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

JAN 12 1988

006546

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 1,3-Dichloropropene (TELONE II): One Pharmacokinetic Study, Three Mutagenicity Studies and Twelve Reprints.

FROM: Alan C. Levy, Ph.D.
Toxicologist, Review Section V
Toxicology Branch/HED (TS-769C)
Alan C. Levy
Jan. 11, 1988

TO: Bruce Kapner - PM # 70
Registration Division (TS-767C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V
Quang Q. Bui 1/11/88

and

Theodore M. Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Dow Chemical Company

Action Requested: Review the pharmacokinetic study, three mutagenicity studies and 12 reprints pertaining to 1,3-dichloropropene (TELONE II).

Recommendations:

I. Review of studies submitted.

A. Pharmacokinetics Study: Accepted as Core Minimum Data.

B. Mutagenicity Studies:

1. Chinese Hamster Ovary Cell Gene Mutation Assay: Supplemental Data. This study will be considered as Acceptable provided acceptable statistical analyses and historical values are received.

2. Bacterial Gene Mutation Test System: Acceptable. The relatively poor translation makes it difficult to obtain several details. There were positive responses in the B. subtilis rec-assay and Salmonella reversion mutation assay. The E. coli reversion mutation assay was negative. The host-mediated assay with Salmonella is Unacceptable as the dose levels were not high enough. No Quality Assurance statement was provided, but the study may have been conducted prior to GLP guidelines.

3. Rat Hepatocyte Unscheduled DNA Synthesis Assay: Supplemental Data. This study will be considered as Acceptable provided the following are acceptable - verification that the second assay had a lower cytoplasmic background than what was observed in the first assay; and, if available, an analysis of the percent of cells with greater than 5 net nuclear grains (i.e. % of cells in repair should be provided).

C. Twelve Publications: All are classified as Supplemental Data:

1. Structure-Activity Relationship in Halogen and Alkyl Substituted Allyl and Allylic Compounds: Correlation of Alkylating and Mutagenic Properties.

2. Mutagenicity of Pesticides Containing 1,3-Dichloropropene.

3. Mutagenic Impurities in 1,3-Dichloropropene Preparations.

4. Investigation of the Potential Genotoxicity of CIS(Z)-1,3-Dichloropropene (Z-DCP).

5. The Protective Action of Glutathione on the Microbial Mutagenicity of the Z- and E-Isomers of 1,3-Dichloropropene.

6. Glutathione Conjugation in the Detoxication of (Z)-1,3-Dichloropropene (a Component of the Nematocide D-D) in the Rat.

7. The Excretion and Retention of Components of the Soil Fumigant D-D and Their Metabolites in the Rat.

8. Urinary Excretion of the N-Acetyl Cysteine Conjugate of Cis-1,3-Dichloropropene by Exposed Individuals.

9. Fate of [^{14}C] Vinyl Chloride after Single Oral Administration in Rats.

10. Fate of [^{14}C] Vinyl Chloride Following Inhalation Exposure in Rats.

11. Hepatic Macromolecular Binding Following Exposure to Vinyl Chloride.

12. Inhalation Pharmacokinetics of Cis- and Trans- 1,3-Dichloropropene inj Rats Exposed to TELONE* II Vapors.

II. Response to the registrant.

With the submission of the aforementioned studies, the Registrant:

A. Questioned the premise that the bacterial mutagenicity data reported to date provides evidence of oncogenic potential in mammals. This was based on a report that a mutagenic contaminant/degradation product of 1,3-D was responsible for the mutagenic activity and that there was a lack of purity data for the 1,3-D used in earlier mutagenicity studies (the known mutagen epichlorohydrin was a stabilizing agent).

B. Stated that if it is assumed that 1,3-D is the mutagenic component of 1,3-D formulations, there are data to suggest the inappropriateness of bacterial mutagenicity assays to predict the potential mammalian mutagenicity of the compound. There is evidence of the protective role of glutathione.

C. Noted that although there is some structural similarity between 1,3-D and vinyl chloride, the metabolic and excretory pathways are different. They indicated that the epoxide of vinyl chloride was believed to be responsible for its toxicity; whereas, 1,3-D does not go to an epoxide.

The Toxicology Branch has addressed these points as presented in Dr. Dearfield's memo of November 25, 1987 (attached).

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Primary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy
Review Section V/HED (TS-769C) Jan. 11, 1988

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q. Bui
Acting Section Head, Review Section V

I. Study Type: Metabolism Study
(Guideline § 85-1)

Study Title: 1,3-Dichloropropene: Pharmacokinetics, Effect on Tissue Non-Protein Sulfhydryls, and Macromolecular Binding in Fischer-344 Rats and B6C3F1 Mice Following Oral Administration

EPA Identification Numbers:

EPA Identification: 029001
EPA Accession: 264936
EPA Record: 184439
Caswell: 324A
Tox. Branch Project: 7-0143

Sponsor: Dow Chemical USA
Midland, MI 48640

Testing Laboratory: Mammalian and Environmental Toxicology Research Laboratory
Health and Environmental Sciences, U.S.A.
Dow Chemical U.S.A.
1803 Building
Midland, MI 48640

Study Number: Lab. No. 155; Problem No. 3140911
Laboratory Report Code: HET:K-6409-11

Study Date: March 12, 1985

Study Authors: F. K. Diez Ph.D., E. A. Hermann, B.S., P. E. Kastl, B.S., D. A. Dittenber, B.S., M.T.(ASCP), and C. J. Ramsey, Ph.D.

Recommendation: This study is accepted as Core Minimum Data.

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TEST MATERIAL:

A mixture of uniformly labeled ^{14}C-cis,trans-1,3-D (Ref. No. 881-117; 1.15 mCi/mmole) with an isomer distribution of 61.6±1.0% cis:38.4±1.0% trans was obtained from the Agricultural Products Department of Dow Chemical U.S.A., Midland, MI.

[2-^{14}C]-Cis-1,3-D was synthesized by Pathfinder Laboratories Inc., St. Louis, MO (Lot No. 820535; 8.9mCi/mmole). As determined by chemical ionization gas chromatography/mass spectrometry interfaced with a Panax Radiogas Beta detector, the radio chemical purities of the ^{14}C-cis,trans-1,3-D and [2-^{14}C]-cis-1,3-D were 97.75% and 97.83%, respectively.

Cis (AGR 164300) and trans (AGR 164302) isomers of 1,3-D were obtained from the Agricultural Products Department of Dow Chemical U.S.A., Midland, MI. Analysis of these materials by gas chromatography using flame ionization detection indicated that they were greater than 96% and 98% 1,3-D, respectively. The distribution of cis and trans isomers within each of the unlabeled materials was 94.1% cis:2.5% trans (AGR 164300) and 0.7% cis:97.3% trans (AGR 164302).

Empirical Formula:	$C_3H_4Cl_2$
Specific Gravity:	1.217 at 20/4°C (cis); 1.224 at 20/4°C (trans)
Molecular Weight:	111
Boiling Points:	104°C (cis); 112°C (trans)
Solubility:	Insoluble in water; soluble in ether or chloroform.

II. Materials and Methods

A. Test Species - Male Fischer-344 rats weighing approximately 225-260 g and male and female B6C3F1 mice weighing approximately 20-30 g were obtained from Charles River Breeding Laboratories. The animals were acclimated for at least 7 days and randomly assigned to experimental groups.

B. Dose Solutions and Dose Levels - Corn oil was the vehicle and the dosing solution was administered by gavage at a volume of 5 ml/kg body weight. Animals were deprived of food for up to 12 hours post-dosing.

Radiolabeled dosing solutions were prepared by mixing various amounts of labeled and unlabeled material. All dose solutions were assayed for radio-activity by liquid scintillation counting. Targeted dose levels included 1 and 50 mg/kg for rats, pharmacokinetics; 1 and 100 mg/kg for mice, pharmacokinetics; and 1, 50 and 100 mg/kg for rats and mice, macromolecular binding. For studying urinary metabolites, the animals were given a minimum of 3.0 uCi of ^{14}C-activity in single oral dose targeted at 30.5 mg/kg.

Target dose levels (corn oil vehicle) for the Non-Protein Sulfhydryl (NPS) experiments were 0 and 100 mg/kg (time-course study) and 0, 1, 5, 25, 50 or 100 mg/kg (dose-response study).

C. Pharmacokinetic and Metabolism Studies - Three male rats and mice/dose level were acclimated for 1-2 days in glass Roth-type metabolism cages (1 animal/cage). Each animal was returned to its cage following a single oral dose of ^{14}C-cis,trans-1,3-D (1 or 50 mg/kg to rats and 1 or 100 mg/kg to mice). Urine, feces, CO_2 and volatiles in expired air were collected at 6, 12, 24, 36 and 48 hours post-dosing.

Rats and mice were sacrificed by exsanguination 48 hours post-dosing. The following tissues were analyzed for radioactivity: non-glandular stomachs, glandular stomachs, livers, kidneys and bladders as well as samples of blood, perirenal fat, skin and carcass.

Two male rats were given 30.5 mg [2-^{14}C]-cis-1,3-D/kg in order to compare profiles of excreted urinary radioactivity to the activity observed after ^{14}C-cis, trans-1,3-D. This amount (30.5 mg) approximates the amount of cis-1,3-D given to rats at 50 mg/kg of 1,3-D as a 62%: 38% mixture of cis and trans. Urine samples were collected at various intervals until the rats were sacrificed 24 hours post-dosing.

