Image 1

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: September 19, 2000

MEMORANDUM

SUBJECT: **1, 3-Dichloropropene (Telone) Metabolites**: Review of the Mutagenicity Studies with two 1,3-dichloropropene metabolites (3-chloroacrylic acid and 3-chloroallyl alcohol) and Review Data Waiver Request for 84-2 Guidelines

TO: Michelle Centra, Ph.D.,
Reregistration Branch 3
Health Effects Division (7509C)

and

Philip Budig
Product Manager
Special Review and Reregistration Division (7508C)

FROM: Nancy E. McCarroll Nancy E. McCarroll 9/21/00
Toxicology Branch 1
Health Effects Division (7509C)

THRU: Alberto Protzel, Ph.D. Alberto Protzel 9/21/00
Branch Senior Scientist
Toxicology Branch 1
Health Effects Division (7509C)

Registrant: Dow AgroSciences
Chemical: 1,3-Dichloropropene
DP Barcode: D264693/D269007

Submission No. S570701/S584008
PC Code: 029001

ACTION: Review and prepare Data Evaluation Reports (DER) for the mutagenicity studies with 1,3-dichloropropene metabolites: (3-chloroacrylic acid and 3-chloroallyl alcohol) and review Data Waiver Request for 84-2 Guidelines.

CONCLUSIONS: Based on our review of the mutagenicity studies submitted on the two Telone metabolites, it was concluded that the data for both metabolites are not adequate to satisfy the FIFRA test guidelines for mutagenicity. The mouse lymphoma assay was not conducted with 3-chloroacrylic acid and the mouse micronucleus assay with 3-chloroallyl alcohol is considered unacceptable. Until acceptable studies for the above assays are submitted, no further action on the Data Waiver request can be taken. Additionally, the positive mouse lymphoma assay with 3-chloroallyl alcohol increases the concern for this compound since the parent is also positive in this test system.

Executive Summaries and Genetic Toxicology Profiles for the metabolites are presented below; the full DERs are attached.

CHLOROACRYLIC ACID

CITATION: Lawlor, T.E. (1999) *Salmonella - Escherichia coli*/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with 3-chloroacrylic acid. Covance Laboratories Inc. (Covance), 9200 Leesburg Pike, Vienna, Virginia 22182. Laboratory Project ID: Covance Study No.: 20270-0-422OECD, September 17, 1999. MRID 44940327. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 44940327), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to 3-chloroacrylic acid (batch No. E0436-65, 100% a.i.) in water at concentrations of 50, 100, 500, 1000, 3330 and 5000 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix). The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

3-Chloroacrylic acid was tested up to a limit concentration of 5000 µg/plate. No cytotoxicity or test material precipitation was seen in a preliminary cytotoxicity assay at any concentration up to 5000 µg/plate in either TA100 or WP2(uvrA), with or without S9-mix. Two independent mutation assays were conducted with all plating in triplicate. Slight thinning of the background lawn was seen with all tester strains at 5000 µg/plate with S9-mix in both assays but in the absence of S9-mix in the first assay, thinning was only in WP2(uvrA) at 5000 µg/plate. The WP2(uvrA) results in the confirmatory assay, with and without S9-mix, were discarded because the mutant frequency of the solvent control was too high (mean number of revertants per plate of 102 with S9 and 90 without compared to the historical values of 13.2 with and 12.5 without S9-mix). No cytotoxicity was seen in a repeat assay with WP2(uvrA). No increase in mutant frequency over solvent control values was seen in either mutation assay at any 3-chloroacrylic acid concentration, in any tester strain, with or

without S9-mix. The positive and solvent control values were appropriate for the respective strains and within historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5265 (§84-2)] for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

CITATION: Day, S.J. (1999) Evaluation of 3-chloroacrylic acid in the mouse bone marrow micronucleus test. Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Project Study ID: 991001, July 8, 1999. MRID 44940312. Unpublished.

EXECUTIVE SUMMARY: In a CD-1 mouse bone marrow micronucleus assay (MRID 44940312), 5 mice/sex/dose were treated by oral gavage on two consecutive days with 3-chloroacrylic acid (Batch No. E0436-65, 100% a.i.) at doses of 62.5, 125 or 250 mg/kg/day (males) and 62.5, 125 or 200 mg/kg/day (females). Bone marrow cells were harvested at 24 hours after the second dose. The vehicle was distilled water.

There were signs of toxicity during the study. Six concentrations of 3-chloroacrylic acid ranging from 31.25 to 2000 mg/kg/day were tested in a preliminary toxicity assay using four mice/sex/dose. All mice treated at 500 mg/kg/day or higher doses died on the first or second day of the study and most showed one or more clinical signs prior to death. Clinical signs included decreased activity; deep, slow, labored respiration; blood in the urine and blood around the snout area. All mice treated at 250 mg/kg/day showed decreased activity following treatment, one female was pale and shaky and two other females died on day two. All mice treated with the two lower doses appeared normal. In the micronucleus assay, all mice of both sexes in the two lower dose groups appeared normal throughout the study. One male in the 250 mg/kg/day group died on the first day of the study and three additional males died on the third day of the study. No clinical signs were seen in this dose group. One female in the 200 mg/kg/day group showed decreased activity following treatment and one other female died on the third day. The remaining females in this dose group appeared normal throughout the study. 3-Chloroacrylic acid did not increase the number of micronucleated PCEs over the solvent control values in either sex at any dose tested in this study. The percentage of PCEs in the total erythrocyte population was not significantly different from the control values, indicating no bone marrow cytotoxicity. The mean number of micronucleated PCEs per 1000 PCEs was 0.6 and 0.9 in the male and female solvent control groups, respectively. Comparable values for the cyclophosphamide positive control groups were 41.8 and 46.6 for males and females, respectively. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

3

CHLOROALLYL ALCOHOL

CITATION: Lawlor, T.E. (1999) *Salmonella - Escherichia coli*/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with 3-chloroallyl alcohol. Covance Laboratories Inc. (Covance), 9200 Leesburg Pike, Vienna, Virginia 22182. Laboratory Project ID: Covance Study No.: 20212-0-422OECD, July 27, 1999. MRID 44940326. Unpublished.

EXECUTIVE SUMMARY In a reverse gene mutation assay in bacteria (MRID 44940326), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to 3-chloroallyl alcohol (lot No. TSN1016921, 98% a.i.), in ethanol at concentrations of 33.3, 100, 333, 1000, 3330 and 5000 µg/plate in the presence of mammalian metabolic activation (S9-mix). WP2(uvrA) was also exposed to the same six concentrations in the absence of S9-mix.. The *S. typhimurium* strains were exposed to 3-chloroallyl alcohol concentrations of 10.0, 33,3, 100, 333, 1000 and 3330 µg/plate in the absence of S9-mix. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

3-Chloroallyl alcohol was tested up to cytotoxic concentrations. In a preliminary cytotoxicity assay, evidence of cytotoxicity (a reduction in the number of revertants per plate and/or thinning or absence of the background lawn of bacteria) was seen in TA100 at 3330 and 5000 µg/plate with S9-mix and at 1000 µg/plate and higher concentrations without S9-mix. No cytotoxicity was seen in WP2(uvrA) with S9-mix but in the absence of S9-mix, thinning of the background lawn was seen at 3330 and 5000 µg/plate and a reduction in the number of revertants per plate was seen at 5000 µg/plate. No precipitates were seen at any concentration. Two independent mutagenicity assays were conducted using the preincubation procedure with all plating in triplicate. In the presence of S9-mix, cytotoxicity was apparent in both assays at 5000 µg/plate in all TA-strains but not in WP2(uvrA) and at 3330 µg/plate in all TA-strains except TA1535. In the absence of S9-mix in the first assay, cytotoxicity was seen at 3330 µg/plate in all *S. typhimurium* strains and at 5000 µg/plate in WP2(uvrA). In the absence of S9-mix in the confirmatory assay, cytotoxicity was seen at 3330 µg/plate in TA98, TA100, TA1537 and WP2(uvrA) and at 5000 µg/plate in WP2(uvrA). No evidence of mutagenicity was seen at any 3-chloroallyl alcohol concentration in any tester strain, with or without S9-mix, in either assay. The solvent and positive control values were appropriate for the respective strains and within the testing laboratory's historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5265 (84-2) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

CITATION: Linscombe, V.A., K.M. Jackson and B.E. Kropscott (1999) Evaluation of 3-chloroallyl alcohol in the mouse lymphoma (L5178Y $TK^{+/-}$) forward mutation assay. Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Project ID: 981203, July 13, 1999. MRID 44940311. Unpublished.

4

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the TK locus (MRID 44940311), mouse lymphoma L5178Y $TK^{+/-}$ cells cultured *in vitro* were exposed to 3-chloroallyl alcohol (lot No. TSN101692, ID No. 6823-MI, notebook reference No. 199801576-46, 98.6% a.i.) in DMSO at concentrations of 12.5, 25, 50, 100, 250, 500, 700, 925 µg/mL in the absence of an exogenous metabolic activation system (S9-mix) and at concentrations of 1.5, 3, 6.1, 12.5, 25, 37.5, 50, 100 µg/mL in the presence of S9-mix. A confirmatory assay was conducted at concentrations of 12.5, 25, 50, 100, 200, 300, 400, 500 µg/mL without S9-mix and at concentrations of 3, 6.25, 12.5, 25, 37.5, 50, 75, 100 µg/mL in the presence of S9-mix. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

3-Chloroallyl alcohol was tested up to cytotoxic concentrations. A preliminary cytotoxicity assay was conducted at nine 3-chloroallyl alcohol concentrations ranging from 3.6 to 925.2 µg/mL in the absence and presence of S9-mix. Excessive cytotoxicity was seen at 925.2 µg/mL in the absence of S9-mix (1.7% day-2 relative suspension growth) and at 115.7 µg/mL and higher concentrations in the presence of S9-mix (3.6% day-2 relative suspension growth at 115.7 µg/mL and 0.0% day-2 relative suspension growth at all higher concentrations).

