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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SEP 12 1997

MEMORANDUM

SUBJECT: Review of Inhalation Dominant Lethal Assay in Rats with 1,3-Dichloropropene

FROM: Nancy E. McCarroll Nancy E. McCarroll 9/4/97
Toxicology Branch 1,
Health Effects Division (7509C)

and

Byron T. Backus, Ph.D. Byron T. Backus 9/16/97
Toxicology Branch 2
Health Effects Division (7509C)

TO: Karen Whitby, Ph.D
Branch Chief, Risk Characterization
and Analysis Branch
Health Effects Division (7509C)

Christina Scheltema
Branch Chief, Risk Characterization
and Analysis Branch
Health Effects Division (7509C)

and

Lisa Nisenson
Chemical Review Manager
Special Review Branch
Reregistration Division (7508W)

THRU: Alberto Protzel, Ph.D. Alberto Protzel 9/16/97
Senior Scientist, Toxicology Branch 1
Health Effects Division (7509C)

Registrant: DowElanco
Chemical: CIS/TRANS 1,3-Dichloropropene (Telone II)
Case No.: 838282 Submission No.: S525896
Identifying No.: 029001 DP Barcode: D237003
MRID No.: 44302801 PC Code: 029001
Action:

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Review of the inhalation dominant lethal assay with CIS/TRANS 1,3-Dichloropropene (Telone II).

Conclusion: Presented below is the Citation and Executive Summary for the reviewed study; Data Evaluations Report is attached.

CITATION: Gollapudi, B.B, Cieszlak, F.S. and Lick, S.J. (1997). Telone* II Soil Fumigant (Cis/Trans 1,3-Dichloropropene): Inhalation Dominant Lethal Mutagenicity Study in the CD (Sprague Dawley Derived) Rat; Toxicology Research Laboratories, The Dow Chemical Co., Midland, MI; Study No. 960035; Study Completion Date: May 29, 1997. (Unpublished) MRID NUMBER: 44302801

SPONSOR: DowElanco, Indianapolis, IN

EXECUTIVE SUMMARY: In a dominant lethal study (MRID No.44302801), groups of 30 male Sprague Dawley rats were exposed via inhalation (whole body) to vapor concentrations of 0, 10, 60 or 150 ppm (equivalent to ≈45, 272 or 682 mg/m^3) 1,3-dichloropropene [Lot No. TSN101035: 96% (49.3% cis + 46.7% trans isomer) and Lot No. TSN101299: 96.5% (49.87% cis + 46.59% trans isomers)] 6 hours/day, 7 days/week for 10 weeks. An additional group of 30 untreated males ("pairfed" controls) was placed on dietary restriction for 10 weeks to control for effects associated with decreased feed consumption in the high-dose group. Following exposure, males were mated at a 1:2 ratio with untreated females over two 1-week mating intervals. Females were sacrificed 13 days after mating and uterine contents were examined for evidence of dominant lethality.

Body weight of the high-dose males was significantly ($p \leq 0.05$) lower than the "pairfed" control group throughout the course of the study and significantly ($p \leq 0.05$) lower than the air control group from study Day 8 until termination. Cumulative weight gain for the 150-ppm, "pairfed" control and air control groups was 7, 20 and 37%, respectively. No clear effects on body weight were seen in the group receiving 60 ppm and no other signs of compound toxicity were noted in any treatment group. **Based on adverse effects on body weight, the LOEL was set at 150 ppm; the NOEL is 60 ppm.** The positive control induced the expected high incidence of dominant lethal mutations. **There was, however, no indication that 1,3-dichloropropene induced a mutagenic effect in male germinal cells.**

This study is classified as Acceptable and satisfies the guideline requirement for a dominant lethal assay (§84-2).

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1,3-DICHLOROPROPENE **RAT DOMINANT LETHAL (84-2)**

EPA Reviewer: Nancy McCarroll
Toxicology Branch 1/HED (7509C)

Signature: Nancy E. McCarroll
Date: 8/6/97

Secondary Reviewer: Byron T. Backus, Ph.D.
Toxicology Branch 2/HED (7509C)

Signature: Byron T. Backus
Date: 8/6/97

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Dominant lethal assay in rats; OPPTS 870.5450 [§84-2]

DP BARCODE: D237003 SUBMISSION NO.: S525896

PC CODE: 029001 TOX. CHEM. NO.: 184 MRID NO: 44302801

TEST MATERIAL (PURITY): CIS/TRANS 1,3-Dichloropropene [Lot No. TSN101035: 96% (49.3% cis + 46.7% trans isomers) and Lot No. TSN101299: 96.5% (49.87% cis + 46.59% trans isomers)]