Separation of Urinary Metabolites. Aliquots of urine were analyzed (individually or pooled) by liquid chromatography (LC). Radiolabeled urinary metabolites were separated by ion exclusion LC and were quantitated by an on-line radioactivity detector or by liquid scintillation counting.

Identification of Urinary Metabolites. The residues of the radioactive LC fractions were either derivatized and analyzed by direct probe chemical ionization mass spectrometry (CI/MS) or analyzed without derivatization by fast atom bombardment mass spectrometry (FAB/MS).

Radioactivity Analyses. Determinations of ^{14}C-activity were made by liquid scintillation counting. Blood, urine, cage washings, aliquots of CO_2 trap solutions and fractions of LC eluent were counted with no further treatment. Aqueous homogenates of feces, livers, kidneys and carcasses were prepared, with aliquots being oxidized. Non-glandular stomachs, glandular stomachs, bladders, fat and skin were oxidized directly. The $^{14}CO_2$ produced during oxidation was counted directly.

D. Time-Course of Non-Protein Sulfhydryl (NPS) Depletion - Male mice were given a single oral dose of 0 or 100 mg 1,3-D/kg and sacrificed by cervical dislocation at 2, 4, 8, 12 or 24 hours post-dosing (9 mice/dose/time interval). Tissue NPS was determined on non-glandular stomachs, glandular stomachs, livers, kidneys and bladders.

E. Dose-Response of Non-Protein Sulfhydryl (NPS) Depletion - NPS were determined in non-glandular stomachs, glandular stomachs, livers, kidneys and bladders of male rats and mice 2 hours after a single oral dose of 0, 1, 5, 25, 50 or 100 mg 1,3-D/kg. The effects of a single oral dose of 0 or 100 mg kg of 1,3-D on NPS in livers and bladders of female B6C3F1 mice were also determined.

F. Macromolecular Binding - Male rats (4/dose) and mice (12 dose) were sacrificed 2 hours after a single oral dose of 1, 50 or 100 mg ^{14}C-cis,trans-1,3-D/kg. The following tissues were removed, frozen and stored at -70°C until analyzed for macromolecular binding: non-glandular stomachs, glandular stomachs, livers, kidneys and bladders. Livers and bladders were analyzed from female mice having received 100 mg ^{14}C-cis,trans-1,3-D/kg and sacrificed 2 hours post-dosing.

Detailed statistical methodology was described.

A Quality Assurance statement was included.

A copy of the Materials and Methods section from the report is appended.

The reviewer has no comments regarding this section.

III. Results

A. Recovery and Excretion of ^{14}C-Activity - At 48 hours following a single dose of 1 or 50 mg/kg of 14C-cis,trans-

1,3-D to male rats, the average recoveries were 96 and 101%, respectively, with urine being the major route of excretion (51-61%). About 20% was excreted via feces and 15-18% as $^{14}CO_2$ in expired air. In male mice dosed at 1 or 100 mg/kg, average recoveries were 112 and 97%, respectively, with percent in the urine being 79 and 63 (1 and 100 mg/kg). Feces and expired $^{14}CO_2$ accounted for about 13-16%. [See Table 1.]

Table 1

PERCENT OF ADMINISTERED ^{14}C-ACTIVITY RECOVERED IN THE EXCRETA AND CARCASSES OF MALE RATS AND MICE 48 HOURS AFTER A SINGLE ORAL DOSE OF ^{14}C-CIS,TRANS-1,3-D[a]

	Percent of Administered ^{14}C-Activity Recovered			
	Rats		Mice	
Sample	1 mg/kg	50 mg/kg	1 mg/kg	100 mg/kg
Urine	50.9±4.6	61.3±4.3	78.6±6.7	62.5±3.6
Feces	20.5±0.3	17.1±1.6	15.7±2.7	14.5±3.0
CO_2	17.6±0.4	15.1±0.9	14.4±0.4	13.7±2.0
Tissues and Remaining Carcass[b]	6.0±1.0	5.6±1.0	2.0±0.2	1.8±0.2
Cage Wash	1.0±0.3	1.0±0.5	3.9±0.5	4.0±2.8
Charcoal Trap	0.1±0.04	0.6±0.8	0.1±0.1	0.2±0.1
TOTAL RECOVERY	96.0±5.5	100.6±4.6	111.7±7.3	96.7±7.8

a = Each value represents the mean ± SD for 3 animals.
b = Represents the sum of all tissues analyzed; individual tissue concentrations are shown in Table 2.
These data are extracted from Table 1 of the report.

In both rats and mice, ^{14}C-activity was excreted via urine in an apparent monophasic fashion with excretion half-lives of 5-6 hours. Data from only 2 of 3 mice given 1 mg/kg were used for the 24-hour interval (3rd mouse data low and was reported not to have fit with other data collected from this animal). [See Figure 1.]

The investigation indicated that most fecal radioactivity excreted by rats occurred 12-24 hours post-dosing while in mice, fecal excretion was consistent throughout all collection periods (report indicated, "data not shown"). The authors stated that most of the $^{14}CO_2$ expired by both rats and mice occurred between 0-12 hours post-dosing (report indicated, "data not shown").

In rats, there was a considerably higher concentration of radioactivity in non-glandular stomachs, glandular stomachs, livers, kidneys and bladders than in fat, skin, blood or remaining carcasses. In mice, the greatest amount of activity was found in the non-glandular stomach, with lesser amounts in other tissues. [See Table 2.]

Table 2

TISSUE CONCENTRATIONS OF ^{14}C-ACTIVITY IN MALE RATS AND MICE 48 HOURS AFTER A SINGLE ORAL DOSE OF ^{14}C-CIS,TRANS -1,3-D[a]

		Microgram Equivalents of ^{14}C-1,3-D/g Tissue	
Rats	Tissue	1 mg/kg	50 mg/kg
	Non-Glandular Stomach	0.29±0.07	12.07±1.13
	Glandular Stomach	0.23±0.03	10.23±1.37
	Liver	0.24±0.02	9.92±0.38
	Kidney	0.27±0.02	10.91±1.13
	Bladder	0.31±0.07	7.12±1.45
	Fat	0.08±0.01	2.62±1.45
	Skin	0.09±0.04	3.78±0.97
	Blood	0.05[b]	1.88±0.82
	Remaining Carcass	0.04±0.01	1.80±0.14
Mice	Tissue	1 mg/kg	100 mg/kg
	Non-Glandular Stomach	0.23±0.08	15.25±1.70
	Glandular Stomach	0.04±0.01	3.15±0.29
	Liver	0.10±0.02	5.79±0.91
	Kidney	0.09±0.01	7.28±0.85
	Bladder	0.13±0.10	3.27±0.65
	Fat	0.04±0.03	4.65±1.29
	Skin	0.02±0.01	1.64±0.83
	Blood	ND[c]	0.79±0.21
	Remaining Carcass	0.01±0.002	1.31±0.20

a = Each value represents the mean ± SD for 3 animals unless otherwise noted.
b = Detectable in only one of 3 rats.
c = ND = Not detectable.

This table was reproduced from the table in the report.

B. Separation and Identification of Urinary Metabolites - Chromatograms of urine excretions from rats given 1 or 50 mg/kg of ^{14}C-cis,trans-1,3-D, indicated four peaks and none was considered to represent the parent compound (based on retention time of unmetabolized 1,3-D). Most of the 1 mg/kg was excreted as metabolites C and D; whereas, for 50 mg/kg, most was excreted as metabolite D (up to 22.5%), less than 9% as metabolite C and minimal amounts as metabolites A or B. [See Figures 2 and 3.]

Metabolite D was identified as N-acetyl-S-(3-chloroprop-2-enyl)cysteine [the mercapturic acid of 1,3-D]. The ions indicated a molecular weight of 251. [See Figure 4.]

Metabolite C was tentatively identified as a sulfoxide or sulfone of N-acetyl-S-(3-chloroprop-2-enyl) cysteine. No attempt was made to identify metabolites A and B.

The primary urinary metabolites excreted by rats after a single oral dose of 30.5 mg/kg (^{14}C-cis-1,3-D) were also D and C. As a percentage of ^{14}C-activity, their excretion was similar to that observed in rats given 50 mg/kg of radiolabeled cis,trans mixture. [See Figure 5.]

Urinary metabolites in male mice were the same as for rats except that B and C were not clearly separated. Mice excreted primarily 1,3-D mercapturic acid and its sulfoxide or sulfone (same as rats). [See Figure 6.]

C. Effects on Non-Protein Sulfhydryl Content -

Time-Course Studies in Mice - In male mice, 100 mg of 1,3-D/kg decreased non-glandular stomach NPS to 20% of control values within 2 hours post-dosing. NPS levels returned to control levels after about 8 hours and increased to almost 250% of control by 24 hours. There was a considerably less effect in the glandular stomach (40% of control) and liver (50% of control) with essentially no effect in kidneys and bladders. [See Figure 8.]