In the first mutation assay, the day-two relative total growth (RTG) was 11% at 500 µg/mL without S9-mix and 12% at 100 µg/mL with S9-mix. In the absence of S9-mix, there was no statistically significant (significance defined at alpha = 0.05) increase in the mutant frequency (MF) over the solvent control value at any dose; however there was a statistically significant increasing linear trend with increasing dose. The average MF of the solvent control without S9-mix was 104×10^{-6} while the average MF at 500 µg/mL without S9-mix was 228×10^{-6}.

In the presence of S9-mix, the results were similar, with no statistically significant increase in the MF over the solvent control value at any evaluated concentration but with a statistically significant increasing linear trend with increasing dose. The average MF of the solvent control with S9-mix was 130×10^{-6} while that at 37.5, 50 or 100 µg/mL was 221, 247 or 365×10^{-6}, respectively. These values represent dose-related MF increases of 1.7-, 1.9- and 2.8-fold. Distribution analysis of large and small colonies revealed no conclusive evidence of a preferential increase in either size.

In the confirmatory assay, the day-two RTG averaged 30% at 500 µg/mL in the absence of S9-mix and approximately 10% at 100 µg/mL in the presence of S9-mix. Small, but statistically significant increases in mutant frequencies over the solvent control value were seen at 400 and 500 µg/mL in the absence of S9-mix. The average mutant frequencies at 400 and 500 µg/mL were 168×10^{-6} and 208×10^{-6}, respectively, compared to the solvent control value of approximately 100×10^{-6} (a 1.68- and 2.08-fold increase, respectively). Both small and large colonies were similarly increased. In the presence of S9-mix, statistically significant increases in mutant frequencies over the solvent control value of 134.5×10^{-6} were seen at the two highest doses, 75 and 100 µg/mL. The average mutant frequencies at these concentrations were 275×10^{-6} (a 2.0-fold increase) and 391×10^{-6} (a 2.9-fold increase), respectively. Both small and large colonies were increased to a similar extent. Control values in both assays, with and without S9-mix, were appropriate and consistent with the laboratory's historical control ranges.

Based on the overall findings, 3-chloroallyl alcohol induced a dose-related and reproducible increase in the mutagenic response of this mammalian cell line in both the presence and absence of S9 activation at severely cytotoxic but also at moderately cytotoxic doses. The study authors questioned the biological relevance of the results because the mutagenic effect was seen mostly at concentrations resulting in high levels of cytotoxicity, because both small and large colonies were equally increased (implying both a point mutation and a chromosome breakage event) and because 3-chloroallyl alcohol was found to be negative in the *Salmonella*/microsome assay (Lawlor, 1999) and negative in the mouse bone marrow micronucleus assay (Day, 1999). The cytotoxicity was not excessive according to the EPA guidelines for *in vitro* mammalian cell gene mutation assays as published in the Federal Register (vol. 62, No. 158, p. 43847). The guidelines state that if the maximum concentration of test material is based upon cytotoxicity, the RTG should be between 10 and 20% but not less than 10% at this concentration.

This study is classified as **Acceptable/Guideline.** It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5300 (84-2) for *in vitro* mutagenicity (mammalian forward gene mutation)data.

CITATION: Day, S.J. (1999) Evaluation of 3-chloroallyl alcohol in the mouse bone marrow micronucleus test. Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Project Study ID: 981207, July 8, 1999. MRID 44940313. Unpublished.

EXECUTIVE SUMMARY: In a CD-1 mouse bone marrow micronucleus assay (MRID 44940313), 5 mice/sex/dose were treated by oral gavage on two consecutive days with 3-chloroallyl alcohol (Lot No. 199801576-46, 98.6% a.i.) at doses of 31.25, 62.5 or 125 mg/kg/day. Bone marrow cells were harvested at 24 hours after the second dose. The vehicle was distilled water.

There were no signs of toxicity during the micronucleus study. Six concentrations of 3-chloroallyl alcohol ranging from 31.25 to 1000 mg/kg/day were tested in a preliminary toxicity assay using four mice/sex/dose. All mice treated at 250 mg/kg/day or higher died on the first or second day of the study and most showed some clinical signs prior to death. Clinical signs seen in one or more mice included decreased activity; slow, labored respiration and dragging hindquarters and rigid limbs. One male treated at 125 mg/kg/day died on the third day of the study. All other mice treated (7/8) at this dose were normal. In the micronucleus assay, all mice of both sexes in all dose groups appeared normal throughout the study. 3-Chloroallyl alcohol did not increase the number of micronucleated PCEs over the solvent control values in either sex at any dose tested in this study. The percentage of PCEs in the total erythrocyte population was not significantly different from the control values, indicating no bone marrow cytotoxicity. The mean number of micronucleated PCEs per 1000 PCEs was 0.9 in the male solvent control group and 0.6 in the female solvent control group, respectively. Comparable values for the cyclophosphamide positive control groups were 49.1 and 50.7 for males and females, respectively. **Based on the lack of evidence showing that the mice were exposed to a level that was overtly toxic to the test animals or to a dose that produced a**

cytotoxic response in the bone marrow, there is no confidence in the negative results of this study.

This study is classified as **Unacceptable/Guideline.** It does not satisfy the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

GENETIC TOXICOLOGY PROFILES ON TWO 1,3-DICHLOROPROPENE METABOLITES--DATA WAIVER REQUEST

BACKGROUND

On November 25, 1998 and December 23, 1998, a Data Waiver Request was submitted for Mutagenicity Guideline 84-2, Chromosomal Aberration in Chinese Hamster Lung Assay. A testing program was presented, based on an agreement between the Agency [Nancy E. McCarroll (USEPA/OPPTS/OPP/HED)] and Dow Agro Sciences (Bhaskar Gollapudi), for the testing of two metabolites of 1,3-dichloropropene (3-chloroacrylic acid and 3-chloroallyl alcohol) in the FIFRA mutagenicity test battery. The following tests were proposed by the Registrant:

Proposed Test	**Guideline**
Salmonella typhimurium reverse gene mutation assay	84-2/870.5100 (OPPTS)
Mouse Lymphoma forward gene mutation assay	84-2/870.5300 (OPPTS)
In vivo cytogenetics: mammalian erythrocyte micronucleus assay	84-2/870.5395 (OPPTS)

It was further agreed (see letter dated December 23, 1998, to Lisa Nisenson, SRRD/OPP/USEPA from Bruce A. Houtman, Dow Agro Sciences) that a positive mouse lymphoma assay with an increase in small colony mutants would trigger the need to perform an *in vitro* cytogenetic assay with Chinese hamster lung (CHL) cells.

SUMMARY

Five mutagenicity studies were submitted for the Telone metabolites: 3-chloroacrylic acid and 3-chloroallyl alcohol. Summarized results were as follows:

3-Chloroacrylic acid

1. *Salmonella typhimurium/Escherichia coli* mammalian-microsome reverse mutation assay (MRID No. 44940327). Independent trials using the preincubation procedure were negative in *S. typhimurium* TA1535, TA1537, TA98 and TA100 and *E. coli* WP2 *uvrA* up to the limit dose (5000 μg/plate +/-S9). The study is **acceptable** and satisfies the guideline requirements for a bacterial gene mutation assay.

2. Mouse micronucleus assay (MRID No. 44940312). The test was negative in CD-1 mice orally administered 62.5, 125 or 250 mg/kg once daily for 2 consecutive days. Overt toxicity at the high dose included deaths and decreased activity; there was, however, no indication of bone marrow cytotoxicity. The study is **acceptable** and satisfies the guideline requirements for an *in vivo* micronucleus assay.

3-Chloroallyl alcohol

1. *Salmonella typhimurium/Escherichia coli* mammalian-microsome reverse mutation assay (MRID No. 44940326). Independent trials using the preincubation procedure were negative in *S. typhimurium* TA1535, TA1537, TA98 and TA100 and *E. coli* WP2 *uvrA* up to a dose that was reproducibly cytotoxic for all *Salmonella* strains (5000 μg/plate +/-S9). The study is **acceptable** and satisfies the guideline requirements for a bacterial gene mutation assay.

2. Mouse lymphoma L5187Y $TK^{+/-}$ cell forward mutation assay (MRID No. 44940311). The test was positive both with and without S9 activation. Mutation frequencies (MFs) approaching or >2-fold higher than the solvent control were seen without S9 activation at 500 μg/mL (11% survival--Trial 1) or at 400 and 500 μg/mL (44 and 30% survival--Trial 2). In the presence of S9 activation, increased MFs approaching or >2-fold were noted at 37.5, 50 or 100 μg/mL (48, 28 or 12% survival, respectively --Trial 1) or at 50, 75 or 100 μg/mL (35, 19 or 10% survival, respectively--Trial 2). Findings for Trial 2, which used narrower concentration ranges with or without S9 activation, were dose related and generally significant. Colony size analysis revealed a relatively equal distribution of small and large colonies. The study is **acceptable** and satisfies the guideline requirements for a mammalian cell gene mutation assay.

3. Mouse micronucleus assay (MRID No. 44940313). The test was negative in CD-1 mice orally administered 31.5, 62.5 or 125 mg/kg once daily for 2 consecutive days. No toxicity to the test animals or cytotoxicity to the bone marrow was apparent at any dose. This study is classified as **unacceptable** because the maximum tolerated dose (MTD) was not achieved.

DISCUSSION

Results from the above studies indicate that 3-chloroacrylic acid, a degradate of Telone is not mutagenic in bacteria and is neither clastogenic nor aneugenic *in vivo*. However, a data gap exists (there is no mouse lymphoma assay), which was part of the agreed regimen for mutagenicity testing and is also part of the initial test battery required to fulfill FIFRA test guidelines for mutagenicity. Therefore, until an acceptable mouse lymphoma assay is submitted for 3-chloroacrylic acid, no further action can be taken.

3-Chloroallyl alcohol was also nonmutagenic in bacterial but induced a reproducible dose-related increase in the MF of L5187Y mouse lymphoma cells in both the presence and absence of S9 activation. We disagree with the investigators' comments regarding the biological relevance of the increased MFs because they occurred "at concentrations yielding relative high levels of cytotoxicity". In contrast, we noted that increased MFs were seen not only at severely cytotoxic doses but also at moderately cytotoxic levels (nonactivated doses caused 11 to 44% survival and S9-activated doses caused 10-48% survival). Thus, there is sufficient mutagenic activity at acceptable concentrations to warrent our concern. Since there was a relatively equal distribution of small and large colonies, further characterization of the response in this test system is not possible. However, no additional work is required at this time to determine if the mutagenic response is associated with point mutation or chromosome breakage events.

