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

FILE

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

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

JAN 11 1985

MEMORANDUM

SUBJECT: Review of metabolism, inhalation, mutagenicity, and reproduction studies with 1,3-Dichloropropene
Accession No. 235250, 235251 Caswell No. 324, 324 A
#201-119

TO: Henry Jacoby, PM # 21
Registration Division (TS 767)

FROM: Quang Q. Bui, Ph.D.
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THRU: Laurence D. Chitlik, DABT
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THRU: Theodore Farber, Ph.D.
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Hazard Evaluation Division (TS 769C)

Registrant:
Shell Oil Company
Washington D.C. 20036

Action Requested:

Review of metabolism, acute inhalation, mutagenicity, subchronic inhalation, and three generation reproduction studies with 1,3-dichloropropene and D-D® vapor.

Background:

Telone II is a fumigant with the following formulation:

cis-1,3-Dichloropropene	45.7%
trans-1,3-Dichloropropene	46.5%
[redacted]	

MANUFACTURING PROCESS INFORMATION HAS BEEN REMOVED

Telone II was referred to SPRD in 1977 as Pre-RPAR. From the preliminary NTP reports, positive oncogenic findings were found in both rats and mice (see memos of J. Seifter dated 7/16/80; G. Burin dated 8/14/80 and 11/27/81; and W. Teeters dated 9/24/84) dosed with Telone II. Dated 1/8/80, Mr. Burin also indicated that Telone II showed a positive mutagenic response in B. Substilis (rec assay) and E. Coli (reversion assay).

In this action, additional toxicology data was submitted and consisted of:

Accession 235250:

Metabolism study, rat
Acute inhalation study, rat
Mutagenicity studies
12-week inhalation, rat and mouse

Accession 235521:

3 generation reproduction, rat

The rat 3-generation reproduction study (IBT #621-06002, 3/2/79) was previously reviewed by EPA on 3/26/82 and classified as Invalid Data and, hence, was not reviewed in this memo.

RECOMMENDATION:

In this action, different test materials were investigated. (Z)-1,3-dichloropropene was used in both the metabolism and acute inhalation studies. 1,2,3- Trichloropropane, which is not a component of Telone II formulation, was assayed in all three mutagenicity studies whereas D-D® was tested in two 12-week inhalation studies. D-D ® is a mixture of (Z)-1,3-dichloropropene, (E)-1,3-dichloropropene, and 1,2-dichloropropane.

1. Metabolism study, rat: Tunstall Lab. #TLGR.0101.78, 6/78

Chemical tested: (Z)-1,3-Dichloropropene
Classification: Supplementary Data

This study was designed to identify the urinary metabolites of (Z)-1,3-dichloropropene in 2 female rats and included an in-vitro study using rat liver cytosol. However, this study did not meet the requirements of a metabolism study since determination and measurement of the test substance in different target organs were not investigated and, hence, cannot be used to meet regulatory requirements.

2. Acute inhalation, rat: Tunstall Lab. #TLTR.0002.77, 3/77

Chemical tested: 1,3-Dichloropropene
Classification: Supplementary Data

Supporting data for the calculation of the chamber concentratio (actual and nominal), body weights, and necropsy findings were not submitted.

The LC50 was reported as 729 ppm/4 hours (males and females)

3. Mutagenicity, microorganisms: Tunstall Lab. #TLGR.79.073, 6/79

Chemical tested: 1,2,3-Trichloropropane
(not a component of Telone II formulation)
Classification: Acceptable

Positive mutagenic response in Salmonella TA-1535 in the absence and presence of metabolic activation. Positive mutagenic response in Salmonella TA-100, TA-1537, TA-98 and E. Coli WP2 uvrA in the presence of metabolic activation.
The registrant is requested to provide clarification regarding:

a. The lack of response observed in strain Salmonella TA-100 in the absence of the S-9 fraction although both strains TA-100 and TA-1535 are derived from the same parental strain and both were used to detect base-pair substitution.

b. The low background spontaneous rate of strains TA-100 and TA-1535 in comparison to acceptable spontaneous ranges (see "Discussion" section on page 13).

c. The lack of response of the positive control selected, BP, in strain TA-100 in the absence of metabolic activation.

d. Techniques used to verify the functional integrity of the strains selected.

e. The purity of the test material.

4. Mutagenicity, Saccharomyces: Tunstall Lab. #TLGR 79.073, 6/79

Chemical tested: 1,2,3-Trichloropropane
(not a component of Telone II formulation)
Classification: Acceptable

Under the conditions of this study, 1,2,3-TCP is a positive mutagen in Saccharomyces cerevisiae JD1. However, the registrant is requested to provide clarification regarding the purity of the test chemical.

5. Mutagenicity, chromosome: Tunstall Lab. #TLGR 79.073, 6/79

Chemical tested: 1,2,3-Trichloropropane
(not a component of Telone II formulation)
Classification: Inconclusive

The mutagenic potential of 1,2,3-TCP cannot be assessed with certainty. The purity of the test chemical was not indicated and sister chromatid exchanges were found at two different dose levels in one experiment but were absent in a subsequent study. To fully assess the mutagenic potential of 1,2,3-TCP in chromosome, a new study at higher dosage levels is recommended.

6. 12-week inhalation, mouse: Hazleton #776-132, 5/20/79

Chemical tested: D-D® soil fumigant, vapor
Classification: Supplementary Data

A systemic NOEL cannot be demonstrated from the dosage levels selected (5, 15, and 50 ppm/6 hours/day). Significant increases in absolute and relative liver weights were noted at 5 ppm (lowest dose used). Clinical measurements of Ca^{++}, K^{+}, total bilirubin, total protein, total cholesterol, SGOT, and food consumption were not determined.