Dose-Response Studies in Rats and Mice - Tissue NPS were determined at 2 hours post-dosing (studies indicated maximal depletion occurred at this time). There was a statistically significant depletion of NPS in the rat non-glandular stomach at doses of 5, 25, 50 and 100 mg/kg (reduction to 74, 27, 19 and 17 % of control, respectively). No reduction was observed at 1 mg/kg. Statistically significant decreases in tissue NPS were found in the glandular stomach (5 mg/kg and above) and in the liver (25 mg/kg and above); these were less severe depletions (40-50% of control at 100 mg/kg) than in the non-glandular stomach. Isolated relatively small

decreases of NPS levels in kidneys and bladders were not considered to be treatment related. [See Figure 9.]

Similar effects were observed in male mice. In non-glandular (to 51, 37 and 27% of control) and glandular stomachs, there was a dose-dependent reduction of NPS at 25, 50 and 100 mg/kg; whereas, in the liver, only 100 mg/kg appeared to cause a statistically significant reduction. No reduction was observed in kidneys or bladders. In female mice given 100 mg/kg, the results were similar to those observed in males. [See Figure 10.]

D. Macromolecular Binding - Two hours after dosing with 1, 50 or 100 mg/kg, the macromolecular binding of ^{14}C-activity was similar in rats and mice and approximately proportionate to the increase in dose. The most binding was observed in the non-glandular stomachs. The least amount of binding was noted in livers, kidneys and bladders. [See Table 3.] Female mice receiving 100 mg/kg showed no binding to bladder molecules, but liver binding was similar to males (report indicated, "data not shown").

Table 3

EFFECTS OF VARYING DOSES OF 1,3-D ON MACROMOLECULAR BINDING IN TISSUES FROM MALE RATS AND MICE [a]

Species	Tissue[b]	Microgram Equivalents of 1,3-D Bound g Tissue Protein		
		1 mg/kg	50 mg/kg	100 mg/kg
Rats	Non-Glandular Stomach (4)	2.1±1.0	172.3±27.1	206.7±37.9
	Glandular Stomach (4)	1.1±0.5[c]	76.1± 2.8	132.3±21.0
	Liver (4)	0.6±0.2	19.7± 2.8	23.8± 8.4
	Kidney (4)	0.6±0.2	17.3± 3.0	25.5± 2.9
	Bladder (2)[d]	0.2±0.2	25.4± 2.8	22.0± 4.9
Mice	Non-Glandular Stomach (4)[e]	5.7±0.4	199.2±26.2	301.4±35.0
	Glandular Stomach (4)[e]	0.9±0.1	29.6± 7.1	60.3±19.8
	Liver (12)	0.7±0.2	13.6± 2.8	24.7± 7.4
	Kidney (12)	1.0±0.3	16.0± 4.4	24.9± 5.9
	Bladder (4)[e]	ND[f]	ND[f]	21.2±18.9

a=Each value represents the mean ± SD.
b=Values in parentheses refer to the number of tissues examined.
c=N equals 3 due to loss of one sample during tissue preparation.
d=N equals 2 due to pooling of bladders from 4 rats/dose level into 2 groups of 2 bladders/group.
e=N equals 4 due to pooling of tissues from 12 mice/dose level into 4 groups of 3 tissues/group.
f=ND=Not detected.
Data reproduced from Table 3 of the report.

IV. Discussion/Conclusions

The primary routes of ^{14}C excretion in rats and mice following a single oral dose of ^{14}C-cis,trans-1,3-D were via urine, feces and expiration of $^{14}CO_2$. Based upon the LC data, no urinary excretion of unchanged parent compound was observed. Radioactive urinary metabolites measured 69-76% in rats and 76-93% in mice within 48 hours of dosing.

Of all tissues investigated, radioactivity was mostly found in the non-glandular stomachs in rats and mice, but the total amount of radioactivity in tissues and carcass never exceeded 6% (rats) and 2% (mice) of the administered dose. Therefore, of 100% of the radioactivity given, approximately 1.2% was found in the non-glandular stomach of rats (20% in this tissue x 6% in all tissues) and 0.7% in the non-glandular stomach of mice (35% in this tissue x 2% in all tissues).

Rats and mice excreted two major urinary metabolites: D = N-acetyl-S-(3-chloroprop-2-enyl)cysteine or a 1,3-D mercapturic acid; C = N-acetyl-S-(3-chloroprop-2-enyl)cysteine sulfoxide or sulfone. The amount of metabolites excreted following administration of ^{14}C-cis-1,3-D alone was enough to account for most of the major urinary metabolites found after dosing with ^{14}C-cis,trans-1,3-D.

Excretion and tissue distribution data in rats and mice (at the doses tested) suggested that the major metabolic pathways followed "linear" pharmacokinetics.

The non-glandular stomach was most sensitive to the NPS-depleting effects of 1,3-D and the results observed showed a dose-response effect.

Covalent binding was determined, and the most pronounced effect was observed in the stomach (50 or 100 mg/kg). There was the suggestion that macromolecular alkylation occurred to a great extent in the stomach.

Core Classification: Core Minimum Data.

Telone

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Pages 13 through 33 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

____ A draft product label.

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Primary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy Jan. 7, 1988
Review Section V/HED (TS-769C)

Secondary Reviewer: Kerry Dearfield, Ph.D. Kerry Dearfield 1/7/88
Mission Support Staff
Toxicology Branch/HED (TS-769C)

I. Study Type: Mutagenicity (Bacterial Test Systems)
(Guideline § 84-1)

Title: The Mutagenicity Test on 1,3-dichloropropene in Bacteria Test System

EPA Identification Numbers:

EPA Identification: 029001
EPA Accession: 264936
EPA Record: 184439
Caswell: 324A
Tox. Branch Project: 7-0143
Document:

Sponsor: Dow Chemical Japan
Dow Chemical U.S.A. (submitted report)
1803 Building
Midland, MI 48640

Testing Laboratory: Nomura Sogo Research Institute

Study Number: NRI-78-2819

Study Date: July 4, 1979

Study Author: S. Sudo, Y. Kimura, K. Yamamoto and Y. Kubota

Recommendation: Acceptable.

The relatively poor translation makes it difficult to obtain several details. No Quality Assurance statement was provided, but the study may have conducted prior to GLP guidelines. The test compound induced positive responses in the Bacillus subtilis rec-assay and a strong positive response in the Salmonella reversion mutation assay. The test compound was negative in the E. coli reversion test. The host-mediated assay with Salmonella is Unacceptable as the dose levels were not high enough.

Test Material:

Name: 1,3-dichloropropene (supplied by Dow Chemical Japan)
Lot No.: none given
Purity: 49.8% cis-1,3-dichloropropene
46.3% trans-1,3-dichloropropene

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II. Materials and Methods

A. Rec-assay: DNA damage was studied using strains H 17 and M 45 of B. subtilis. Fifty uL of test substance dissolved in DMSO were added to the culture and incubated overnight. The amount of inhibition was measured. Hydrochloride (HCL) and Kanamycin (KM) were used as the negative controls and AF-2 as the positive control.

B. Reversion Test with E. coli: E. coli, B/r, Wp 2 and Try- tryptophan-requiring mutants were incubated overnight ($3.3x10^9$/ml). Aliquots of this solution were mixed with the test sample dissolved in agar and plated. The number of colonies were counted after three days of incubation (mean count of 2 plates). The solvent was the negative control and AF-2 was the positive control. This assay was conducted with and without S-9.

C. Reversion test with S. typhimurium: TELONE II was tested in the following strains of S. typhimurium : G 46, TA 1535, TA 100, TA 1537, TA 1538 and TA98. Concentrations of the test material in agar were mixed with each strain. The solvent served as a negative control and positive controls were as follows: MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) for G 46; beta-PL (beta-propiolactone) and 2 AA (2-aminoanthracene) for TA 1535; AF-2 and 2-AA (2-aminoanthracene) for TA 100; 9 AC (9-aminoacridine) and 2 AA for TA 1537; 2 NF (nitrofluorene) and 2 AA for TA 1538; and AF-2 and 2 AA for TA 98. The number of colonies was counted after two days of incubation. The reversion test with S. typhimurium was conducted with and without S-9.

D. Host-Mediated Assay: The assay followed the publication of Legator (in Chemical Mutagens, Ed. A. Hollaender, 1971, Vol. 2, p. 569). Doses of 0, 30 and 60 mg/kg in corn oil were administered by gavage to 7 week old ICR male mice. The positive control was 50 mg/kg of dimethylnitrosamine (DMNA). Immediately after compound administration, 2 ml of S. typhimurium (G 46) were injected i.p. Two more injections were given at hourly intervals. Thirty minutes after the third administration the animals were killed and 2 ml of saline were given i.p. One ml of intraperitoneal fluid was withdrawn and was inoculated on media without histidine. After 2 days of incubation, the numbers of reversion mutants, surviving bacteria and colonies were determined.

A copy of Materials and Methods from the report is appended.

No Quality Assurance statement was included.

There was no reference to statistical methodology.

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The reviewer feels that the apparent translation should have been more accurate and reviewed by Dow Chemical U.S.A. before submission to the Agency.

III. Results

A. Rec-assay: A concentration of 25 mg/ml appeared to cause a difference between the inhibitory areas of H 17 and M 45, thus indicating a positive effect. AF-2 (positive control) caused an even greater difference in zones of inhibition. [See Table 1.]

B. Reversion test with E. coli: The compound, with or without S-9, did not appear to be mutagenic. AF-2 (positive control) was considered mutagenic. [See Table 2.] Toxicity was seen at $\geq$ 1000 ug/plate.