The *in vivo* mouse micronucleus assay with 3-chloroallyl alcohol is deemed unacceptable because the MTD was not achieved. The "negative" results of this test were used by the Registrant to question the biological relevance of the positive findings with the mouse lymphoma assay. To support the registrant's position, it is, therefore, prudent to conduct a repeat study with doses that include the MTD. Hence, the submitted studies for 3-chloroallyl alcohol do not satisfy the FIFRA test guidelines for mutagenicity.

CONCLUSIONS

Overall, the data for both metabolites are not adequate to satisfy the FIFRA test guidelines for mutagenicity or to meet the conditions of the agreed testing program. Missing studies include a mouse lymphoma assay for 3-chloroacrylic acid and an acceptable mouse micronucleus assay for 3-chloroallyl alcohol. In addition, the positive mouse lymphoma assay with 3-chloroallyl alcohol increases the concern for this compound.

DATA EVALUATION REPORT

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3-CHLOROACRYLIC ACID/3-CHLOROALLYL ALCOHOL (DEGRADATES OF TELONE, 1,3-DICHLOROPROPENE)

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS - MICRONUCLEUS ASSAY IN MOUSE BONE MARROW CELLS (OPPTS 870.5395 [§84-2]) (MRID 44940313)

Prepared for

Health Effects Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
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Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 00-22

Primary Reviewer: B.L. Whitfield, Ph.D.	Signature: Date:	B L Whitfield MAR 2 8 2000
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast MAR 2 8 2000
Robert H. Ross, Group Leader	Signature: Date:	Robert H Ross MAR 2 8 2000
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson MAR 2 8 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

*Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Nancy E. McCarroll Nancy E. McCarroll, Date 8/9/00
Toxicology Branch I (7509C)
EPA Work Assignment Manager: Sanjivani Diwan Sanjivani Diwan, Date 8/10/00
Reregistration Branch IV (7509C)

014337

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mouse bone marrow [OPPTS 870.5395 (§84-2)]

DP BARCODE: D264693 SUBMISSION CODE: S570701
P.C. CODE: 029001 TOX. CHEM. NO.: not provided

TEST MATERIAL (PURITY): 3-Chloroallyl alcohol 98.6% a.i. (ratio of E/Z = 1.26/1)

SYNONYMS: Telone Alcohol

CITATION: Day, S.J. (1999) Evaluation of 3-chloroallyl alcohol in the mouse bone marrow micronucleus test. Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Project Study ID: 981207, July 8, 1999. MRID 44940313. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLC, 9330 Zionsville Rd., Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a CD-1 mouse bone marrow micronucleus assay (MRID 44940313), 5 mice/sex/dose were treated by oral gavage on two consecutive days with 3-chloroallyl alcohol (Lot No. 199801576-46, 98.6% a.i.) at doses of 31.25, 62.5 or 125 mg/kg/day. Bone marrow cells were harvested at 24 hours after the second dose. The vehicle was distilled water.

There were no signs of toxicity during the micronucleus study. Six concentrations of 3-chloroallyl alcohol ranging from 31.25 to 1000 mg/kg/day were tested in a preliminary toxicity assay using four mice/sex/dose. All mice treated at 250 mg/kg/day or higher died on the first or second day of the study and most showed some clinical signs prior to death. Clinical signs seen in one or more mice included decreased activity; slow, labored respiration and dragging hindquarters and rigid limbs. One male treated at 125 mg/kg/day died on the third day of the study. All other mice treated (7/8) at this dose were normal. In the micronucleus assay, all mice of both sexes in all dose groups appeared normal throughout the study. 3-Chloroallyl alcohol did not increase the number of micronucleated PCEs over the solvent control values in either sex at any dose tested in this study. The percentage of PCEs in the total erythrocyte population was not significantly different from the control values, indicating no bone marrow cytotoxicity. The mean number of micronucleated PCEs per 1000 PCEs was 0.9 in the male solvent control group and 0.6 in the female solvent control group, respectively. Comparable values for the cyclophosphamide positive control groups were 49.1 and 50.7 for males and females, respectively. **Based on the lack of evidence showing that the mice were exposed to a level**

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that was overtly toxic to the test animals or to a dose that produced a cytotoxic response in the bone marrow, there is no confidence in the negative results of this study.

This study is classified as **Unacceptable/Guideline.** It does not satisfy the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

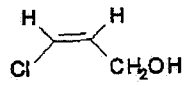
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

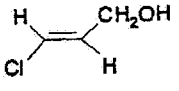
A. MATERIALS

1. Test material: 3-chloroallyl alcohol

Description: amber liquid
Lot/Batch #: 199801576-46; TSN101692
Purity: 98.6% a.i.
Stability of compound: stable
CAS #: 29560-84-7
Structure:



Cis



Trans

Solvent used: distilled water
Other comments: Ratio of E/Z = 1.26/1

2. Control materials

Negative *(if not vehicle)*/Route of administration: none

Vehicle/Final volume/Route of administration: distilled water / 10 mL/kg / oral gavage

Positive/Final dose/Route of administration: Cyclophosphamide / 120 mg/kg / oral gavage

3. Test compound administration

Volume of test substance administered: 10 mL/kg/body weight
Route of administration: oral gavage
Dose levels used:
Preliminary range-finding test: 31.25, 62.5, 125, 250, 500, 1000 mg/kg/day
Micronucleus assay: 31.25, 62.5, 125 mg/kg/day

12

4. Test animals

a. Species mouse Strain CD-1 Age 9 weeks
Weight male 31.1 - 31.9 g (group means) female 26.2 - 27.7 g (group means)
Source: Charles River Laboratories, Portage, MI

b. No. animals used per dose: _5_ males _5_ females

c. Properly maintained? Y

B. TEST PERFORMANCE

1. Treatment and sampling times

a.. Test compound

Dosing: ___ once _x_ twice (24 hr apart)
Sampling (after last dose): ___ 6 hr ___ 12 hr _x_ 24 hr ___ 48 hr ___ 72 hr (mark all that are appropriate), ___ other (describe):

b. Negative and/or vehicle control

Dosing: ___ once _x_ twice (24 hr apart)
Sampling (after last dose): ___ 6 hr ___ 12 hr
_x_ 24 hr ___ 48 hr ___ 72 hr (mark all that are appropriate), other (describe):

c. Positive control

Dosing: _x_ once ___ twice (24 hr apart)
Sampling (after last dose): ___ 6 hr ___ 12 hr
_x_ 24 hr ___ 48 hr ___ 72 hr (mark all that are appropriate), other (describe):

2. Tissues and cells examined

_x_ bone marrow ___ other (list)

No. of polychromatic erythrocytes (PCE) examined per animal: 2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:
the number of PCEs in 200 total bone marrow erythrocytes was recorded

13

3. Details of slide preparation

At the desired harvest time, mice were killed by CO_2 inhalation, both femurs were removed from each mouse, the distal end of each femur was cut off and a 25-gauge needle was use to aspirate the bone marrow into a 3 mL disposable plastic syringe containing 0.5 mL of fetal bovine serum. The contents of the syringe were put into a centrifuge tube containing 0.5 mL of serum, the cells resuspended and then centrifuged at 1000 rpm (about 80 g) for five minutes. Most of the supernatant was removed and the pellet resuspended in the remaining serum. Wedge smears were prepared on microscope slides, air dried and stained with Wright-Giemsa. Slides were coded prior to analysis.

4. Statistical methods

The raw micronuclei count from each mouse was transformed by adding 1 to the count and then taking the natural log of the new number. The transformed micronuclei data and the percent PCE data were analyzed separately by a three-way analysis of variance (sex, dose and time) assuming the three-way interaction was zero. Two-way interactions were reviewed for significance and the data then analyzed by one-, two- or three-way analysis of variance for main effects only. Treated vs. control group comparisons were done, if necessary, by Dunnett's t-tests, one sided (upper) for micronucleated PCEs and two-sided for percent PCE. If significant differences were found in the pairwise comparisons, linear dose-related trend tests were performed. All tests were conducted at an alpha level of 0.01. When different dose levels were used for each sex, the data were analyzed separately by sex.

5. Evaluation criteria

Two thousand PCE per mouse were examined for the presence of micronuclei (darkly stained bodies with smooth contours and varying shapes such as round, almond, or ring (Schmid, 1976)). Results were reported as the number of micronucleated PCEs per 1000 PCEs. The percentage of PCEs per 200 bone marrow erythrocytes was also reported. No criteria were provided to judge assay validity or the biological significance of the findings.

II. REPORTED RESULTS

A. PRELIMINARY TOXICITY ASSAY

Six concentrations of 3-chloroallyl alcohol ranging from 31.25 to 1000 mg/kg/day were tested in the preliminary toxicity assay using four mice/sex/dose. All mice treated at 250, 500 and 1000 mg/kg/day died on the first or second day of the study and most showed some clinical signs prior to death. Clinical signs seen in one or more mice included decreased activity; slow, labored respiration and dragging hindquarters and rigid limbs. One male mouse treated at 125 mg/kg/day died on the third day of the study but all other mice at this dose level and all mice treated at lower doses appeared normal. Results of

the preliminary toxicity study are presented in Appendix Table 2 (MRID 44940313, p. 19). Test material concentrations used in the toxicity assay were analytically determined (HPLC with UV detection) to be 104 to 111% of the target concentrations.

B. MICRONUCLEUS ASSAY

Groups of five mice/sex/dose were treated with doses of 31.25, 62.5 or 125 mg/kg/day (five additional mice were treated at the high dose as backups if needed). All mice of both sexes in all dose groups appeared normal throughout the study.

3-Chloroallyl alcohol did not increase the number of micronucleated PCEs over the solvent control values in either sex at any dose tested in this study. The percentage of PCE in the total erythrocyte population was not significantly different from the control values, indicating no bone marrow cytotoxicity. The mean number of micronucleated PCEs per 1000 PCEs was 0.9 and 0.6 in the male and female solvent control groups, respectively. Comparable values for the positive control groups were 49.1 and 50.7 for males and females, respectively. Results of the micronucleus assay are presented in Appendix Tables 1 (males) and 2 (females) (MRID 44940313, pp. 21 and 22).

