[redacted] SR-1,3 Dichloropropen

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

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JUL 15 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Mutagenicity, Mechanism and Metabolism Studies with Telone II (1,3-Dichloropropene)

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THRU: Alberto Protzel, Ph.D. Alberto Protzel 7/13/99
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cc: Vicki Dellarco, Ph.D.
Immediate Office
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Registrant: DowElanco
Chemical: CIS/TRANS 1,3-Dichloropropene (Telone II)
Case No.: 838282 Submission Nos.: S531185, S535155
Identifying No.: 029001 DP Barcode: D233640, D242582, D242785
MRID Nos.: 44460501, 44460502, 44460503, 44470501, 44446301, 44446302
PC Code: 029001

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ACTION:

Review of the mutagenicity, mechanism and metabolism studies with CIS/TRANS 1,3-Dichloropropene (Telone II).

I. BACKGROUND:

In response to the conclusion presented in the Cancer Peer Review Committee's Reports classifying Telone II as a Group B2 carcinogen, probable human carcinogen, which have been reiterated in the Draft Health Effects Division (HED) Reregistration Eligibility Decision (RED) for 1,3-dichloropropene (Telone II), the registrant submitted studies to demonstrate that Telone II is not mutagenic and that the earlier findings of mutagenesis were associated with impurities, stabilizers and autooxidation products. Mechanistic data to demonstrate that the tumors seen in the 2-year bioassays (both oral and inhalation) were not associated with the mutagenicity of Telone II were also submitted. These new data consist of *in vitro* and *in vivo* genetic toxicology studies and an *in vivo* cell proliferation/apoptosis/DNA adduct mechanistic study. In addition, two metabolism studies (glutathione transferase activities in several mammalian cell lines and bioavailability of microencapsulated Telone II) were also included in the submissions.

II. CONCLUSIONS: Based on our review of these newly submitted studies and the weight-of-the-evidence, we conclude that the assays do not support the registrant's claims that Telone II is not mutagenic. Furthermore, there are studies from the open literature showing the *in vivo* mouse liver conversion of 1, 3-D to mutagenic *cis* and *trans* epoxides, the *in vitro* formation of four DNA adducts when 1, 3-D epoxides are reacted with 2'-deoxyguanosine and the *in vivo* formation of DNA lesions in the stomach, colon, liver, kidneys, bladder, lungs, brain and bone marrow harvested 3 or 24 hours after mice received a single intraperitoneal (ip) dose of 150 mg/kg 1,3-D . We, therefore, conclude that the registrant's claim that a mutagenic mode of carcinogenic action does not account for the liver adenomas in rats and lung adenomas in mice in the dietary and inhalation bioassays, respectively, is unsupportable by the weight-of-the-evidence. The investigators also claim that Telone II does not present a carcinogenic hazard to humans because the protective mechanism, such as glutathione (GSH), is sufficient to completely conjugate and detoxify the administered dose. We disagree with this claim since tumors were recorded at levels that do not deplete GSH. Similarly, the conflicting evidence from several mammalian cell lines demonstrated no clear correlation between physiological levels of GSH and attenuation and mitigation of mutagenicity. Furthermore, depletion of GSH is not regarded as a mode of carcinogenic action (MOA). As defined by the Agency, a MOA is a description of key events and processes starting with the interaction of an agent with a cell, through operational and anatomical changes, resulting in cancer formation (USEPA, 1996). Under this definition, depletion of GSH does not qualify as a MOA, it is part of the physiological defense system that determines the biologically effective dose interacting with cellular macromolecules. We conclude that no convincing data have

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been presented to identify the MOA for tumor formation, other than mutagenicity. At this time, the mutagenicity of Telone II, in conjunction with the observation of tumor induction at multiple sites in two species, and the absence of an acceptable alternative MOA support the Agency's position that a linear approach for the cancer dose response extrapolation remains reasonable and is, therefore, not changed.

III. CITATION and EXECUTIVE SUMMARIES

Citation and Executive Summaries for the reviewed study followed by the Agency's comments on each study are presented below; Data Evaluation Reports (DERs) are attached (see Attachment 1).

CITATION: Lawlor, T.E. (1996). Evaluation of 1,3-Dichloropropene for Mutagenic Potential in Salmonella in the Presence of Mouse Lung Homogenate (S9); Coming Hazleton Inc. (CHV), Vienna, VA; CHV Study No. 17037-0-401; Dow Study No. M-003993-036; Study Completion Date: November 26, 1996 (Study Report Reformatted January 22, 1997). (Unpublished) MRID NUMBER: 44460501

EXECUTIVE SUMMARY: In a series of microbial preincubation mutagenicity studies (MRID No. 44460501), *Salmonella typhimurium* strain TA100 was exposed to 1,3-dichloropropene (unspecified purity). The test substance was purified by passage through a silicic acid column, did not contain an epoxidized soybean oil stabilizer and was stored at 5±3°C under nitrogen. Trials were conducted in the absence of exogenous metabolic activation (100-450 μg/plate); with mouse lung S9 derived from control mice in both the presence and absence of glutathione (GSH) at doses of 150-1000 μg/plate (-GSH) or 75-1000 μg/plate (+GSH); and in the presence of mouse lung S9 derived from mice pretreated by inhalation with 63 ±7 ppm 1,3-D, 5 days/week for 2.5 weeks both with and without GSH at doses of 75-1000 μg/plate. The mouse lung S9 fractions were characterized prior to use and found to convert the known promutagen, 2-aminoanthracene to an active form. The test material was delivered to the test system in ethanol.

