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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

**DATE:** September 26, 2019

**SUBJECT:** **1,3-Dichloropropene:** Report of the Cancer Assessment Review Committee

**PC Code**: 029001
**Decision No.:** N/A
**Petition No.:** N/A
**Risk Assessment Type:** NA
**TXR No**.: 0057949
**MRID No.:** N/A

**DP Barcode:** N/A
**Registration No.:** N/A
**Regulatory Action:** N/A
**Case No.:** N/A
**CAS No.:** 542-75-6
**40 CFR:** N/A

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On May 22, 2019, the Cancer Assessment Review Committee (CARC) of the Health Effects Division, evaluated the carcinogenic potential of 1,3-dichloropropene in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document. This report supersedes the previous reviews (J. Quest, 10-NOV-1985, TXR 0057324 and K. Dearfield, 08-DEC-1989, TXR 0056176).



ED_006472_00000731-00001

*CANCER ASSESSMENT DOCUMENT*

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
**1,3-Dichloropropene**

PC CODE 029001

September 26, 2019

**C**ANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS



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# I. EXECUTIVE SUMMARY

The Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met on May 22, 2019 to re-evaluate the carcinogenic potential of 1,3-dichloropropene (1,3-D), also known as Telone. 1.3-D was previously peer-reviewed in 1985 and in 1989. In 1985, the Toxicology Branch Peer Review Committee classified 1,3-D as a "Group B2 – Probable Human Carcinogen" based on increased tumors following oral administration. As a result, an estimated unit risk ($Q_1$*) of $1.22x10^{-1}$ $(mg/kg/day)^{-1}$ in human equivalents, was calculated based on combined tumors in male rats (forestomach, liver, adrenal, and thyroid). This classification was based on an NTP oral (gavage) toxicity study in rats that used a formulation of 1,3-D that contained 1% epichlorohydrin as a stabilizer, which is a known mutagen and carcinogen. It should be noted that since 1983, 1,3-D formulations no longer contain epichlorohydrin. In 1989, the HED Peer Review Committee evaluated inhalation cancer studies in mice and rats conducted with 1,3-D that did not contain epichlorohydrin as a stabilizer. That review committee retained the Group B2 classification and calculated an inhalation estimated unit risk of $1.30x10^{-5}$ $(\mu g/m^3)^{-1}$ in human equivalents based on lung adenoma rates in male mice. Since that time, Dow has submitted additional studies including two inhalation pharmacokinetic studies, an *in vitro* bacterial reverse gene mutation assay, and an *in vivo* transgenic rodent mutagenicity assay for the agency to consider. In 2019, the CARC convened to evaluate these submissions in the context of the of the entire database as part of a re-evaluation of 1,3-D. In total, six carcinogenicity studies were considered in this evaluation: oral gavage studies in the mouse and rat, oral dietary studies in the mouse and rat, and inhalation studies in the mouse and rat. The NTP rodent cancer bioassays conducted with 1,3-D containing epichlorhydrin are not included in this evaluation. The CARC concluded that the interpretation of the findings in those studies are confounded by the presence of a known mutagen. The CARC determined that the database of studies conducted with 1,3-D without epichlorohydrin is adequate and appropriate for assessing the carcinogenic potential of 1,3-D.

The CARC considered the following in its weight of evidence determination of the carcinogenic potential of 1,3-D:

## Rats

In an oral (dietary) combined chronic/carcinogenicity study in F344 rats, male rats showed a statistically significant ($p<0.05$) increase in hepatocellular adenomas at the high dose (25 mg/kg/day). Although there was no clear evidence of supporting non-neoplastic lesions, the incidence of these benign tumors at the high dose exceeded the expected historical control range. The doses tested were considered to be adequate and not excessive for assessing the carcinogenic potential of 1,3-D in both sexes. **CARC concluded that the liver adenomas in male rats are treatment-related. There were no treatment-related tumors in female rats in the dietary study.**

In an oral (gavage) combined chronic/carcinogenicity study in CD-1 rats, the CARC determined that although the animals could have tolerated higher doses, the committee concluded that the high dose (25 mg/kg/day) was adequate to assess carcinogenicity. **There were no treatment-related tumors in male or female rats in this study.**



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In an inhalation combined chronic/carcinogenicity study in F343 rats, the CARC determined that the concentrations tested were adequate and not excessive to assess carcinogenicity. **There were no treatment-related tumors observed in male or female rats via the inhalation route.**

**Mice**

In a 2-year dietary carcinogenicity study in B6C3F1 mice, the CARC concluded that the animals likely could have tolerated a higher dose; however, the committee concluded that the doses evaluated are adequate to assess the carcinogenic potential of 1,3-D in mice. **There were no treatment-related tumors in male or female mice.**

In an oral gavage carcinogenicity study in CD-1 mice, CARC determined the doses tested to be adequate and not excessive. **There were no treatment-related tumors seen in male or female mice.**

In an inhalation carcinogenicity study in B6C3F1 mice, there was a statistically significant trend ($p<0.01$) and pairwise ($p<0.05$) increase in benign bronchioloalveolar adenomas in male mice at the highest test concentration (60 ppm). CARC considered the concentrations to be adequate and not excessive based on toxicological endpoints; however, the registrant conducted a toxicokinetic study evaluating the relationship between 1,3-D concentration in male mice blood and inhalation exposure. These data indicated that the relationship was non-linear at exposure levels of 40 ppm and above. **Based on these findings, the CARC concluded that there were no treatment-related tumors in male mice via the inhalation route at concentrations below the kinetically derived maximum tolerated dose (KMD). There was no evidence of carcinogenicity in female mice.**

Based on a weight of evidence of the available genotoxicity studies, the CARC concluded that there is low concern for mutagenicity in vivo.

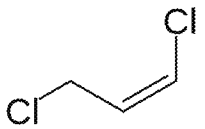
In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the **CARC classified 1,3-Dichloropene (Telone) as "Suggestive Evidence of Carcinogenic Potential"** based on the presence of liver tumors by the oral route in male rats only.

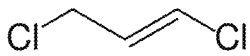
Quantification of human cancer risk is not required. The CARC recommends using a non-linear approach (i.e., reference dose (RfD)) that will adequately account for all chronic toxicity including carcinogenicity, that could result from exposure to 1,3-dichloropropene.

## II. INTRODUCTION

On May 22, 2019, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to re-evaluate the carcinogenic potential of 1,3-dichloropropene (1,3-D), also known as Telone.

## III. BACKGROUND




trans-1,3-dichloropropene cis-1,3-dichloropropene

1,3-D is a soil fumigant that is registered for pre-plant and for post-plant applications to control nematodes and/or garden symphylans. It contains approximately equal proportions of the *cis* and *trans* isomers. It is formulated as a pressurized gas, liquid ready to use, emulsifiable concentrate, or a flowable concentrate. The liquid concentrates are intended for direct metering into drip irrigation systems, and other formulations are used in more conventional soil fumigation applications (i.e., shank/chisel/plowsole pre-plant soil treatments). Registered products for pre-plant applications may be applied by drip irrigation, soil injection and row and broadcast applications. The only post-plant use registered is for drip irrigation to established vineyards.

The carcinogenic potential of 1,3-D has never been evaluated by the CARC. In 1985, the Toxicology Branch Peer Review Committee (J. Quest, 10-NOV-1985, TXR 0057324) classified 1,3-D as a "Group B2 – Probable Human Carcinogen" based on increased tumors following oral administration. As a result, an estimated unit risk ($Q_1$*) of $1.22 \times 10^{-1}$ $(mg/kg/day)^{-1}$ in human equivalents, was calculated based on combined tumors in male rats (forestomach, liver, adrenal, and thyroid) and converted from animals to humans using ¾ body weight scaling. This classification was based on an NTP oral (gavage) toxicity study in rats that used a formulation of 1,3-D that contained 1% epichlorohydrin as a stabilizer, which is a known mutagen and carcinogen. However, commercial formulations of 1,3-D have used epoxidized soybean oil as the stabilizer since 1983.

In 1989, the HED Peer Review Committee (K. Dearfield, 08-DEC-1989, TXR 0056176) met to evaluate inhalation cancer bioassays in mice and rats that had been submitted. The Committee concluded that the new data reaffirmed the Group B2 classification. The potency estimate for the inhalation route was based on a study by Lomax *et al.* from 1987 that used a formulation of 1,3-D that did not contain epichlorohydrin. For the inhalation route, an estimated unit risk of $1.30 \times 10^{-5}$ $(\mu g/m^3)^{-1}$ in human equivalents (converted from animals to humans using ¾ body weight scaling) was calculated based on lung adenoma rates in male mice.

1,3-D is currently undergoing registration review. Dow AgroSciences submitted a position paper "1,3-Dichloropropene Cancer Classification Weight of Evidence" (MRID 49889503) along with additional studies for the agency to consider. In response to Dow's submissions, the CARC met to re-evaluate the carcinogenic potential of 1,3-D. This review includes the



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evaluation of six rodent carcinogenicity studies conducted with 1,3-D that did not contain epichlorohydrin as a stabilizer.

**Rat Oral Studies:**

Stott, WT, Johnson, KA, Jeffries, TK, et al. (1995). Telone II Soil Fumigant: Two-Year Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats. Dow Chemical Co. Lab Project No. M-003993-031. August 15, 1995. MRID 43763501.

Kelly, CM. (1998) A chronic toxicity and oncogenicity study with DD-92 in the rat via oral gavage administration. Huntingdon Life Sciences (East Millstone, NJ). Laboratory Project ID 95-2379, January 21, 1998. MRID 49889501.

**Mouse Oral Studies:**

Redmond, JM, Stebbins, KE, Stott, WT. (1995) Telone® II Soil Fumigant: Two-year dietary chronic toxicity/oncogenicity study in B6C3F1 mice- Final report. Dow Chemical Co. (Midland, Michigan). Laboratory Project ID M-003993-032, August 9, 1995. MRID 43757901.

Kelly, CM. (1997) An oncogenicity study with DD-92 in the mouse via oral gavage administration. Huntingdon Life Sciences (East Millstone, New Jersey). Laboratory Project ID 95-2378, December 17, 1997. MRID 49889502.

**Rat Inhalation Study**:

Lomax, LG, Calhoun, LL, Stott, WT, et al. (1987). Telone II Soil Fumigant: Two-Year Inhalation Chronic Toxicity/Oncogenicity Study in Rats. Dow Chemical USA (Midland, MI). Lab Project No. M-003993-009R. July 13, 1987. MRID 40312201.

**Mouse Inhalation Study:**

Stott, WT, Johnson, KA, Calhoun, LL, et al. (1987). Telone II Soil Fumigant: Two-Year Inhalation Chronic Toxicity/Oncogenicity Study in Mice. Dow Chemical USA (Midland, MI). Lab Project No. M-003993-009. July 13, 1987. MRID 40312301.

This review also includes the following additional studies: two inhalation pharmacokinetic studies, and an *in vivo* transgenic rodent mutagenicity assay.



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# IV. EVALUATION OF CARCINOGENICITY STUDIES VIA ORAL EXPOSURE

*Note: The NTP oral gavage studies in mice and rats were previously evaluated and the findings are presented in the Toxicology Branch Peer Review Committee Report (J. Quest, 10-NOV-1985, TXR 0057324). The CARC agreed that the findings in these studies are confounded by the use of the known mutagen and carcinogen epichlorohydrin to stabilize the test formulations used in those studies. Epichlorohydrin is no longer used commercially to stabilize 1,3-D, and therefore the CARC concluded that only studies without epichlorohydrin are relevant for the current evaluation.*

## A. Combined Chronic Toxicity/Carcinogenicity Study in Fischer 344 Rats - Dietary

Citations: Stott, WT, Johnson, KA, Jeffries, TK, et al. (1995). Telone II Soil Fumigant: Two-Year Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats. Dow Chemical Co. Lab Project No. M-003993-031. August 15, 1995. MRID 43763501.

**Experimental Design:**

In a chronic/carcinogenicity study (MRID 43763501), TELONE II (96.0% purity, 50.7 cis/ 45.1trans isomers) as microcapsules was administered by dietary admix to 60 Fischer 344 rats/sex/group at doses of 0, 2.5, 12.5, or 25 mg/kg/day (achieved doses were 0/0, 2.5/2.5, 12.7/12.7, 25.4/24.8 mg/kg/day [M/F]) for 2 years. Ten rats/sex/dose were sacrificed at 12 months for interim evaluation. The test article in this study did not contain epichlorohydrin.

**Survival Analysis:**

There were no statistically significant survival disparities among the dose groups in male or female rats (Tables 1-2).



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Table 1. Telone – Fischer 344 Rat Study (Stott *et al.* 1995)
(MRID No. 43763501)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

| Dose (mg/kg/day) | Weeks 1-26 | 27-52 | 53[i] | 53-78 | 79-105[f] | Total |
|---|---|---|---|---|---|---|
| 0 | 0/60 | 1/60 | 10/59 | 1/49 | 12/48 | 14/50 (28) |
| 2.5 | 0/60 | 0/60 | 10/60 | 2/50 | 14/48 | 16/50 (32) |
| 12.5 | 0/60 | 0/60 | 10/60 | 1/50 | 15/49 | 16/50 (32) |
| 25.0 | 0/60 | 2/60 | 10/58 | 0/48 | 14/48 | 16/50 (32) |

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval. Percent animals died in parenthesis.

[i]Interim sacrifice at week 53.
[f]Final sacrifice at week 105.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If $^{*}$, then $p < 0.05$. If $^{**}$, then $p < 0.01$.



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Table 2. Telone – Fischer 344 Rat Study (Stott *et al.* 1995)
(MRID No. 43763501)

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

| Dose (mg/kg/day) | Weeks 1-26 | 27-52 | 53[i] | 53-78 | 79-105[f] | Total |
|---|---|---|---|---|---|---|
| 0 | 1/60 | 0/59 | 10/59 | 0/49 | 10/49 | 11/50 (22) |
| 2.5 | 0/60 | 0/60 | 10/60 | 3/50 | 12/47 | 15/50 (30) |
| 12.5 | 0/60 | 0/60 | 10/60 | 3/50 | 16/47 | 19/50 (38) |
| 25.0 | 0/60 | 0/60 | 10/60 | 1/50 | 10/49 | 11/50 (22) |

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval. Percent animals died in parenthesis.

[i]Interim sacrifice at week 53.
[f]Final sacrifice at week 105.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If $^{*}$, then $p < 0.05$. If $^{**}$, then $p < 0.01$.



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**Discussion of Tumor Findings:**

**(i) Liver Tumors in Male and Female Rats**

Male rats had statistically significant trends at $p < 0.01$, and significant pair-wise comparisons of the 25 mg/kg/day dose group with the controls at $p < 0.05$, for hepatocellular adenomas, and adenomas and/or carcinomas combined. The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test and the Exact Test for Trend (Table 3). Historical control data from nine studies from 1991-1997 indicated a background range of 0-16% for hepatocellular adenomas in male Fischer 344 rats; with a mean of 4.4%. The incidence of hepatocellular adenomas in male rats at 25 mg/kg/day (19%) was outside the historical control range of the testing laboratory. Historical control data from the same studies indicated a control range of 0-2% for hepatocellular carcinomas in male rats; with a mean of 0.2%.