D-D® is a mixture of (Z)-1,3-dichloropropene, (E)-1,3-dichloropropene, and 1,2-dichloropropane. However, the purity and the concentration of each constituent in the test material were not indicated.

7. 12-week inhalation, rat: Hazleton #776-132, 5/20/79

Chemical tested: D-D® soil fumigant, vapor
Classification: Supplementary Data

Under the conditions of this study, a systemic NOEL was demonstrated at 15 ppm with increased liver weight and kidney weight observed at 50 ppm (highest dose tested). Clinical measurements of Ca^{++}, K^{+}, total bilirubin, total protein, total cholesterol, SGOT, and food consumption were not determined.

D-D® is a mixture of (Z)-1,3-dichloropropene, (E)-1,3-dichloropropene, and 1,2-dichloropropane. However, the purity and the concentration of each constituent in the test material were not indicated.

DATA REVIEW:

STUDY I

Study Title: Metabolism studies on (Z)-1,3-dichloropropene in the rat.
Testing Facility: Tunstall Lab. (England)
Report No.: TLGR. 0101.78
Report Date: 6/78

I. Procedures

A copy of the methods used is appended.

The procedures employed are adequate to identify and quantify the metabolites recovered in the urine. However, this study cannot satisfy the requirements for a metabolism study since determination and measurement of the test substance in different targets organs were not undertaken. Furthermore, the following comments are noted:

a) Only a single dose was used. At least two dose levels should be tested.

b) Only two female rats were treated. At least five animals per sex per test group should be tested.

c) Age and body weight of the test animals were not indicated.

II. Results

1. In-vivo Study:

Thin layer chromatography followed by radioscanning and autoradiography techniques indicated the presence of 3 metabolites in the urine.

U1 = 92% U2 = 3% U3 = 5%

The main metabolite, U1, was isolated as its methyl ester and identified as methyl-N-acetyl-S-[(Z)-3-chloroprop-2-enyl] cysteine. This metabolite, hence, is a derivative of mercapturic acid. The structure of this metabolite was further confirmed as a derivative of mercapturic acid by nuclear magnetic resonance spectroscopy.

The other metabolites, U2 and U3, were present in small amounts in comparison with U1 and chromatographed poorly.

2. In-vitro Study:

One conjugate was obtained from the incubations of ^{14}C (Z)-DCP with glutathione and rat liver cytosol. This conjugate was identified as S-[(Z)-3-chloroprop-2-enyl) glutathione.

III. Discussion and Conclusions

From the submitted data, it is suggested that (Z)-DCP was biotransformed by a glutathione-dependent system in rats. The enzyme glutathione transferase may be involved in this system to form an intermediate conjugate. This intermediate glutathione conjugate then undergoes mercapturic acid biotransformation to yield the main polar metabolite U1, N-acetyl-S-[(Z)-3-chloroprop-2enyl] cysteine.

The involvement of the glutathione system was further confirmed by the identification of S-[(Z)-3-chloroprop-2-enyl] glutathione from the in-vitro incubation studies.

In the rat, the formation of polar metabolites through the glutathione and mercapturic acid pathways thus appears as detoxifying mechanisms for (Z)-DCP.

IV. Core Classification:

This study is classified as Core Supplementary Data. The procedures used were adequate to identify the metabolites from both in-vitro and in-vivo studies. However, the study protocol was not designed to meet the requirements of a metabolism study since determination of the test material at different target organs was not undertaken. This study does not fulfill regulatory requirements for a metabolism study.

STUDY II

Study Title: Acute inhalation toxicity of 1,3-dichloropropene in the rat.
Testing Facility: Tunstall Lab. (England)
Final Report No.: TLTR. 0002.77
Report Date: 3/77

I. Procedures

The test material used in this study consisted of:

1,3-dichloropropene, E isomer (cis) : 51.0% (m/m)
1,3-dichloropropene, Z isomer (trans): 43.4% (m/m)
epichlorhydrin: 1.0% (m/m)

Groups of 5 Wistar rats per sex each were exposed to a mixture of 1,3-dichloropropene/air at 454, 647, 699, 762, 832, or 958 ppm for 4 hours. All animals were observed daily for clinical signs and mortality for 14 days post-treatment. Food and water were available at all times except for the exposure period.

II. Results

Piloerection, lacrymation, nasal discharge, diarrhea, and peripheral vascular dilation were the clinical signs reported. The mortality rates were as follows : 0, 20, 30, 50, 100, and 100% for the groups receiving 454, 647, 699, 762, 832, and 958 ppm, respectively.

III. Discussion and Conclusions

The LD50 was calculated to be 729 ppm/4 hours (95% CL= 690-766 ppm) for both males and females.

There were no individual clinical observation and body weight data. Necropsy apparently was not performed by study termination.

IV. Core Classification: Supplementary Data

Supporting data for the calculation of the chamber concentrations (nominal and actual), body weights, and necropsy findings were not submitted.

STUDY III

Study Title: In-vitro mutation studies with 1,2,3-trichloropropane (1,2,3-TCP).
Testing Facility: Tunstall Lab. (England)
Final Report No.: TLGR 79.073
Report Date: 6/79

I. Procedures

The mutagenic potential of 1,2,3-TCP (of unknown purity and not a component of Telone II formulation) was investigated in two systems described below:

A. Gene Mutation

In this system, Salmonella Typhimurium TA 98, TA 100, TA 1535, TA 1537, and TA 1538, Escherischia Coli WP2 and WP2 uvr A, and Saccharomyces Cerevisiae JD1, were the microorganisms used.

