic activity was observed in the presence of S9 mix in TA 1535 and TA 100. In TA 1535 responses were observed at 10, 33 and 333 ug/plate; in TA 100 at 333 ug per plate only. TA 1538 in the presence of S9 mix did not meet study criteria for acceptability.

In the absence of S9 mix, no mutagenic activity was observed in any strain. Toxicity to the bacteria occurred at 333 and 1000 ug in the presence of S9 mix and at 500 ug per plate in the absence of S9 mix.

The response observed in TA 1535 was not reproduced in the second experiment. In the presence of S9 mix, TA 100 showed significant dose related increases. Mutagenic activity was also observed in TA 100 in the absence of S9 mix at 500 ug per plate only. (Toxicity was also noted at this dose.)

TA 1535, TA 1538 and TA 98 were all rejected because they did not meet the criteria for acceptance under this protocol. Toxicity to the bacteria was similar to that observed in the first experiment.

A third experiment was undertaken. TA 1535 in the absence, and TA 1538 in the presence and absence of S9 mix, gave results very similar to previous acceptable tests in that no mutagenic activity was observed. Toxicity to the bacteria was also very similar. TA 98 in the absence of S9 did not meet the study criteria for acceptance.

In a forth experiment, TA 98 again did not meet the criteria for acceptance.

A fifth experiment in this strain and activation condition gave acceptable results and the findings were similar to those obtained in previous studies (e.g. no mutagenic activity was observed).

Following review by regulatory authorities (US EPA), further work was undertaken using a new batch of test material(9011-6630). This included retesting in *S. typhimurium* TA 100 and also *E. coli* WP2uvrA- (pKM101), which is a strain with more sensitivity in detecting mutagenicity of this type.

A narrower range of dose levels was employed in both the presence and absence of S9 mix. Selected plates were also subjected to replicate plating to ensure that all colonies identified as being phenotypically his+ or trp+ were also genetically his+ or trp+ respectively. (This work was carried out under IRI project no. 751220.) Clear dose-related mutagenic responses were observed in all the tests and in both of the strains employed.

Toxicity to the bacteria was observed from 350 ug per plate in the presence of S9 mix and from 500 ug per plate in the absence of S9 mix. The results from all plates subjected to replicate plating confirmed the genotypes of the revertants present.

**Conclusions**

It was concluded that 2-[(Hydroxymethyl) amino] ethanol, Batch numbers 8904-5016 and 9011-6630 were mutagenic to *S. Typhimurium* TA 100 and *E.coli* WP2uvrA- (pKM101) when tested in sterile, ultra-pure water at concentrations extending into the toxic range. (Study author)

**Reliability**

Klimisch Code = 1

This study is reliable without restriction. This study was conducted in compliance with Good Laboratory Practice regulations as described by EPA (40 CFR Parts 160 and 792). (Study author) Test substances ranged in purity from 98.2 – 98.5 %. Copies of test substance receipts and supplier certificates of analysis are included in the study report. (Reviewer)

## References

Inveresk Research International, Scotland, "2[(Hydroxymethyl) amino] ethanol (HMAE), Batch 8904-5016: Testing for Mutagenic Activity With *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100 and 2[(hydroxymethyl)amino] ethanol (HMAE) Batch No. 9011-6630: Testing for Mutagenic Activity with *Salmonella typhimurium* TA 100 and *Escherichia coli* WP2uvrA$^-$ (pKM101)," IRI Project Nos. 740837 and 751220, April 20, 1991.

## 11.0 Repeated Dose Toxicity

### Test Substance

Identity: Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
Test substance purity not specified (Reviewer)

### Method

| | |
|---|---|
| Method/guideline followed: | EPA Guideline F 82-3 |
| Test type: | Repeat dose, dermal |
| GLP: | Yes |
| Year (*study performed*): | 1990 |
| Species: | Rat |
| Strain: | Sprague-Dawley |
| Route of administration: | Dermal |
| Duration of test: | 13 weeks |
| Doses/concentration levels: | 50, 250, 1000 mg/kg/day |
| Sex: | Male and Female |
| Exposure period: | 13 weeks |
| Frequency of treatment: | 6 hours daily (Monday-Friday) for 13 weeks |
| Control group and treatment: | Distilled water, 10 Male and 10 Female, same treatment |

Statistical methods:

Haematology, clinical chemistry, organ weight and body weight data were statistically analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous, then a non-parametric test such as a Kruskal-Wallis ANOVA was used and pairwise comparisons made via Dunn Z test where considered appropriate.

Organ weights were also analysed conditional on body weight (i.e. analysis of covariance).

Histopathology data were analysed using Fisher's Exact Probability test.

**Test Conditions**

No. of animals per sex per dose: 10 Males/10 Females per dose
Vehicle: Water
Clinical observations performed and frequency (clinical pathology, functional observations, etc.):
Opthalmoscopic examination was carried out on all animals in the Control and High dose groups before treatment commenced and during Week 12 of the study. Blood samples were taken for Hematology and clinical chemistry screens during Week 13.