Concentrations of test material used in the micronucleus assay were analytically determined to be 106 to 110% of the target concentrations.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an unacceptable study. 3-Chloroallyl alcohol was not tested to a concentration that caused overt toxicity in the test animals or cytotoxicity in the target tissue. As shown in Study Report Table 2, all animals administered 250 mg/kg displayed physical signs of toxicity and died by study day 2. In contrast, 7/8 animals in the 125-mg/kg group were normal throughout the observation period and a single male succumbed to treatment on study day 3. The marked difference between these dose groups relative to compound toxicity should have prompted the selection of an intermediate dose between 125 and 250 mg/kg as the high level for the micronucleus assay. Without a clear indication that the animals were tested to the maximum tolerated dose, there is no confidence in the negative results of this study.

This study is classified as **Unacceptable/Guideline.** It does not satisfy the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

B. STUDY DEFICIENCIES: See above.

15

REFERENCES

Schmid, W. (1976) The micronucleus test for cytogenetic analysis. In: Hollaender (ed), "Chemical mutagens: Principles and methods for their detection." Vol. 4, Plenum, New York, NY, pp. 31-53.

16

APPENDIX

(MRID 44940313)

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Page_____ is not included in this copy.

Pages 19 through 21 are not included.
18 20

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_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__✓__ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

014337

DATA EVALUATION REPORT

3-CHLOROACRYLIC ACID/3-CHLOROALLYL ALCOHOL (DEGRADATES OF TELONE, 1,3-DICHLOROPROPENE)

SALMONELLA/ESCHERICHIA/MAMMALIAN ACTIVATION GENE MUTATION ASSAY; [OPPTS 870.5265[1] (§84-2)]

MRID 44940326

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis
Life Sciences Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 00-22

Primary Reviewer: B.L. Whitfield, Ph.D.	Signature: Date:	B L Whitfield MAR 2 8 2000
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast MAR 2 8 2000
Robert H. Ross, Group Leader	Signature: Date:	Robert H Ross MAR 2 8 2000
Quality Assurance: LeeAnn Wilson, M.A.	Signature: Date:	L. A. Wilson MAR 2 8 2000

Disclaimer

This review may have been altered subsequent to the contractor's signature above.

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[1] 870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

EPA Reviewer: Nancy E. McCarroll Nancy E. McCarroll, Date 8/9/00
Toxicology Branch I (7509C)
EPA Work Assignment Manager: Sanjivani Diwan Sanjivani Diwan, Date 8/10/00
Reregistration Branch IV (7509C)

014337

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella/Escherichia*/mammalian activation gene mutation assay; [OPPTS 870.5265[2] (§84-2)]

DP BARCODE: D264693
P.C. CODE: 029001

SUBMISSION CODE: S570701
TOX. CHEM. NO.: not provided

TEST MATERIAL (PURITY): 3-Chloroallyl alcohol (degradate of Telone, 1,3-dichloropropene; 98% a.i.)

SYNONYMS: 3-Chloro-2-propen-1-ol, 3-Chloro-2-en-1-ol, and 3-Chloroprop-2-en-1-ol isomer mix [trans (E)/cis (Z) isomers tenatively assigned a ratio of 1.14:1]

CITATION: Lawlor, T.E. (1999) *Salmonella - Escherichia coli*/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with 3-chloroallyl alcohol. Covance Laboratories Inc. (Covance), 9200 Leesburg Pike, Vienna, Virginia 22182. Laboratory Project ID: Covance Study No.: 20212-0-422OECD, July 27, 1999. MRID 44940326. Unpublished.

SPONSOR: The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences (DAS) LLC, 9330 Zionsville Rd., Indianapolis, IN 46268

EXECUTIVE SUMMARY In a reverse gene mutation assay in bacteria (MRID 44940326), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to 3-chloroallyl alcohol (lot No. TSN1016921, 98% a.i.), in ethanol at concentrations of 33.3, 100, 333, 1000, 3330 and 5000 µg/plate in the presence of mammalian metabolic activation (S9-mix). WP2(uvrA) was also exposed to the same six concentrations in the absence of S9-mix.. The *S. typhimurium* strains were exposed to 3-chloroallyl alcohol concentrations of 10.0, 33,3, 100, 333, 1000 and 3330 µg/plate in the absence of S9-mix. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

3-Chloroallyl alcohol was tested up to cytotoxic concentrations. In a preliminary cytotoxicity assay, evidence of cytotoxicity (a reduction in the number of revertants per plate and/or thinning

[2]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

or absence of the background lawn of bacteria) was seen in TA100 at 3330 and 5000 µg/plate with S9-mix and at 1000 µg/plate and higher concentrations without S9-mix. No cytotoxicity was seen in WP2(uvrA) with S9-mix but in the absence of S9-mix, thinning of the background lawn was seen at 3330 and 5000 µg/plate and a reduction in the number of revertants per plate was seen at 5000 µg/plate. No precipitates were seen at any concentration. Two independent mutagenicity assays were conducted using the preincubation procedure with all plating in triplicate. In the presence of S9-mix, cytotoxicity was apparent in both assays at 5000 µg/plate in all TA-strains but not in WP2(uvrA) and at 3330 µg/plate in all TA-strains except TA1535. In the absence of S9-mix in the first assay, cytotoxicity was seen at 3330 µg/plate in all *S. typhimurium* strains and at 5000 µg/plate in WP2(uvrA). In the absence of S9-mix in the confirmatory assay, cytotoxicity was seen at 3330 µg/plate in TA98, TA100, TA1537 and WP2(uvrA) and at 5000 µg/plate in WP2(uvrA). No evidence of mutagenicity was seen at any 3-chloroallyl alcohol concentration in any tester strain, with or without S9-mix, in either assay. The solvent and positive control values were appropriate for the respective strains and within the testing laboratory's historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline.** It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5265 (84-2) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

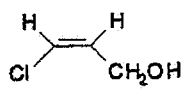
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: 3-chloroallyl alcohol

Description: described as a transparent, colorless liquid in the main text and as an amber liquid in the appendix
Lot/Batch #: TSN101692, ID No. 6823-MI, notebook reference No. 199801576-46
Purity: 98% a.i. [trans (E)/cis (Z) isomers tenatively assigned a ratio of 1.14:1]
Stability of compound: stable
CAS #: 29560-84-7
Structure:



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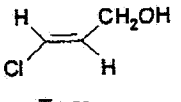

Trans

23

Solvent used: ethanol
Other comments: none

2. Control materials

Negative: none
Solvent/final concentration: ethanol / 50 µL/plate
Positive:
Nonactivation:
Sodium azide 2.0 µg/plate TA100, TA1535
2-Nitrofluorene 1.0 µg/plate TA98
ICR-191 2.0 µg/plate TA1537
4-Nitroquinoline-N-Oxide 0.4 WP2(uvrA)

Activation:
2-Aminoanthracene 2.5 µg/plate TA100, TA1535, TA1537
2-Aminoanthracene 25 µg/plate WP2(uvrA)
Benzo(*a*)pyrene 2.5 µg/plate TA98

3. Activation: S9 derived from male Sprague-Dawley rats

x Aroclor 1254	x induced	x rat	x liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none	__ other	__ other	

S9 mix composition: S9-homogenate purchased from Molecular Toxicology, Inc., batch 0883 (35.9 mg protein/mL)

H_2O	0.70 mL
1 M NaH_2PO_4/Na_2HPO_4, pH 7.4	0.10 mL
0.25 M Glucose-6-phosphate	0.02 mL
0.10 M NADP	0.04 mL
0.825 M KCl/0.2M $MgCl_2$	0.04 mL
S9-homogenate	0.10 mL

4. Test organisms: *S. typhimurium* strains

__ TA97 x TA98 x TA100 __ TA102 __ TA104
x TA1535 x TA1537 __ TA1538;
list any others: *E. coli* WP2(uvrA)

Properly maintained? Y
Checked for appropriate genetic markers (rfa mutation, R factor)? Y

5. Test compound concentrations used:

Preliminary cytotoxicity test: TA100 and WP2(uvrA) (single plating)
Nonactivated and activated conditions: 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate

First and confirmatory mutagenicity assays: (all platings in triplicate)
Nonactivated conditions: 10.0, 33.3, 100, 333, 1000, 3330 µg/plate (all *Salmonella* strains)
Nonactivated conditions: 33.3, 100, 333, 1000, 3330, 5000 µg/plate (WP2(uvrA)
Activated conditions: 33.3, 100, 333, 1000, 3330, 5000 µg/plate (all strains)

B. TEST PERFORMANCE

1. Type of Salmonella assay:

___ standard plate test
_x_ pre-incubation (20 ± 2 minutes)
___ "Prival" modification *(i.e. azo-reduction method)*
___ spot test

2. Protocol: Each assay was conducted by adding 0.5 mL of S9-mix (or 0.5 mL of 0.1 M phosphate buffer for assays without metabolic activation) to a glass culture tube preheated to 37±2°C followed by the addition of 100 µL of the desired tester strain of bacteria and by 50 µL of the desired concentration of test material or control. The contents were mixed by vortexing and incubated for 20±2 minutes at 37±2°C. Two mL of molten top agar was then added, the mixture vortexed and poured onto 25 mL of minimal bottom agar in a petri dish. After the molten agar had solidified, the plates were inverted and incubated for 52±4 hours at 37±2°C. Three plates were used at each experimental point. Culture medium was Vogel-Bonner salt solution supplemented with 2.5% (w/v) Oxoid Nutrient Broth No. 2. Bottom agar was Vogel-Bonner minimal medium E supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose. Overlay agar was 0.7% agar (w/v) and 0.5% NaCl (w/v) supplemented with 10 mL of 0.5 mM histidine/biotin solution per 100 mL agar for the *Salmonella* strains or with 0.5 mM tryptophan solution per 100 mL agar for WP2(uvrA).

Each plate was evaluated for evidence of cytotoxicity (the background lawn of bacteria was examined) and for evidence of test material precipitation. The number of revertants per plate was counted manually for all test material and solvent control plates. An automatic colony counter was used for positive control plates except for strain TA1535 with S9-mix which was counted manually. The mean number of revertants per plate and the standard deviation were calculated for each series of triplicate plates.