C. Reversion test with S. typhimurium: Positive results were noted with and without S-9 in assays with G 46, TA 1535, TA 100 and TA 98. There were also positive results observed only in the presence of S-9 in the TA 1537 and TA 1538 strains. In some assays concentrations as low as 0.1 mg/ml (10 ug/plate) were positive. All positive controls showed mutagenicity. [See Tables 3-8.]

D. Host-mediated Assay: The doses of 30 or 60 mg/kg did not appear to increase the number of revertants over the control value. The positive control (50 mg/kg DMNA) showed 344$\pm$106 (mean $\pm$ S.D.) revertants compared with a range of 3.01-4.85 for the control and test groups. [See Table 9.] Higher doses could have been tested in order to possibly obtain toxicity.

IV. Conclusions

In the rec-assay, the highest dose was considered to be positive. Weak mutagenicity appeared in Salmonella strains TA 1537 (only with S-9: 2.5-25 mg/ml, 1.5-2 x control), TA 1538 (only with S-9: 2.5-50 mg/ml, 1.5-2.1 x control; 100 mg/ml, 7.7 x control) and TA 98 (without S-9: 0.25-2.5 mg/ml, 1.9-3.4 x control with no increase at 5 or 10 mg/ml; with S-9: 5 and 10 mg/ml, 1.8 and 2.6 x control). Strong mutagenicity was observed in Salmonella strains TA 1535 (without S-9: 0.25-10 mg/ml, 2.2-21.1 x control; with S-9: 5-100 mg/ml, 3.3-24.8 x control) and TA 100 (without S-9: 0.1-5 mg/ml, 2.2-23.6 x control with no increase over control at 10 mg/ml; with S-9: 2.5-10 mg/ml, 4.4-5.7 x control). With Salmonella strain G 46, results were positive, but the degree of mutagenicity was weak (without S-9: 2.5-10 mg/ml, 1.8-3.0 x control; with S-9: 10-100 mg/ml, 1.9-2.8 x control). The compound did not appear to cause a positive effect in the host-mediated assay.

V. Recommendations: Acceptable.

1. The relatively poor translation makes it difficult to obtain several details.

2. Gaps in report/errors:

A. No Quality Assurance statement - This study may have been conducted prior to GLP guidelines.

B. Table 1, columns H 17 and M 45, 3rd line from bottom: H 17=1, M 45=7; difference should be 6 (report says 5).

C. Reference list, No. 8: no year of publication mentioned (should be 1971).

Telone

Page_____ is not included in this copy.

Pages 38 through 59 are not included.

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____ Identity of product inert ingredients.

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006546

Primary Reviewer: Alan C. Levy, Ph.D.
Review Section V/HED (TS-769C)
Alan C. Levy Jan. 7, 1988

Secondary Reviewer: Kerry Dearfield, Ph.D.
Mission Support Staff
Toxicology Branch/HED (TS-769C)
1/7/88

I. Study Type: Mutagenicity (Chinese Hamster Ovary Cell) (Guideline § 84-1)

Study Title: The Evaluation of TELONE II Soil Fumignat in the Chinese Hamster Ovary Cell/Hypoxanthine (Guanine) Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay

EPA Identification Numbers:

EPA Identification: 029001
EPA Accession: 264936
EPA Record: 184439
Caswell: 324A
Tox. Branch Project: T-0143
Document:

Sponsor: Dow Chemical U.S.A.
1803 Building
Midland, MI 48640

Testing Laboratory: Dow Chemical U.S.A.
1803 Building
Midland, MI 48640

Study Number: Lab. No. 208; Problem No. 8007395
Laboratory Report Code: HET M-003993-016

Study Date: February 27, 1986

Study Author: Alan L. Mendrala, M.S.

Recommendation: Supplemental Data.

This study will be considered as Acceptable provided acceptable statistical analyses and historical values are received for review.

Test Material:

Name: TELONE II (technical grade 1,3-dichloropropene)
Lot No.: TB 331213-4 (Drum #2)
Purity: (determined by gas chromatography)
48.9% cis-1,3-dichloropropene
43.2% trans-1,3-dichloropropene

II. Materials and Methods: Dose selection was based on results of preliminary cytotoxicity experiments both with and without the S9 fraction of a rat liver homogenate metabolic activation system (Data not provided, but appear to be in appropriate range based on results in mutagenicity experiments). Due to the volatility of TELONE II, exposure of cell cultures to the chemical was conducted in tissue culture flasks which were tightly capped. Five dose levels of TELONE II were tested in the high density monolayers (with and without metabolic activation). Concentrations were chosen which reduced the survival from 100% to approximately 10% of control cultures.

Growing culture cells were counted, diluted to the desired concentration and plated at a density of $2x10^4$ cells/cm^2 approximately 16-18 hours prior to chemical treatment. The number of culture vessels was adjusted to yield $1x10^6$ surviving cells. Flasks were also plated with 100-5000 cells (number of cells varied due to expected survival). The next day, the media was aspirated off, the cultures rinsed with saline and the culture media replaced with serum-free F-12 media. Ten uL of test chemical was introduced into each flask, the flasks were capped and the cultures incubated for 4-5 hours. After chemical treatment, solution was removed, the cultures washed with saline, fed complete medium and allowed to grow at 5% CO_2 in air at 37°C in a humidified incubator. The flasks with 100-5000 cells were incubated to allow colony development and the determination of toxicity. After 7 days the colonies were fixed, stained and counted to determine the % survival (compared to the negative control).

Sixteen to 24 hours after removal of the treatment media, the high density monolayers were trypsinized and replated for phenotypic expression. The cells were subcultured until the eighth day (at days 4 and [illegible] of the expression period in the first two assays and at day 5 in the third non-activation assay). After the expression time, the cells were trypsinized, counted and seeded ($2x10^5$ cells/each of 5 dishes) in selection media for detection of $HGPRT^-$ mutants, and at 200 cells/each of 5 dishes in media without 6-thioguanine for cloning efficiency determination. The dishes were incubated for about 7 days and then fixed and stained. The mutation frequency was calculated by dividing the total number of mutant colonies by the total number of cells plated, and corrected by the cloning efficiency. The mutation frequency was expressed as mutants/million clonable cells.

For the activation assay, an S9 fraction of rat liver homogenate was added (necessary cofactors also added). Two ml of the S9 mixture was added to 8 ml of serum-free media. During the phenotypic expression period the cells were subcultured on days 1 and 5 and selection of TG-resistant mutants was begun on day 8.

TELONE II was dissolved and diluted in DMSO. The concentrations were tested at 50, 100, 150, 200 and 250 uM in the first two non-activated assays; and at 50, 100, 125, 150 and 200 uM in the third non-activated assay as well as in the S9 activated assay. The concentrations were determined analytically with the observed concentrations being 89-100% of the targeted concentrations.

Table I

Analytical Determination of the Concentration of TELONE II in DMSO Solutions

Targeted Concentration (uM)	(mg/ml)[a]	Observed Concentration[b] (mg/ml)	Percent Observed Target
60	6.16	6.16	100.0
80	8.19	7.99	97.6
100	10.21	9.11	89.2
125	12.79	11.73	91.7
150	15.36	14.46	94.1
200	20.42	18.85	92.3
250	25.58	23.91	93.5
300	30.64	28.53	93.1
350	35.70	32.48	91.0
400	40.85	37.44	91.6

DMSO=Dimethylsulfoxide a=mg TELONE II/ml DMSO
b=Mean of two injections per solution. Samples were analyzed by gas chromatography.
This table was copied from Table I of the report (p. 13).

The negative controls consisted of 0.1% DMSO. Ethyl methanesulfonate (EMS) was diluted in DMSO and used as the positive control at a concentration of 3 mM (370 ug/ml) in the non-activation assay. In the activation assay, the positive control was 3-methylcholanthrene (MCA) at a concentration of 18.6 uM (5 ug/ml).

A copy of the description of materials and methods from the Dow Chemical Company report is appended.

No statistical methodology was described.

A Quality Assurance statement was included.

The reviewer has no comments regarding the description of materials and methods.

III. Results:

TELONE II was tested three times in the non-activated mutagenicity assay. In the first assay, the survival values were approximately 105, 55, 18, 3 and <1% of the negative control at concentrations of 50, 100, 150, 200 and 250 uM of TELONE II, respectively. An apparent mutagenic response was observed only at 200 and 250 uM (Table II). There is questionable biological significance because of the low cell survival rate. EMS (positive control) increased the mutation frequency to about ten times the negative control value (DMSO).

A second non-activated assay was performed, "to determine whether this observation was reproducible." [low survival and a mutagenic response observed in the first assay.] Survival was 122, 37, 12, 11, and 9% of the negative control at concentrations of 50, 100, 150, 200 and 250 uM, respectively (same as in first assay). However, in the second assay, no increase in mutation frequency was observed. The positive control was approximately eleven times the negative control (mutation frequency). See Table II.