1. Bacterial Mutation

1,2,3-TCP was diluted into DMSO and 20 ul of this solution was mixed with 2 ml of molten top agar and 0.1 ml of an overnight bacterial culture (nutrient broth) in an overlay tube. For activation studies, 0.5 ml of the S-9 mix collected from liver cells of male Wistar ICI rats treated with Arochlor 1254 was added to each overlay tube.

The concentrations of 1,2,3-TCP used were 0, 0.2, 2.0, 20, 200, and 2000 ug per plate both in the presence and absence of the S-9 mix. All plates were incubated at 37°C for 48 hours and then scored for the number of revertants per plate.

3,4-benzo(a)pyrene (BP), 4-nitroquinoline oxide (NQO), and ethyl-methanesulphonate (EMS) served as positive control agents for both activation and non-activation assays.

2. Saccharomyces Gene Conversion Assay

20 ul of the test material (1,2,3-TCP in DMSO) or solvent (DMSO) were added to 2 ml aliquots of the cell suspension (2.5×10^7 cells/ml). The cell suspensions were then exposed at room temperature for 1 hour without the S-9 mix (experiments # 1 and 3) or for 1 hour (experiments # 2b and 4b) and 4 hours (experiments # 2a and 4a) with 0.5 ml of the S-9 mix in a shaking water bath at 37°C. 0.1 ml of each sample was then seeded in appropriate culture medium. The number of mutant colonies were counted after 4 days incubation at 28°C and the

the number of prototrophes per 10^6 survivors were determined. The concentrations of 1,2,3-TCP used were 0, 0.01, 0.1, 0.5, 1.0, and 5.0 mg/ml. EMS and cyclophosphamide (CP) served as positive control agents for non-activation and activation assays, respectively.

B. Chromosomal Mutation Assays.

Slide cultures were prepared by adding 2 x 10^5 rat liver cells (collected from 1 Catworth Farm E rat) in 0.5 ml of culture medium onto microscopic slides. After 24 hours incubation for commencement of active growth in appropriate medium, 1,2,3-TCP was added at 0, 250, 500, or 1000 ug/ml. After a further 22 hours incubation, colcemid (0.3 ug/ml) was added to each culture and the slides were processed for chromosomal analysis according to routine chromosomal mutation assay techniques. 100 cells from each of 3 slides per dose level were analyzed microscopically for chromosomal changes.

II. Results

1. Bacterial Mutation Without Activation

Positive mutagenic activity was noted only in strain TA-1535, which was used to detect base-pair substitution. The lowest concentration of 1,2,3-TCP that induced a positive response in this strain was 200 ug/plate. However, evidences of positive mutagenicity were not found in all other strains tested including strain TA-100, which was also sensitive to base-pair substitution.

Microorganisms	Dose levels (ug/plate)				
	0.2	2	20	200	2000
TA-100	0.8	1.3	1.0	1.7	1.5
TA-1535	1.6	0.7	1.4	2.7*	4.6*

Results are expressed as : $\frac{\text{Mean \# revertant colonies per treated plate}}{\text{Mean \# revertant colonies per control plate}}$

* reproducible values of 2.5 x the control value or greater are considered to indicate a mutagenic response.

Positive mutagenic responses were also observed with EMS (500 ug) in E. Coli WP 2 and WP2 uvr A and NQO (20 ug) in both strains of E.Coli and Salmonella TA-1535.

2. Bacterial Mutation With Activation

The reverse mutation rates in microorganisms after treatment with 1,2,3-TCP or positive control agents are listed as follows:

Microorganisms	Experiment number	1,2,3-TCP (ug/plate)					EMS	BP	NQO
		0.2	2.0	20	200	2000	500ug	20ug	20ug
E.Coli WP2	3	1.4	1.7	1.3	1.6	1.1	-	-	34.1*
E.Coli WP2 uvrA	1	0.6	1.1	0.9	1.0	6.0*	2.4	-	-
	3	1.1	1.0	1.1	1.1	3.3*	-	-	22.4*
TA - 98	4	1.3	1.1	1.3	1.6	(a)	-	5.2*	-
	6	0.8	0.9	2.1	5.3*	3.6*	-	22.0*	-
TA - 1537	2	0.9	0.9	2.2	7.3*	(a)	-	0.6	-
	6	1.1	1.2	4.1*	17.6*	(a)	-	-	2.2
TA - 100	2	1.3	1.5	3.0*	6.8*	8.1*	-	3.5*	
TA - 1535	5	0.6	1.4	1.2	16.6*	35.5*	-	-	92.2*

Data extracted from TLGR.79.073

Results expressed as ratio: $\frac{\text{Mean \# revertant colonies per treated plate}}{\text{Mean \# revertant colonies per control plate}}$

(a) : cytotoxic effect

* : reproducible values of 2.5 x the control value or greater are considered to indicate a mutagenic response.

No evidence of mutation was detected with E. Coli WP2, the DNA-repair proficient strain, and Salmonella TA-1538. However, the frequency of mutant colonies increased with E. Coli WP2 uvr A, Salmonella TA-98, TA-1537, TA-100, and TA-1535. The lowest concentration of 1,2,3-TCP which induced mutation was 20 ug per plate with the strains TA-100 and TA-1537. Cytotoxic effects were also observed at the highest concentration used (2000 ug/plate) with the strains TA-98 (one experiment) and TA-1537 (both experiments). The references agents, BP and NQO, also induce positive mutagenic response.