Criteria for a positive response were a dose related and reproducible increase in the number of revertants per plate in at least one tester strain over the respective solvent control value with at least a 3-fold increase in TA98, TA1535, TA1537 and WP2(uvrA) or at least a 2-fold increase in TA100.

II. REPORTED RESULTS

Stock solution concentrations of 3-chloroallyl alcohol for the inital and confirmatory trials were analytically determined (HPLC with UV detection) to be within 88 to 108% of the target doses.

A. PRELIMINARY CYTOTOXICITY ASSAY

Ten concentrations of 3-chloroallyl alcohol ranging from 6.67 to 5000 µg/plate were tested in a preliminary range-finding assay using strains TA100 and WP2(uvrA). A single plate was used per dose. Cytotoxicity, as based on thinning of the background lawn and/or by a reduction in the number of revertants per plate, was seen in TA100 at 3330 and 5000 µg/plate with S9-mix and at 1000 µg/plate and higher concentrations without S9-mix. No cytotoxicity was seen in WP2(uvrA) with S9-mix but in the absence of S9-mix, thinning of the background lawn was seen at 3330 and 5000 µg/plate and a reduction in the number of revertants per plate was seen at 5000 µg/plate. No precipitates were observed at any concentration. Results of the preliminary cytotoxicity assay are presented in Appendix Tables 1 and 2 (MRID 44940326, pp. 30 and 31).

B. MUTAGENICITY ASSAY

Six concentrations of 3-chloroallyl alcohol ranging from 33.3 to 5000 µg/plate were tested, with and without S9-mix, in WP2(uvrA) and with S9-mix in the *Salmonella* strains. Six concentrations ranging from 10.0 to 3330 µg/plate were tested without S9-mix in the TA-strains. Two independent assays were conducted using the preincubation procedure with all plating in triplicate. In the presence of S9-mix, cytotoxicity was apparent in both assays at 5000 µg/plate in all Salmonella strains but not in WP2(uvrA) and at 3330 µg/plate in all TA-strains except TA1535. In the absence of S9-mix in the first assay, cytotoxicity was noted at 3330 µg/plate in TA98, TA100 and TA1535 and at 5000 µg/plate in WP2(uvrA). In the absence of S9-mix in the confirmatory assay, cytotoxicity was seen at 3330 µg/plate in TA98, TA100, TA1537 and TA1535 and WP2(uvrA) and at 5000 µg/plate in WP2(uvrA).

No evidence of mutagenicity was seen at any 3-chloroallyl alcohol concentration in any tester strain, with or without S9-mix, in either assay. The solvent and positive control values were appropriate for the respective strains and within the testing laboratory's historical control ranges. Results of the first assay are summarized in Appendix Tables 3 and 4 (MRID 44940326, pp.33 and 34) and results of the confirmatory assay are summarized in Appendix Tables 5 and 6 (MRID 44940326, pp. 36 and 37).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. 3-Chloroallyl alcohol was tested up to cytotoxic concentrations, proper experimental protocol was followed and the solvent and positive control values were appropriate for the respective strains. 3-Chloroallyl alcohol was not mutagenic as tested in this study.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5265 (84-2) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

APPENDIX

(MRID 44940326)

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APPENDIX

(MRID 44940327)

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DATA EVALUATION REPORT 014337

3-CHLOROACRYLIC ACID/3-CHLOROALLYL ALCOHOL (DEGRADATES OF TELONE, 1,3-DICHLOROPROPENE)

STUDY TYPE: *SALMONELLA/ESCHERICHIA*/MAMMALIAN ACTIVATION GENE MUTATION ASSAY; [OPPTS 870.5265[1] (§84-2)]
MRID 44940327

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 00-22

Primary Reviewer: B.L. Whitfield, Ph.D.	Signature: Date:	B L Whitfield MAR 2 8 2000
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast MAR 2 8 2000
Robert H. Ross, Group Leader	Signature: Date:	Robert H Ross MAR 2 8 2000
Quality Assurance: LeeAnn Wilson, M.A.	Signature: Date:	L. A. Wilson MAR 2 8 2000

Disclaimer

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Managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464

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[1] 870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

EPA Reviewer: Nancy E. McCarroll, Date 8/9/00
Toxicology Branch I (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D., Date 8/10/00
Reregistration Branch IV (7509C)

014337

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella/Escherichia*/mammalian activation gene mutation assay [OPPTS 870.5265[2] (§84-2)]

DP BARCODE: D264693
P.C. CODE: 029001

SUBMISSION CODE: S570701
TOX. CHEM. NO.: not provided

TEST MATERIAL (PURITY): 3-chloroacrylic acid (100% (49.5% cis-3-chloroacrylic acid, 50.5% trans-3-chloroacrylic acid))

SYNONYMS: Telone Acid, 3-chloroacrylic acid mix

CITATION: Lawlor, T.E. (1999) *Salmonella - Escherichia coli*/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with 3-chloro-acrylic acid. Covance Laboratories Inc. (Covance), 9200 Leesburg Pike, Vienna, Virginia 22182. Laboratory Project ID: Covance Study No.: 20270-0-422OECD, September 17, 1999. MRID 44940327. Unpublished.

SPONSOR: The Dow Chemical Company, Midland, Michigan 48674 for Dow AgroSciences (DAS) LLC, 9330 Zionsville Rd., Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 44940327), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to 3-chloroacrylic acid (batch No. E0436-65, 100% a.i.) in water at concentrations of 50, 100, 500, 1000, 3330 and 5000 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix). The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

3-Chloroacrylic acid was tested up to a limit concentration of 5000 µg/plate. No cytotoxicity or test material precipitation was seen in a preliminary cytotoxicity assay at any concentration up to 5000 µg/plate in either TA100 or WP2(uvrA), with or without S9-mix. Two independent mutation assays were conducted with all plating in triplicate. Slight thinning of the background

[2]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

36

lawn was seen with all tester strains at 5000 µg/plate with S9-mix in both assays but in the absence of S9-mix in the first assay, thinning was only in WP2(uvrA) at 5000 µg/plate. The WP2(uvrA) results in the confirmatory assay, with and without S9-mix, were discarded because the mutant frequency of the solvent control was too high (mean number of revertants per plate of 102 with S9 and 90 without compared to the historical values of 13.2 with and 12.5 without S9-mix). No cytotoxicity was seen in a repeat assay with WP2(uvrA). No increase in mutant frequency over solvent control values was seen in either mutation assay at any 3-chloroacrylic acid concentration, in any tester strain, with or without S9-mix. The positive and solvent control values were appropriate for the respective strains and within historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5265 (§84-2)] for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

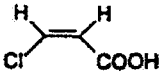
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

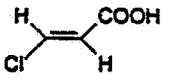
A. MATERIALS

1. Test material: 3-Chloroacrylic acid

Description: white powder
Lot/Batch #: E0436-65
Purity: 100% a.i.
Stability of compound: stable
CAS #: 625-40-1
Structure:



Cis



Trans

Solvent used:
Other comments: 49.5% cis-3-chloroacrylic acid, 50.5% trans-3-chloroacrylic acid

37

2. Control materials

Negative: none
Solvent/final concentration: water (cell culture grade) / 50 µL/plate
Positive:
Nonactivation:
Sodium azide _2.0_ µg/plate TA100, TA1535
2-Nitrofluorene _1.0_ µg/plate TA98
ICR-191 _2.0_ µg/plate TA1537
4-Nitroquinoline-N-Oxide _0.4_ WP2(uvrA)

Activation:
2-Aminoanthracene _2.5_ µg/plate TA100, TA1535, TA1537
2-Aminoanthracene _25_ µg/plate WP2(uvrA)
Benzo(*a*)pyrene _2.5_ µg/plate TA98

3. Activation: S9 derived from male Sprague-Dawley rats

x Aroclor 1254	x induced	x rat	x liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none	__ other		__ other

S9 mix composition: S9-homogenate purchased from Molecular Toxicology, Inc., batch 0883 (35.9 mg protein/mL)

H_2O	0.70 mL
1 M NaH_2PO_4/Na_2HPO_4, pH 7.4	0.10 mL
0.25 M Glucose-6-phosphate	0.02 mL
0.10 M NADP	0.04 mL
0.825 M KCl/0.2M $MgCl_2$	0.04 mL
S9-homogenate	0.10 mL

4. Test organisms: *S. typhimurium* strains

__ TA97 _x_ TA98 _x_ TA100 __ TA102 __ TA104
_x_ TA1535 _x_ TA1537 ___ TA1538;

Properly maintained? Y
Checked for appropriate genetic markers (rfa mutation, R factor)? Y

38

5. Test compound concentrations used:

Preliminary cytotoxicity assay: TA100 and WP2(uvrA) (single plating)
Nonactivated and activated conditions: 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate

Mutagenicity assays: all strains, both assays, triplicate plating
Nonactivated and activated conditions: 50, 100, 500, 1000, 3330, 5000 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay

__ standard plate test
_x_ pre-incubation (20 ± 2 minutes)
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test

2. Protocol: Each assay was conducted by adding 0.5 mL of S9-mix (or 0.5 mL of 0.1 M phosphate buffer for assays without metabolic activation) to a glass culture tube preheated to 37±2°C followed by the addition of 100 µL of the desired tester strain of bacteria and by 50 µL of the desired concentration of test material or control. The contents were mixed by vortexing and incubated for 20±2 minutes at 37±2°C. Two mL of molten top agar was then added, the mixture vortexed and poured onto 25 mL of minimal bottom agar in a petri dish. After the molten agar had solidified, the plates were inverted and incubated for 52±4 hours at 37±2°C. Three plates were used at each experimental point. Culture medium was Vogel-Bonner salt solution supplemented with 2.5% (w/v) Oxoid Nutrient Broth No. 2. Bottom agar was Vogel-Bonner minimal medium E supplemented with 1.5% (w/v) agar and 0.2% (w/v) glucose. Overlay agar was 0.7% agar (w/v) and 0.5% NaCl (w/v) supplemented with 10 mL of 0.5 mM histidine/biotin solution per 100 mL agar for the TA-strains or with 0.5 mM tryptophan solution per 100 mL agar for WP2(uvrA).