The third non-activated assay utilized concentrations of 50, 100, 125, 150 and 200 uM of TELONE II. [The report stated that the highest concentration in the two previous assays (250 uM) was not repeated in this final assay based on the recommendation of the USEPA GENE-TOX Work Group on Specific Gene Mutations in Chinese Hamster Ovary Cells, that survival lower than 10% might prevent an accurate determination of mutation frequency - Hsie, A.W., D.A. Casciano, D.B. Couch, D.F. Krahn, J.P. O'Neill, B.L. Whitfield (1981), The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals: a report of the U.S. EPA's Gene -Tox Program. Mutat. Res., 86:193-214.] There was a decrease in survival to about 70% of the control cultures at 200 uM TELONE II. The report stated that the reason for the lower percent of cell killing in the third assay (70% vs 18 and 11% relative survival in the previous assays) was not clear. Mutation frequencies were similar to the negative control value; the positive control was approximately 45 times the negative control. See Table II.

In the activation assay, TELONE II was evaluated at 50, 100, 125, 150 and 200 uM (Table II). Survival rates were 98, 69, 68, 48 and 14%, respectively. Mutation frequencies were similar to the negative control value at all concentrations; the positive control was approximately 7.6 times the negative control.

Table 2
TELONE II - CHO/HGPRT Results

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	Cytotoxicity Assay				Mutagenicity Assay			
	Cells Seeded for Analysis (per dish)	Colonies per dish avg.	Absol. Clon. Effic. (%)	Rel. Surv. (% of Con.)	Mutant Colonies per dish $2x10^5$	Total Mutant Colon.	Absol Clon Effic. (%) at Time of Mut. Sel.	TG-Resis. Mutants/ 10^6 Clon. Cells
Neg. Con. 0.1% DMSO	100	58	58	100	0-3	6	74	8.1
Pos. Con. 3 mM EMS	100	64	64	110†	70-91	396	46	853.4*
TELONE II 50 uM a	100	61	61	105	0-4	12	75	16.0
100 uM	150	48	32	55	0-4	10	91	11.0
150 uM	200	21	10	18	0-3	10	61	16.5
200 uM	500	8	2	3	3-14	37	52	71.8*
250 uM	500	0	0	<1	2-5	19	43	44.3*
Neg. Con. 0.1% DMSO	1000	229	23	100	2-4	16	61	26.5
Pos. Con. 3 mM EMS	1000	31	3	14	36-78	271	96	282.3*
TELONE II 50 uM b	1000	279	28	122†	1-3	8	73	11.0
100 uM	1500	126	8	37	2-5	17	98	17.3
150 uM	2000	55	3	12	0-3	7	102	6.9
200 uM	5000	127	3	11	2-4	15	102	14.8
250 uM	5000	98	2	9	1-5	13	116	11.2
Neg. Con. 0.1% DMSO	100	72	72	100	0-4	9	69	13.1
Pos. Con. 3 mM EMS	100	75	75	104†	64-77	357	62	580.5*
TELONE II 50 uM c	100	68	68	94	0-3	4	84	4.8
100 uM	100	86	86	119	2-6	21	76	27.8
125 uM	200	153	77	106	0-2	6	73	8.3
150 uM	200	159	80	110	1-4	12	86	14.0
200 uM	600	304	51	70	3-6	20	86	23.2
Neg. Con. 0.1% DMSO	100	89	89	100	0-6	18	95	19.0
Pos. Con. 18.6uM MCA	100	75	75	84	18-30	112	78	142.9*
TELONE II 50 uM d	100	88	88	98	1-4	10	78	12.8
100 uM	100	61	61	69	1-4	11	74	15.0
125 uM	200	120	60	68	2-10	27	91	29.7
150 uM	200	85	43	48	1-4	11	71	15.4
200 uM	600	77	13	14	1-7	14	75	18.7

a=Non-Activation Assay #1 b=Non-Activation Assay #2 c=Non-Activation Assay #3
d=Activation Assay *=Positive Response †=Values greater than 100% can be obtained due to small errors during cell counting, dilution and delivery.
DMSO=Dimethylsulfoxide EMS=Ethyl Methanesulfonate MCA=3-methylcholanthrene
ABBREVIATIONS: Absol. Clon. Effic.=Absolute Cloning Efficiency; Rel. Surv. % of Con.= Relative Survival % of Control; Total Mutant Colon.=Total Mutant Colonies; Absol Clon Effic. (%) at Time of Mut. Sel.=Absolute Cloning Efficiency (%) at Time of Mutant Selection; TG-Resis. Mutants 106 Clon. Cells=TG-Resistant Mutants/106 Clonable Cells
Data extracted from Tables II, III, IV & V of the report (pages 14-17).

IV. Discussion/Conclusions:

First Non-Activation Assay: Increased mutation frequencies were noted at the two highest concentrations (200 and 250 uM); but these levels were too toxic to have any biological relevance. Lower doses have mutant frequencies and absolute total numbers of mutant colonies about twice those of the background, indicating the possibility of activity (still within some historical control values).

Second Non-Activation Assay: This assay is considered to be unacceptable due to a high negative control mutant frequency (26.5 x 10^6). Assays 1 and 3 were 8.1 and 13.1 x 10^6, respectively.

Third Non-Activation Assay: Weak activity is suggested by a doubling of the background count and total mutant colonies at 100 and 200 uM, although 100 uM did not appear to be positive in the first study.

Activated Assay: Concentrations of TELONE II appear to be negative, although the negative control borders on the high end of the acceptable range.

NOTE: There appears to be variable negative controls with unacceptability in the second study. Although there was no reproducible dose response, sporatic increases indicate the possibility of some activity.

V. Recommendation: Supplemental Data.

This study will be considered acceptable provided statistical analyses and historical values are received for review.

Telone

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Pages 66 through 75 are not included.

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____ Identity of product inert ingredients.

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Structure-Activity Relationship in Halogen and Alkyl Substituted Allyl and Allylic Compounds: Correlation of Alkylating and Mutagenic Properties - T. Neudecker, D. Lutz, E. Eder and D. Henschler (Institute of Toxicology, University of Wurzburg, D-8700 Wurzburg, Federal Republic of Germany) - Biochemical Pharmacology, Vol. 29, 1980, 2611-2617.

In a series of allylic chloroolefins and their non-allylic isomers the significance of the allylic structure and the influence of methyl and chlorine substituents on the direct mutagenic activity in Salmonella typhimurium (TA-100) was tested. The direct mutagenic potentials correlate well with the alkylating activities as measured in the nitrobenzyl-pyridine (NBP) test. In contrast to allyl chloride, the vinylic chloroolefins 2-chloro-1-propene and 1-chloro-1-propene did not show any direct mutagenic and alkylating properties. Monomethylated allylic chlorides are six to thirty times more mutagenic: 3-chloro-2-methyl-1-propene <3-chloro-1-butene <1-chloro-2-butene. The non-allylic isomers 2-chloro-2-butene and 4-chloro-1-butene, however, are not directly mutagenic. In spite of a higher alkylating potency, bimethylated allylic chlorides did not show an increase in mutagenicity if compared with monomethylated derivatives: 3-chloro-2-methyl-1-butene <1-chloro-2-methyl-2-butene. 1-Chloro-1-cyclohexene lacks mutagenic and alkylating activity, whereas 3-chloro-1-cyclohexene is comparable to allyl chloride in both respects. Dichloropropenes are also more directly mutagenic than allyl chloride: 2,3-dichloro-1-propene <<trans-<cis-1,3-dichloropropene. Benzyl chloride exerted the highest alkylating activity of all substances tested in this survey, and is about fifty times more mutagenic than allyl chloride. Addition of rat liver S-9 mix was followed by a distinct decrease in the mutagenicity of directly mutagenic substances, the only exception being 2,3-dichloro-1-propene, which demonstrated an increase by a factor of 35. Under the same conditions, vinylic chloroolefins are activated and become mutagenic to various degrees. Only 1-chloro-1-cyclohexene and the homoallylic compound 4-chloro-1-butene are negative both in the presence and absence of S-9 mix.

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Mutagenicity of Pesticides Containing 1,3-Dichloropropene - F. DeLorenzo, S. Degl'Innocenti, A. Ruocco, L. Silengo and R. Cortese (I and II Cattedra di Chimica Biologica, II Facolta di Medicina e Chirurgia, University of Naples, Via Sergio Pansini 5, 80131 Naples, Italy) - Cancer Research, 37 June 1977, 1915-1917.

D.D. soil fumigant (40% 1,3-dichloropropene, 40% 1,2-dichloropropane and 20% other unknown chemicals) and Telone (30% cis-1,3-dichloropropene, 30% trans-1,3-dichloropropene, 20% 1,2-dichloropropane, 5% 2,3-dichloro-1-propene, 2% allyl chloride and about 15% unknown compound) were tested for possible mutagenic activity. Both isomers of 1,3-dichloropropene were mutagenic in Salmonella strains TA 1535 and TA100 as was 2,3-dichloro-1-propene (about 5% of Telone). These compounds were structurally similar to vinyl chloride, a known mutagen and carcinogen.

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Mutagenic Impurities in 1,3-Dichloropropene Preparations - R. Talcott and J. King (Northern California Health Center, University of California at San Francisco and Clinical Toxicology Laboratory, Building 30, 5th Floor, San Francisco General Hospital, San Francisco, Calif. 94110) - JNCI, Vol. 72, No. 5, May 1984, 109-112.