Dose related experiments using 250, 500, 1000, 2000 and 5000 ug/plate of 1,2,3-TCP were further assessed with strains TA-100 and TA-1535, the strains used for base-pair substitution detection.

Dose-related experiment without S-9 activation

ug/plate	TA-100	TA-1535
0	24.8 +/- 1.5*	3.0 +/- 1.0
250	25.5 +/- 5.2	1.5 +/- 1.7
500	35.5 +/- 11.8	2.3 +/- 1.9
1000	42.8 +/- 9.9	5.0 +/- 1.6
2000	57.5 +/- 8.4	4.3 +/- 1.5
5000	111.0 +/- 14.3	38.0 +/- 5.7
BP (20)	22.3 +/- 6.3	-
NaN_3 (20)	-	829.3 +/- 51.5

Data extracted from TLGR. 79.073
* : expressed as mean number of revertants per plate

Positive mutagenic responses were observed with strains TA-100 and TA-1535 at 5000 ug of 1,2,3-TCP per plate (2.5 x the control value or greater). The positive control agent BP failed to induce a positive mutagenic response with TA-100.

Dose-related experiment with S-9 activation

ug/plate	TA - 100	TA - 1535
0	63.5 +/- 13.3*	28.0 +/- 5.7
250	205.5 +/- 13.3	89.8 +/- 18.2
500	385.8 +/- 41.8	180.8 +/- 17.6
1000	307.8 +/- 82.2	223.3 +/- 32.7
2000	(a)	360.8 +/- 33.4
5000	(a)	(a)
BP (20)	90.5 +/- 17.4	-
NaN_3 (20)	-	1167.5 +/- 128.3

Data extracted from TLGR. 79.073
* : expressed as mean number of revertants per plate
(a): cytotoxic effect

Positive mutagenic responses were observed at all dosage levels tested in both strains TA-100 and TA-1535 in the presence of S-9 activation. A dose-related increase in the number of revertants was noted with strains TA-1535. The mutagenic potency calculated from the dose-response curve with TA-1535 was 130 revertants/ 3 micromoles of 1,2,3-TCP or 0.04 revertants per nanomole.

The positive control agent, BP, failed to induce a positive mutagenic response in strains TA-100 in the presence of metabolic activation.

3. Saccharomyces gene conversion

a) Exposure for 1 hour without S-9 activation at room temperature

In the absence of metabolic activation, increased gene conversion rates were observed at the highest dose level used (5 mg/ml) at both histidine and tryptophan loci. Positive responses were also noted with the reference agent NQO (0.0001 mg/ml) at both loci.

b) Exposure for 1 hour with S-9 activation at 37°C

Increased gene conversion rates were observed at both loci at dose levels of 0.1 mg/ml and above.

c) Exposure for 4 hours with S-9 activation at 37°C

The conversion frequency was increased when the incubation was extended to 4 hours at 37°C. Increased gene conversion rate at the histidine locus was noted at all dosage levels tested including the lowest dose used (0.01 mg/ml). Positive mutagenic responses were observed at the tryptophan locus at dose levels of 0.1 mg/ml and above.

4. Chromosomal mutation assay

In the cytotoxic assay, a concentration of 1000 ug/ml of 1,2,3-TCP was required to induce a detectable effect on cell growth.

Chromosomal analysis of slide cultures of rat liver cells exposed to culture medium containing 250, 500, or 1000 ug of 1,2,3-TCP are summarized as follows:

Dose-levels (ug/ml)	Chromatid Abberations	Chromosome Abberations
0	1.3 *	0
250	0.3 [a]	0
500	0.3 [a]	0.3
1000	0.0	0.8
MMS (10)	12.0	3.0

* : percentage of cells examined (100 cells from each of 3 slides)

(a): Chromatid abberations in the 250 and 500 ug/ml were due to the presence of 2 cells with chromatid exchange.

The observation of 2 cells with chromatid exchange in cultures exposed to 250 and 500 ug/ml suggested a marginal effect. A second experiment was subsequently carried out with 500 ug/ml. Of the 400 cells analysed for chromosomal mutation, chromatid exchange was not detected.

III. Discussion and Conclusions

a) Baterial Mutation

In the absence of S-9, mutagenic response was observed in strain TA-1535 at dose levels of 200 ug/plate and above. Positive mutagenic responses were noted, in the presence of S-9, with strains E. Coli WP2 uvrA, Salmonella TA-100, TA-1535, TA-1537 and TA-98 in one experiment. The lowest concentration of 1,2,3-TCP which induced a detectable mutagenic response was 20 ug/plate. The effects with strain TA-1535 were dose-related.

Two strains were used in this report to detect base-pair substitution mutation type, namely TA-100 and TA-1535. The strains TA-100 and TA-1535 are derived from the same parental strain (G-46) and strain TA-100 is regarded by many investigators as more sensitive that TA-1535. For these reasons, (a) similar pattern of response should be expected from both strains for the same mutagen. However, from the submitted data, (b) positive mutagenic response was observed only in one strain (TA-1535) in the absence of S-9.

It is unclear why strain TA-100 did not respond in this study. Also, the functional integrity and sensitivity of the strains used are questionable due to the following reasons:

i. The backgroud of the mean number of revertants per plate for TA-100 in the absence of S-9 varied from 24.8 (experiment 1) to 43.5 (experiment 2). The ranges of spontaneous revertants per plate considered acceptable for TA-100 were 60 - 150 (Science 103, pp. 563-563, 1979). These values are much higher than those reported.