Table 3. Telone – Fischer 344 Rat Study (Stott *et al.* 1995)
(MRID No. 43763501)

Male Liver Tumor Rates[+] and
Fisher's Exact Test and Exact Trend Test Results

| | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2.5 | 12.5 | 25 |
| Adenomas (%) | 2/49 (4) | 1/50 (2) | 6[a]/50 (12) | 9/48 (19) |
| P = | 0.0014** | 0.8825 | 0.1409 | 0.0233* |
| Carcinomas (%) | 0/49 (0) | 0/50 (0) | 0/50 (0) | 1[a]/48 (2) |
| P = | 0.2437 | 1.0000 | 1.0000 | 0.4949 |
| Combined (%) | 2/49 (4) | 1/50 (2) | 6/50 (12) | 10/48 (21) |
| P = | 0.0005** | 0.8825 | 0.1409 | 0.0124* |

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 67 in the 12.5 mg/kg/day dose group.
[b]First carcinoma observed at week 105 in the 25 mg/kg/day dose group.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Female rats had a statistically significant trend at $p < 0.01$ for hepatocellular adenomas. The statistical analyses of the tumors in female rats were based upon Fisher's Exact Test and the Exact Test for Trend (Table 4). Historical control data from nine studies from 1991-1997



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indicated a control range of 0-8% for hepatocellular adenomas in female Fischer 344 rats; with a mean of 1.5%. The incidence of hepatocellular adenomas in female rats at 25 mg/kg/day (8%) was at the high end of the historical control range of the testing laboratory.

Table 4. Telone – Fischer 344 Rat Study (Stott *et al.* 1995)
(MRID No. 43763501)

Female Liver Tumor Rates[+] and
Fisher's Exact Test and Exact Trend Test Results

| | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2.5 | 12.5 | 25 |
| Adenomas[#] (%) | 0/49 (0) | 0/50 (0) | 0/50 (0) | 4[a]/50 (8) |
| P = | 0.00363** | 1.0000 | 1.0000 | 0.06118 |

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[#]No hepatocellular carcinomas were observed.

[a]First adenoma observed at week 99 in the 25 mg/kg/day dose group.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Non-Neoplastic Lesions in the Liver: Relevant non-neoplastic lesions in the liver of male and female rats are presented in Table 5.



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| Table 5: Incidence and severity of non-neoplastic microscopic findings in the liver of male and female rats[ab] | | | | |
|---|---|---|---|---|
| Parameter: | Dose (mg/kg/day) | | | |
| | 0 | 2.5 | 12.5 | 25 |
| Main Study: 24 Months | | | | |
| Males -# animals evaluated | 50 | 50 | 50 | 50 |
| Focus of altered cells – basophilic, hepatocellular | | | | |
| -very slight | 24 | 23 | 28 | 29 |
| -slight | 13 | 11 | 4 | 1 |
| -moderate | 0 | 1 | 0 | 0 |
| -any | 37 | 35 | 32 | 30 |
| Focus of altered cells – eosinophilic, hepatocellular | | | | |
| -very slight | 29 | 25 | 18 | 11 |
| -slight | 3 | 11 | 23 | 24 |
| -moderate | 0 | 0 | 2 | 1 |
| -any | 32 | 36 | 43 | 36 |
| Females - # animals evaluated | 50 | 50 | 50 | 50 |
| Focus of altered cells – eosinophilic, hepatocellular | | | | |
| -very slight | 12 | 24 | 20 | 32 |
| -slight | 0 | 3 | 3 | 1 |
| -moderate | 0 | 0 | 0 | 0 |
| -any | 12 | 27 | 23 | 33 |
| Hyperplasia – Often accompanied by chronic inflammation, bile duct (s) | | | | |
| -very slight | 39 | 35 | 27 | 31 |
| -slight | 5 | 9 | 18 | 11 |
| -moderate | 0 | 0 | 1 | 3 |
| -any | 44 | 44 | 46 | 45 |

a Data obtained from pages 1624-1664 and 2395-2414, MRID 49889501.

### (ii) Uterine/Cervical Tumors in Female Rats

In the upper uterus, female rats had statistically significant trends at $p < 0.01$, and significant pair-wise comparisons of the high dose group with the controls at $p < 0.05$, for (1) uterine endometrial stromal polyps; and (2) endometrial stromal polyps, endometrial stromal sarcomas, leiomyosarcomas and undifferentiated sarcomas combined; The statistical analyses of the tumors in the upper uterus of female rats were based upon Fisher's Exact Test and the Exact Test for Trend (Tables 6-8).

For female rats, cervical tumors included endometrial stromal polyps (0/58, 0/15, 0/19, 1/59), endometrial stromal sarcomas (0/58, 0/15, 1/19, 0/59), and leiomyosarcomas (0/58, 0/15, 1/19, 1/59). However, not all the animals were examined for the cervix in the low- and mid-dose groups. Therefore, the cervical tumors were not combined with the uterine tumors.

Historical control data from nine studies from 1991-1997 indicated a control range of 18-46% for endometrial stromal polyps in female Fischer344 rats.

Historical control data from the same studies indicated a control range of 0-2% for the following tumor types: stromal cell sarcomas, leiomyosarcomas, undifferentiated sarcomas, and polypoid adenomas.

Historical control data indicated a control range of 0-6% for carcinomas.

Historical control data indicated a control range of 0-8.2% for adenocarcinomas.

Table 6. Telone – Fischer 344 Rat Study (Stott *et al.* 1995)
(MRID No. 43763501)

Female Uterine Sarcoma and Polyp Tumor Rates[+] and
Fisher's Exact Test and Exact Trend Test Results
Dose (mg/kg/day)

| | 0 | 2 | 10 | 25 |
|---|---|---|---|---|
| Endometrial Stromal Polyps (%) | 15[a]/59 (25) | 13/60 (22) | 13[a]/60 (22) | 27[a]/60 (45) |
| P = | 0.0032** | 0.7576 | 0.7576 | 0.0202* |
| Endometrial Stromal Sarcomas (%) | 1/59 (2) | 0/60 (0) | 2[b]/60 (3) | 0/60 (0) |
| P = | 0.3558 | 1.0000 | 0.5064 | 1.0000 |
| Leiomyosarcomas (%) | 0/59 (0) | 0/60 (0) | 0/60 (0) | 2[c]/60 (3) |
| P = | 0.0622 | 1.0000 | 1.0000 | 0.2521 |
| Undifferentiated Sarcomas (%) | 0/59 (0) | 1[d]/60 (2) | 0/60 (0) | 0/60 (0) |
| P = | 0.4979 | 0.5042 | 1.0000 | 1.0000 |
| Combined (%) | 16/59 (27) | 14/60 (23) | 14[e]/60 (23) | 29/60 (48) |
| P = | 0.0020** | 0.7537 | 0.7537 | 0.0137* |

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.

[a]First endometrial stromal polyps observed simultaneously at the interim sacrifice in the control, 10 and 25 mg/kg/day dose groups.
[b]First endometrial stromal sarcoma observed at week 104 in the 10 mg/kg/day dose group.
[c]First leiomyosarcoma observed at week 66 in the 25 mg/kg/day dose group.
[d]First undifferentiated sarcoma observed at week 97 in the 2.5 mg/kg/day dose group.
[e]One animal in the 10 mg/kg/day dose group had both an endometrial stromal polyp and an endometrial stromal sarcoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.



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Table 7. Telone – Fischer 344 Stott 1995 Rat Study
(MRID No. 43763501)

Female Uterine Adenoma and Carcinoma Rates[+] and
Fisher's Exact Test and Exact Trend Test Results

| | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2 | 10 | 25 |
| Polypoid Adenomas (%) | 0/59 (0) | 0/60 (0) | 0/60 (0) | 1[a]/60 (2) |
| P = | 0.2510 | 1.0000 | 1.0000 | 0.5042 |
| Carcinomas (%) | 1/59 (2) | 0/60 (0) | 2[b]/60 (3) | 0/60 (0) |
| P = | 0.3558 | 1.0000 | 0.5064 | 1.0000 |
| Adenocarcinomas (%) | 0/59 (0) | 1[c]/60 (2) | 0/60 (0) | 2/60 (3) |
| P = | 0.1098 | 0.5042 | 1.0000 | 0.2521 |
| Combined (%) | 1/59 (2) | 1/60 (2) | 2/60 (3) | 3/60 (5) |
| P = | 0.1269 | 0.7563 | 0.5064 | 0.3157 |

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.

[a]First polypoid adenoma observed at week 105 in the 25 mg/kg/day dose group.
[b]First carcinoma observed at week 95 in the 10 mg/kg/day dose group.
[c]First adenocarcinoma observed at week 84 in the 2 mg/kg/day dose group.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If $^{*}$, then $p < 0.05$. If $^{**}$, then $p < 0.01$.



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Table 8. Telone – Fischer 344 Stott 1995 Rat Study
(MRID No. 43763501)

Female Cervical Tumor Rates[+] and
Fisher's Exact Test and Exact Trend Test Results

| | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2[#] | 10[#] | 25 |
| Endometrial Stromal Polyps (%) | 0/58 (0) | 0/15 (0) | 0/19 (0) | 1[a]/59 (2) |
| P = | 0.3907 | 1.0000 | 1.0000 | 0.5043 |
| Endometrial Stromal Sarcomas (%) | 0/58 (0) | 0/15 (0) | 1[b]/19 (5) | 0/59 (0) |
| P = | 0.5166 | 1.0000 | 0.2468 | 1.0000 |
| Leiomyosarcomas (%) | 0/58 (0) | 0/15 (0) | 1[c]/19 (5) | 1/59 (2) |
| P = | 0.2501 | 1.0000 | 0.2468 | 0.5043 |
| Combined (%) | 0/58 (0) | 0/15 (0) | 2/19 (11) | 2/59 (3) |
| P = | 0.1796 | 1.0000 | 0.0584 | 0.2521 |

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.

[#]Not all animals were examined in the 2 and 10 mg/kg/day dose groups.

[a]First endometrial stromal polyp observed at week 105 in the 25 mg/kg/day dose group.
[b]First endometrial stromal sarcoma observed at week 76 in the 10 mg/kg/day dose group.
[c]First leiomyosarcoma observed at week 70 in the 10 mg/kg/day dose group.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

Non-Neoplastic Lesions in the Uterus: There were no treatment-related increases in the incidence of non-neoplastic lesions in the uterus.



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**CARC Conclusions on the Tumor Findings in the Rat (Dietary Administration).** The CARC concluded that the liver adenomas in male rats are considered to be treatment-related. This is based on a statistically significant ($p<0.05$) pairwise increase in hepatocellular adenomas observed at the high dose (25 mg/kg/day). Although there was little convincing evidence of pre-neoplastic lesions in the liver, the incidence of adenomas (19%) at the high does exceeded the historical control range (0-16%) for this tumor type and strain of rat. The incidence of hepatocellular adenomas in female rats was not considered treatment-related. The incidence at the high dose (4%) was within the expected historical control range (0-4%). The CARC concluded that there were no treatment-related uterine tumors in the rat. There was a high incidence of uterine polyps at the high dose; however, this is a common finding in this strain of rat and the incidence at the high dose fell within expected historical control range. There were no treatment-related adenomas, carcinomas or sarcomas seen in the uterus and there were no treatment-related findings pre-neoplastic or supporting non-neoplastic lesions seen in the uterus.

**Registrant's argument for exceedance of maximum tolerated dose (MTD) in high-dose male rats.** The Registrant proposed that the decreased mean body weight gains for the 25 mg/kg/day males and females (average reductions of 19% and 17% in males and females, respectively) indicated that the maximum tolerated dose (MTD) was exceeded. Therefore, the significant increased incidence of liver tumor in high-dose males (18%) only occurred at a dose exceeding the MTD at which excessive toxicity was evident.

**CARC conclusions on the adequacy of dosing**
The CARC considered the doses used in the male and female rat dietary carcinogenicity studies to be adequate and not excessive. This is based on statistically significant ($p<0.05$) lower group mean body weights in males at 12.5 and 25 mg/kg/day throughout the study. Terminal absolute body weights for males and females in the 25 mg/kg/day group were decreased relative to the control animals by 13% and 14%, respectively. Histopathology revealed a treatment-related increase in the incidence of histopathological findings in the nonglandular mucosa of the stomach (basal cell hyperplasia) at both the interim sacrifice and the main study animals. For females, there were also treatment-related findings in the liver (very slight to slight: increased incidences of eosinophilic focus of altered cells in both sexes and hyperplasia). These findings were not considered to be excessive for assessing carcinogenic potential in male or female rats.

### B. Combined Chronic Toxicity/Carcinogenicity Study in Sprague Dawley Rats - Gavage

Citation: Kelly, CM. (1998) A chronic toxicity and oncogenicity study with DD-92 in the rat via oral gavage administration. Huntingdon Life Sciences (East Millstone, NJ). Laboratory Project ID 95-2379, January 21, 1998. MRID 49889501. Unpublished.

**Experimental Design:**

In a combined chronic toxicity/carcinogenicity study (MRID 49889501), DD-92 (94.8% a.i., Lot # AC 8879-141; 1,3-D) was administered in corn oil by oral gavage to 75 Sprague-

Dawley rats, /sex/dose at dose levels of 0, 2, 10, or 25 mg/kg/day for up to 24 months (due to problems with survivorship in the low-dose males, all surviving males were necropsied after 23 months of treatment). Up to 10 animals/sex/dose were sacrificed after 12 months for interim evaluations. The test article in this study did not contain epichlorohydrin.

**Survival:**

Animal survival was low for the treatment groups, including for the control female animals. The percent survival of the control, LDT, MDT, or HDT for the males were 34, 17, 32, or 22% and for the females were 24, 28, 31, or 38%.

**Discussion of Tumor Data:**

There were no treatment-related tumors in male or female rats.

**Non-Neoplastic Lesions:**

As reported in Table 9, there were some dose-dependent increases in non-neoplastic lesions in the forestomach. The lesions were only observed at the interim sacrifice.

**Table 9: Incidence and severity of non-neoplastic microscopic findings in the stomach of male and female rats[ab]**

| Parameter: | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2 | 10 | 25 |
| Interim Sacrifice: 12 Months | | | | |
| Males - # animals evaluated | 9 | 10 | 9 | 9 |
| Forestomach: Squamous cell hyperplasia | | | | |
| -minimal | 2 | 0 | 3 | 7 |
| -slight | 1 | 0 | 2 | 0 |
| -any | 3 | 0 | 5 | 7 |
| Forestomach: Hyperkeratosis | | | | |
| -minimal | 3 | 0 | 4 | 6 |
| -slight | 0 | 0 | 1 | 2 |
| -any | 3 | 0 | 5 | 8 |
| Females - # animals evaluated | 7 | 10 | 10 | 9 |
| Forestomach: Squamous cell hyperplasia | | | | |
| minimal | 0 | 1 | 2 | 3 |
| -slight | 1 | 0 | 2 | 5 |
| -any | 1 | 1 | 4 | 8 |
| Forestomach: Hyperkeratosis | | | | |
| minimal | 1 | 1 | 3 | 3 |
| -slight | 0 | 0 | 1 | 5 |
| -any | 1 | 1 | 4 | 8 |

a Data obtained from pages 2395-2414, MRID 49889501.



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**Adequacy of Dosing for Assessment of Carcinogenicity:**

The percent survival of the control, low-, mid-, or high-dose males were 34, 17, 32, or 22%, respectively; and 24, 28, 31, or 38%, respectively for females.