Four preparations of 1,3-Dichloropropene (DCP) were purchased from Pfaltz & Bauer (catalog #D16890, cis-trans) and K&K Laboratories (ICN Pharmaceuticals catalog #9986, #22891 and #22892: cis-trans DCP, low boiling DCP and high boiling DCP) and were assayed for mutagenic activity before and after silicic acid chromatography. The different preparations displayed mutagenic activities ranging from undetectable levels to 15,600 TA100 revertants/mg. After purification by chromatography, none of the four preparations retained mutagenic activity. The yellow color of the preparations was lost and the specific DCP content was increased after column chromatographic separation of the DCP from its polar impurities. The fractions of polar impurities, eluted from the silicic acid columns with methanol, displayed mutagenic activities ranging from 1,900 to 549,000 TA100 revertants/mg. The authors therefore suggested that the reported mutagenic activity of DCP, might actually be caused by its impurities.

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Investigation of the Potential Genotoxicity of CIS(Z)-1,3-Dichloropropene (Z-DCP)-T.M. Brooks, K.R. Huckle, D.H. Hutson, K.L. Lang, C.J. Logan, W.P. Watson and A.S. Wright (Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG, U.K.)- The Toxicologist, 5 (1), 1985, 77.

The direct mutagenic action of Z-DCP against bacteria has previously been ascribed to Z-DCP itself (Creedy, et al., Chem. Biol. Interact., 50:39, 1984). However, the results of microbial mutation studies have confirmed a recent report (Talcott and King, JNCI., 72:1113, 1984) that this activity is due to the presence of a potent mutagenic contaminant(s). The intrinsically reactive mutagen(s) is effectively deactivated by reaction with glutathione. Nevertheless, freshly purified Z-DCP undergoes a bioactivation reaction catalysed by both the S9 fraction and washed microsomes/NADPH from rat liver. The putative bioactivation products, Z-DCP oxide and 2-chloroacrolein, have been synthesized and shown to be effective microbial mutagens. Comparative DNA-binding studies are in progress using bacteria and the rat with a view to determining whether or not the potential genotoxicity of purified preparations of [^{14}C]Z-DCP is realized in vivo.

The Protective Action of Glutathione on the Microbial Mutagenicity of the Z- and E-Isomers of 1,3-Dichloropropene- C.L. Creedy, T.M. Brooks, B.J. Dean, D.H. Hutson and A.S. Wright (Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME 9 8AG, U.K.) - Chem. Biol. Interactions, 50(1984)39-48.

The Z(cis)- and E(trans)-isomers of 1,3-dichloropropene (DCP), in confirmation of previous reports, caused dose-dependent increases in the number of reverse mutations in Salmonella typhimurium TA100 in the presence and absence of S9 from the livers of Aroclor-treated rats. There was a reduction in the microbial mutagenicity of both Z- and E-DCP when the concentration of glutathione (GSH) in the microbial assays was adjusted to a normal physiological concentration (5mM). The protective action of GSH was at least as effective in the absence of S9 as in its presence, suggesting that it was not mediated by mammalian GSH transferase. The results of this study are reported to indicate the efficiency of GSH-linked systems in affording protection against the genotoxic action of the DCP's.

Glutathione Conjugation in the Detoxication of (Z)-1,3-Dichloropropene (a Component of the Nematocide D-D) in the Rat - I.J.G. Climie, D.H. Hutson, B.J. Morrison and G. Stoydin (Shell Research Limited, Shell Toxicology Laboratory (Tunstall), Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, U.K.)-Xenobiotica, vol. 9, no. 3, 1979, 149-156.

The (Z(cis)1,3-Dichloro[2-^{14}C]propene([^{14}C](Z)-DCP when dosed orally to rats gave 82-84% of the radioactivity in the urine 24 hours after treatment. Ninety-two percent of the ^{14}C was present as N-acetyl-S-((Z)-3 chloroprop-2-enyl)cysteine((Z)-DCP mercapturic acid). This was attributed to an efficient glutathione-dependent biotransformation. The authors indicated that the results of the in vivo study point to the existance of a highly effective detoxification mechanism which was felt likely to afford protection against a direct mutagenic action.

When (Z)-DCP was incubated with glutathione and rat liver cytosol (containing glutathione s-alkyl transferase), very rapid loss of (Z)-DCP was observed. The product of this reaction was S-[(Z)-3-chloroprop-2-enyl)] glutathione. It was concluded that the glutathione-dependent detoxification of the (E)-isomer (trans) was 4-5 fold less rapid than that of the (Z) isomer.

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The Excretion and Retention of Components of the Soil Fumigant D-D and Their Metabolites in the Rat - D. Hutson, J. Moss and B. Pickering (Shell Research Ltd., Broad Oak Road, Sittingbourne, Kent, England)- Fd Cosmet. Toxicol., Vol. 9, 1971, 677-680.

The major components of the soil fumigant D-D are: 1,2-dichloropropane (30%), cis-1,3-dichloropropene (27.7%) and trans-1,3-dichloropropene (27.4%). The labelled components were: 1,2-dichloro[1-^{14}C]propane (0.88 mg, 8.5 uC) and cis- and trans-1,3-dichloro-[2-^{14}C]propenes (2.53 mg, 7.68 uC and 2.70 mg, 8.50 uC, respectively). Rats were dosed by stomach intubation For all three compounds, 80-90% of the radioactivity administered was eliminated in the feces, urine and expired air during the first 24 hours. The urine was the major route of excretion: 1,2-dichloropropane 50.2%, cis- 1,3-dichloropropene 80.7% and trans-1,3-dichloropropene 80.5% (all during the first 24 hours). After four days, carcass and skin had relatively little radioactivity (3.2% for 1,2-dichloropropane, 1.2% for cis- and 1.5% for trans-1,3-dichloropene).

Urinary Excretion of the N-Acetyl Cysteine Conjugate of Cis-1,3-Dichloropropene by Exposed Individuals - J. Osterloh [a], B. Cohen[b], W. Popendorf[c] and S. Ponda [a] ([a]Northern California Occupational Health Center and the Medical Service San Francisco General Hospital Medical Center and Department of Medicine, University of California, San Francisco, CA; [b]Department of Industrial Hygiene, School of Public Health, University of California, Berkeley, CA; and [c]Institute of Agricultural Medicine, Department of Preventive Medicine and Environmental Health, University of Iowa, Iowa City, IA) - Archives of Environmental Health, Vol. 39, No. 4, July/August 1984, 271-275.

The purpose of the study was to determine whether exposure of individuals to 1,3-dichloropropene (DCP) in the workplace led to systemic absorption and metabolism of DCP. During three separate 8-hour field applications of DCP (Telone II), 24-hour urine collections were obtained from five men: two were commercial applicators; one investigator at one site; and two collections from a second investigator at two sites. The applicators wore no special protective clothing or devices (no mention regarding investigators). All applicators and investigators excreted detectable quantities of the N-acetyl cysteine conjugate of cis-1,3-dichloropropene.

Fate of [^{14}C] Vinyl Chloride after Single Oral Administration in Rats - P. Watanabe, G. McGowan and P. Gehring (Toxicology Research Laboratory and Analytical Laboratory, The Dow Chemical Company, Midland, MI 48640)-Toxicology and Applied Pharmacology, 36, 1976, 339-352.

Male rats were given single oral doses of 0.05, 1, and 100 mg/kg of [^{14}C] vinyl chloride (VC), and the routes and rates of elimination of ^{14}C activity followed for 72 hr. Following 0.05 and 1 mg/kg, excretion in the urine as nonvolatile metabolites and as $^{14}CO_2$ in expired air accounted for 59-68% and 9-13%, respectively of the administered dose. Only 1-2% of the dose was expired by the lungs as VC. Conversely, after 100 mg/kg, 67% of the dose was eliminated by the lungs as VC, while urinary nonvolatile metabolites and $^{14}CO_2$ comprised 11 and 3%, respectively. Pulmonary elimination after 100 mg/kg showed an apparent biphasic clearance with half-times ($t_{1/2}$) of 14.4 and 40.8 min for the respective fast and slow phases. Following 0.05 and 1 mg/kg the pulmonary clearance of VC was monophasic with $t_{1/2}$ of 53.3 and 57.8 min. The percentage of the dose remaining in the carcass after 72 hr was 10, 11, and 2% for the 0.05-, 1- and 100-mg/kg doses, respectively. The urinary radioactivity was separated by high pressure liquid chromatography into three major metabolites. Two of the three major urinary metabolites have been identified as N-acetyl-S-(2-hydroxyethyl)-cysteine and thiodiglycolic acid by gas chromatography-mass spectrometry. The proportions of the urinary metabolites were not influenced by the dose. The fate of VC following an oral dose between 1 and 100 mg/kg was dose-dependent. Consistent with the authors' previous studies on the fate of VC following inhalation exposure in rats, the metabolism of VC appears to be a saturable process.

Fate of [^{14}C] Vinyl Chloride following Inhalation Exposure in Rats - P. Watanabe, G. McGowan, E. Madrid and P. Gehring (Toxicology Research Laboratory, Health and Environmental Research and Analytical Laboratory, Dow Chemical U.S.A., Midland, MI 48640) - Toxicology and Applied Pharmacology, 37, 1976, 49-59.