Similarly, the range of spontaneous revertants reported for TA-1535 (1.8 - 3.0) was considerably lower than that accepted by many laboratories (5 - 25).

ii. The reference agent selected, BP at 20 ug/plate, failed to produce a positive response in strain TA-100 in the absence of S-9 (experiments # 1 and 2) and in the presence of S-9 (experiment # 1).

iii. The number of spontaneous revertants for the S-9 mixture was not indicated.

b) Saccharomyces gene mutation

The addition of 1,2,3 TCP to cultures of Saccharomyces cerevisiae JD1 consistently increased the rate of mitotic gene conversion in the absence or presence of the S-9 fraction. In the absence of S-9, increased gene conversion was observed only at the highest dose used (5 mg/ml). In the presence of metabolic activation and exposure for 4 hours, increased mutation rates were noted at 0.01 and 0.1 mg/ml for the histidine and tryptophan locus, respectively. Both positive control substances cyclophosphamide and 4-nitroquinoline oxide, induced a significant increase in gene conversion rates.

Under the conditions of this study, 1,2,3-TCP can be classified as a mutagen in Saccharomyces.

c) Rat liver cells chromosomal mutation

In the first experiment, chromatid exchanges were observed in 2 cells each in the 250 and 500 ug/ml dosage levels. However, the percentage of cells showing chromatid and chromosomal abberations between the treated and control cultures were similar. The finding of chromatid exchange in the treated cultures was subsquently checked by another experiment in which a dose of 500 ug/ml of 1,2,3-TCP was used. In this second experiment, no cells with chromatid exchanges were found and the percentage of cells with chromatid and chromosomal abberations were negative for both control and treated cells analyzed.

Significant increases in chromatid aberrations were observed with both positive control agents used, methylmethanesulphonate and dimethylbenzathracene.

IV. Core Classification

1. The bacterial portion of this study is acceptable. Under the conditions of this study, mutagenic response was observed in Salmonella TA-1535 in the absence of S-9. Positive mutagenic responses were noted, in the presence of S-9, with strains E. Coli WP2 uvrA, Salmonella TA-100, TA-1535, TA-1537, and TA-98. However, due to questionable functional integrity and sensitivity of the strains Salmonella TA-100 and TA-1535 used, the registrant is requested to submit additional information concerning:

a. The lack of response observed in strain TA-100 in the absence of the S-9 fraction although both strains TA-100 and TA-1535 are derived from the same parental strain and both were used to detect base pair substitution mutation type.

b. The low background spontaneous rate of strains TA-100 and TA-1535 in comparison to acceptable spontaneous ranges.

c. Techniques of the testing laboratory used to verify the funtional integrity of the strains tested.

d. Spontaneous rate of revertants for cultures containing only S-9 were not reported.

e. The lack of response of the positive control selected, BP, in strain TA-100 in the absence of metabolic activation.

f. The purity of the test material

2. The Saccharomyces portion of this study is acceptable and 1,2,3-TCP is a positive mutagen in Saccharomyces cerevisiae JD1. However, the registrant is requested to provide clarification regarding the purity of the test chemical.

3. The results from the two chromosomal investigations are inconclusive. Chromatid exchanges were found at two different dosage levels in one experiment but were absent in a subsequent study. The following comments are noted:

a. Cells should be exposed to the test chemical in the presence of a S-9 metabolic activation system.

b. Data supporting the selection of dosage levels were not submitted.

c. The 500 ug/plate dosage level in the second experiment apparently was not a maximum tolerated dose.

To fully assess the mutagenic potential of 1,2,3-TCP in chromosomes, a new study at higher dosage levels is recommended.

STUDY IV

Study Title : Subacute Inhalation Toxicity Study of D-D Soil Fumigant in CD-1 Mice and Albino Fischer 344 Rats.
Testing Facility : Hazleton Lab.
Final Report No. : 776-132
Report Date : May 20, 1979

I. MATERIAL :

D-D® Soil Fumigant, Vapor (SD-14647)
(A mixture of (Z)-1,3-dichloropropene, (E)-1,3-dichloropropene, and 1,2-dichloropropane)
Purity : unknown
Concentration of each constituent: unknown

Rats and mice were exposed to 5, 15, or 50 ppm of D-D® for 6 hours/day, 5 days/week for 12 weeks, a total of 60 exposures. The mean analytical concentrations were determined as 4.66, 14.4, and 53.72 ppm and, hence, were within 10% of the target concentrations.

II. PROCEDURES :

Copies of the procedures are appended. The methods used are unexceptionally different from those listed in the EPA Proposed Guidelines of 1978. However, the following comments are noted :

a) The purity of D-D vapor used was not indicated.

b) Hematological and clinical determinations should also be performed at study initiation. Clinical measurements of calcium, K^+, SGOT, total protein, total cholesterol, and total bilirubin apparently were not studied.

c) Food consumption apparently was not measured.

d) Treated animals were inadvertently exposed to Telone II vapor instead of D-D vapor on 12/18/82 (45th exposure) and the registrant was notified of this error.

III. RESULTS

A. MOUSE STUDY

1) Clinical Observations and Mortality

No compound-related clinical signs were evident from the submitted data. Lacerations, scratches, swollen legs and paws were the only signs observed and were attributed to fighting injuries by the investigators.

Respective mortality rates for the groups receiving 0, 5, 15, or 50 ppm were 1.8, 12.5, 5.4, and 8.9%. The majority of

these deaths were attributed to fighting by the investigators except for two mice (# 377 and 477) whose deaths were accidental.

Necropsy observations of the dead (or moribund sacrifice) animals did not reveal any compound-related effects.