Mortality did not appear to be attributed to treatment. There was no adverse effect on absolute body weight for either sex under the conditions of the study. For males in the high dose group, there was a maximum decrease in mean absolute body weight at week 72 (9%) and body weight gain for weeks 0-72 was decreased 11% compared to the control. There were no treatment-related impacts on body weight or body weight gain for females, even at the highest dose tested. There were no adverse effects on clinical chemistry or hematological parameters. There was evidence of a dose-related increase in hyperplasia in the forestomach in both sexes. Although the animals could have tolerated higher doses, the bodyweight decrements in males and the evidence of forestomach lesions in both sexes support that the doses used in the study were adequate to assess carcinogenicity.

**C. Carcinogenicity Study in B6C3F1 Mice - Dietary**

Citation: Redmond, JM, Stebbins, KE, Stott, WT. (1995) Telone® II Soil Fumigant: Two-year dietary chronic toxicity/oncogenicity study in B6C3F1 mice- Final report. Dow Chemical Co. (Midland, Michigan). Laboratory Project ID M-003993-032, August 9, 1995. MRID 43757901. Unpublished.

**Experimental Design:**

In a carcinogenicity study (MRID 43757901), TELONE II (95.8% purity, 50.7% cis and 45.1% trans) as a microencapsulated formulation was administered by dietary admix to 50 B6C3F1 mice /sex/group for 2 years at doses of 0, 2.5, 25, or 50 mg/kg/day (achieved doses were 0/0, 2.7/2.5, 27/26, or 53/51 mg/kg/day [M/F]). An interim sacrifice group (10/sex/dose level) was necropsied following a 12-month dosing period.

**Survival Analysis:**

There were no treatment-related survival disparities in male or female mice

**Discussion of Tumor Data:**

There were no treatment-related tumors in male or female mice.

**Non-Neoplastic Lesions:**
There were no relevant treatment-related increases in the incidence of non-neoplastic lesions in any of the groups in male or female mice.

**Adequacy of Dosing for Assessment of Carcinogenicity:**

There was a statistically significant decrease in absolute body weights for the male and

female mice treated with 25 or 50 mg/kg/day of 1,3-D. Compared to the control animals, body weights of 25 and 50 mg/kg/day males were decreased 11% and 23%, respectively. There was an accompanying decrease in food consumption at the same doses. For females, the 25 and 50 mg/kg/day mice had group mean body weights about 7-9% below the control during most of the study, for a few weeks. While the animals could have tolerated a higher dose, the CARC concluded that the doses evaluated are adequate to be used in a weight of evidence evaluation of the carcinogenic potential of 1,3-D.

### D. Carcinogenicity Study in CD Mice - Gavage

Citation: Kelly, CM. (1997) An oncogenicity study with DD-92 in the mouse via oral gavage administration. Huntingdon Life Sciences (East Millstone, New Jersey). Laboratory Project ID 95-2378, December 17, 1997. MRID 49889502. Unpublished.

**Experimental Design:**

In a carcinogenicity study (MRID 49889502), DD-92 (1,3-D); (94.8% a.i.; Lot No. AC 8879-141) was administered in corn oil by oral gavage to 65 CD-1 mice /sex/dose by oral gavage at dose levels of 0, 2, 10, or 25 mg/kg/day for up to 79 weeks.

**Survival Analysis:**

There were no treatment-related survival disparities in male or female mice.

**Discussion of Tumor Data:**

There were no treatment-related increases in tumor incidences in male or female mice.

**Non-Neoplastic Lesions:**

The only clear treatment-related histopathological findings were in the urinary bladder of the 25 mg/kg/day females (Table 10).



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| Table 10: Incidence and severity of non-neoplastic microscopic findings in female mice | | | | |
|---|---|---|---|---|
| Parameter: | Dose (mg/kg/day) | | | |
| | 0 | 2 | 10 | 25 |
| Terminal Sacrifice: 24 Months | | | | |
| Females - # animals (severity) | 50 | 42 | 43 | 41 |
| Urinary Bladder | 50 | 41 | 42 | 41 |
| Hyaline change | | | | |
| -minimal | 1 | 0 | 0 | 4 |
| -slight | 0 | 0 | 1 | 10 |
| -moderate | 0 | 0 | 1 | 9 |
| -any | 1 | 0 | 2 | 23 |
| Urothelium (transitional epithelium): Hyperplasia | | | | |
| -minimal | 0 | 0 | 0 | 4 |
| -slight | 0 | 0 | 2 | 16 |
| -moderate | 0 | 0 | 1 | 9 |
| -any | 0 | 0 | 3 | 29 |
| Subacute (chronic active)/Chronic inflammation | | | | |
| -minimal | 0 | 0 | 2 | 9 |
| -slight | 0 | 0 | 1 | 15 |
| -moderate | 0 | 0 | 1 | 4 |
| -moderately severe (marked) | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 4 | 29 |
| Lymphoid cell infiltrate/aggregate(s) | | | | |
| -minimal | 25 | 17 | 20 | 11 |
| -slight | 5 | 5 | 8 | 12 |
| -moderate | 0 | 0 | 1 | 3 |
| -moderately severe (marked) | 0 | 0 | 1 | 0 |
| -any | 30 | 22 | 30 | 26 |
| Stromal hyperplasia | | | | |
| -minimal | 0 | 0 | 0 | 1 |
| -slight | 0 | 0 | 0 | 4 |
| -moderate | 0 | 0 | 0 | 3 |
| -moderately severe (marked) | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 9 |
| Stromal hyperplasia | | | | |
| -minimal | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 1 |
| Reticuloendothelial cells: brown pigment accumulation | | | | |
| -slight | 0 | 0 | 0 | 5 |
| -any | 0 | 0 | 0 | 5 |
| Erosion(s)/ulcer(s) | | | | |
| -slight | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 1 |
| Urothelium: squamous/ squamoid metaplasia | | | | |
| -slight | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 1 |
| Urothelium: intracytoplasmic eosinophilic inclusions | | | | |
| minimal | 0 | 0 | 0 | 2 |
| -slight | 0 | 0 | 0 | 0 |
| -moderate | 0 | 0 | 1 | 0 |
| -any | 0 | 0 | 1 | 2 |



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**Adequacy of Dosing for Assessment of Carcinogenicity:**

There were no treatment-related effects on mortality/survival; clinical signs; body weight or body weight gain; food consumption or efficiency; hematology parameters; organ weights; or macroscopic pathology findings. Treatment-related microscopic findings were only observed in the urinary bladders of the 25 mg/kg/day female mice. The doses tested in this study are considered to be adequate and not excessive to assess the carcinogenicity of 1,3-D.

## E. Chronic Toxicity/Carcinogenicity Study in Fischer 344 Rats - Inhalation

Citations: Lomax, LG, Calhoun, LL, Stott, WT, et al. (1987). Telone II Soil Fumigant: Two-Year Inhalation Chronic Toxicity/Oncogenicity Study in Rats. Dow Chemical USA (Midland, MI). Lab Project No. M-003993-009R. July 13, 1987. MRID 40312201. Unpublished.

**Experimental Design:**

In a chronic/carcinogenicity study (MRID 40312201), TELONE II (92.1% purity, 49.5% cis and 42.6% trans isomers, Lot No TB831213-4) was administered by whole-body inhalation to 70 Fischer 344 rats /sex/group at doses of 0, 5, 20, or 60 ppm (achieved doses were 0, 4.6, 18.4, or 55.2 ppm) for 6 hours/day, 5 days/week for a total of 509 days of exposure in 2 years. These doses converted to mg/kg/day based on an average 400 g rat were approximately 0, 2.68, 10.73, or 32.20 mg/kg/day, respectively. Ten rats/sex/exposure level were randomly predesignated to the 6- and 12-month interim sacrifice groups. The test article in this study did not contain epichlorohydrin.

**Survival Analysis:**

There were no compound related effects on mortality.

**Discussion of Tumor Data:**

Under the conditions of the study, 1,3-D did not produce evidence of compound related increased incidences of any tumor types examined.

**Non-Neoplastic Lesions**

The only non-neoplastic findings in the main study animals were in the nasal tissues (Table 11). High-dose males and females showed decreased thickness and erosion of the olfactory epithelium as well as minimal submucosal fibrosis. There were no histopathological findings in the nasal tissues for male or females of the interim sacrifice groups at 6- or 12-months.

| Table 11: Incidence and severity of non-neoplastic microscopic findings in male and female rats[ab] | | | | |
|---|---|---|---|---|
| Parameter: | Dose (ppm) | | | |
| | 0 | 5 | 20 | 60 |
| Main Study: 24 Months | | | | |
| Males -# animals (severity) | 50 | 50 | 50 | 50 |
| Nasal Tissues | 50 | 50 | 50 | 50 |
| Decreased thickness, olfactory epithelium, unilateral or bilateral | | | | |
| -slight | 0 | 1 | 0 | 2 |
| -moderate | 0 | 0 | 1 | 12 |
| -severe | 0 | 0 | 0 | 3 |
| -any | 0 | 1 | 1 | 20 |
| Erosion(s), olfactory epithelium, unilateral, multifocal | | | | |
| -slight | 0 | 0 | 0 | 2 |
| -moderate | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 3 |
| Erosion(s), olfactory epithelium, bilateral, multifocal | | | | |
| -slight | 0 | 0 | 1 | 3 |
| -moderate | 0 | 0 | 0 | 7 |
| -severe | 0 | 0 | 0 | 2 |
| -any | 0 | 0 | 1 | 12 |
| Fibrosis, submucosa, unilateral or bilateral, multifocal | | | | |
| -slight | 0 | 0 | 0 | 3 |
| -moderate | 0 | 0 | 0 | 3 |
| -any | 0 | 0 | 0 | 6 |
| Females - # animals (severity) | 50 | 50 | 50 | 50 |
| Nasal Tissues | 50 | 50 | 50 | 50 |
| Decreased thickness, olfactory epithelium, unilateral or bilateral | | | | |
| -slight | 0 | 0 | 0 | 8 |
| -moderate | 0 | 0 | 0 | 7 |
| -any | 0 | 0 | 0 | 15 |
| Degeneration, olfactory epithelium, bilateral, multifocal | | | | |
| -moderate | 0 | 0 | 0 | 2 |
| -any | 0 | 0 | 0 | 2 |
| Erosion(s), olfactory epithelium, unilateral, multifocal | | | | |
| -slight | 0 | 0 | 0 | 1 |
| -moderate | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 2 |
| Erosion(s), olfactory epithelium, bilateral, multifocal | | | | |
| -slight | 0 | 0 | 0 | 3 |
| -moderate | 0 | 0 | 0 | 0 |
| -severe | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 4 |
| Fibrosis, submucosa, unilateral or bilateral, multifocal | | | | |
| -slight | 0 | 0 | 0 | 1 |
| -moderate | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 2 |



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**Adequacy of the Dosing for Assessment of Carcinogenicity:**

During a previous review (K. Dearfield, 08-DEC-1989, TXR 0056176), the HED Peer Review Committee agreed that there was no evidence of carcinogenicity in this Fischer 344 rat study; however, the Committee concluded that the study did not test up to adequately high doses to assess the carcinogenic potential. There were no compound related effects on mortality, clinical signs, organ weights, urinalysis, or hematology. Slight (approximately 5% in 60 ppm males and females) decreases in body weight gain were observed during the first year of study, but weights were similar to controls for the remainder of the study. Animals exposed at the 60 ppm dose (not lower doses) showed decreased thickness of the olfactory epithelium of the nasal cavity (40% incidence in males, 31% in females) as well as minimal submucosal fibrosis; however, the nasal histopathology findings were not considered to be significant enough to be of real toxicological concern or adequate for assessing oncogenic potential. The CARC agreed with the previous assessment that animals could have tolerated testing at a higher dose; however, the committee concluded that the doses evaluated are adequate to be used in a WOE evaluation of the carcinogenic potential of 1,3-D.

## F. Chronic Toxicity/Carcinogenicity Study in B6C3F1 Mice - Inhalation

Citations: Stott, WT, Johnson, KA, Calhoun, LL, et al. (1987). Telone II Soil Fumigant: Two-Year Inhalation Chronic Toxicity/Oncogenicity Study in Mice. Dow Chemical USA (Midland, MI). Lab Project No. M-003993-009. July 13, 1987. MRID 40312301. Unpublished.

**Experimental Design:**

In a chronic/carcinogenicity study (MRID 40312301), TELONE II (92.1% purity, 49.5% cis and 42.6% trans isomers, Lot No TB831213-4) was administered by whole-body inhalation to 50 B6C3F1 mice /sex/group at doses of 0, 5, 20, or 60 ppm (achieved doses were 0, 4.6, 18.4, or 55.2 ppm) for 6 hours/day, 5 days/week for a total of 510 days of exposure in 2 years. These doses converted to mg/kg/day based on an average 30 g rat were approximately 0, 6.88, 27.51, or 82.54 mg/kg/day, respectively. An additional 10 mice/sex/exposure level were randomly predesignated to the 6- and 12-month interim sacrifice groups.

**Survival Analysis:**

As previously reviewed (K. Dearfield, 08-DEC-1989, TXR 0056176), there were no statistically significant effects on mortality for male or female mice after inhalation of 1,3-D.

**Discussion of Tumor Data:**

**(i) Bronchioloalveolar Tumors in Male Mice**

*The following information is summarized from the second Peer Review Committee meeting (August 23, 1989) report (K. Dearfield, 08-DEC-1989, TXR 0056176).*

1,3-D was associated with a significant positive dose-related trend in benign lung bronchioloalveolar adenomas in male mice. A pair-wise comparison at the top dose, 60 ppm, showed a significant ($p<0.05$) increase of bronchioloalveolar adenomas from control (22/60 animals (37%) versus control of 9/57 animals (16%)). Historical control data from two chronic inhalation studies from 1994 and 1998 reported control incidences 20% and 18%, respectively. The incidence of lung adenomas in male mice in this study was outside the historical range. At 20 ppm, the tumor incidence (13/49 animals; 27%) was above the concurrent control and outside of the historical control range, however, the pair-wise comparison was not statistically significant.

Table 12. 1,3-D – B6C3F1 Mouse Inhalation Study
(MRID No. 40312301)
Male Bronchioloalveolar Tumor Rates

| | Dose (ppm) | | | |
|---|---|---|---|---|
| | 0 | 5 | 20 | 60 |
| Adenomas | 9/57 | 6/51 | 13/49 | 22/60 |
| (%) | (16) | (12) | (27) | (37) |
| P= | 0.001** | 0.374 | 0.132 | 0.009** |

**=p<0.01

**Non-Neoplastic Lesions**

Tables 13-17 provide summaries of the gross and histopathological findings at the interim sacrifices.

There were no treatment-related histopathological findings in the lung. Treatment related histopathological findings in the respiratory tract included hypertrophy and hyperplasia of respiratory (nasal) epithelium in mid-dose females and high-dose males and female, and degeneration of olfactory epithelium in high-dose males and female (Tables 13, 15, and 17).