Inhalation exposure to vinyl chloride (VC) has been shown to be carcinogenic in rats and man. It is important in assessing the toxicological potential of inhaled VC to understand the disposition of VC in the body. Therefore, the objective of the present study was to determine the fate of inhaled [^{14}C]VC at different exposure concentrations in rats. Male rats were exposed to 10 or 1000 ppm [^{14}C]VC for 6 hr and the routes and rates of elimination of ^{14}C activity were followed for 72 hr after termination of exposure. Following exposure to 10 ppm of VC, urinary ^{14}C activity and expired VC comprised 68 and 2%, respectively, of the recovered radioactivity. After exposure to 1000 ppm of VC, the proportion of the radioactivty in the urine decreased while that expired as VC increased representing 56 and 12%, respectively. The pattern of pulmonary elimination of VC per se was described by similar apparent first-order kinetics following 10 or 1000 ppm with respective half-lives of 20.4 and 22.4 min. The elimination of ^{14}C activity in the urine occurred in accordance with a two-exponential equation; the half-lives for the initial phase of excretion were 4.6 and 4.1 hr following 10 and 1000 ppm, respectively. The percent of the recovered ^{14}C activity remaining in the carcass after 72 hr was 14 and 15% at the respective low and high exposure level. VC per se was not found in tissues. The urinary ^{14}C activity was separated by high pressure liquid chromatography into three major metabolites corresponding to N-acetyl-S-(2-hydroxyethyl)cysteine, thiodiglycolic acid, and a third unidentified metabolite. The proportions of the urinary metabolites were not markedly influenced by the exposure magnitude. The fate of inhaled [^{14}C]VC was shown to be dose-dependent; this is consistent with previous studies on the fate of VC following ingestion as well as inhalation.

Hepatic Macromolecular Binding Following Exposure to Vinyl Chloride - P.G. Watanabe, J.A. Zempel, D.G. Pegg and P.J. Gehring (Toxicology Research Laboratory, Health and Environmental Research, 1803 Building, The Dow Chemical Company, Midland, MI 48640)- Toxicology and Applied Pharmacology, 44, 1978, 571-579.

Covalent binding of radioactivity to hepatic macromolecules in rats exposed to ^{14}C-labeled vinyl chloride (VC) was studied to determine if VC-induced carcinogenesis may be related to electrophilic alkylation of macromolecules in vivo. Male Sprague-Dawley rats were exposed to 1, 10, 25, 50, 100, 200, 500, 1000, or 5000 ppm of [^{14}C]VC for 6 hr. Following exposure, radioactivity covalently bound to hepatic macromolecules and purified nucleic acids (RNA, DNA) was determined. The total amount of [^{14}C]VC metabolized and hepatic glutathione (GSH) content were also determined. The total amount of radioactivity bound to macromolecules in the liver did not increase proportionately to the increase in the exposure concentration of VC. A disproportionate decrease in macromolecular binding was observed as the concentration of VC increased. The covalent binding to hepatic macromolecules was related to the amount of VC metabolized. At exposures greater than 50 ppm, the amount of ^{14}C bound to macromolecules in the liver correlates with induction of hepatic angiosarcoma. There was no detectable binding of radioactivity to either DNA or RNA in the liver. Hepatic glutathione content was significantly depressed only at exposure concentrations greater than 100 ppm.

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Inhalation Pharmacokinetics of Cis- and Trans- 1,3-Dichloropropene in Rats Exposed to TELONE* II Vapors. W.T. Stott, P.E. Kastl and M.J. McKenna (Mammalian and Environmental Toxicology Research Laboratory, Dow Chemical Company, Midland, MI 48640)- The Toxicologist, 5, No. 1, 1985, 110.

The absorption of 1,3-dichloropropene (DCP), DCP effects upon respiratory physiology, and blood concentrations of DCP were determined in rats exposed to 30, 90, 300 or 900 ppm TELONE II vapors (91% DCP) for 3 hrs. The absorption of DCP by rats was not observed to increase proportionately with increasing exposure level. An observed 40-50% depression in the RMV of rats exposed to 300 or 900 ppm TELONE II was partially responsible for this finding. Steady-state blood levels of cis-DCP attained in exposed rats were 20% lower than levels of trans-DCP even though the cis:trans isomer ratio in the TELONE II used was 1:1.2. Post-exposure elimination curves for both isomers displayed an initial rapid and dose-dependent elimination phase (2-3 min half-life) followed by a slower elimination phase (38-44 min half-life). The DCP dosages calculated from the areas under these blood concentration curves (AUC) were roughly proportional to all but the high exposure levels. However DCP dosages calculated from AUC were 8-13x lower than those determined by vapor uptake measurements, suggesting a significant portal-of-entry effect. Overall, these data demonstrate that a complex interaction of DCP-induced changes in respiratory physiology and isomer-specific, saturable, elimination mechanism(s) determine the ultimate body burden of DCP in rats exposed to TELONE II vapors.

[This article was an abstract only and the above is a copy.]

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 1,3-Dichloropropene (1,3-D): Comments on Mutagenicity

FROM: Kerry L. Dearfield, Ph.D.
Geneticist
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Alan Levy, Ph.D.
Section V
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Reto Engler, Ph.D.
Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

I. Introduction

I have recently been asked by Dr. Levy to perform a secondary review of mutagenicity studies submitted to OPP concerning 1,3-D. This has been completed and returned to Dr. Levy. In addition to these studies was a letter from Dow Chemical in which they offered comments and accompanying information to address some of the Agency's concerns regarding 1,3-D. This memo will in turn comment and present information addressing the matters concerning mutagenicity that Dow has presented in the letter.

II. Outline of Issues Presented by Dow

1. No convincing evidence is available that unequivocally identifies 1,3-D as a mutagen. Dow suggests that the bacterial mutagenic activity of 1,3-D may be due to impurities and/or oxidative degradation product(s).

2. Even if 1,3-D is intrinsically mutagenic, Dow suggests that reaction with glutathione in mammalian systems would provide a level of protection against 1,3-D inherent activity.

3. Although structural similarity exists between 1,3-D and

vinyl chloride, vinyl chloride may not be a relevant analogue to 1,3-D based on mechanism(s) of action and excretory pathways.

The remainder of this memo will address these issues and present further relevant information. An overall review will follow the responses section.

III. Responses

1. Inherent genotoxicity of 1,3-dichloropropene

Recently, a series of studies has been submitted to OPP. The results are briefly: a. Positive in the Salmonella assay in strains G46, TA98, TA100 and TA1535 ± activation and in strains TA1538 and TA1537 with activation. Responses up to approximately 100X and 10X background in strains TA1535 and TA100, respectively, were seen. b. Positive in the B. subtilis rec-assay at one concentration (25 mg/ml). c. Negative in the E. coli reversion test ± activation up to a toxic concentration of 1000 ug/plate. d. Negative in a host-mediated assay with Salmonella exposed to compound in host mice. However, dosing of the mice up to 60 mg/kg may not be high enough (adequate toxicity not reported). e. Negative in an unscheduled DNA synthesis (UDS) assay with primary rat hepatocytes. f. Very slight increases (just at 2X background) at unique concentrations were seen in the Chinese hamster ovary (CHO)/HPRT gene mutation assay. However, no reproducible dose responses were noted.

It is clear from these submitted data that the 1,3-D preparations used in these assays were bacterial mutagens. Little evidence for an effect in mammalian cells is found based on these submitted studies.

The National Toxicology Program (NTP) has sponsored many studies which have included 1,3-D as one of the test compounds. These are summarized in Tennant et al. (1987). Briefly, 1,3-D is positive in the Salmonella assay ± activation, the mouse lymphoma assay without activation, and the sister chromatid exchange (SCE) assay in V79 cultured cells ± activation. 1,3-D is negative for aberration induction in V79 cells. Valencia et al. (1985) demonstrated that 1,3-D induced sex-linked recessive lethal mutations in Drosophila after feeding exposure (NTP sponsored study). The SCE activity is confirmed by von der Hude et al. (1987) in cultured V79 cells without activation; little activity with activation was found.

It is clear from these additional data that the tested 1,3-D preparations not only produce mutations in bacteria, but also exert effects in mammalian cells in culture. The Drosophila data demonstrate that 1,3-D has an in vivo effect in an eukaryote as well as effects on germ cells.

2. Bacterial studies: activity by 1,3-D or impurities

Many studies using the Salmonella/mammalian activation assay have demonstrated that 1,3-D has induced large increases in

revertant frequencies (e.g. see DeLorenzo et al., 1977; Neudecker et al., 1980; Haworth et al., 1983; Creedy et al., 1984). However, Talcott and King (1984) suggest that the responses seen in many studies are variable and may be due to the presence of various amounts of impurities. After removing polar impurities by silicic acid column chromatography, none of the 1,3-D preparations they examined exhibited mutagenic activity in Salmonella strain TA100 without activation. The polar impurities were assayed and proved mutagenic in Salmonella; these were tentatively identified as epichlorohydrin and 1,3-dichloro-2-propanol, among others. After purified 1,3-D was refluxed, mutagenic activity was regenerated. Talcott and King suggest that 1,3-D autooxidizes easily to mutagenic products (not identified).

Dow states that these authors and Brooks et al. (1985) showed that gas chromatographic purification procedures utilized by the other researchers above are able to catalyze mutagenic activity. An examination of these papers do not appeqr to support this statement. They do not specifically address whether the mode of purification is able to catalyze mutagenic activity. Talcott and King did use a different purification process and reported no activity. It appears that freshly purified 1,3-D may not be mutagenic in their hands, but 1,3-D that may not be freshly purified has mutagenic activity.