SYNONYM(S): TELONE II; Soil Fumigant; 1,3-D

CITATION: Gollapudi, B.B, Cieszlak, F.S. and Lick, S.J. (1997). Telone* II Soil Fumigant (Cis/Trans 1,3-Dichloropropene): Inhalation Dominant Lethal Mutagenicity Study in the CD (Sprague Dawley Derived) Rat; Toxicology Research Laboratories, The Dow Chemical Co., Midland, MI; Study No. 960035; Study Completion Date: May 29, 1997. (Unpublished) MRID NUMBER: 44302801

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Body weight of the high-dose males was significantly ($p \leq 0.05$) lower than the "pairfed" control group throughout the course of the study and significantly ($p \leq 0.05$) lower than the air control group from study Day 8 until termination. Cumulative weight gain for the 150-ppm, "pairfed" control and air control groups was 7, 20 and 37%, respectively. No clear effects on body weight were seen in the group receiving 60 ppm and no other signs of compound toxicity were noted in any treatment group. **Based on adverse effects on body weight, the LOEL was set at 150 ppm; the NOEL is 60 ppm.** The positive control induced the expected high incidence of dominant lethal mutations. **There was, however, no indication that 1,3-dichloropropene induced a mutagenic effect in male germinal cells.**

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This study is classified as Acceptable and satisfies the guideline requirement for a dominant lethal assay (§84-2).

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Cis/Trans-1,3-Dichloropropene (1,3-D)

Description: Liquid
Lot/batch number: Lot Nos. TSN101035 and TSN101299
Purity: TSN101035: 96% (49.3% cis + 46.7% trans isomer) and Lot No. TSN101299: 96.5% (49.87% cis + 46.59% trans isomers)
Receipt dates: Not reported
Stability: Not reported
CAS number: 6055-19-2
Vehicle used: Compressed/room air
Other provided information: Storage conditions for the test material were not reported. Compressed air and test vapors were diluted with room air to achieve a flow rate of 2900 L/min and the desired test concentrations. Analytical determinations were performed to verify achieved concentrations (see below).

2. Control Substances:

Vehicle control/route of administration: Compressed air was diluted with room air to achieve a flow rate of 2900 L/min.

"Pairfed" control group: An additional group consisting of 30 males received daily food rations equivalent to the estimated daily food consumption of the high concentration treatment group (based on the daily food consumption of the high-dose males in the previous week). The study authors stated that the group on restricted diets was included in the study "to control for any effects of decreased feed consumption observed in the 150 ppm 1,3-D dose group".

Positive control/concentration/route of administration: Cyclophosphamide (CP) was prepared in normal saline and administered once by oral gavage at a dose of 75 mg/kg, 48 hours prior to mating.

4. Test Compound:

Route of administration: Inhalation (whole body)

Dose levels: 10, 60 or 150 ppm

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Note: Dose selection was based on the findings of a preliminary study conducted with 90, 150 or 200 ppm 1,3-D administered via inhalation 6 hours per day, 7 days/week for 4 weeks. Body weight reductions of 6, 19 or 25% were reported for the low-, mid- or high-treatment groups, respectively, at the end of the 4-week exposure. Accordingly, 150 ppm was selected as the highest concentration for the dominant lethal assay.

5. Test Animals:

(a) Species: Rat; Strain: Sprague-Dawley;
Age (at initiation): 10 weeks males
12 weeks (females)
Male Weight ± S.D. (at initiation):
362.6±18 g to 365.7±14 g (Main Groups)
363.8 ± 17 g ("Pairfed" Control)
Source: Charles River Breeding Laboratory, Portage MI.

(b) Number of animals/dose: Animals were randomly assigned to the study groups shown in Table 1.

Table 1. Study Group Assignments

Test Group	Dose	Males/ Group	Females/ Matings[a]
Vehicle Control			
Air	--	30	60
Dietary Control			
Pairfed Group	--[b]	30	60
Positive Control			
Cyclophosphamide	75 mg/kg[c]	30	60
Test Groups			
Low dose	10 ppm	30	60
Mid dose	60 ppm	30	60
High dose	150 ppm	30	60

[a] Each male was mated with 2 females for 1 week and remated with new females for a second 1-week mating period.
[b] Males in this group were placed on restricted diets for 10 weeks. Diets were adjusted based on the daily food consumption of the high-dose males in the previous week.
[c] Males in this group received a single oral gavage dose, 48 hours prior to the first mating.

(c) Animals properly maintained? Yes.

Environmental Conditions: Temperature -- 22± 3°C
Humidity -- 40-60%
Air changes -- 12/hour
Photoperiod -- 12 hrs. light/12 hrs. dark

Housing: Males were housed individually except during the mating periods.

B. TEST PERFORMANCE

1. EXPOSURE PHASE

(a) Inhalation Chamber: Whole-body exposures were performed in 14.5-cubic meter exposure chambers (2.4 m high x 2.4 m wide x 2.4 m deep with a pyramidal top) under dynamic air flow conditions. Chambers were also operated under a slightly negative pressure. Air flow was maintained at 2900 L/min (≈12 air changes/hr.) and monitored once per hour. Chamber humidity and temperature were monitored at least once per hour.