At the 12-month and 24-month sacrifices, there were treatment-related increases in gross findings in the urinary bladder. At the 12-month sacrifice, high-dose females were found to have thickened mucosa, diffuse (Table 14). At the terminal sacrifice, the mid-dose females and high-dose males had urinary bladders that had a roughened, irregular and opaque surface (Table 16). At the terminal sacrifice, mid-dose females and high-dose males and females also showed a treatment-related increase in hyperplasia of the transitional epithelium of the urinary bladder (Table 17). Similar effects were also observed in high-dose females at the 6- and 12-month sacrifice (Tables 13 and 15).

The only other findings at the interim and terminal sacrifices included increased hyperplasia of nonglandular stomach in 60 ppm males, decreased vacuolation of kidney tubular epithelial cells in 60 ppm males, and decreased vacuolation of liver cells in 60 ppm females (Tables 13,



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15, and 17).

| Table 13: Incidence and severity of non-neoplastic microscopic findings in male and female mice | | | | |
|---|---|---|---|---|
| Parameter: | Dose (ppm) | | | |
| | 0 | 5 | 20 | 60 |
| Interim Sacrifice: 6 Months | | | | |
| Males -# animals | 10 | 10 | 10 | 10 |
| Nasal Tissues, # animals evaluated | 10 | 10 | 10 | 10 |
| Hypertrophy and hyperplasia, respiratory epithelium mucosa, focal | | | | |
| -very slight | 1 | 0 | 3 | 2 |
| -slight | 0 | 0 | 0 | 8 |
| -any | 1 | 0 | 3 | 10 |
| Urinary Bladder, # animals evaluated | 10 | 10 | 10 | 9 |
| Hyperplasia, epithelial cells | | | | |
| -moderate | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 1 |
| Inflammation, subacute to chronic | | | | |
| -very slight | 0 | 0 | 0 | 0 |
| -slight | 0 | 0 | 0 | 1 |
| -moderate | 0 | 0 | 0 | 0 |
| -any | 0 | 0 | 0 | 1 |
| Liver, # animals evaluated | 10 | 10 | 10 | 10 |
| Vacuolation – decreased, hepatocellular | | | | |
| -slight | 1 | 0 | 0 | 7 |
| Kidney, # animals evaluated | 10 | 10 | 10 | 10 |
| Vacuolation - decreased, convoluted tubule - proximal | | | | |
| -slight | 4 | 1 | 2 | 9 |
| Females - # animals | 10 | 10 | 10 | 10 |
| Nasal Tissues, # animals evaluated | 10 | 10 | 10 | 10 |
| Hypertrophy and hyperplasia, respiratory epithelium mucosa, focal | | | | |
| -very slight | 0 | 0 | 0 | 7 |
| -any | 0 | 0 | 0 | 7 |
| Urinary Bladder, # animals evaluated | 10 | 10 | 10 | 10 |
| Hyperplasia, epithelial cells | | | | |
| -moderate | 0 | 0 | 0 | 4 |
| -any | 0 | 0 | 0 | 4 |
| Inflammation, subacute to chronic | | | | |
| very slight | 0 | 0 | 0 | 1 |
| -slight | 0 | 0 | 0 | 0 |
| -moderate | 0 | 0 | 0 | 1 |
| -any | 0 | 0 | 0 | 2 |



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| Table 14: Incidence and severity of gross findings in male and female mice | | | | |
|---|---|---|---|---|
| Parameter: | Dose (ppm) | | | |
| | 0 | 5 | 20 | 60 |
| Interim Sacrifice: 12 Months | | | | |
| Females - # animals | 10 | 10 | 10 | 10 |
| Urinary Bladder, # animals evaluated | | | | |
| Thickened, Mucosa, Diffuse | 0 | 1 | 1 | 3 |
| Focus – Pale Elevated, Mucosa, Lateral, Focal | 1 | 1 | 4 | 0 |

| Table 15: Incidence and severity of non-neoplastic microscopic findings in male and female mice | | | | |
|---|---|---|---|---|
| Parameter: | Dose (ppm) | | | |
| | 0 | 5 | 20 | 60 |
| Interim Sacrifice: 12 Months | | | | |
| Males -# animals | 10 | 10 | 10 | 10 |
| Nasal Tissues, # animals evaluated | 10 | 10 | 10 | 10 |
| Hypertrophy and hyperplasia, respiratory epithelium mucosa, focal | | | | |
| -very slight | 1 | 0 | 7 | 3 |
| -slight | 0 | 0 | 0 | 7 |
| -any | 1 | 0 | 7 | 10 |
| Liver | 10 | 10 | 10 | 10 |
| Vacuolation – decreased, hepatocellular | | | | |
| -slight | 0 | 0 | 0 | 5 |
| -severe | 1 | 0 | 1 | 0 |
| -any | 1 | 0 | 1 | 5 |
| Kidney | 10 | 10 | 10 | 10 |
| Vacuolation – Decreased, convoluted tubule – proximal | | | | |
| -slight | 1 | 0 | 0 | 4 |
| -moderate | 0 | 0 | 0 | 5 |
| -any | 1 | 0 | 0 | 9 |
| Females - # animals | 10 | 10 | 10 | 10 |
| Nasal Tissues, # animals evaluated | 10 | 10 | 10 | 10 |
| Hypertrophy and hyperplasia, respiratory epithelium mucosa, focal | | | | |
| -very slight | 0 | 0 | 0 | 8 |
| -any | 0 | 0 | 0 | 8 |
| Urinary Bladder, # animals evaluated | 10 | 10 | 10 | 10 |
| Hyperplasia, epithelial cells | | | | |
| -slight | 0 | 0 | 1 | 5 |
| -moderate | 0 | 0 | 0 | 4 |
| -any | 0 | 0 | 1 | 9 |
| Inflammation, subacute to chronic | | | | |
| very slight | 0 | 0 | 0 | 1 |
| -slight | 0 | 0 | 0 | 3 |
| -any | 0 | 0 | 0 | 4 |



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| Table 16: Incidence and severity of gross findings in male and female mice | | | | |
|---|---|---|---|---|
| Parameter: | Dose (ppm) | | | |
| | 0 | 5 | 20 | 60 |
| Main Study: 24 Months | | | | |
| Males -# animals | 50 | 50 | 50 | 50 |
| Urinary Bladder, # animals evaluated | 50 | 50 | 50 | 50 |
| Roughened, Irregular and opaque surface | | | | |
| -slight | 0 | 1 | 0 | 2 |
| -moderate | 0 | 0 | 0 | 3 |
| -marked | 0 | 0 | 0 | 1 |
| -any | 0 | 1 | 0 | 6 |
| Females - # animals | 50 | 50 | 50 | 50 |
| Urinary Bladder, # animals evaluated | 50 | 50 | 50 | 50 |
| Roughened, Irregular and opaque surface | | | | |
| -slight | 3 | 4 | 7 | 14 |
| -moderate | 0 | 1 | 11 | 14 |
| -marked | 0 | 0 | 2 | 2 |
| -any | 3 | 5 | 20 | 30 |

| Table 17: Incidence and severity of non-neoplastic microscopic findings in male and female mice | | | | |
|---|---|---|---|---|
| Parameter: | Dose (ppm) | | | |
| | 0 | 5 | 20 | 60 |
| Main Study: 24 Months | | | | |
| Males -# animals | 50 | 50 | 50 | 50 |
| Nasal Tissues, # animals evaluated | 50 | 50 | 50 | 50 |
| Degeneration, olfactory, epithelium, bilateral | | | | |
| -very slight | 1 | 0 | 1 | 32* |
| -slight | 0 | 0 | 0 | 16* |
| -any | 1 | 0 | 1 | 48* |
| Hyperplasia and hypertrophy, respiratory epithelium, bilateral | | | | |
| -very slight | 5 | 1 | 4 | 38* |
| -slight | 0 | 0 | 0 | 10* |
| -any | 5 | 1 | 4 | 48* |
| Urinary Bladder, # animals evaluated | 50 | 50 | 50 | 50 |
| Hyperplasia, mucosa | | | | |
| -very slight | 4 | 7 | 7 | 16*T |
| -slight | 0 | 0 | 3 | 18* |
| -moderate | 0 | 0 | 0 | 2 |
| -any | 4 | 7 | 10 | 36 |
| Hyperplasia, mucosa (simple or nodular) | | | | |
| -any | 4 | 7 | 11 | 37* |
| Kidney, # animals evaluated | 50 | 50 | 50 | 50 |
| Vacuolation – Decreased, convoluted tubule - proximal | 9 | 8 | 8 | 29 |
| Stomach, # animals evaluated | 50 | 50 | 50 | 50 |
| Hyperplasia, often accompanied by choric inflammation, nonglandular mucosa, multifocal or focal | 0 | 3 | 1 | 8 |



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| Table 17: Incidence and severity of non-neoplastic microscopic findings in male and female mice | | | | |
|---|---|---|---|---|
| Parameter: | Dose (ppm) | | | |
| | 0 | 5 | 20 | 60 |
| Females - # animals | 50 | 50 | 50 | 50 |
| Nasal Tissues, # animals evaluated | 50 | 50 | 50 | 50 |
| Degeneration, olfactory, epithelium, bilateral | | | | |
| -very slight | 0 | 0 | 1 | 29* |
| -slight | 0 | 0 | 0 | 16* |
| -any | 0 | 0 | 1 | 45* |
| Hyperplasia and hypertrophy, respiratory epithelium, bilateral | | | | |
| -very slight | 4 | 4 | 28* | 39*T |
| -slight | 0 | 0 | 0 | 10* |
| -any | 4 | 4 | 28* | 49* |
| Urinary Bladder, # animals evaluated | 50 | 50 | 50 | 50 |
| Hyperplasia, mucosa | | | | |
| -very slight | 1 | 3 | 13* | 5 |
| -slight | 0 | 1 | 6* | 18*T |
| -moderate | 0 | 0 | 0 | 19* |
| -any | 1 | 4 | 19 | 32 |
| Hyperplasia, mucosa (simple or nodular) | | | | |
| -any | 1 | 4 | 21* | 44* |
| Inflammation – Chronic or Chronic Active | 0 | 1 | 6* | 8*T |
| Liver, # animals evaluated | 50 | 49 | 50 | 50 |
| Vacuolation – Decreased Hepatocellular, diffuse | 10 | 9 | 11 | 24 |

CARC concluded that the benign lung adenomas in male mice are treatment-related based on a statically significant pair-wise ($P<0.05$) comparison at the top concentration of 60 ppm (22/60 animals (37%) versus control of 9/57 animals (16%)). The incidence at the high concentration exceeded the historical control ranges for this strain of male mice.

**Adequacy of the Dosing for Assessment of Carcinogenicity:**

There were no statistically significant effects on mortality on male and female mice after inhalation of 1,3-D. Decreases in body weight gain were seen (3-9% in 60 ppm males and 2-11% in 60 ppm females: although the approximate 10% values at the high end of the range may be approaching adequate decrements). Non-neoplastic histopathology indicated some hyperplasia of the transitional epithelium of the urinary bladder in high dose males and females (lesser effect at 20 ppm), hypertrophy and hyperplasia of respiratory epithelium and degeneration of olfactory epithelium in high dose males and females (considered to be of minimal severity: lesser effect at 20 ppm females), hyperplasia of nonglandular stomach in 60 ppm males only and decreased vacuolation of kidney tubular epithelial cells in high dose males and of liver cells in high dose females. The CARC considered the doses adequate to assess the carcinogenic potential of 1,3-D based on toxicological endpoints. However, the likelihood that the highest test concentration in male mice exceeded a kinetically derived maximum tolerated dose (KMD) is discussed below.

**Exceedance of a kinetically derived maximum tolerated dose (KMD) at the tumorigenic test concentration in male mice.**

The registrants position paper argues that the increase in benign bronchioloalveolar adenomas observed in male mice was the result of nonlinear kinetics by the inhalation route. The data submitted to support this argument are presented and reviewed below:

**1,3-Dichloropropene: Inhalation, Absorption and Systemic Bioavailability in B6C3F1 Mice (MRID 49889504)**

An acute inhalation study (MRID 49889504) was conducted to determine the respiratory response and systemic absorption / bioavailability of inhaled 1,3-D in male B6C3F1 mice after a single, acute inhalation exposure. In the study, male B6C3F1 mice (n = 4/concentration) were administered 1,3-D vapors (97.5% a.i.; 48.1 % cis and 49.4% trans) by inhalation for a single, 6-hour exposure period at nominal concentrations of 4.3, 5.7, 10.5, 22.7, 65.0, and 156.8 ppm. The test atmosphere concentration was determined at least once per hour, and then, the measurements were averaged over the 6-hour exposure period to calculate a time-weighted average (TWA) for each group (TWA exposure concentrations of 2.5, 4.8, 10.5, 19.8, 59.8, or 150.0 ppm). Single mice were exposed in whole-body plethysmography chambers attached to individual ports of a nose-only exposure system. Plethysmography was performed to determine the respiration rate (f), tidal volume (Tv), and minute volume (MV) for each mouse and these functional parameters were recorded continuously during the time in the exposure chamber. Animals were anesthetized with isoflurane/O2 and blood samples were collected by cardiac puncture within 8 minutes after exposure cessation prior to sacrifice. Samples were processed to determine the blood levels of the parent molecule (cis- and trans-1,3-D) using gas chromatography with tandem mass spectrometry (GC/MS-MS) detection.

Results for the respiratory rates were reported as maximum exposure-dependent decreases in respiration rate for the average maximum value of 5-minute intervals during exposure compared to each animal's average baseline. There was a concentration-related decrease in respiratory rate, and thereby also minute volume. An $R^2$ of 0.8217 was observed for the effects on respiratory frequency which EPA reviewers consider a reliable correlation, and this portion of the study was evaluated to be well-designed and performed. The respiratory rate depression exposure-response regression indicated the $RD_{50}$ (concentration of inhaled test material resulting in a 50% depression in respiration rate) of inhaled 1,3-D was 17.9 ppm. Mice exposed to 150.0 ppm 1,3-D showed a statistically significant decrease (52% reduction) in the minute volume area under the curve ($AUC_{MV}$) ($p<0.05$) compared to the mice exposed to 2.5 ppm. The decrease at the 150 ppm exposure level was also statistically different in comparison to the 4.8, 10.5, and 19.8 ppm exposure levels. However, the 59.8 ppm exposure $AUC_{MV}$ was not statistically different from either the lower 2.5, 4.8, 10.5, or 19.8 ppm exposure groups nor the higher 150.0 ppm as analyzed by the registrant.

The study report provided blood concentration levels for the cis- and trans-1,3-D isomers as well as the summed levels for both. However, it is important to note that the cis-isomer was present at levels below the limit of quantification (LOQ = 0.482 ng/g) for the three lowest

exposure groups. While the study report considered the cis- concentration to be zero for these groups, it is possible that the cis-isomer levels could contribute quantitatively to the total 1,3-D concentration for the lowest doses (LOQ is 7-27% of the measured trans-isomer concentration at these concentrations). This uncertainty prevents any interpretation of the summed concentrations at those doses. Further, it was not possible to discern between the 19.8 and 59.8 ppm treatment groups due to the high level of variability.