2. Body Weight Data

No treatment-related differences were noted among males and females of the test groups.

3. Hematology

Hematological determinations at study termination (week 12) are presented in the following table.

	0 ppm	5 ppm	15 ppm	50 ppm
Hematocrit (%)				
Males	48.3	45.1	47.2	45.7
Females	48.3	46.6	48.7	48.1
Hemoglobin (gm/dl)				
Males	15.4	14.0	15.1	14.8
Females	15.6	14.6	16.0	15.5
RBC ($10^6/mm^3$)				
Males	8.39	7.87	8.52	8.11
Females	9.09	8.29	8.79	8.60
WBC ($10^3/mm^3$)				
Males	11.4	12.0	7.6*	8.0
Females	12.5	10.0	10.4	7.1*

Data extracted from pages 68-74
* : significantly different from control at $p < 0.05$

The mean WBC counts were significantly lower than the controls for males and females of the groups receiving 15 and 50 ppm, respectively. All other hematological parameters investigated were comparable to control values.

4. Clinical Chemistry

At the 6-week interim sacrifice, no significant differences in all parameters investigated were noted in males among the test groups. However, significant increases in albumin levels were found in females of the 15 ppm in comparison with the female control values. The biological significance of this finding is unclear in the absence of a dose-response relationship.

Clinical studies at study termination are summarized as follows :

	0 ppm	5 ppm	15 ppm	50 ppm
Albumin (g/dl)				
Males	2.6	2.3*	2.5	2.6
Females	2.8	2.7	2.8	2.8
BUN (mg/dl)				
Males	26.1	26.8	23.4	34.6*
Females	23.2	24.7	22.8	28.6
SGPT (IU/L)				
Males	23	23	31	28
Females	34	40	19*	19*
ALP (IU/L)				
Males	22	23	26	29
Females	31	24	34	27

* Significantly different from control at $P < 0.05$

In the males, the albumin level was significantly decreased in the 5 ppm group. However, this finding was considered as biologically insignificant by this reviewer due to the lack of a dose-response relationship. Statistically significant increases in BUN were observed in males of the highest dose group (50 ppm) in comparison with control values. The mean SGPT values for the 15 and 50 ppm females were significantly lower than those of the control group. No compound induced effects were noted in the treated females with respect to albumin, BUN, and alkaline phosphatase levels.

5. Urinalysis

No apparent trends or differences between the control and treated groups were seen for both males and females.

6. Gross Pathology

a) Week-6 Interim Sacrifice

Higher incidences of enlarged peribronchial lymph nodes were noted in the treated groups when compared to the control group. These incidences were 0, 31.3, 20.0, and 17.6% for the groups receiving 0, 5, 15, and 50 ppm, respectively. No other differences were seen.

b) Week-12 Sacrifice

The findings of enlarged peribronchial lymph nodes observed at the 6-week interim sacrifice were not evident by final sacrifice. Isolated incidences of pale liver and foci on kidneys occurred with comparable frequency in both the control and treated groups.

7. Histopathology

Histopathological evaluations of selected tissues taken from mice after 60 exposures to D-D® are summarized in the following table:

	0 ppm		5 ppm		15 ppm		50 ppm	
	M	F	M	F	M	F	M	F
Number of animals examined	18	18	21	19	18	18	21	18
Adrenal								
focal suppurative inflammation	0	0	0	0	0	0	1/P	0
Heart								
aortitis	0	0	0	0	0	0	1/P	0
non-suppurative myocarditis	1/2(a)	0	0	0	0	0	0	2/1
Lungs								
peribronchial lym.hyperplasia	14/2	6/2	2/1	1/1	0	1/2	8/2	7/1
perivascular lym. hyperplasia	17/1	10/1	2/1	1/1	1/1	1/1	13/2	10/1
pneumonitis	4/P	2/P	0	0	0	0	5/P	4/P
congestion	0	3/P	2/P	0	2/P	0	7/P	3/P
Spleen								
increased extramed.hematopoiesis	0	1/P	3/P	1/P	0	0	2/P	0
Liver								
microgranulomas	6/P	12/P	6/P	9/P	5/P	8/P	8/P	10/P
diffuse hepatocytic enlarg.	4/2	0	0	1/2	1/3	1/2	12/3	6/3
congestion	0	1/3	6/P	1/P	3/P	0	4/P	0
Kidneys								
non-suppurative pyelitis	3/2	3/2	2/2	2/2	0	3/2	3/2	2/2
focal interstitial nephritis	7/1	2/2	2/1	3/1	6/1	3/1	5/1	3/1
perivascular lymphoid foci	11/1	10/P	11/1	6/P	9/1	15/1	8/P	12/P
Uterus								
hydrometra		3/P		0		1/P		2/P
cystic endometrial hyperplasia		0		1/P		0		0
Ovary								
paraovarian cysts		1/P		1/P		2/P		0

Data extracted from pp. 165-184
(a): Number of animals affected/grading of severity
P : present ; 1 : minimal ; 2 : slight ; 3 : moderate

Compound related histopathological alterations were observed in the liver of the highest dose treated animals. The incidences of "diffuse hepatocytic enlargement" were found in 12 males (57.1%) and 6 females (33.3%) of the highest dose group (50 ppm) and in 4 males (22.2%) of the control group. Furthermore, the severity of the findings in the 50 ppm group was greater than that of the control group (grading of 3 vs. 2 of the control).

Other histopathological findings in the liver, such as, microgranulomas and congestion, occurred in similar frequency and severity in all test groups.

Focal non-suppurative myocarditis was present in a single control male and two highest dose level females.