(b) Generation of Test Atmospheres: Test vapors were generated using a glass J-tube. The test material was vaporized by introducing a metered volume of the test material into a J-tube through which a preheated stream of ≈90 L/min. compressed air was passed. The high treatment dose was heated to 95°C; lower treatment doses were not preheated. Test vapors/compressed air mixtures were diluted and remixed with room air to achieve a total flow rate of 2900 L/min. and the target concentrations of the test substance.

(c) Analytical Determinations: Samples of the test vapors were taken from the center of the breathing zone of the animals approximately twice per hour and analyzed for actual concentration.

2. ANIMALS (MALES) OBSERVATIONS:

(a) Clinical signs: Treated males were observed twice daily for mortality, morbidity and moribundity and behavior changes that would indicate compound toxicity.

(b) Body weight and feed consumption: Body weights for the male rats were recorded prior to treatment and weekly during the 10-week exposure period and the 2-week posttreatment mating period. Feed consumption for the males was measured weekly throughout the exposure interval.

(c) Necropsy: Males in all experimental groups were sacrificed at the conclusion of the 2-week breeding sequence and subjected to gross necropsies. Testes and epididymides were removed and preserved in Bouin's fixative.

3. DOMINANT LETHAL PHASE:

(a) Mating: At the end of the 10-week exposure, male rats in the vehicle or three treatment groups were mated with untreated virgin females for 1 week at a mating ratio of 1:2. The mating sequence was repeated once with new virgin females. The above mating sequence was initiated for males in the "pairfed" group, following 10 weeks on the rationed diets. Males receiving the positive control were mated as above 48 hours postdosing with 75 mg/kg CP.

(b) Examination of uterine contents: Females were sacrificed by CO_2 asphyxiation 13 days after completion of the 7-day mating period. Corpora lutea were counted and uteri were examined for total number and position of implants, viable implants, and the number and position of dead implants. Uteri that appeared nongravid were stained with 10% aqueous sodium sulfide and examined for evidence of early resorptions.

(c) Calculated indices: The following indices were calculated:

$$\text{Male Fertility Rate (\%)} = \frac{\text{No. fertile males}}{\text{No. of males mated}} \times 100$$

$$\text{Pregnancy Rate (\%)} = \frac{\text{No. pregnant females}}{\text{No. females mated}} \times 100$$

$$\text{Preimplantation Loss (\%)} = \frac{\text{Total corpora lutea-total implantations}}{\text{Total corpora lutea}} \times 100$$

$$\text{Postimplantation Loss (\%)} = \frac{\text{Total resorptions}}{\text{Total implantations}} \times 100$$

(d) Statistical analysis: Body weights were evaluated by Bartlett's test for equality of variances and/or a parametric or nonparametric ANOVA, Dunnett's test, or Bonferroni corrected Wilcoxon test. Fertility and pregnancy indices were evaluated using Fisher exact test with a Bonferroni correction. Corpora lutea, total implants and live implants were averaged across the two females mated with a given male, averaged for all males in a given group and analyzed using nonparametric ANOVA and a Bonferroni corrected Wilcoxon test. Preimplantation and postimplantation loss were evaluated using a censored Wilcoxon test with a Bonferroni correction. With the exception of Bartlett's test ($p \leq 0.01$), significance was established at $p \leq 0.05$ for all statistical procedures.

(e) Evaluation criteria: It was stated (p. 19) that "... the final interpretation of numerical data considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in light of other biological findings."

C. REPORTED RESULTS:

1. Chamber Monitoring/Analytical Determinations: Summarized results from the monitoring of chambers for temperature, humidity and air flow are presented in Study Report, Table 2, p.28 (see Attachment 1). Ranges for these parameters were:

Temperature	-- 21.9-22.4°C
Humidity	-- 51.2-51.4%
Air Flow	-- 2920-2940 L/min

Summarized results from the analysis of chamber concentrations are also presented in Study Report, Table 2, p.28 (see Attachment 1). As shown, time-weighted averages were within ±10% of the nominal levels which indicates that test material losses in the vapor generation and exposure systems were within acceptable limits. Mean nominal concentrations for the 70 exposures of the low, intermediate and high doses were also within ± 10% of the target concentrations. With a single exception (≈65% of target during Day 53), actual concentrations for the individual high-dose exposures were also within ±10% of the target concentrations.

2. Animal (Males) Observations:

(a) Clinical signs: No signs of compound toxicity were noted in the male rats exposed by inhalation to target concentrations of 10, 60 or 150 ppm 1,3-D 6 hours/day, 7 days/week for 10 weeks. The single death (1 male in the 60-ppm group during week 2) was not attributed to test material exposure.