Each plate was evaluated for evidence of cytotoxicity (the background lawn of bacteria was examined) and for evidence of test material precipitation. The number of revertants per plate was counted manually for all test material and solvent control plates. An automatic colony counter was used for positive control plates except for strain TA100 with S9-mix which was counted manually. The mean number of revertants per plate and the standard deviation were calculated for each series of triplicate plates.

Criteria for a positive response were a dose related and reproducible increase in the number of revertants per plate in at least one tester strain over the respective solvent

control value with at least a 3-fold increase in TA98, TA1535, TA1537 and WP2(uvrA) or at least a 2-fold increase in TA100.

II. REPORTED RESULTS

Stock concentrations of 3-chloroacrylic acid were analyzed using HPLC with UV detection and found, with two exceptions, to be within 10% of the target concentrations for the initial, confirmatory and the repeat confirmatory assay with WP2(uvrA). The stock concentrations of 0.666 mg/mL and 6.66 mg/mL from the first assay were 150% and 143% of the target values, respectively.

A. PRELIMINARY CYTOTOXICITY ASSAY

Ten concentrations of 3-chloroacrylic acid ranging from 6.67 to 5000 µg/plate were tested, with and without S9-mix, in a preliminary range-finding assay using strains TA100 and WP2(uvrA). A single plate was used per dose. Cytotoxicity was determined by thinning of the background lawn of bacteria and by a reduction in the number of revertants per plate compared to the solvent control. No cytotoxicity or test material precipitation was seen at any concentration in either strain, with or without S9-mix.

B. MUTAGENICITY ASSAY

Six concentrations of 3-chloroacrylic acid ranging from 50 to 5000 µg/plate were tested, with and without S9-mix, in two independent mutation assays. All plating was in triplicate. Slight thinning of the background lawn was seen with all tester strains at 5000 µg/plate with S9-mix in both assays but only with WP2(uvrA) at 5000 µg/plate without S9-mix in the first assay. The WP2(uvrA) results in the confirmatory assay with and without S9-mix were discarded because the mutant frequency of the solvent control was too high (mean number of revertants per plate of 102 with S9 and 90 without compared to the historical values of 13.2 with and 12.5 without S9-mix). No cytotoxicity was seen when this part of the assay was repeated.

The number of revertants per plate was not increased over solvent control values at any 3-chloroacrylic acid concentration, in any tester strain, with or without S9-mix in either mutation assay. The positive and solvent control values were acceptable and within historical control ranges with the exception of the WP2(uvrA) solvent control in the confirmatory assay as mentioned in the preceding paragraph. Results of the mutation assays are summarized in Appendix Tables 1 - 3 (MRID 44940327, pp. 33, 35 and 36).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. 3-Chloroacrylic acid was tested to a limit concentration of 5000 µg/plate, proper experimental protocol was followed and the solvent and positive control values were appropriate for the respective strains and within the laboratory's

historical control ranges. There was no evidence that 3-chloroacrylic acid increased the number of revertants per plate over solvent control values as tested in this study.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5265 (§84-2)] for *in vitro* mutagenicity (bacterial reverse gene mutation)

B. STUDY DEFICIENCIES

No study deficiencies were identified.

Page _____ is not included in this copy.

Pages 43 through 45 are not included.
42 44

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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_____ Identity of the source of product ingredients.

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DATA EVALUATION REPORT 014337

3-CHLOROACRYLIC ACID/3-CHLOROALLYL ALCOHOL (DEGRADATES OF TELONE, 1,3-DICHLOROPROPENE)

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS - MICRONUCLEUS ASSAY IN MOUSE BONE MARROW CELLS (OPPTS 870.5395 [§84-2])
MRID 44940312

014337

Prepared for

Health Effects Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 00-22

Primary Reviewer: B.L. Whitfield, Ph.D.	Signature: Date:	B L Whitfield MAR 2 8 2000
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast MAR 28 2000
Robert H. Ross, Group Leader	Signature: Date:	Robert H. Ross MAR 2 8 2000
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson MAR 2 8 2000

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

45

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

46

EPA Reviewer: Nancy E. McCarroll, Date 8/9/00
Toxicology Branch I (7509C)
EPA Work Assignment Manager: Sanjivani Diwan, Date 8/10/00
Reregistration Branch IV (7509C)

014337

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mouse bone marrow [OPPTS 870.5395 (§84-2)]

DP BARCODE: D264693
P.C. CODE: 029001

SUBMISSION CODE: S570701
TOX. CHEM. NO.: not provided

TEST MATERIAL (PURITY): 3-Chloroacrylic acid; (100% a.i. (49.5% cis-3-chloroacrylic acid, 50.5% trans-3-chloroacrylic acid))

SYNONYMS: Telone Acid, 3-chloroacrylic acid mix

CITATION: Day, S.J. (1999) Evaluation of 3-chloroacrylic acid in the mouse bone marrow micronucleus test. Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Project Study ID: 991001, July 8, 1999. MRID 44940312. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLC, 9330 Zionsville Rd., Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a CD-1 mouse bone marrow micronucleus assay (MRID 44940312), 5 mice/sex/dose were treated by oral gavage on two consecutive days with 3-chloroacrylic acid (Batch No. E0436-65, 100% a.i.) at doses of 62.5, 125 or 250 mg/kg/day (males) and 62.5, 125 or 200 mg/kg/day (females). Bone marrow cells were harvested at 24 hours after the second dose. The vehicle was distilled water.

There were signs of toxicity during the study. Six concentrations of 3-chloroacrylic acid ranging from 31.25 to 2000 mg/kg/day were tested in a preliminary toxicity assay using four mice/sex/dose. All mice treated at 500 mg/kg/day or higher doses died on the first or second day of the study and most showed one or more clinical signs prior to death. Clinical signs included decreased activity; deep, slow, labored respiration; blood in the urine and blood around the snout area. All mice treated at 250 mg/kg/day showed decreased activity following treatment, one female was pale and shaky and two other females died on day two. All mice treated with the two lower doses appeared normal. In the micronucleus assay, all mice of both sexes in the two lower dose groups appeared normal throughout the study. One male in the 250 mg/kg/day group died on the first day of the study and three additional males died on the third day of the study. No clinical signs were seen in this dose group. One female in the 200 mg/kg/day group showed decreased activity following treatment and one other female died on the third day. The remaining females in this dose group appeared normal throughout the study. 3-Chloroacrylic

46
47

acid did not increase the number of micronucleated PCEs over the solvent control values in either sex at any dose tested in this study. The percentage of PCEs in the total erythrocyte population was not significantly different from the control values, indicating no bone marrow cytotoxicity. The mean number of micronucleated PCEs per 1000 PCEs was 0.6 and 0.9 in the male and female solvent control groups, respectively. Comparable values for the cyclophosphamide positive control groups were 41.8 and 46.6 for males and females, respectively. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as **Acceptable/Guideline.** It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: 3-chloroacrylic acid

Description: amber liquid
Lot/Batch #: E0436-65; TSN101700
Purity: 100% a.i.
Stability of compound: stable
CAS #: 625-40-1
Structure:

Cis Trans

Solvent used: distilled water
Other comments: 49.5% cis-3-chloroacrylic acid, 50.5% trans-3-chloroacrylic acid

2. Control materials:

Negative *(if not vehicle)*/Route of administration: none

Vehicle/Final volume/Route of administration: distilled water / 10 mL/kg / oral gavage

47

Positive/Final dose/Route of administration: Cyclophosphamide / 120 mg/kg / oral gavage

3. Test compound administration:

Volume of test substance administered: 10 mL/kg/body weight

Route of administration: oral gavage

Dose levels used:
Preliminary range-finding test: 31.25, 125, 250, 500, 1000, 2000 mg/kg/day

Micronucleus assay: 62.5, 125, 200 mg/kg/day (females); 62.5, 125, 250 mg/kg/day (males)

4. Test animals

a. Species mouse Strain CD-1 Age 9 weeks

Weight male 30.1 - 30.8 g (group means); female 25.4 - 26.3 g (group means)
Source: Charles River Laboratories, Portage, MI

b. No. animals used per dose: 5 males 5 females

c. Properly maintained? Y

B. TEST PERFORMANCE

1. Treatment and sampling times

a. test compound

Dosing: ___ once _x_ twice (24 hr apart)

Sampling (after last dose): ___ 6 hr ___ 12 hr _x_ 24 hr ___ 48 hr ___ 72 hr

b. Negative and/or vehicle control
Dosing: ___ once _x_ twice (24 hr apart)

Sampling (after last dose): ___ 6 hr ___ 12 hr
_x_ 24 hr ___ 48 hr ___ 72 hr

48 (?)

c. Positive control

Dosing: _x_ once ___ twice (24 hr apart)

Sampling (after last dose): ___ 6 hr ___ 12 hr
_x_ 24 hr ___ 48 hr ___ 72 hr (mark all that are appropriate), other (describe):

2. Tissues and cells examined:

_x_ bone marrow ___ other

No. of polychromatic erythrocytes (PCE) examined per animal: 2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: the number of PCEs in 200 total bone marrow erythrocytes was recorded

3. Details of slide preparation

At the desired harvest time, mice were killed by CO_2 inhalation, both femurs were removed from each mouse, the distal end of each femur was cut off and a 25-gauge needle was use to aspirate the bone marrow into a 3 mL disposable plastic syringe containing 0.5 mL of fetal bovine serum. The contents of the syringe were put into a centrifuge tube containing 0.5 mL of serum, the cells resuspended and then centrifuged at 1000 rpm (about 80 g) for five minutes. Most of the supernatant was removed and the pellet resuspended in the remaining serum. Wedge smears were prepared on microscope slides, air dried and stained with Wright-Giemsa. Slides were coded prior to analysis.

4. Statistical methods

The raw micronuclei count from each mouse was transformed by adding 1 to the count and then taking the natural log of the new number. The transformed micronuclei data and the percent PCE data were analyzed separately by a three-way analysis of variance (sex, dose and time) assuming the three-way interaction was zero. Two-way interactions were reviewed for significance and the data then analyzed by one-, two- or three-way analysis of variance for main effects only. Treated vs. control group comparisons were done, if necessary, by Dunnett's t-tests, one sided (upper) for micronucleated PCEs and two-sided for percent PCE. If significant differences were found in the pairwise comparisons, linear dose-related trend tests were performed. All tests were conducted at an alpha level of 0.01. When different dose levels were used for each sex, the data were analyzed separately by sex.