In the lung, observations of peribronchial and perivascular lymphoid hyperplasia and pneumonitis were similar among the test groups. However, increased incidences of lung congestion were noted in the 50 ppm group (10/39 = 25.6%) when compared to control values (3/36 = 8.3%).

"Increased extramedullary hematopoiesis" in the spleen was observed in 1, 4, 0, and 2 animals in the 0, 5, 15, and 50 ppm groups, respectively.

In the kidneys, the incidences of non-suppurative pyelitis, focal interstitial nephritis, and perivascular lymphoid foci occurred with essentially comparable frequency and severity in the control and treated mice.

A variety of other findings (in the uterus, salivary gland, ovary, adrenal, and heart) occurred infrequently and apparently were not compound related.

8. Organ Weights

No significant differences were noted among the test groups with respect to the brain, heart, kidneys, testes, and ovary weights at final sacrifice. However, changes in liver and adrenal weights were found in the treated groups and were summarized as follows:

		Liver (g)		Adrenal (g x 100)	
		weight	ratio(a)	weight	ratio
Control	Males	1.67 +/- 0.18	5.00 +/- 0.45	0.52 +/- 0.11	1.57 +/- 0.32
	Females	1.40 +/- 0.20	5.26 +/- 0.75	1.19 +/- 0.30	4.42 +/- 0.91
5 ppm	Males	1.90* +/- 0.21	5.71* +/- 0.39	0.52 +/- 0.16	1.60 +/- 0.60
	Females	1.42 +/- 0.11	5.40 +/- 0.65	1.33 +/- 0.23	5.05* +/- 0.86
15 ppm	Males	1.83 +/- 0.15	5.18 +/- 0.44	0.50 +/- 0.10	1.42 +/- 0.35
	Females	1.47 +/- 0.23	5.30 +/- 0.71	1.31 +/- 0.23	4.73 +/- 0.72
50 ppm	Males	1.99* +/- 0.25	5.56* +/- 0.48	0.56 +/- 0.14	1.56 +/- 0.37
	Females	1.49 +/- 0.14	5.48 +/- 0.47	1.30 +/- 0.40	4.86 +/- 1.87

Data extracted from pp. 121-124; all values given are Mean +/- SD
(a) : organ/body weight ratio
* : statistically different from control at $P < 0.05$.

The absolute and relative liver weights of all treated males were higher than those of the control group with statistically significant differences found at the 5 and 50 ppm dosage levels. Although these effects were not clearly dose-related, they were apparently compound-related since similar higher than control liver weight findings were also observed in females.

Significant increase in the relative adrenal weight was noted in females of the lowest dose group. However, this finding was of questionable biological significance.

B. RAT STUDY

1. Clinical Observations and Mortality

Isolated incidences of soft stool and urine stains of the fur were noted in a few animals of each test group. No unusual appearance or deleterious effects were due to D-D® exposure.

Only one unscheduled death occurred in the 50 ppm group (female # 322). The cause of death was cited by the investigators as accidental. Necropsy of this animal did not reveal any compound related effect.

2. Body Weights

Scattered instances of significant differences in mean body weights were noted in males of the treated groups when compared to control values. Males in the 5 ppm group had significantly lower mean body weight at weeks 1 through 5, the 15 ppm group at weeks 1 and 3, and the 50 ppm group at weeks 1, 3, 4, 5, and 6. All male rats gained weight throughout the entire study. However, the mean body weight gain in the treated groups was less than that of the control group being 153, 138, 145, and 134 grams for the groups receiving 0, 5, 15, and 50 ppm, respectively.

No significant differences were apparent from the female data.

3. Hematology

No biologically significant alterations were observed in both males and females of all test groups at either the 6-week interim or final sacrifice.

4. Clinical Chemistry

Serum determinations of albumin, alkaline phosphatase, BUN, glucose, and SGPT were measured on both males and females at weeks 6 and 12. Analysis of variance indicated no significant differences among groups for both sexes at either interval. However, the SGPT levels of the treated females were relatively lower than those of control females at final sacrifice. Respective SGPT values of 51, 46, 33, and 31 IU/L were obtained from females of the control, 5, 15, and 50 ppm groups. The biological significance of this finding

is unclear. However, it is noteworthy since statistically significant decreases in SGPT levels were noted in female mice of the 15 and 50 ppm groups.

5. Urinalysis

No apparent trends or differences between the control and treated groups were seen for both males and females.

6. Gross Pathology

Isolated incidences of pale lung, granular liver, enlarged peribronchial lymph nodes, and cystic ovary were found in all test groups. No compound related effects were evident from the submitted data.

7. Organ Weights

The following table summarizes the changes observed in liver and kidney weights of the treated groups:

		Liver (g)		Kidneys (g)	
		Weight	Ratio (a)	Weight	Ratio
Males					
	Control	6.67 +/- 0.82	2.43 +/- 0.11	1.71 +/- 0.25	0.62 +/- 0.08
	5 ppm	6.40 +/- 0.39	2.45 +/- 0.08	1.71 +/- 0.12	0.69 +/- 0.03
	15 ppm	6.63 +/- 0.58	2.48 +/- 0.17	1.69 +/- 0.12	0.63 +/- 0.03
	50 ppm	6.80 +/- 0.55	2.61* +/- 0.09	1.73 +/- 0.14	0.66 +/- 0.03
Females					
	Control	3.95 +/- 0.37	2.64 +/- 0.19	1.03 +/- 0.06	0.69 +/- 0.02
	5 ppm	3.97 +/- 0.28	2.58 +/- 0.14	1.08 +/- 0.09	0.70 +/- 0.05
	15 ppm	4.07 +/- 0.29	2.62 +/- 0.14	1.10 +/- 0.09	0.71 +/- 0.04
	50 ppm	4.11 +/- 0.25	2.71 +/- 0.13	1.10 +/- 0.08	0.73* +/- 0.04

Data extracted from pp. 129-132; all values given are Mean +/- SD.
(a) : organ/body weight ratio
* : significantly different from control at $P < 0.05$

No other significant differences were indicated.