(b) Body Weights: Summarized body weight data, presented in Study Report, Table 3, pp. 29 and 30 (see Attachment 2), indicate that the body weight for the high-dose males was significantly ($p \leq 0.05$) lower than the "pairfed" controls throughout the course of the study and significantly ($p \leq 0.05$) lower than the air control group from study Day 8 until termination. Body weight decrements between the high-dose group and the air control group ranged from 9% at Day 8 to 22% at Day 70. High-dose group body weights were generally ≥10% lower than the "pairfed" control from Day 8 to termination. Cumulative weight gain for the 150-ppm, "pairfed" control and air control was 7, 20 and 37%, respectively. Significant but <10% lower body weights (compared to the air control group) were noted for the males in the 60-ppm group at Days 8, 22-50 and 70. No adverse effects on body weight were observed in the lowest dose group.

(b) Feed Consumption: Feed consumption in the 150-ppm group was markedly lower that the air control group during the first 3 weeks of exposure (31% less than control--Days 1-8, 17% less than control--Days 8-15 and 13% less than control--Days 15-22). Feed consumption by the high-dose males was generally <8% of control for the remainder of the study. In the mid-dose males, feed consumption was 18% less than control for Days 1-8 but comparable

to control throughout the remainder of the study. No treatment-related effects on feed consumption occurred at the lowest assayed concentration.

(c) Gross Necropsy: No information was provided from the gross necropsy of the treated males. Our reviewers assume, therefore, that no treatment-related lesions were found.

3. Dominant Lethal Parameters: Data from the mating phase of the study were evaluated using both the male or the female as the experimental unit; these data are summarized in Study Report Tables 7 and 8, pp. 34 and 35 (male-based analysis) and Study Report Tables 9 and 10, pp. 36 and 37 (female-based analysis). As shown, neither the fertility rates nor the pregnancy rates were significantly affected by treatment with 1,3-D. When the data were analyzed using the male as the experimental unit (i.e., averaging the data from a given parameter across the two females mated to a given male and then averaging the resulting value for all males in a group), a significantly ($p \leq 0.05$) lower mean for corpora lutea was calculated for the week 1 high-dose mating (Study Report Table 7). The study authors concluded that the finding was "a chance occurrence because of the lack of reproducibility in Week 2 matings". Our reviewers agree with the study authors that the finding has no biological relevance and further note that calculating mean corpora lutea/female, which is the more conventional approach, yielded no significant or appreciable differences between the 150-ppm test group and the air or restricted dietary control group values. Similarly, no significant difference in the endpoints considered indicative of dominant lethality (i.e., decreased live implants accompanied by increased postimplantation deaths) was noted at any 1,3-D dose or mating week using either the male or the female as the experimental unit. By contrast, the positive control (75 mg/kg CP) induced a clear positive increase in dominant lethal mutations at both mating intervals.

Based on the overall data, the study authors concluded that 1,3-D was not mutagenic to the male germ cells at exposure levels up to and including 150 ppm, the highest dose tested.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: We agree with the study authors' conclusion that 1,3-D was negative in this inhalation dominant lethal assay. Exposure to the high dose (150 ppm) resulted in significantly reduced body weight and markedly depressed body weight gain compared to both the vehicle control and "pairfed" control groups. Our reviewers noted that reduced body weight was also reported at 150 ppm in the preliminary study and adverse effects on body weight have been repeatedly observed in other toxicology studies submitted for 1,3-D. On this basis, we assess that the high dose was adequate for the assessment of the *in vivo* mutagenic potential of 1,3-D. Accordingly, the LOEL is set at 150 ppm based on decreased body weight and body weight gain; the NOEL is 60 ppm. In addition, the sensitivity of the test system to detect a dominant lethal effect was demonstrated by the results achieved with the positive control (75 mg/kg CP). From the above considerations, we assess that the study

provided acceptable evidence of a nonmutagenic response in this whole animal test system.

E. STUDY DEFICIENCIES: None

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ATTACHMENT 1

CHAMBER SUMMARY DATA
STUDY REPORT TABLE 2, p. 28

12 B

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_____ Identity of product impurities.

_____ Description of the product manufacturing process.

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_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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ATTACHMENT 2

MALE BODY WEIGHT SUMMARY
STUDY REPORT TABLE 3, pp. 29-30

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ATTACHMENT 3

SUMMARIZED DATA FROM THE DOMINANT LETHAL PHASE OF TESTING
(MALE BASED ANALYSIS)
STUDY REPORT TABLES 7 AND 8, pp. 34 AND 35

SUMMARIZED DATA FROM THE DOMINANT LETHAL PHASE OF TESTING
(FEMALE BASED ANALYSIS)
STUDY REPORT TABLES 9 AND 10, pp. 36 AND 37

17 18

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