5. Evaluation criteria

Two thousand PCE per mouse were examined for the presence of micronuclei (darkly stained bodies with smooth contours and varying shapes such as round, almond, or

ring (Schmid, 1976)). Results were reported as the number of micronucleated PCEs per 1000 PCEs. The percentage of PCEs per 200 bone marrow erythrocytes was also reported. No criteria were provided to assess the validity of the assay or the biological relevance of the findings.

II. REPORTED RESULTS

A. PRELIMINARY TOXICITY ASSAY

Six concentrations of 3-chloroacrylic acid ranging from 31.25 to 2000 mg/kg/day were tested in the preliminary toxicity assay using four mice/sex/dose. All mice treated at 500 mg/kg/day or higher doses died on the first or second day of the study and most showed one or more clinical signs prior to death. Clinical signs included decreased activity; deep, slow, labored respiration; blood in the urine and blood around the snout area. All mice treated at 250 mg/kg/day showed decreased activity following treatment, one female was pale and shaky and two other females died on day two. All mice treated with the two lower doses appeared normal. Test material concentrations used in the toxicity assay were analytically determined (HPLC with UV detection) to be 81 to 109% of the target concentrations.

B. MICRONUCLEUS ASSAY

Groups of five male mice/dose were treated with 3-chloroacrylic acid at doses of 62.5, 125 or 250 mg/kg/day (five additional mice were treated at the high dose as backups if needed). Groups of five females/dose were treated with doses of 62.5, 125 or 200 mg/kg/day (five additional mice were treated at the high dose as backups if needed). All mice of both sexes in the two lower dose groups appeared normal throughout the study. One male in the 250 mg/kg/day group died on the first day of the study and three additional males died on the third day of the study. No clinical signs were seen in this dose group. One female in the 200 mg/kg/day group showed decreased activity following treatment and one other female died on the third day. The remaining females in this dose group appeared normal throughout the study.

3-Chloroacrylic acid did not increase the number of micronucleated PCEs over the solvent control values in either sex at any dose tested in this study. The percentage of PCEs in the total erythrocyte population was not significantly different from the control values, indicating no bone marrow cytotoxicity. The mean number of micronucleated PCEs per 1000 PCEs was 0.5 and 0.9 in the male and female solvent control groups, respectively. Comparable values for the positive control groups were 41.8 and 46.6 for males and females, respectively. Results of the micronucleus assay are presented in Appendix Tables 1 (males) and 2 (females) (MRID 44940312, pp. 24 and 25).

Concentrations of test material used in the micronucleus assay were analytically determined to be 100 to 109% of the target concentrations.

50 51

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. 3-Chloroacrylic acid was tested to cytotoxic concentrations, proper experimental protocol was followed and the solvent and positive control values were appropriate. As tested in this study, 3-chloroacrylic acid did not increase the frequency of micronucleated PCEs over solvent control values.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

REFERENCES

Schmid, W. (1976) The micronucleus test for cytogenetic analysis. In: Hollaender (ed), "Chemical mutagens: Principles and methods for their detection." Vol. 4, Plenum, New York, NY, pp. 31-53.

51
52

APPENDIX

(MRID 44940312)

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DATA EVALUATION REPORT **014337**

3-CHLOROACRYLIC ACID/3-CHLOROALLYL ALCOHOL (DEGRADATES OF TELONE, 1,3-DICHLOROPROPENE)

STUDY TYPE: MAMMALIAN CELLS IN CULTURE GENE MUTATION ASSAY IN MOUSE LYMPHOMA L5178Y TK$^{+/-}$ CELLS [OPPTS 870.5300 (§84-2)]

MRID 44940311

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 00-22

Primary Reviewer:
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Date: MAR 2 8 2000

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAR 2 8 2000

Quality Assurance:
LeeAnn Wilson, M.A.

Signature: L. A. Wilson
Date: MAR 2 8 2000

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

55

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

56

EPA Reviewer: Nancy E. McCarroll, Date 8/9/00
Toxicology Branch I (7509C)
EPA Work Assignment Manager: Sanjivani Diwan, Date 8/10/00
Reregistration Branch IV (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma L5178Y $TK^{+/-}$ cells; OPPTS 870.5300 [§84-2]

DP BARCODE: D264693
P.C. CODE: 029001

SUBMISSION CODE: S570701
TOX. CHEM. NO.: not provided

TEST MATERIAL (PURITY): 3-Chloroallyl alcohol (98.6% a.i).; [trans (E)/cis (Z) isomers tentatively assigned a ratio of 1.26:1)

SYNONYMS: 3-Chloro-2-propen-1-ol, 3-Chloroprop-2-en-1-ol, and 3-Chloroprop-2-en-1-ol cis and trans isomer mix

CITATION: Linscombe, V.A., K.M. Jackson and B.E. Kropscott (1999) Evaluation of 3-chloroallyl alcohol in the mouse lymphoma (L5178Y $TK^{+/-}$) forward mutation assay. Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Project ID: 981203, July 13, 1999. MRID 44940311. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLC, 9330 Zionsville Rd., Indianapolis, IN 46268

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the TK locus (MRID 44940311), mouse lymphoma L5178Y $TK^{+/-}$ cells cultured *in vitro* were exposed to 3-chloroallyl alcohol (lot No. TSN101692, ID No. 6823-MI, notebook reference No. 199801576-46, 98.6% a.i.) in DMSO at concentrations of 12.5, 25, 50, 100, 250, 500, 700, 925 µg/mL in the absence of an exogenous metabolic activation system (S9-mix) and at concentrations of 1.5, 3, 6.1, 12.5, 25, 37.5, 50, 100 µg/mL in the presence of S9-mix. A confirmatory assay was conducted at concentrations of 12.5, 25, 50, 100, 200, 300, 400, 500 µg/mL without S9-mix and at concentrations of 3, 6.25, 12.5, 25, 37.5, 50, 75, 100 µg/mL in the presence of S9-mix. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

3-Chloroallyl alcohol was tested up to cytotoxic concentrations. A preliminary cytotoxicity assay was conducted at nine 3-chloroallyl alcohol concentrations ranging from 3.6 to 925.2 µg/mL in the absence and presence of S9-mix. Excessive cytotoxicity was seen at 925.2 µg/mL in the absence of S9-mix (1.7% day-2 relative suspension growth) and at 115.7 µg/mL and higher concentrations in the presence of S9-mix (3.6% day-2 relative suspension growth at 115.7 µg/mL and 0.0% day-2 relative suspension growth at all higher concentrations).

56

In the first mutation assay, the day-two relative total growth (RTG) was 11% at 500 µg/mL without S9-mix and 12% at 100 µg/mL with S9-mix. In the absence of S9-mix, there was no statistically significant (significance defined at alpha = 0.05) increase in the mutant frequency (MF) over the solvent control value at any dose; however there was a statistically significant increasing linear trend with increasing dose. The average MF of the solvent control without S9-mix was 104 x 10^{-6} while the average MF at 500 µg/mL without S9-mix was 228 x 10^{-6}.

In the presence of S9-mix, the results were similar, with no statistically significant increase in the MF over the solvent control value at any evaluated concentration but with a statistically significant increasing linear trend with increasing dose. The average MF of the solvent control with S9-mix was 130 x 10^{-6} while that at 37.5, 50 or 100 µg/mL was 221, 247 or 365 x 10^{-6}, respectively. These values represent dose-related MF increases of 1.7-, 1.9- and 2.8-fold. Distribution analysis of large and small colonies revealed no conclusive evidence of a preferential increase in either size.

In the confirmatory assay, the day-two RTG averaged 30% at 500 µg/mL in the absence of S9-mix and approximately 10% at 100 µg/mL in the presence of S9-mix. Small, but statistically significant increases in mutant frequencies over the solvent control value were seen at 400 and 500 µg/mL in the absence of S9-mix. The average mutant frequencies at 400 and 500 µg/mL were 168 x 10^{-6} and 208 x 10^{-6}, respectively, compared to the solvent control value of approximately 100 x 10^{-6} (a 1.68- and 2.08-fold increase, respectively). Both small and large colonies were similarly increased. In the presence of S9-mix, statistically significant increases in mutant frequencies over the solvent control value of 134.5 x 10^{-6} were seen at the two highest doses, 75 and 100 µg/mL. The average mutant frequencies at these concentrations were 275 x 10^{-6} (a 2.0-fold increase) and 391 x 10^{-6} (a 2.9-fold increase), respectively. Both small and large colonies were increased to a similar extent. Control values in both assays, with and without S9-mix, were appropriate and consistent with the laboratory's historical control ranges.