As an additional approach for analyzing the data and to provide mechanistic insights, the EPA reviewers calculated the ratio of cis- and trans-isomers (C/T ratio) as shown in Table 18. These C/T ratio data also do not support a role of either decreased respiratory delivery or saturated systemic clearance until exposures greater than 59.8 ppm. The value for the trans-isomer, which is the only available isomer at all exposure concentrations, shows an increase in blood concentration with exposure concentration at greater than 59.8 ppm. This suggests that the respiratory rate is not the only contribution to absorption, and thus, the change at exposure concentrations greater than 59.8 ppm might represent a decrease in absorption in the respiratory tissues due to saturation of GST metabolism or changes in distribution (including additional exhalation and elimination) between 59.8 and 150 ppm. However, without a proper mass balance analysis, it is hard to ascertain what the ADME determinants actually are, and additional empirical data are needed to support any hypothesis.

**Table 18. Cis- or trans-1,3-D blood concentration (ng/g ± s.d.) and the ratio of cis- and trans-isomers from the acute inhalation bioavailability study in male B6C3F1 mice.**

| Exposure Concentration (ppm) | Total 1,3-D (ng/g) | Cis (ng/g) | Trans (ng/g) | C/T Ratio |
|---|---|---|---|---|
| 2.5 | 1.80 ± 0.185 | NQ | 1.80 ± 0.185 | -- |
| 4.8 | 1.77 ± 0.337 | NQ | 1.77 ± 0.337 | -- |
| 10.5 | 6.92 ± 2.32 | NQ | 6.92 ± 2.32 | -- |
| 19.8 | 21.2 ± 14.4 | 2.16 ± 1.68 | 19.0 ± 12.8 | 0.11 |
| 59.8 | 17.7 ± 10.2 | 1.75 ± 1.14 | 15.9 ± 9.16 | 0.11 |
| 150.0 | 831 ± 138 | 157 ± 61.1 | 674 ± 85.5 | 0.23 |

The registrant used a least squares regression to analyze each of the blood concentration (*cis*-, *trans*-, or total 1,3-D) data sets. The independent variables in the model were the total inhaled mass (dose) of the test material and the quadratic term (the total inhaled mass (dose) of the test material squared). However, concentration data typically follows a lognormal distribution and such data should be log-transformed prior to the modeling, which was not conducted for the registrant's analysis. Therefore, EPA's Health Effects Division (HED) HED conducted an independent analysis (Memo, J. Nguyen, 18-DEC-2017; D444774) of the statistical methods utilized in the study. The approach selected was a linear effects model where log (blood concentration) was set as the dependent variable in the model. HED then used the same least squares regression analysis as the registrant. This analysis indicated that the only dose level that caused nonlinearity in the dose response curve was 150.0 ppm. However, an analysis including the highest dose level as 59.8 ppm would not result in a nonlinear dose response curve. Therefore, the results from this study do not adequately support Dow's proposal that the dose level of 59.8 ppm should be excluded because it exceeds the KMD for B6C3F1 mice. Furthermore, neither a mass balance analysis of the

parent 1,3-D and metabolites nor a time course analysis, in either the respiratory tract tissues or blood, for pharmacokinetic characterization of ADME were performed. Without a time-course analysis, it is unclear if a steady state was reached for either isomer at the end of the exposure period in this study.

**1,3-Dichloropropene: Steady State Pharmacokinetics Study**

In a steady-state pharmacokinetic study (MRID 50715302), male B6C3F1 mice (n=6-34/concentration) were administered 1,3-dichloropropene vapors by nose-only inhalation at 6 h/day for 15 days at nominal concentrations of 0, 10, 20, 40, 60, 90, or 120 ppm (equivalent to mg/L). An additional group of male mice (n=4) was administered 1,3-dichloropropene in corn oil by oral gavage at a nominal concentration of 100 mg/kg on Day 15. The respiration rate, tidal volume, and minute volume of selected mice (n=4) in the 20, 60, and 120 ppm exposure groups were measured on Days 1 and 14. Animals were euthanized at various time points after the end of exposure on Day 15, and blood samples were collected and processed to determine the concentrations of *cis*- and *trans*-1,3-dichloropropene.

There were no treatment-related effects on mortality, clinical signs, or absolute body weights.

On exposure Day 1, there were dose-related decreases in mean respiratory rate (168 to 124 breaths/min) and minute volumes (30 to 23 mL/min) as exposure concentrations increased from 20 to 120 ppm; however, there was no effect on mean tidal volumes. On Day 14, respiratory rates were decreased by 10% and 33% relative to the Day 1 values for the 60 and 120 ppm exposure groups, respectively. Day 14 mean tidal volumes decreased by 11% compared to Day 1 in the 20 ppm exposure group and increased by 22% compared to Day 1 in the 120 ppm exposure group, with no change at 60 ppm. The variation in mean tidal volumes were not considered to be treatment-related due to the lack of dose-response. The resulting minute volume values on Day 14 showed a similar pattern to Day 1. There was a dose-dependent decrease in MV with the 60 and 120 ppm groups showing a 9% and 22% decrease in MV compared to the 20 ppm animals. The blood concentrations of *cis*- and *trans*-1,3-dichloropropene were not determined for the animals that had the plethysmography measurements. Therefore, the estimates of inhaled dose were considered to be informative, but they were not relied on for other calculations in this DER.

For the target exposure concentrations of 20, 60, and 120 ppm, the mean inhaled doses were estimated to be 35, 102 and 168 mg/kg/day, respectively, on Day 1. On Day 14, the mean inhaled doses were estimated to be 31, 90 and 146 mg/kg/day for the target exposure concentrations of 20, 60, and 120 ppm, respectively. The ratios of inhaled dose to nominal exposure concentration for 20 and 60 ppm were 1.70-1.75 on Day 1 and 1.5-1.55 on Day 14. There was an approximately 20% decrease in the ratio of inhaled dose to nominal exposure concentration (1.40 on Day 1; 1.22 on Day 14) for the 120 ppm animals, however, the decrease correlated with the decrease in respiration rate.

Estimates of $T_{max}$ were reported as 90 min for the 20 and 60 ppm exposure groups and 360 min for the 120 ppm group. However, the blood concentrations for the 20 and 60 ppm groups were decreasing throughout the exposure period and the 120 ppm group blood



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concentration was increasing throughout the exposure period. Therefore, $T_{max}$ cannot be definitively determined for any of the exposure groups. Blood concentrations declined rapidly upon cessation of exposure, with $t_{1/2}$ estimates of approximately 12-13 min for the 20 and 120 ppm groups and approximately 6 min for the 60 ppm group.

In the study report, the primary finding from the piecewise linear (hockey-stick) modeling that examined dose proportionality was that the estimated exposure concentrations ($X_0$) at which blood concentrations transition from linear to non-proportionality were very consistent. Estimates of $X_0$ for total 1,3-dichloropropene (and the individual isomers) ranged from 26.9-30.8 ppm, with 95% lower and upper confidence intervals of 16.8-39.1 ppm. Application of a power model to the individual isomer or total 1,3-dichloropropene blood concentration data from the 10, 20, and 40 ppm groups further corroborated those of the hockey-stick model and indicated that the departure from dose-proportionality occurred even at low concentrations (up to 40 ppm). The EPA reviewed the statistical analyses and concluded that the statistical methods used were appropriate. To validate the results in the report, EPA statisticians also used hockey-stick models to analyze the log-transformed CIS, TRANS, TOTAL 1,3-D blood concentrations vs. log-transformed dose data. From the analyses of the 3-parameter model, there was high confidence that the relationship between 1,3-D concentration in male mice blood and inhalation exposure was non-linear (and non-proportional) at exposure levels of 40 ppm and above. The study report also presented results from the ratio analysis of CIS indicated the relationship between blood concentration and exposure dose was not proportional at 60 ppm and greater exposure level (non-proportional relationship could start at a lower exposure level, but there was no evidence). However, the results of ratio analyses of TRANS and TOTAL indicated the relationship was non-proportional at 40 ppm and greater exposure dose level. See Appendix A for additional information.

**CARC's conclusion on the exceedance of a kinetically derived maximum tolerated dose (KMD): The CARC concluded that based on the agency's independent evaluation and validation of the hockey-stick model, there is high confidence that the relationship between 1,3-D concentration in male mice blood and inhalation exposure was non-linear (and non-proportional) at exposure levels of 40 ppm and above. This supports that the concentration where benign lung adenomas were observed in male mice exceeded the KMD.**

## V. TOXICOLOGY

### A. Metabolism and Pharmacokinetics (Oral)

A number of 1,3-D absorption, metabolism, distribution, and excretion studies have been submitted to the EPA. However, the description below summarizes the findings from a 2004 literature study by Bartels because the results were in agreement with the submitted studies and the analysis was more robust, assessing a greater number of metabolites. Bartels (2004) showed that a single oral gavage dose of radiolabeled 1,3-D is rapidly absorbed and eliminated in both male F344 rats (1 or 50 mg/kg) and B6C3F1 mice (1 or 100 mg/kg). Following oral administration, the major route of excretion for both species was the urine (51-79% of the administered dose) with the feces and expired $CO_2$ also being predominant

routes of excretion (15-20% and 14-18% of the administered, respectively). At 48 hours post-dosing, the remaining radioactivity in the carcass was slightly higher in the rat than in the mouse (6% vs. 2% of the administered dose). Three metabolic pathways have been identified for 1,3-D. While the relative contribution of each pathway is consistent between rats and mice, there are quantitative differences in the metabolic profiles. The major pathway for metabolism in both rodents involves glutathione conjugation, accounting for the metabolism of 36-55% and 48-50% of the administered dose from rats and mice, respectively.

In a second metabolism study (MRID 40959801), rats were dosed for 14 consecutive days with 5 mg/kg of non-radiolabeled 1,3-D followed by a single 5 mg/kg dose of radiolabeled 1,3-D. In addition, two rats per sex that had not been previously dosed received a single 5 mg/kg dose of radiolabeled 1,3-D. The percent of the administered dose excreted in the urine for the singly dosed rats (54-61%) was similar to the levels measured in the previous study. In this study, there were no major differences in excretion routes between singly dosed and repeatedly dosed rats. 1,3-D was distributed to all tissues examined with highest concentrations found in the nonglandular stomach and urinary bladder in both sexes at 48 hours after dosing.

Following oral administration, 1,3-D is extensively metabolized and the metabolite profiles were qualitatively similar for rats and mice.

## B. Tissue Glutathione Depletion Following 1,3-Dichloropropene Exposures

### 1,3-Dichloropropene: Pharmacokinetics, Effect on Tissue Non-protein Sulfhydryls, and Macromolecular Binding in Fischer-344 Rats and B6C3F1 Mice following Oral Administration (MRID 00155846)

This study (MRID 00155846) included evaluations of the pharmacokinetics and metabolism of 1,3-D as well as the effects on tissue non-protein sulfhydryls (NPS) and macromolecular binding.

The assessment of NPS depletion included a time-course and dose-response assessment. Male mice 9/dose/sacrifice time were given a single oral dose of 0 or 100 mg/kg 1,3-D and sacrificed at 2, 4, 8, 12, or 24 hours post-dosing. All animals were dosed between 0600-0800 hrs in order to normalize for possible diurnal variation in tissue NPS content. In addition, the depletion of NPS was determined in the nonglandular stomach, glandular stomach, liver, kidney, and bladder of male rats and mice (animal numbers not specified) at 2 hrs after a single oral dose of 0, 1, 5, 25, 50, or 100 mg/kg 1,3-D. The effects of a single oral dose of 0 or 100 mg/kg of 1,3-D on NPS in the livers and bladders of female B6C3F1 mice (animal numbers not specified) were also determined.

In the time-course studies, the oral administration of 100 mg/kg 1,3-D to male mice was shown to decrease nonglandular stomach NPS to 20% of control values within 2 hrs post-dosing. Nonglandular stomach NPS levels did not return to control values until 8 hrs after dosing, and then, increased to almost 250% of control values thereafter. NPS in the glandular stomach and liver were less affected; maximal depletion in these tissues approached 66% and



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42%, respectively. In mice, NPS levels in the kidney and bladder were generally unaffected by the oral administration of 1,3-D.

In the dose-response studies, the nonglandular stomach was most sensitive to the NPS-depleting effects of orally administered 1,3-D in mice and the depletion was dose-related. The no-observable-effect level (NOEL) for NPS depletion in the nonglandular stomach was 1 mg/kg in the rat and 5 mg/kg in the mouse. NPS in the glandular stomach and liver of both species was also depleted in a dose-related fashion; however, the magnitude of depletion was substantially less than that noted in the nonglandular stomach. Renal and bladder NPS in both species were relatively unaffected by the oral administration of 1,3-D. Male and female mice were shown to have a similar response for NPS depletion. These effects occurred independent of any marked dose-dependencies in the pharmacokinetic fate of 1,3-D.

**1,3-Dichloropropene: Mechanism of Tumorigenicity Studies in Male B6C3F1 Mice and Fischer 344 Rats (MRID 44446302)**

In a repeated oral gavage study (MRID 44446302), Fischer 344 male rats 6/dose were administered 0, 5, 12.5, 25, or 100 mg/kg/day, 5 days/week for 3, 12, or 26 days for a total of 3, 9, or 20 exposures. In addition, ancillary groups of rats (6/dose) were included; these animals were sacrificed on study day 12 (9 exposures over 11 days and sacrificed 24 hours after the final dosing) to examine rebound levels of liver GSH (increased synthesis to compensate for GSH loss due to conjugation with 1,3-D).

The effect of oral dosing with 1,3-D on liver GSH levels is summarized in Table 19. These data support a role for GST metabolism in the liver. Following 3 days of administration, there were statistically significant decreases in hepatic GSH levels at 25 and 100 mg/kg/day (decreased 22.3% and 60.4% compared to controls, respectively). However, following 12 or 26 days of administration, there were only minimal changes in GSH levels. Based on the data for the rebound group, there was a statistically significant increase in GSH levels at 25 mg/kg/day (111.7%) and at 100 mg/kg/day (138.1%).

**Table 19. Liver GSH levels (% of control level) in rats after repeated-oral gavage dosing [a] (MRID 44446302).**

| Exposure Group (mg/kg/day) | 3-Day Period (3 exposure days) | 12-Day Period (9 exposure days) | 26-Day Period (20 exposure days) | "Rebound" (1-day post 9 exposure days) |
|---|---|---|---|---|
| 0 | 7.75 ± 0.45 | 7.78 ± 0.15 | 7.42 ± 0.40 | 7.09 ± 0.41 |
| 5 | 7.46 ± 0.42 (96.3) | 7.36 ± 0.49 (94.6) | 7.45 ± 0.58 (100.4) | 7.46 ± 0.43 (105.2) |
| 12.5 | 6.83 ± 0.49 (88.1) | 7.30 ± 0.45 (93.8) | 7.35 ± 0.15 (99.1) | 7.23 ± 0.43 (102.0) |
| 25 | 6.02* ± 1.17 (77.7) | 7.28 ± 0.73 (93.6) | 6.71 ± 0.48 (90.4) | 7.92* ± 0.74 (111.7) |
| 100 | 3.07* ± 1.16 (39.6) | 7.08 ± 0.83 (91.0) | 7.95 ± 1.16 (107.1) | 9.79* ± 0.45 (138.1) |

a 5 d/w; taken from pages 59-59 of MRID 44446302

* $p < 0.05$

In a repeated inhalation exposure study (MRID 44446302), B6C3F1 male mice 6/dose were exposed to target concentrations of 0, 10, 30, 60, or 150 ppm 1,3-D for 6 hours/day, 5 days/week for a total of 3, 9, or 20 exposures over 3-, 12-, or 26-day periods, and then, sacrificed 1-2 hours post-dosing. In addition, ancillary groups of mice (6/dose) were included; these animals were sacrificed on study day 12 (9 exposures over 11 days and sacrificed 24 hours after the final dosing) to examine the rebound levels of lung GSH.