The following hematological parameters were measured: hemoglobin, total red blood cell count, total white blood cell count, differential white cell count, hematocrit, calculations of absolute indices, and Hepato Quick test (on sample obtained by tail snip without anesthesia).

The following parameters were measured on plasma from whole blood taken into tubes containing heparin: blood urea nitrogen, glucose, aspartate aminotransferase, alanin aminotransferase, sodium, potassium, calcium, chloride, total protein, albumin, albumin-globulin ratio, alkaline phosphatase, Creatinine, phosphate, total bilirubin.

Organs examined at necropsy (macroscopic and microscopic):
After 13 weeks of dosing all rats were killed and necropsied and selected organs were weighed. All Control and High dose rats underwent histopathological examination of a comprehensive list of tissues. Rats from the Low and Intermediate dose groups had only lungs, liver, kidneys and skin sections examined.

| | |
|---|---|
| Organs weighed: | Adrenals, kidneys, liver, ovaries (with fallopian tubes), testes (plus epididymides) |

Tissues examined *in situ* and fixed:
Adrenals, aortic arch, any abnormal tissue, bladder, bone (sternum and rib), brain, eyes, femur, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidneys, liver, lungs (perfused), mammary gland, mesenteric lymph node, muscle (thigh), nasal cavity, esophagus, ovaries (with fallopian tubes), pancreas, pituitary, prostate, sciatic nerve, seminal vesicles, skin (normal), skin (treated), spleen, stomach (glandular and nonglandular), spinal cord (cervical, thoracic and lumbar portions), submaxillary salivary gland, submandibular lymph node, testes (plus epididymides), thymus, thyroids, tongue, trachea, uterus

Test material was applied daily (Monday – Friday) at a constant volume of 2 ml/kg over an area of approximately 10% of the total body surface area. The treated area was protected for approximately 6 hours by an occlusive dressing held in place by means of nonirritating tape.

In order to gain an overall impression of the severity of the localized lesions at the dosing site, a nonspecific term was used – i.e. 'Reaction' – and this was graded according the severity of the overall lesion. The term 'Reaction' included inflammation, dermal fibrosis, epithelial hyperplasia and ulceration. A (+/-) grade was assigned to indicate the severity of the reactions.

**Results**

| | |
|---|---|
| NOAEL (NOEL): | 1000 mg/kg/day |
| LOAEL (LOEL): | > 1000 mg/kg/day |

Actual dose received by dose level by sex:
50, 250, 1000 mg/kg/day in males and females

Toxic response/effects by dose level:
There was scabbing on and yellow staining around the dosing site, which were seen in all dose groups that received the test material.

Body weight:
There were no consistent dose related effects seen in body weight gain.

Food/water consumption:
There were no notable intergroup differences in food or water consumption.

Description, severity, time of onset and duration of clinical signs:
There were no notable intergroup differences.

Ophthalmologic findings (incidence and severity):
There were no abnormal opthalmoscopic findings.

Hematological findings incidence and severity:
There were no notable hematological intergroup differences.

Clinical biochemistry findings incidence and severity:
The most notable clinical signs included scabbing on and yellow staining around the dosing site, which were seen in all dose groups that received the test material.

Mortality and time to death:
There was one premature death (male at 250 mg/kg/day dose), which was not considered due to treatment with the test material.

## Conclusion

Dermal treatment of Sprague-Dawley rats for a period of 13 weeks with up to 1000 mg/kg/day of 2HMAE produced signs of a reaction at the site of administration with no evidence of systemic toxicity. (Study author)

## Reliability

Klimisch Code = 1

This study is reliable without restriction. This study was conducted in compliance with the Good Laboratory Practice regulations as described by the EPA (40 CFR Parts 160 and 792). (Study author) This study was conducted according to US EPA Pesticide Assessment Guidelines Subdivision F, 82-3. (Study author)

## References

Inveresk Research International, Scotland, “2-[(Hydroxymethyl)amino] ethanol 13 Week Dermal Toxicity Study in Rats,” IRI Project No. 438047, January 5, 1990.

## 12.0 Developmental Toxicity/Teratogenicity

### Test Substance

Identity (purity): Troysan 174, (2[(Hydroxymethyl)amino] ethanol)
98.5% Purity, Batch 8904-5016

### Method

| | |
|---|---|
| Method/guideline followed: | EPA Guideline 83-3(a) |
| GLP: | Yes |
| Year *(study performed):* | 1990 |
| Species: | Rat |
| Strain: | Sprague-Dawley |
| Route of administration: | Oral, gavage |
| Doses/concentration levels: | 0, 100, 250 and 500 mg/kg/day |
| Sex: | Female |
| Exposure period: | Days 6-16 inclusive of gestation, where Day 0 was the day of detection of mating |
| Frequency of treatment: | Once daily |
| Control group of treatment: | Distilled water, similar treatment |
| Duration of test: | 20 days after gestation |

Statistical methods:
Maternal body weight gains were analyzed by analysis of variance, treatment groups being compared using an F-protected Least Significant Difference (LSD) procedure.