Based on the overall findings, 3-chloroallyl alcohol induced a dose-related and reproducible increase in the mutagenic response of this mammalian cell line in both the presence and absence of S9 activation at severely cytotoxic but also at moderately cytotoxic doses. The study authors questioned the biological relevance of the results because the mutagenic effect was seen mostly at concentrations resulting in high levels of cytotoxicity, because both small and large colonies were equally increased (implying both a point mutation and chromosome breakage event) and because 3-chloroallyl alcohol was found to be negative in the *Salmonella*/microsome assay (Lawlor, 1999) and negative in the mouse bone marrow micronucleus assay (Day, 1999). The cytotoxicity was not excessive according to the EPA guidelines for *in vitro* mammalian cell gene mutation assays as published in the Federal Register (vol. 62, No. 158, p. 43847). The guidelines state that if the maximum concentration of test material is based upon cytotoxicity, the RTG should be between 10 and 20% but not less than 10% at this concentration.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5300 (84-2) for *in vitro* mutagenicity (mammalian forward gene mutation)

57

data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

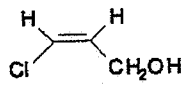
58

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: 3-Chloroallyl alcohol

Description: amber liquid
Lot/Batch #: TSN101692, ID No. 6823-MI, notebook reference No. 199801576-46
Purity: 98.6% a.i.
Stability of compound: stable
CAS #: 29560-84-7
Structure:



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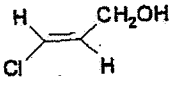

Trans

Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: none

2. Control materials

Negative: none
Solvent/final concentration: DMSO / 1%
Positive (concentrations/solvent):
Nonactivation: Methyl methanesulfonate / 10 and 15 µg/mL / phosphate buffered saline
Activation: 20-Methylcholanthrene / 2.5, 5.0 and 7.5 µg/mL / DMSO

3. Activation: S9 derived from male Sprague-Dawley rats (unspecified commercial source)

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	_ non-induced	_ mouse	_ lung
___ none	_ hamster	_ other	
_ other	_ other		

S9 mix composition:

NADP	2.0 mg/mL
Sodium isocitrate	11.25 mg/mL
S9-fraction	5% v/v

59

4. Test cells: mammalian cells in culture

_x_ mouse lymphoma L5178Y cells
___ Chinese hamster ovary (CHO) cells
___ V79 cells (Chinese hamster lung fibroblasts)

Properly maintained? Y
Periodically checked for Mycoplasma contamination? Y
Periodically checked for karyotype stability? Y
Periodically "cleansed" against high spontaneous background? Y

Media: Cells were maintained in Fischer's medium (F_{10P}) containing 204 mg/mL L-glutamine, 10% heat-inactivated horse serum, penicillin G (20 units/mL), streptomycin sulfate (20 µg/mL), 0.22 mg/mL sodium pyruvate and 10 mg/mL Plurionic F68®. F_{10P} without serum (F_{0P}) was used during treatment. Cloning medium was F_{10P} containing 0.28% granulated agar. Selection medium for the detection of $TK^{-/-}$ mutants was cloning medium supplemented with 1 µg/mL trifluorothymidine (TFT).

5. Locus examined

_x_ thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
(give concentr.) _____ fluorodeoxyuridine (FdU)
_1 µg/mL_ trifluorothymidine (TFT)

___ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentr. _____ 6-thioguanine (6-TG)

___ Na^+/K^+ ATPase
Selection agent: _____ ouabain
(give concentration)

___ other (locus and/or selection agent; give details):

6. Test compound concentrations used:

Preliminary cytotoxicity test:
Nonactivated and activated conditions: 3.6, 7.2, 14.5, 28.9, 57.8, 115.7, 231.3, 462.6, 925.2 µg/mL
First mutation assay:
Nonactivated conditions: 12.5, 25, 50, 100, 250, 500, 700, 925 µg/mL
Activated conditions: 1.5, 3, 6.1, 12.5, 25, 37.5, 50, 100 µg/mL

Confirmatory mutation assay: Lo

Nonactivated conditions: 12.5, 25, 50, 100, 200, 300, 400, 500 µg/mL
Activated conditions: 3, 6.25, 12.5, 25, 37.5, 50, 75, 100 µg/mL

B. TEST PERFORMANCE

1. Cell treatment

a. Cells exposed to test compound, negative/solvent or positive controls for:

_4_ hours (nonactivated) _4_ hours (activated)

b. After washing, cells cultured for _2_ days (expression period) before cell selection:

c. After expression, 1 x 10^6 cells/dish (_3_ dishes/ group) were cultured for _12_ days in selection medium to determine numbers of mutants and _200_ cells/dish (_3_ dishes/group) were cultured for _12_ days without selective agent to determine cloning efficiency.

2. Statistical methods

MF (MF) data were evaluated using a weighted analysis of variance where weights were derived from the inverse of the MF variance. The actual plate counts were assumed to follow a Poisson distribution, therefore the mean plate count was used as an estimate of variance. A linear trend test and lack of fit test were employed (alpha = 0.05) as omnibus tests to compare treated groups to the negative control. If there was a significant increasing trend or a significant lack of fit, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one sided). An additional comparison of the positive control to the negative control (alpha = 0.05) was conducted using a linear contrast statement.

3. Evaluation criteria

The mean MF (defined as the number of mutants per 10^6 clonable cells) was determined for each treatment group and the number of small and large colonies was recorded. The following criteria must be met for the results to be considered positive: (1) A statistically significant, dose-related, reproducible increase in MF with a range of dose levels yielding >20% relative total growth. (2) The MF in at least one dose level of the treated cultures (resulting in >205 RTG) is 100 x 10^{-6} above concurrent solvent controls (assuming these to be in the range of 20-80 x 10^{-6}).

II. REPORTED RESULTS

A. ANALYTICAL DETERMINATIONS

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1. Ph and Osmolality Determinations

There was no appreciable difference in the pH or the osmolality of culture medium containing the limit dose (10 mM) of the test material.

2. Analytical Determinations

The concentrations of test material used in the assays were analytically determined (HPLC with UV detection) to be within 98 to 103% of the target values in the first assay and within 98 to 102% of the target values in the confirmatory assay.

A. PRELIMINARY CYTOTOXICITY ASSAY

A preliminary cytotoxicity assay was conducted at nine 3-chloroallyl alcohol concentrations ranging from 3.6 to 925.2 µg/mL in the absence and presence of S9-mix. Excessive cytotoxicity was seen at 925.2 µg/mL in the absence of S9-mix (1.7% day-2 relative suspension growth) and at 115.7 µg/mL and higher concentrations in the presence of S9-mix (3.6% day-2 relative suspension growth at 115.7 µg/mL and 0.0% day-2 relative suspension growth at all higher concentrations). Based on the results of the preliminary cytotoxicity assay, concentrations of test material chosen for the mutagen assay were 12.5 to 925 µg/mL without S9-mix and 1.5 to 100 µg/mL with S9-mix. Results of the preliminary cytotoxicity assay are presented in Appendix Tables 1A and 1B (MRID 44940311, pp. 24 and 25).

B. MUTAGENICITY ASSAY

Two independent mutation assays were conducted using duplicate cultures at each dose. In the first assay, eight concentrations of 3-chloroallyl alcohol ranging from 12.5 to 925 µg/mL were evaluated in the absence of S9-mix and eight concentrations ranging from 1.5 to 100 µg/mL were evaluated in the presence of S9-mix. The two higher levels in the absence of S9-mix, 700 and 925 µg/mL, proved excessively cytotoxic and were not plated for analysis. The day-two RTG was 11% at 500 µg/mL without S9-mix and 12% at 100 µg/mL with S9-mix. In the absence of S9-mix, there were no statistically significant increases in MF over the solvent control value at any dose; however there was a statistically significant increasing linear trend with increasing dose. The average MF of the solvent control without S9-mix was 104 x 10^{-6} while the average MF at 500 µg/mL without S9-mix was 228 x 10^{-6}. Although the MF at this level was increased 2.3-fold, the percent survival was 11%.

In the presence of S9-mix, the results were similar, with no statistically significant increase in the MF over the solvent control value at any evaluated concentration but with a statistically significant increasing linear trend with increasing dose. The average MF of the solvent control with S9-mix was 130 x 10^{-6} while that at 100, 50 or 37.6 µg/mL was 365, 247 or 221 x 10^{-6}, respectively. These values represent dose-related MF increases of 1.7-, 1.9- and 2.8-fold. Analysis of colony size distribution revealed no conclusive

62

evidence of an increase in either large or small colonies. Results of the first mutation assay are presented in Appendix Tables 2A - 2D (MRID 44940311, pp. 26 - 29).

In the confirmatory assay, eight concentrations of 3-chloroallyl alcohol ranging from 50 to 500 µg/mL were initially evaluated in the absence of S9-mix (concentrations of 12.5 and 25 µg/mL were used but considered superfluous and not evaluated) and eight concentrations ranging from 6.25 to 100 µg/mL were evaluated in the presence of S9-mix (a concentration of 3.0 µg/mL was initially used but considered superfluous and not evaluated). RTG averaged 30% at 500 µg/mL in the absence of S9-mix and approximately 10% at 100 µg/mL in the presence of S9-mix. Statistically significant ($p < 0.05$) increases in the MFs over the solvent control value were seen at 400 and 500 µg/mL in the absence of S9-mix. The average MFs at 400 and 500 µg/mL were 168 and 208 x 10^{-6}, respectively, compared to the solvent control value of ≈ 100 x 10^{-6} (a 1.68- and 2.08-fold increase, respectively). Both small and large colonies were similarly increased. In the presence of S9-mix, statistically significant increases in the MFs over the solvent control value of 134.5 x 10^{-6} were seen at the two highest doses, 75 and 100 µg/mL. The average MFs at these concentrations were 275 x 10^{-6} (a 2.0-fold increase) and 391 x 10^{-6} (a 2.9-fold increase), respectively. RTG at 75 and 100 µg/mL was ≈19 and 10%. In agreement with the data from the first assay, both small and large colonies were increased to a similar extent. Control values in both assays, both with and without S9-mix, were appropriate and consistent with the laboratory's historical control ranges. Results of the confirmatory mutation assay are presented in Appendix Tables 3A - 3D (MRID 44940311, pp. 30 - 33).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. 3-Chloroallyl alcohol was tested to cytotoxic concentrations, proper experimental protocol was followed and the solvent and positive control values were appropriate. The test material was weakly mutagenic as tested in this study, both with and without S9-mix. The study authors question the biological relevance of the results because the mutagenic effect was seen mostly at concentrations resulting in high levels of cytotoxicity and because 3-chloroallyl alcohol was found to be negative in the *Salmonella*/microsome assay (Lawlor, 1999) and negative in the mouse bone marrow micronucleus assay (Day, 1999). However, cytotoxicity was not excessive according to the EPA guidelines for *in vitro* mammalian cell gene mutation assays as published in the Federal Register (vol. 62, No. 158, p. 43847). The guidelines state that if the maximum concentration of test material is based upon cytotoxicity, the RTG should be between 10 and 20% but not less than 10% at this concentration. We conclude, therefore, that 3-chloroallyl alcohol induced a dose-related and reproducible mutagenic effect in this test system.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

63

REFERENCES

Lawlor, T.E., Covance Laboratories, Inc.: (1999) Mutagenicity test with 3-chloroallyl alcohol in the *Salmonella-Escherichia* coli/mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay. R&D Report, The Dow Chemical Company.

Day, S.J. (1999) Evaluation of 3-chloroallyl alcohol in the bone marrow micronucleus test. R&D Report, The Dow Chemical Company.

APPENDIX

(MRID 44940311)

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