The effect of inhaled 1,3-D on lung GSH levels is summarized in Table 20. These data support a role for GST metabolism in the uptake of 1,3-D in the respiratory tract tissue as the POE for inhalation exposures. Decreases in lung GSH were dose-related and generally statistically significant for all concentrations and all sampling times in the main study groups. Following 3 consecutive days of treatment, GSH decreases ranged from 5-50%. Comparable responses were sustained following 12 or 26 days of administration. Based on the data for the rebound in GSH, there does not appear to be a difference in the increased level of GSH production for the 10, 30, or 60 ppm groups (110.0-119.6%). However, there is an appreciable increase in GSH rebound for the 150 ppm group.

**Table 20. Lung GSH levels (% of control level) in mice after intermittent inhalation exposure periods [a] (MRID 44446302).**

| Exposure Group (ppm) | 3-Day Period (3 exposure days)[b] | 12-Day Period (9 exposure days) | 26-Day Period (20 exposure days) | "Rebound" (1-day post 9 exposure days) |
|---|---|---|---|---|
| 0 | 1.91 ± 0.22 | 2.49 ± 0.19 | 2.04 ± 0.33 | 2.40 ± 0.30 |
| 10 | 1.75 ± 0.09 (91.6) | 2.09* ± 0.11 (83.9) | 1.74 ± 0.19 (85.3) | 2.64 ± 0.51 (110.0) |
| 30 | 1.29* ± 0.12 (67.5) | 1.92* ± 0.19 (77.1) | 1.63* ± 0.11 (79.9) | 2.86 ± 0.12 (119.2) |
| 60 | 1.38 *± 0.14 (72.3) | 1.46* ± 0.06 (58.2) | 1.19* ± 0.20 (58.3) | 2.87 ± 0.30 (119.6) |
| 150 | 0.96* ± 0.12 (50.3) | 1.19* ± 0.12 (47.8) | 0.88* ± 0.07 (43.1) | 3.52* ± 0.18 (146.7) |

a 6 h/d; 5 d/w; taken from pages 60-63 of MRID 44446302

b Due to methodological problems, the 3-day mouse lung data were obtained using lung tissues from 3-day exposed Ancillary Group Mice.

* $p < 0.05$

## C. Mutagenicity

1,3-D has a long regulatory history and an extensive database of genetic toxicology assays, either sponsored by the registrant (Dow AgroSciences) or found in the open literature. Many of these studies have been evaluated by the EPA (see Quest, 1985, Dearfield, 1989; Shah and McCarroll, 2002, and McCarroll 1999) as well as other regulatory agencies. Over the course of 24 years, issues related to the use of a mutagenic stabilizer (epichlorohydrin), mutagenic impurities, or the use of an inappropriate solvent (Telone II mixed with dimethyl sulfoxide, DMSO), has been shown to yield, the known mutagen and carcinogen, 2-chloroacrolein. Also, the use of an insufficient concentration of glutathione (GSH) to avoid GSH depletion in *in vitro* systems has been raised. Many investigators have reported on the mutagenicity of 1,3-D in the Ames assay, with positive responses in *Salmonella typhimurium* in TA 1535 and TA 100. Some authors

proposed that polar impurities were responsible for the mutagenicity. The agency has determined that overall, the data support the conclusion that 1,3-D when purified appears to be non-mutagenic in the Ames assay in the absence of S9.

For this cancer classification review for 1,3-D, it has been HED's practice to exclude studies from the evaluation that utilized a 1,3-D formulation that either contained epichlorohydrin or used DMSO as the solvent because both substances are known to produce false positive results. One exception is an *in vitro* Chinese hamster ovary (CHO)\HGPRT assay (MRID 49889505) which was negative even though it used dimethyl sulfoxide (DMSO) as the solvent. In this case, however, the results were negative, and there is no evidence that 1,3-D or the interaction of 1,3-D with DMSO induced a mutagenic effect.

A number of *in vivo* genotoxicity and mutagenicity studies were conducted with 1,3-D (not containing epichlorohydrin) as shown in Table 21. 1,3-D was negative for inducing clastogenicity in the mouse micronucleus assay (MRID00146468) and the rat dominant lethal study (MRID 44302801). A "Big Blue" inhalation study (MRID 44470501) was previously submitted to evaluate the potential for 1,3-D to induce mutations in vivo. At the time the Big Blue mouse inhalation study (MRID 44470501) was reviewed, the agency had concerns about the use of lacI as a surrogate target, length of expression time and target dose. There were no EPA or OECD test guidelines available for this assay at the time the study was conducted. Although the study was negative for *in vivo* mammalian mutations, the concern that the expression time was inadequate remained. There was also concern with the experimental design for the $^{32}P$ post labelling (MRID 44446302) study to evaluate the potential for 1,3-D to induce DNA adducts. This study was also negative, but there was uncertainty about achievement of a biological target dose. Due to the conflicting *in vitro* mutagenicity findings and the technical concerns with the previous in vivo Big Blue mouse and *in vivo* adduct study, some uncertainty regarding the *in vivo* mutagenic potential of 1,3-D remained.

To address these concerns, the registrant conducted a new *in vivo* transgenic mutation test (MRID 49889503. In this study, twenty-four F344 homozygous Big Blue male rats (six/group) were exposed daily to the test substance or to placebo (sucrose microcapsules) mixed in diet, for 4 consecutive weeks, at target dose levels of 0, 12.5, 25 or 50 mg/kg/day. Liver and kidney tissue samples from the first five rats/group were processed for DNA isolation and analyzed for cII mutants. There was no statistically significant increase in mutations in the target tissues. A summary of the *in vivo* genotoxicity findings is shown in Table 21.

Based on a weight of evidence of the available data and clear negative findings for mutagenicity *in vivo*, the CARC concluded that 1,3-D is not a mutagenic concern *in vivo*.



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| Table 21: Summary of the *In Vivo* Genotoxicity Studies with 1,3-Dichloropropene | | | | |
|---|---|---|---|---|
| Study Type/Assay | MRID No. (year)/ Classification | Species/Duration/Dose | Result | HED Classification/Comment |
| Mouse micronucleus | Gollapudi, 1985<br>MRID 00146468<br>Acceptable/guideline | Mouse/CD-1<br>24 & 48 hours<br>0, 38, 115, 380 mg/kg | Negative. | Acceptable/guideline: Negative up to a lethal dose (380 mg/kg). |
| ıe™ Mouse | Gollapudi, 1997<br>MRID 44470501<br>Unacceptable | Transgenic Mouse/B6C3F1<br>6 hours/day, 5 days/week for 2 weeks<br>0, 10, 60, or 150 ppm (0, 45, 270, or 682 mg/m$^3$)<br>(Whole body inhalation) | Negative in tumor target tissues (lung/liver); no clear evidence of toxicity to the treated mice. | HED has reservations regarding demonstration of the biological target dose; length of mutation expression time, adequacy of the lacI gene as a surrogate for telone-induced mutagenicity (See McCarroll, 1999; TXR No 0013566). |
| $^{32}$P post labelling assay | Stott, 1997a<br>MRID 44446302<br>Unacceptable | Male Rat/F344<br>Oral gavage: 0, 5, 12.5, or 100 mg/kg/day for 3, 12 or 26 days<br><br>Male Mouse/B6C3F1<br>Inhalation: 0, 30, 60, or 150 ppm (0, 45, 136, 272, or 682 mg/m$^3$)<br>5 days/week for 3, 12 or 26 days | Negative for DNA adducts in rat liver and mouse lung. | HED has reservations regarding demonstration of the biological target dose, (See McCarroll, 1999; TXR No 0013566). |
| Dominant lethal assay | Gollapudi, 1998<br>MRID 44302801<br>Acceptable | Rat/CD<br>0, 10, 60, and 150 ppm<br>7 days/week for 10 weeks<br>(Whole body inhalation) | Negative for induction of dominant lethal mutations in CD rats. | Acceptable: Negative up to the LOAEL of 150 ppm, based on adverse effects on body weight (≥10 % ↓, starting on week 1 thru week 7. |
| Big Blue™ Mouse | Young, 2018 | Male Big Blue (*cII*) transgenic rat F344 | Negative for induction of mutations at the *cII* locus in the liver and kidney. | Acceptable: Negative |



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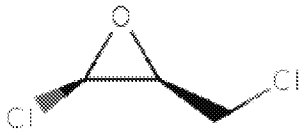
## D. Structure-Activity Relationship

Two metabolites of 1,3-D are the cis- and trans-1,3-D epoxides. As can be seen from the chemical structures below, these metabolites are structurally related to epichlorohydrin, a known mutagen and carcinogen. The only difference between the compounds is that the 1,3-D epoxides have an extra chloride located on a carbon in the epoxide ring.

epichlorohydrin




1,3-dichloropropene epoxide

Studies have confirmed that both cis- and trans-1,3-D epoxide are *in vivo* metabolites of 1,3-D (Schneider, 1998; MRID 45106201). Analysis of the liver and blood of mice exposed to 1,3-D found that the resulting epoxide concentrations were approximately 2 orders of magnitude lower than the corresponding parent isomers. While these studies utilized dosing levels and routes of administration that are not relevant for risk assessment (*e.g.*, 100 or 700 mg/kg *via* i.p. injection), the epoxide levels that resulted from i.p. administration of 100 mg/kg 1,3-D were just above the analytical method detection limit (e.g., 10 ng/g tissue).

While the study by Schneider *et al.* (1998) evaluated cis- and trans-1,3-D epoxide for mutagenicity in *S. typhimurium* TA100 and determined that both produced positive response, the newly submitted transgenic rat bioassay (MRID 50715301) did not indicate an *in vivo* mutagenic concern.

## E. Subchronic and Chronic Toxicity

### Subchronic Oral Toxicity

Repeated Dose 90-day Oral Toxicity Study in Rats (MRID 42954802)
In a subchronic toxicity study, TELONE II (1,3-D - microencapsulated) was administered by dietary admix to Fischer 344 rats (10/sex/group) at doses of 0, 5, 15, 50, or 100 mg/kg/day for 13 weeks with an additional 10/sex in the 0 or 100 mg/kg/ day group given basal food during a 4-week recovery period. The following parameters were examined: mortality, clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, macroscopic pathology, organ weight, and microscopic pathology.

Absolute body weights in the males were decreased 15% or 16% for the males treated with 50 or 100 mg/kg/day, respectively. Absolute body weights for the females were only decreased 11% at 100 mg/kg/day. There was a treatment-related increased incidence of hyperkeratosis (slight) and/or basal cell hyperplasia in the nonglandular portion of the stomach (very slight to slight) for both sexes starting at 15 mg/kg/day. After the 4-week recovery the hyperkeratosis was not observed, and the severity of the basal cell hyperplasia

was only very slight. The NOEL is 5 mg/kg/day and the LOEL is 15 mg/kg/day based on an increased incidence of hyperkeratosis and basal cell hyperplasia in the nonglandular mucosa of the stomach.

Repeated Dose 90-day Oral Toxicity Study in Mice (MRID 42954801)
TELONE II (1,3-D - microencapsulated) was administered by dietary admix to B6C3F1 mice (10/sex/group) at doses of 0, 15, 50, 100, or 175 mg/kg/day for 13 weeks. The following parameters were examined: mortality, clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry, macroscopic pathology, organ weight, and microscopic pathology. There was a treatment-related decrease in absolute body weight for the 175 mg/kg/day males and females (13% and 11%, respectively). Food consumption was comparable for all the treated groups compared to the control animals. There were no ophthalmic findings. For the 175 mg/kg/day males, there was a statistically significant treatment-related decrease in group mean leukocyte counts as compared to the controls (decreased 45%); however, all the other clinical chemistry or hematological parameters were not remarkably different for any of the treated groups compared to the controls. There were no gross necropsy findings which were considered to be treatment-related. Any differences in absolute and/or relative (to-body weight) organ weights appeared to reflect lower nonfasted final body weights in treated mice. There were no microscopic findings that were considered to be of toxicological significance.

**Subchronic Inhalation Toxicity**

Subchronic inhalation toxicity studies with Fischer 344 rats and B6C3F1 mice (MRID 00146461) were previously submitted; however, it should be noted that the formulation contained 1.2% epichlorohydrin.

Repeated Dose 90-day Inhalation Toxicity Study in Rats (MRID 00146461)

1,3-dichloropropene (containing 1.2% epichlorohydrin) was administered by inhalation to Fischer 344 rats 10/sex/dose at concentrations of 0, 10, 30, 90, or 150 ppm (0, 45.4, 136, 409, or 681 mg/m3, respectively) for 6 hr/day, 5 days/week for 13 weeks. Terminal body weights of rats of the highest exposure group were 18-20% lower than control animals. Treatment-related histopathological findings were noted in the nasal mucosa, including degeneration of the olfactory epithelium and/or hyperplasia of the respiratory epithelium in both sexes at 90 and 150 ppm. A minimally detectable level of hyperplasia was observed in 2/10 male rates exposed to 30 ppm 1,3-dichloropropene.

Repeated Dose 90-day Inhalation Toxicity Study in Mice (MRID 00146461)

1,3-dichloropropene (containing 1.2% epichlorohydrin) was administered by inhalation to B6C3F1 mice 10/sex/dose at concentrations of 0, 10, 30, 90, or 150 ppm (0, 45.4, 136, 409, or 681 mg/m3, respectively) for 6 hr/day, 5 days/week for 13 weeks. Terminal body weights of mice of the highest exposure group were 10-12% lower than control animals. Treatment-related histopathological findings were noted in the nasal mucosa of both sexes and urinary bladders of female mice. In the nasal tissues, degeneration of the olfactory epithelium and/or

hyperplasia of the respiratory epithelium were noted in both sexes at 90 and 150 ppm. At 150 ppm, the lesions were accompanied by some focal areas of respiratory metaplasia. In addition, diffuse moderate hyperplasia of the transitional epithelium occurred in the urinary bladders of female mice exposed to 90 and 150 ppm 1,3-dichloropropene.

### F. Mode of Action Studies

No tumor mode of action data were submitted for 1,3-D.

### G. Registrant's Weight of Evidence Analysis of Oral Tumors

The Registrant provided the following weight of evidence rational to support classifying 1,3-D as "Not Likely to be Carcinogenic to Humans at doses below 12.5 mg/kg bw/day via the oral route".

1. The NTP studies in rats and mice are not relevant to assess 1,3-D's tumorigenic potential because the studies were conducted on an antiquated formulation of 1,3-D that contained epichlorohydrin.

2. Following prolonged oral exposure of 1,3-D for two years, only benign liver tumors (hepatocellular adenomas) were observed in both sexes of F344 rats. However, 1,3-D was not tumorigenic in three other rodent cancer bioassays that were conducted in rats (CD) and mice (B6C3F1 and CD-1) at doses equal or above the tumorigenic level (25 mg/kg bw/day) to F344 rats.

3. The tumors in F344 rats were benign only and late onset liver tumors with no observed pre-neoplastic liver lesions. No treatment-related liver tumors were observed following inhalation exposure.