Neudecker et al. (1980) purified their 1,3-D sample by distillation and preparative gas liquid chromatography to a reported 100% purity as confirmed by NMR. If the sample was 100% 1,3-D, then impurities would not have much impact on the subsequent mutagenic activity seen by these researchers in the Salmonella assay. However, Dow implies that epichlorohydrin as a contaminant could contribute to the mutagenic response by 1,3-D preparations. A comparison of results by epichlorohydrin and 1,3-D (as purified by Neudecker et al., 1980) can be made. Epichlorohydrin was tested by the same research group (Eder et al., 1980) under the same conditions in Salmonella TA100 as for 1,3-D (Neudecker et al., 1980). Epichlorohydrin induced 275 revertants/umole without activation and 70 revertants/umole with activation. cis-1,3-D induced 1075 revertants/umole without activation and 450 revertants/umole with activation. trans-1,3-D induced 700 revertants/umole without activation and 340 revertants/umole with activation. It appears that even if epichlorohydrin did contribute some activity to a 1,3-D preparation, it would be very small; especially if it is only a few percent of a 1,3-D preparation. Epichlorohydrin by itself did not appear to induce as large a response as 1,3-D on an equimolar basis.

Neudecker and Henschler (1986) purified 1,3-D by distillation in vacuo to >99.8% pure and tested it in Salmonella TA100 with activation. The different preparations (cis and trans) were tested by liquid suspension incubation for 20 min and 120 min with different concentrations of protein S9 mix. Mutagenic activity was seen with 20 min incubation, but was substantially increased with 120 min incubation. The activity by cis isomer was much greater than by trans isomer. This suggests

that some mutagenic activity by 1,3-D may be generated by S9 mix.

3. Effect of glutathione on bacterial mutagenicity

The addition of glutathione to bacterial mutagenicity assays appears to significantly reduce the mutagenic activity of the tested 1,3-D preparations (Creedy et al., 1984; Neudecker and Henschler, 1986). Creedy et al. (1984) examined the possible effects of glutathione in the Salmonella assay with strain TA100. They saw significant increases in mutation frequency with both isomers of 1,3-D ± activation (cis more active). Glutathione (GSH) added at 5 mM to the incubation eliminated activity for the trans isomer and a good portion of the activity for the cis isomer. This GSH concentration was stated by the authors to be a physiologically relevant concentration. Regardless, there was still a significant increase in mutant frequency (100 - 200 revertants over background, 32 - 71) induced by the cis isomer ± activation at higher concentrations. These results suggest that GSH can provide some "protection", but S9 activates some cis isomer to a form that is not effectively deactivated by GSH.

Creedy et al. (1984) showed that while Salmonella has similar levels of GSH as mammalian cells, Salmonella does not have measurable levels of GSH alkyl or aryl transferases. From in vivo studies with rodents, it appears that cis-1,3-D is biotransformed by glutathione transferases to catalyze conjugation with glutathione; these products are readily excreted in the urine (Climie et al., 1979). It appears then that the effect of GSH on bacterial mutagenicity is a direct acting mechanism in Salmonella and not enzyme mediated.

4. Analogy to vinyl chloride

1,3-D bears a structural similarity to vinyl chloride (see figures, section V). Dow suggests that the metabolic and excretory pathways of these two compounds are different and vinyl chloride would not be an adequate analogue. It is currently believed that vinyl chloride is metabolized via an epoxide moiety and that this is its mechanism for toxicity. The submitted reports do not actually support this argument (although other studies would be more appropriate). 1,3-D, however, does not appear to be metabolized via an epoxide. Neudecker et al. (1980) suggest that epoxidation is not a prerequisite for mutagenic activity and follow up this suggestion in Neudecker and Henschler (1986). By using inhibitors of various metabolic activities, they demonstrate that SKF 525 (an inhibitor of microsomal oxygenase) and 1,1,1-trichloropene-2,3-oxide (TCPO; inhibitor of epoxide hydrolase) do not have an effect on the mutagenicity of 1,3-D in Salmonella strain TA100 with activation. However, cyanamide (an inhibitor of aldehyde dehydrogenase) enhances the mutagenic activity of 1,3-D; presumably by the accumulation of mutagenic aldehydes (chloro- derivative of acrolein) due to inhibition of aldehyde dehydrogenase. This suggests that under activated conditions in the Salmonella assay, 1,3-D is metabolized by the hydrolytic-oxidative pathway via allylic

alcohols and aldehydes. Therefore, epoxidation of 1,3-D does not appear to be responsible for its toxicity, as is suggested for vinyl chloride.

Although Dow suggests that vinyl chloride and 1,3-D have different excretion pathways, the reports they submitted indicate they may be similar. Both compounds conjugate in vivo with glutathione (see above for 1,3-D; for vinyl chloride, see Watanabe et al., 1978) and are excreted in the urine as cysteine conjugates (1,3-D: N-acetyl-S-(Z)-3-chloroprop-2-cysteine (Climie et al., 1979); vinyl chloride: N-acetyl-S-(2-hydroxyethyl)-cysteine (Watanabe et al., 1976)).

IV. Overall Observations

1. It appears that 1,3-D preparations have genotoxic activity, both in bacterial systems and mammalian cells. Furthermore, the Drosophila study indicates an effect in vivo in an eukaryote as well as germ cell interaction. It initially appears from the bacterial studies and the mammalian cultured cell assays that 1,3-D acts via a direct acting mechanism. However, the later Neudecker and Henschler (1980) study indicates that 1,3-D mutagenic activity can be enhanced with longer term incubation with S9 mix. They indicate that the hydrolytic-oxidative pathway via aldehydes and alcohols may be relevant to 1,3-D mutagenicity. So whether 1,3-D is mutagenic directly or indirectly, or both, it appears that there is a concern for the mutagenicity of 1,3-D.

The role of impurities may not play as large a role as Dow suggests (see comparison of epichlorohydrin activity to 1,3-D activity above). It may also be relevant that Talcott and King (1984) demonstrate that 1,3-D readily autooxidizes and this process appears to provide mutagenic activity. Also Brooks et al. (1985) states that one of the detected bioactivation products of freshly purified cis-1,3-D is a chloroacrolein product. This would be consistent with the findings by Neudecker and Henschler for their proposed hydrolytic-oxidative pathway. Therefore, it may be that 1,3-D preparations have some inherent mutagenic activity, but over a period of time, e.g. storage of sample (i.e. autooxidation) or by hydrolytic-oxidative mechanisms, more mutagenic activity becomes apparent.

The result by Talcott and King (1984) is only one full study that demonstrates no mutagenic activity of freshly purified 1,3-D in the face of many other studies. While their technique appears different from the other purification techniques used, this idea of mode of purification should be examined further before casting aside the majority of studies that indicate mutagenicity. The study by Talcott and King itself may be unique.

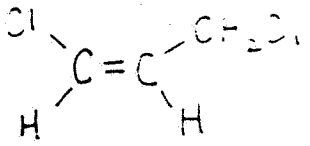
It appears that the Agency should be concerned with the potential mutagenic activity of 1,3-D. While in some cases freshly purified samples may not have mutagenic activity, it appears that these samples regain such activity over time and that metabolic processes are also able to produce mutagenic products. There is no convincing evidence available to suggest that 1,3-D does not have mutagenic activity.

2. Glutathione (GSH) does appear to play a role in the metabolism of 1,3-D. GSH reacts with 1,3-D both in vivo and in vitro and appears to reduce the toxicity of 1,3-D. However, it is important to note that it does not completely eliminate the mutagenicity of 1,3-D (Craedy et al., 1984). Therefore, while GSH may reduce the effect of 1,3-D, it does not appear to provide complete protection from the genotoxic effects of 1,3-D. A similar circumstance can be seen with acrylamide. Acrylamide readily reacts with GSH in in vivo mammalian systems, yet still has a spectrum of toxic effects, including neurotoxicity, cancer and genotoxicity.

3. Although there is structural similarity between 1,3-D and vinyl chloride, they do appear to have different metabolic spectra. However, Dow does not really present any specific information regarding the mechanism of epoxide formation for vinyl chloride and should do so when making such an argument. The proposed differences in excretory pathways between 1,3-D and vinyl chloride does not seem as clear. Both react with glutathione and the major conjugate products are excreted in the urine as cysteine derivatives. Dow should present more information if they feel there is a major difference in excretory pathways for the two compounds.

4. Overall, based on the available evidence, 1,3-D presents a mutagenicity concern, based on bacterial, Drosophila and mammalian cell studies. In addition, there appears to be a concern for germ cell effects that may need to be pursued based on the Drosophila results. While these data do not suggest that 1,3-D is carcinogenic, they do provide support for a carcinogenicity concern in the weight-of-the-evidence evaluation for carcinogenicity. There is a published report of 1,3-D carcinogenicity evidence in exposed mice (Van Duuren et al., 1979).

V. Figures



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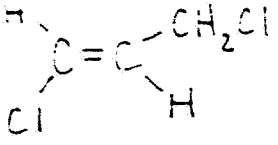

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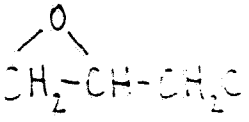

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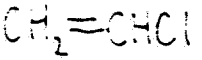

vinyl chloride

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cc: Quang Bui, Ph.D.
Section Chief, Section V
Toxicology Branch