8. Histopathology

Histopathological evaluations of several tissues taken from rats at final sacrifice are summarized as follows:

	Control M	Control F	5 ppm M	5 ppm F	15 ppm M	15 ppm F	50 ppm M	50 ppm F
Number of animals examined:	18	18	18	18	18	18	18	19
Heart								
focal nonsuppurative myocarditis	12/1[a]	8/1	0	0	0	0	11/1	6/1
Lungs								
peribronchial lymphoid hyperplasia	18/2	18/2	3/2	0	4/2	3/2	17/2	19/2
perivascular lymphoid hyperplasia	18/2	18/1	3/1	0	4/2	3/1	18/2	19/1
focal pneumonitis	9/P	9/P	1/P	0	0	2/P	7/P	6/P
congestion	0	0	0	1/P	0	0	2/P	2/P
Liver								
microgranulomas	10/P	13/P	10/P	11/P	6/P	10/P	11/P	13/P
bile duct proliferation	4/1	2/1	2/1	2/1	2/1	2/1	2/1	0
nonsuppurative pericholangitis	6/1	1/1	1/1	0	2/1	0	2/1	1/1
diaphagmatic hernia	0	0	0	0	1/P	1/P	0	2/P
Kidneys								
focal interstitial nephritis	16/1	3/1	16/1	3/1	15/1	5/1	15/1	4/1
Trachea								
nonsuppurative tracheitis	5/2	1/2	0	0	0	0	3/2	4/2
Nasal cavity								
rhinitis	0	0	0	0	0	0	4/2	2/2
Uterus								
hydrometra		5/P		3/P		2/P		6/P

Data extracted from pp. 185-203

(a) : No. of animals affected/ grading of severity

P : present ; 1 : minimal ; 2 : slight ; 3 : moderate

Minimal (grading of 1) focal nonsuppurative myocarditis occurred only in the control and 50 ppm groups. Lesions of chronic respiratory disease, such as, peribronchial and perivascular lymphoid hyperplasia, were noted in almost all animals of the control and highest dose groups with essentially comparable severity.

In the liver, microgranulomas, bile duct proliferation, and nonsuppurative pericholangitis were noted in all test groups at similar incidence and severity. Diaphragmatic hernia was present in 2 animals each in the two highest dose groups (15 and 50 ppm).

Focal interstitial nephritis occurred frequently in males of all test groups with essentially comparable severity and incidence.

Slight nonsuppurative tracheitis were observed in 6 and 7 animals of the control and 50 ppm, respectively, and none in the 5 and 15 ppm groups. Rhinitis of the nasal cavity was found only in the highest dose group and could possibly be compound-related.

Hydrometra was present in uterine sections from occasional animals.

Histopathological examinations of other tissues were essentially unremarkable.

IV. DISCUSSION AND CONCLUSIONS

In mice, exposure to D-D® vapor for 12 weeks may result in several biological and morphological alterations. The absolute and relative (organ to body weight ratio) liver weights of all treated males were higher than control values with statistical significances found at the 5 and 50 ppm dosage levels. Increased incidences of "diffuse hepatocytic enlargement" were found in 57.1 % of males and 33.3% of females treated at the 50 ppm as compared to 22.2% of males and 0% of females in the control group. Furthermore, the incidence of "diffuse hepatocytic enlargement" was more severe in the 50 ppm group than the control (grading of 3 vs. 2 of the control). Consequently, the increases in absolute and relative liver weights observed in the treated males in conjunction with an increase in incidence and severity of liver histopathological alterations may indicate compound-related effects. Significant decreases in WBC counts and SGPT levels were also observed in males and females at the 15 ppm dosage level, respectively.

The rat appears to be less sensitive to the effects of D-D® than the mouse. Exposure to 50 ppm for 12 weeks significantly increased the relative liver and kidney weights in males and females, respectively. These increases were not accompanied by histopathological alterations. Although not statistically different, the SGPT of treated females were still lower than control values. Significant decreases in SGPT levels had been observed in female mice. The biological significance of this finding is unclear. Diaphragmatic hernia was found in 4 animals, 2 each in the 15 and 50 ppm groups. This finding was not considered as compound-related effects by this reviewer but as spontaneous occurrence. The presence of rhinitis in the highest dose group may be compound related and of biological significance.

V. CORE CLASSIFICATION

1. The subacute inhalation toxicity in mouse is classified as Supplementary Data. A systemic NOEL cannot be demonstrated from the dose levels selected. Significant increases in absolute and relative liver weights were noted at the lowest dose used (5 ppm) Furthermore, clinical measurements of Ca++, K+, total bilirubin, total protein, total cholesterol, SGOT, and food consumption were not determined.

2. Under the conditions of the rat study, a systemic NOEL is demonstrated at 15 ppm with increased relative liver and kidney weight observed at 50 ppm (highest dose tested).

This study is classified as Supplementary Data. The purity of the test chemical was not indicated and clinical measurements of Ca^{++}, K^{+}, total bilirubin, total cholesterol, total protein, SGOT, and food consumption were not determined.