4. The increase in benign liver tumors observed in female F344 rats was only at 25 mg/kg/day (high-dose) and in males at 12.5 mg/kg/day (mid-dose) and 25 mg/kg/day (high- dose). However, the tumor incidences in mid-dose males and high-dose females are within the most relevant HCD range and were not statistically significantly increased relative to concurrent controls (12% vs. 16% HCD for males and 8% vs. 8% HCD for females, respectively). Further, the incidence of high dose males although being statistically significant ($p<0.05$, but $p> 0.01$), is just outside HCD (18% vs. 16% HCD) and for a common liver adenoma, $p<0.01$, and not $p<0.05$ has been recommended for decision making per Haseman's rule.

5. Examinations of dose adequacy indicates that 25 mg/kg/day (high-dose) administration in F344 males exceeded the maximum tolerated dose (MTD) as evidenced by sustained reduction in body weight gain of about 20%, a clear indication of excessive toxicity.

### H. Registrant's Weight of Evidence Analysis of Inhalation Tumors

The Registrant provided the following weight of evidence rational to support classifying 1,3-D as "Not Likely to be Carcinogenic to Humans at concentrations below 20 ppm via the inhalation route".

1. The lung tumors were benign only and late onset lung with no observed pre-neoplastic lung lesions. There was no progression to malignancy even at the terminal sacrifice and no tumors were observed following 1-yr treatment.

2. Lung adenomas occur at a high background incidence in male B6C3F1 mice. When the incidence of current cancer bioassay in B6C3F1 male mice was compared with the HCD from the Dow Toxicology Lab and NTP, the combined incidence in male mice at 60 ppm (44%) from the cancer bioassay is just outside the Dow Toxicology Lab (up to 38%) and NTP range (up to 42%), suggesting an equivocal tumorigenic response of 1,3-D.

3. The tumorigenic concentration of 60 ppm in male mice exceeded a kinetically-derived maximum dose (KMD) as indicated by systemic exposure far above dose-proportionality at concentration of 60 ppm results in non-dose proportional (superlinear) systemic exposure levels. This excessive high-dose level would confound the interpretation of study results and is generally not considered suitable for dose-response extrapolation (U.S. EPA, 2005). Based on the results, consistent with OECD GD116 and U.S. EPA (U.S. EPA, 2005), the tumorigenic dose of 60 ppm demonstrates non-relevance of 1,3-D induced benign lung tumors in mice for human health risk assessment.

4. Further evaluations of the second inhalation cancer bioassay in F344 rats, and 4 oral route rodent cancer bioassays in F344 rats, B6C3F1 mice, CD rats, and CD-1 mice, clearly indicate that lung is not a target organ of 1,3-D.

## VI. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

### A. Rats

In an oral (dietary) combined chronic/carcinogenicity study in F344 rats, male rats showed a statistically significant ($p<0.05$) increase in hepatocellular adenomas at the high dose (25 mg/kg/day). Although there was no clear evidence of supporting non-neoplastic lesions, the incidence of these benign tumors at the high dose exceeded the expected historical control range. The doses tested were considered to be adequate and not excessive for assessing the carcinogenic potential of 1,3-D in both sexes. CARC performed statistical analyses on uterine tumors in female rats. The CARC concluded that there were no treatment-related uterine tumors in the rat. There was a high incidence of uterine polyps at the high dose; however, this is a common finding in this strain of rat and the incidence at the high dose fell within expected historical control range. There were no treatment-related adenomas, carcinomas or sarcomas seen in the uterus and there were no treatment-related findings pre-neoplastic or supporting non-neoplastic lesions seen in the uterus. **CARC concluded that the liver adenomas in male rats are treatment-related. There are no treatment-related tumors in female rats.**

In an oral (gavage) combined chronic/carcinogenicity study in CD-1 rats, CARC determined that although the animals could have tolerated higher doses, the committee concluded that the high dose (25 mg/kg/day) was adequate to assess carcinogenicity. **There were no treatment-related tumors in male or female rats in this study.**

In an inhalation combined chronic/carcinogenicity study in F343 rats, CARC determined that the concentrations tested were adequate and not excessive to assess carcinogenicity. **There were no treatment-related tumors observed in male or female rats via the inhalation route.**

### B. Mice

In a 2-year dietary carcinogenicity study in B6C3F1 mice, CARC concluded that the animals likely could have tolerated a higher dose; however, the committee concluded that the doses evaluated are adequate to assess the carcinogenic potential of 1,3-D in mice. **There were no treatment-related tumors in male or female mice.**

In an oral gavage carcinogenicity study in CD-1 mice, CARC determined the doses tested to be adequate and not excessive. **There were no treatment-related tumors seen in male or female mice.**

In an inhalation carcinogenicity study in B6C3F1mice, there was a statistically significant trend ($p<0.01$) and pairwise ($p<0.05$) increase in benign bronchioloalveolar adenomas in male mice at the highest test concentration (60 ppm). CARC considered the concentrations to be adequate and not excessive based on toxicological endpoints; however, the registrant conducted a toxicokinetic study evaluating the relationship between 1,3-D concentration in male mice blood and inhalation exposure. These data indicated that the relationship was non-linear at exposure levels of 40 ppm and above. **Based on these findings, the CARC concluded that there were no treatment-related tumors in male mice via the inhalation route at concentrations below the kinetically derived maximum tolerated dose (KMD). There was no evidence of carcinogenicity in female mice.**

Based on a weight of evidence of the available genotoxicity studies, the CARC concluded that there is no concern for mutagenicity in vivo.

## VII. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC classified 1,3-Dichloropene (Telone) as "Suggestive Evidence of Carcinogenic Potential" based on the presence of liver tumors by the oral route in male rats only.

## VIII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Not required. The CARC recommends using a non-linear approach (i.e., reference dose (RfD)) that will adequately account for all chronic toxicity including carcinogenicity, that could result from exposure to 1,3-dichloropropene.



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## X. APPENDIX A

Extracted from MRID 50715302

To validate the results submitted by the registrant, EPA statisticians also used hockey-stick models to analyze the log-transformed CIS, TRANS, TOTAL 1,3-D blood concentrations vs. log-transformed dose data. EPA only performed analyses using the data as they appeared in the raw data file. EPA did not replicate the analyses using pseudo-random normal data, the analyses using only the 10, 20, and 40 ppm data, nor the ratio of mean responses analyses since the registrant indicated the censoring issue had little effect on the results.

Summary results EPA's analyses

- The animal 613 was an outlier and caused a significant deviation from normality. Due to violation of the normality assumption, the 95% CIs of the estimates of $X_0$ in the analyses including animal 613 were substantially wider than the analyses excluding animal 613.

- Since $b_1$ was not significantly different from 1 in all analysis models, there was no evidence to reject the assumption that the concentration of 1,3-D in B6C3F1 male mice blood is dose-proportional for exposure levels $\leq X_0$.

- Since $b_2$ was significantly greater than 1 in all analysis models, there was evidence that the concentration of 1,3-D in B6C3F1 male mice blood is not dose-proportional at exposure levels $> X_0$.

- The primary interest is $X_0$ (the exposure level at which dose-proportional ceases):

    - $X_0$ ranges from 32.4 ppm to 39.0 ppm (4-parameter model analyses) and the 95% CIs are entirely below 80 ppm if animal 613 was included and entirely below 65 ppm if animal 613 was excluded.

    - $X_0$ ranges from 26.9 ppm to 30.2 ppm (3-parameter model analyses) and the 95% CIs are entirely below 40 ppm (i.e., dose-proportionality does not hold when the exposure level exceeds 40 ppm).

Tables 1 and 2 present the detailed results of EPA analyses and the registrant analyses. There are some discrepancies in the estimates of $X_0$ and $a_1$ between EPA and the registrant's analyses. For those estimates that have discrepancies, the results of registrant analyses are presented in the Tables with *italic and highlighted* font.

The first critical discrepancy was in the confidence interval of parameter $a_1$ in the 4-parameter models. In EPA analyses, $a_1$ was significantly greater than 0 (lower bounds of its 95% CIs > 0) while $a_1$ was not significantly different from 0 (95% CIs include 0) in the registrant's analyses. Note that a proportional dose response relationship exists only if $a_1 > 0$ and $b_1$=1. A second discrepancy was the differences in the estimates of $X_0$, especially its 95% CIs. It is interesting that the all the estimates of $b_1$ and $b_2$ were consistent between EPA and the



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registrant analyses.

Conclusion

From the analyses of the 3-parameter model, there was high confidence that the relationship between 1,3-D concentration in male mice blood and inhalation exposure was non-linear (and non-proportional) at exposure levels of 40 ppm and above.

From the analyses of the 4-parameter model where the animal 613 were excluded, there is high confidence that the relationship between 1,3-D concentration in male mice blood and inhalation exposure was non-linear (and non-proportional) at exposure levels of 60 ppm and above.

**Table 1: Results of EPA and registrant analyses where data of animal=613 were included**

| Model | Parameter | Estimated Parameters | | |
|---|---|---|---|---|
| | | CIS | TRAN | TOTAL |
| **4-parameter models** | $X_0$ | 32.36 (15.97, 65.60)<br>*32.36 (9.50, 55.23)* | 36.97 (17.25, 79.27)<br>*37.72 (9.15, 66.28)* | 36.18 (17.17, 76.28)<br>*36.91 (9.56, 64.27)* |
| | $a_1$ | 0.01 (0.00, 0.18)<br>*0.01 (-0.02, 0.05)* | 0.10 (0.01, 0.67)<br>*0.09 (-0.09, 0.28)* | 0.11 (0.01, 0.82)<br>*0.11 (-0.11, 0.33)* |
| | $b_1$ | 1.40 (0.44, 2.36) | 1.43 (0.71, 2.16) | 1.43 (0.68, 2.18) |
| | $b_2$ | 2.82 (2.24, 3.40) | 2.50 (2.06, 2.94) | 2.55 (2.09, 3.00) |
| **3-parameter models $b_1$=1** | $X_0$ | 26.93 (18.49, 39.23)<br>*26.93 (16.80, 37.06)* | 28.02 (19.94, 39.36)<br>*28.61 (18.94, 38.29)* | 27.87 (19.79, 39.24)<br>*28.46 (18.77, 38.15)* |
| | $a_1$ | 0.04 (0.03, 0.05) | 0.30 (0.24, 0.39) | 0.34 (0.27, 0.45) |
| | $b_2$ | 2.82 (2.24, 3.40) | 2.50 (2.06, 2.94) | 2.55 (2.09, 3.00) |
| Italic font shows the discrepancies between the EPA and registrant analyses | | | | |

| Table 2: Results of EPA and registrant analyses where data of animal=613 were excluded | | | | |
|---|---|---|---|---|
| **Model** | **Parameter** | **Estimated Parameters** | | |
| | | **CIS** | **TRAN** | **TOTAL** |
| **4-parameter models** | $X_0$ | 34.84 (21.73, 55.85)<br>*34.84 (18.40, 51.28)* | 38.95 (23.78, 63.79)<br>*39.72 (20.25, 59.19)* | 38.26 (23.67, 61.86)<br>*39.01 (20.38, 57.65)* |
| | $a_1$ | 0.01 (0.00, 0.10)<br>*0.01 (-0.01, 0.04)* | 0.10 (0.02, 0.42)<br>*0.09 (-0.05, 0.23)* | 0.11 (0.02, 0.50)<br>*0.11 (-0.06, 0.27)* |
| | $b_1$ | 1.40 (0.66, 2.15) | 1.43 (0.88, 1.98) | 1.43 (0.86, 2.00) |
| | $b_2$ | 3.11 (2.64, 3.58) | 2.72 (2.38, 3.07) | 2.78 (2.42, 3.14) |
| **3-parameter models $b_1$=1** | $X_0$ | 29.32 (23.05, 37.29)<br>*29.32 (22.27, 36.37)* | 30.21 (24.28, 37.59)<br>*30.83 (24.13, 37.54)* | 30.09 (24.20, 37.42)<br>*30.72 (24.05, 37.38)* |
| | $a_1$ | 0.04 (0.03, 0.05) | 0.30 (0.25, 0.37) | 0.34 (0.28, 0.42) |
| | $b_2$ | 3.11 (2.64, 3.58) | 2.72 (2.37, 3.08) | 2.78 (2.42, 3.15) |
| Italic font shows the discrepancies between the EPA and registrant analyses | | | | |

| Table 3: Shapiro-Wilk test for normality of residuals | | | | |
|---|---|---|---|---|
| **Model** | **p-value** | | | **Normality assumption** |
| | **CIS** | **TRAN** | **TOTAL** | |
| 4-parameter model | 0.0188 | 0.0042 | 0.0056 | rejected |
| 3-parameter model with b1=1 | 0.0228 | 0.0074 | 0.0106 | rejected |
| 4-parameter model, excluded outlier ID = 613 | 0.2724 | 0.2307 | 0.4490 | not rejected |
| 3-parameter model with b1=1, excluded outlier ID = 613 | 0.3112 | 0.3019 | 0.4953 | not rejected |

Figure 1: predicted curves and residual normal quantile plots of 4-parameter model analyses (animal 613 included) from EPA analyses

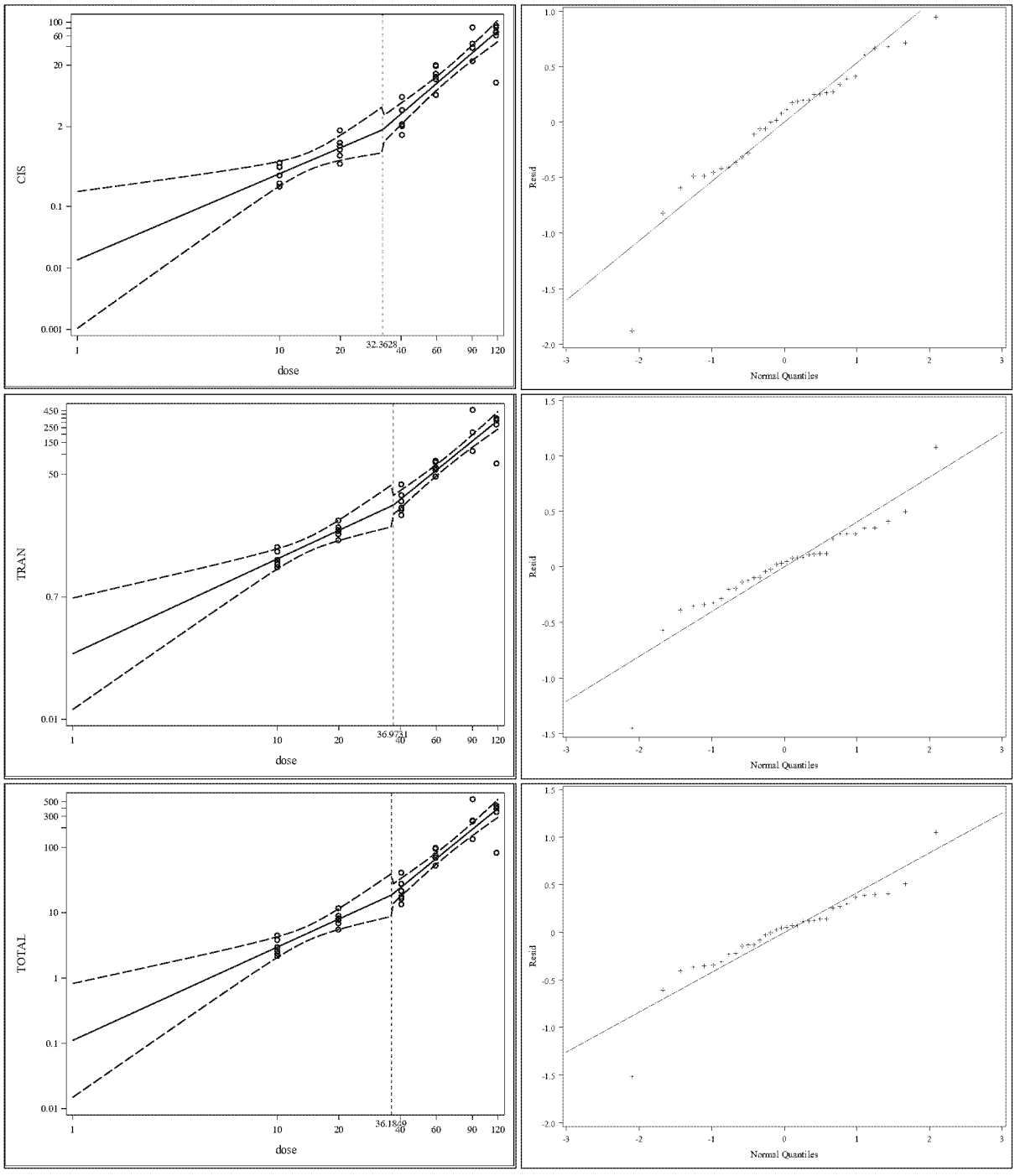


Figure 2: predicted curves and residual normal quantile plots of 3-parameter model analyses (animal 613 included) from EPA analyses