For other parameters no formal statistical analyses were considered necessary, interpretation of the data being based on inspection of the individual and group values.

The dose levels for this study were selected after evaluation of a separate dose range finding study (IRI Project No. 438026).

### Test Conditions

| | |
|---|---|
| Age at study initiation: | Approximately 9 weeks |
| Number of animals per dose per sex*:* | 25, female |
| Vehicle: | Distilled water |

Clinical observations performed and frequency:
All the animals were checked for viability at the beginning of each day, and again as late as possible on each day. All the animals were examined for reaction to treatment on each day. Specific examinations were made 1-1.5 hours after dosing, during which the nature, onset, duration and intensity of any signs were recorded.

Individual body weights were recorded on Days 0, 6, 9, 13, 17 and 20 of gestation.

The weight of food consumed by each mated female was recorded daily throughout the study, commencing on Day 4 of gestation.

Mating procedures:
Mating was on the basis of 2 females to each male, female siblings not being paired with the same male.

For each female, cohabitation with a male was continuous until mating was detected. A vaginal lavage was examined each morning and the day of detection of sperm in the lavage, or of a copulatory plug *in situ*, was considered as Day 0 of gestation. A record was kept of the male, which inseminated each female.

Parameters assessed during study (maternal and fetal):
The animals were monitored during gestation for clinical signs of toxicity and for body weight and food consumption performance. They were killed on Day 20 of gestation and the conceptuses were evaluated. The live fetuses were examined for visceral and skeletal abnormalities.

Organs examined at necropsy (macroscopic and microscopic):
On Day 20 of gestation, the animals were killed by carbon dioxide asphyxiation.

The contents of the thoracic and abdominal cavities were examined macroscopically for abnormalities. The reproductive tract was removed and weighed intact then opened and the contents were examined. The number of corpora lutea graviditatis in each ovary and the number and position of all implantation sites in the uterus were recorded. Each implant was classified as being live, a fetal death (after ca. Day 16 of gestation), a late embryonic death (ca Day 12-16) or an early embryonic death (death judged to have occurred prior to ca Day 12.

Each live fetus was individually identified within the litter and its weight was recorded. The fetuses were examined for externally visible abnormalities. Approximately one half of the fetuses from each uterus were fixed in methylated ethyl alcohol, the remaining half were fixed in Bouin's fluid.

Those fetuses fixed in alcohol were subsequently examined for visceral abnormalities by open dissection. Skeletal structures in these fetuses were examined for abnormalities and variants, including state of ossification. The fetuses fixed in Bouin's fluid were examined for soft tissue abnormalities and variants by means of a free-hand razor blade sectioning technique. The sex of each fetus was determined during the dissection procedures.

Food and water were provided *ad libitum* during the study.

On detection of mating, the females were re-housed one per cage and randomly allocated to treatment groups.

**Results**

NOEL (maternal toxicity): 250 mg/kg/day
LOEL (maternal toxicity): 500 mg/kg/day

Maternal toxicity was observed at 500 mg/kg/day indicated by gastro-intestinal abnormalities, reduced body weight gain and reduced food consumption during the treatment period.

NOEL (developmental toxicity): 250 mg/kg/day
LOEL (developmental toxicity): 500 mg/kg/day

There was a moderately increased incidence of advanced ossification, coupled with a decrease in the incidence of patchy ossification in the fetuses at 500 mg/kg/day. The incidences of other abnormalities and variants were similar to the Control values.

Actual dose received by dose level by sex if available: 0, 100, 250 and 500 mg/kg/day

Analysis of Group 4, High dose samples taken on the first day of dosing indicated a concentration that was 16.6% lower than nominal, resulting in a formulated concentration of 46.7 mg/kg/day rather than 50 mg/kg/day. This type of formulation error did not occur again in the study.

In error, 10 females out of the 25 in Group 2, Low Dose, were not dosed on Day 16 of gestation. The omission is not considered to have affected the validity of the study. (Study author)

**Conclusion**

Under the conditions of this study, no effect was seen on the dam, or conceptus, at dose levels of up to 250 mg/kg/day. Maternal toxicity was observed at 500 mg/kg/day indicated by gastro-intestinal abnormalities, reduced body weight gain and reduced food consumption during the treatment period. There was a moderately increased incidence of advanced ossification, coupled with a decrease in the incidence of patchy ossification in the fetuses at 500 mg/kg/day. (Study author)

## Reliability

Klimisch Code = 1

This study was conducted in compliance with the Good Laboratory Practice regulations as described by the EPA (40CFR Part 160 and Part 792) and was conducted according to EPA Guideline 83-3(a). (Study author) Purity of test substance certified as 98.5% by supplier certificate of analysis, which is included in the test report. (Reviewer)

## References

Inveresk Research International, Ltd., Scotland, "2[(Hydroxymethyl) Amino] Ethanol Teratogencity Study in Rats," IRI Project No. 438031, March 5, 1990.