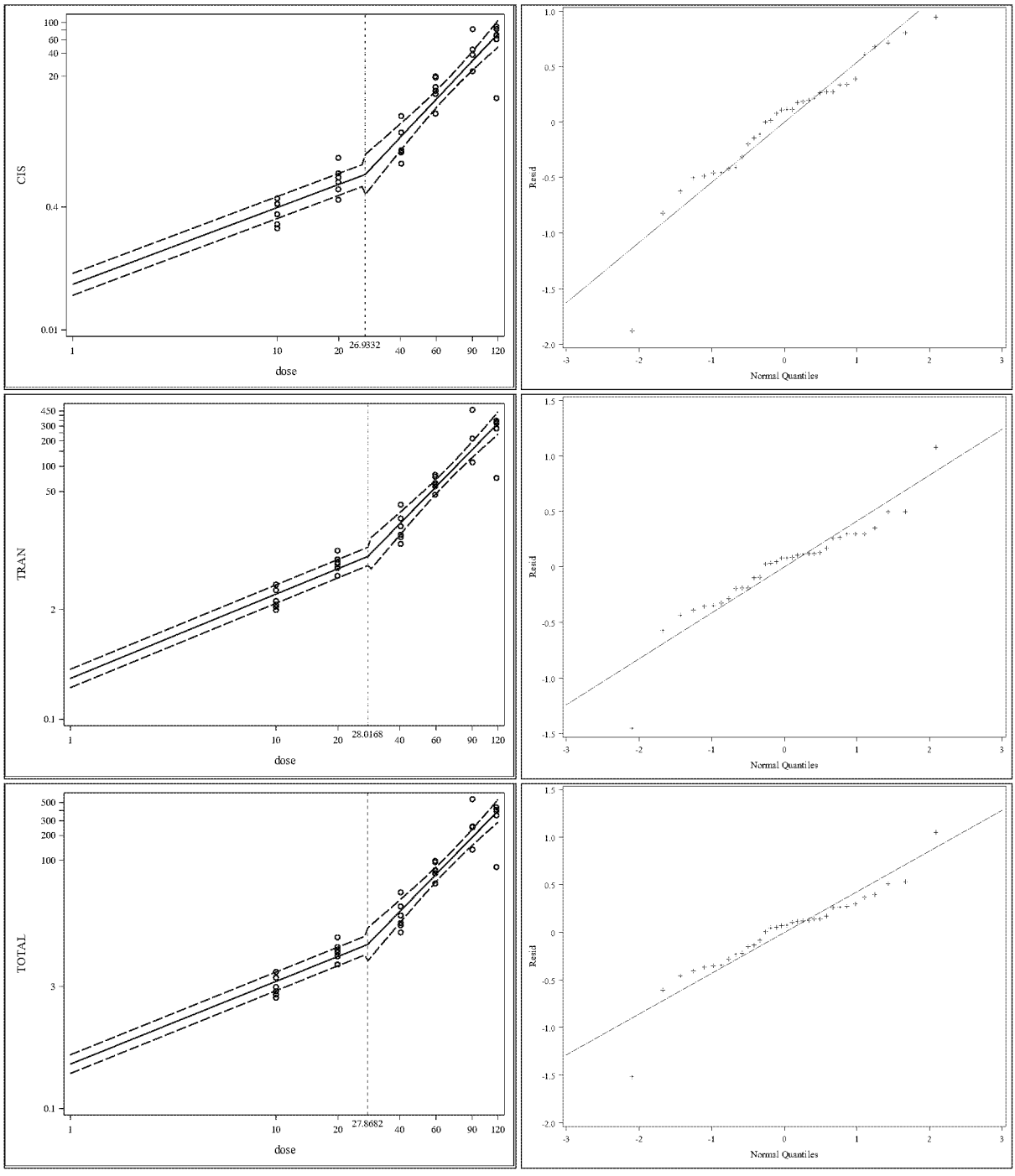



Figure 3: predicted curves and residual normal quantile plots of 4-parameter model analyses (animal 613 excluded) from EPA analyses

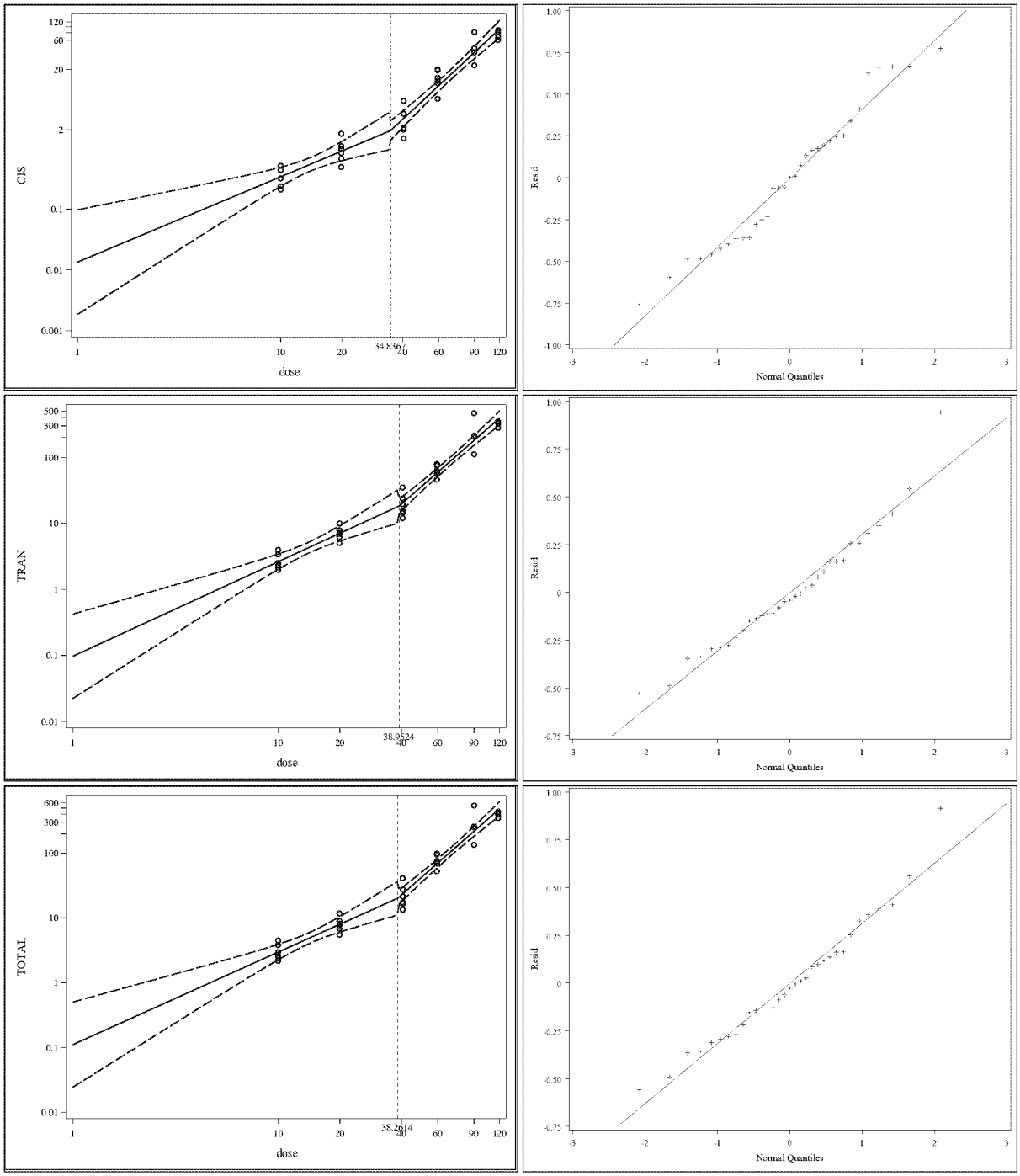
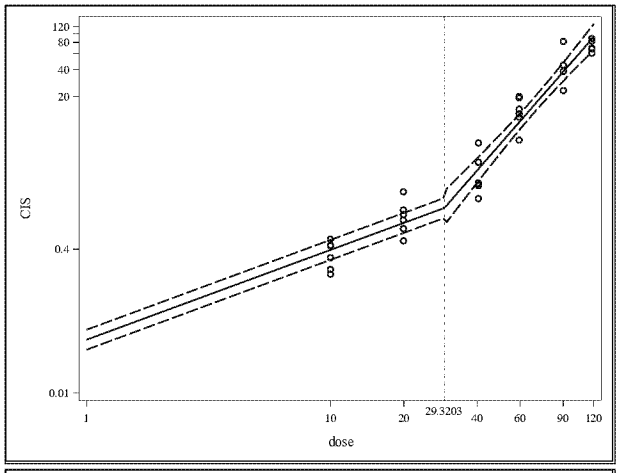



Figure 4: predicted curves and residual normal quantile plots of 3-parameter model analyses (animal 613 excluded) from EPA analyses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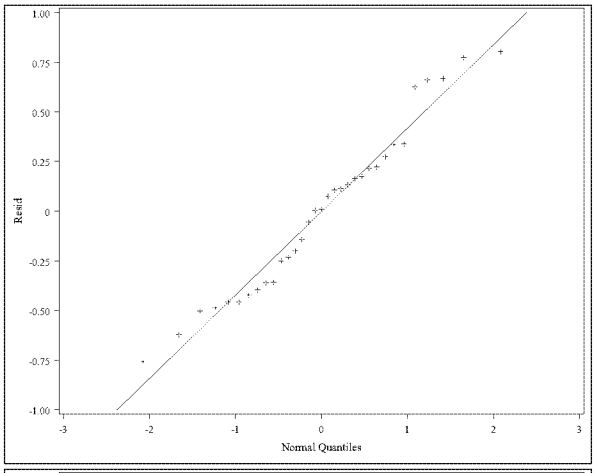
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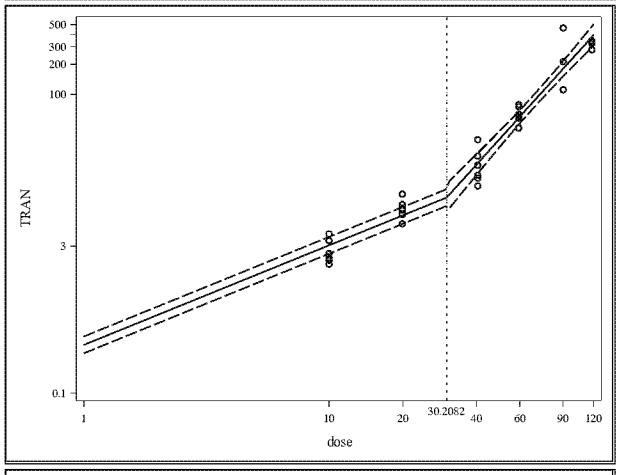
Normal Quantiles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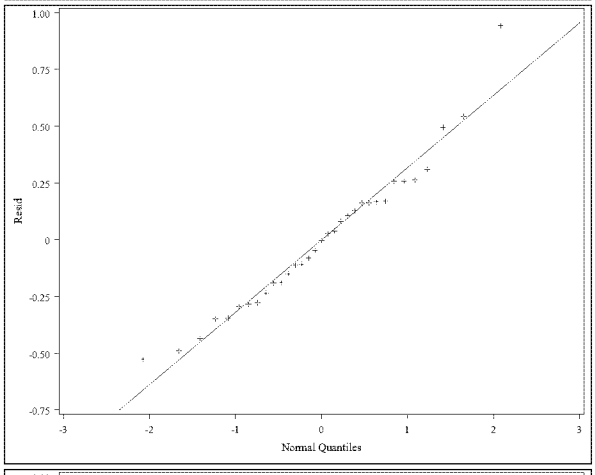
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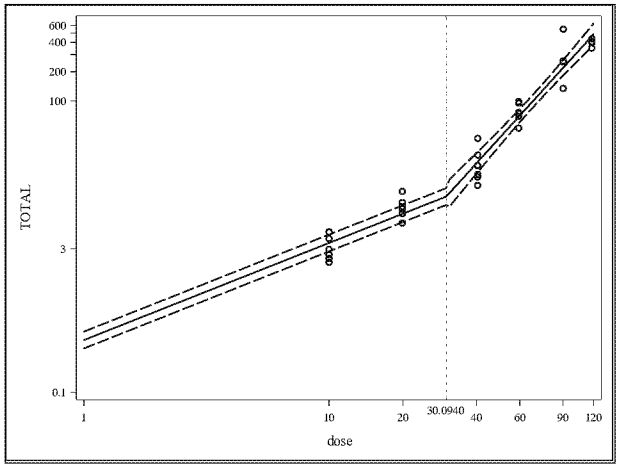
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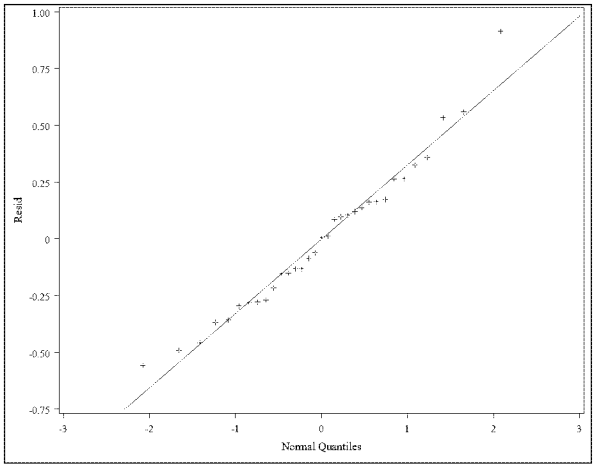
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Normal Quantiles