Cytotoxicity was observed at ≥450 μg/plate -S9. Under all conditions of mouse lung activation (+/-GSH), 1,3-D was lethal at ≥600 μg/plate and caused a marginal cytotoxic effect at 300 μg/plate. The positive controls induced a clear response in *S. typhimurium* TA100 under all assay conditions. **There was, however, no indication that 1,3-dichloropropene induced a mutagenic effect at any dose in either the presence or absence of control or pretreated mouse lung S9 fractions with or without GSH. It was concluded that the physical state of the test material (i.e., without the epoxidized soybean stabilizer), method of purification (passage through silicic acid column) and/or storage conditions (at 5±3°C under N_2) probably accounts for the lack of a mutagenic response in the nonactivated phase of testing. It was further concluded that the absence of mutagenicity in the S9-activated phase of the study was likely associated with the low level of microsomal/oxidative**

proteins in the lung preparations since there is independent confirmation from Watson et al., (1987)[1] and Schneider et al., (1998a)[2] that 1,3-D is mutagenic in *S. typhimurium* TA100 (See Section D. Reviewers' Discussion/Conclusions).

The study is classified as Unacceptable (Nonguideline) for a negative response and does not contribute to the MOA understanding for carcinogenesis.

CITATION: Gollapudi, B.B, and Cieszlak, F.S. (1997). Telone® II Soil Fumigant: Evaluation in an In Vivo Assay for Gene Mutations Using Transgenic Big Blue Mice; Health & Environmental Research Laboratories, The Dow Chemical Co., Midland, MI; Laboratory Project Study ID K-006409-017; Study Completion Date: February 10, 1997. (Unpublished) MRID NUMBER: 44470501

EXECUTIVE SUMMARY: In an *in vivo* gene mutation assay (MRID No.44470501), groups of five male transgenic Big Blue B6C3F1 mice were exposed by inhalation (whole body) to vapor concentrations of 0, 10, 60, or 150 ppm Telone II (equivalent to ≈45, 270 or 682 mg/m^3) Telone II 6 hours/day, 5 days/week for 2 weeks. Mice were sacrificed 17 days following the end of exposure and genomic DNA from lung and liver samples of vehicle and high concentration animals was isolated. The target gene (*lac*I) was packaged into lambda phage, mixed with *Escherichia coli* SCS-8 and plated, and the mutation frequency (MF) was determined.

No clear evidence of an overt toxic effect was observed at the highest dose tested. The positive controls induced marked increases in the MF. **There was no evidence that Telone II induced a mutagenic response in either liver or lung tissue harvested from the treated mice. It was concluded, however, that concerns related to the demonstration of a biological target dose, length of the mutation expression time, adequacy of the lacI gene as a surrogate for Telone-induced mutagenicity, if any, raise doubts regarding test system sensitivity. These reservations weaken the understanding of the negative findings of this study (See Section D., Reviewers' Discussion/Conclusions). Moreover, the results of this study need to be viewed in the context of the weigh-of-the-evidence considerations.**

This study is Unacceptable (Nonguideline) for a negative response, based on the above reservations.

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[1]Watson, W.P., Brooks, T.M., Huckle, K.R., Hutson, D.H., Lang, K.L., Smith, R.J. and Wright, A.S. (1987). Microbial mutagenicity studies with (Z)-1,3-dichloropropene. Chem. Biol. Interactions 61:17-30.

[2] Schneider, M., Quistad, G.B., and Casida, J.E. (1998a). 1,3-Dichloropropene epoxides: Intermediates in Bioactivation of the Promutagen 1,3-Dichloropropene. Chem. Res. Toxicol. 11, 1137-1144.

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CITATION: Stott, W.T., Miller, T.J. and Wardynski, A.K. (1997). 1,3-Dichloropropene: In Vitro DNA Binding; Health & Environmental Research Laboratories, The Dow Chemical Co., Midland, MI; Project Study ID 970180; Study Completion Date: December 12, 1997. (Unpublished) MRID NUMBER: 44446301

EXECUTIVE SUMMARY: In a series of in vitro DNA binding assays (MRID No. 44446301), 2-μL samples of racemic ^{14}C-1,3-Dichloropropene (>99% radiochemical purity and 0.22 mCi/mmole specific activity) at a final concentration of 11 mM (equivalent to ≈1220 mg/mL) were reacted with calf thymus DNA in the presence or absence of S9 activation and/or glutathione (GSH). Six to seven assays were performed per reaction mixture. After 4 hours, DNA was recovered and the DNA concentration was determined spectrophotometrically. The total radioactivity of the recovered DNA was determined by liquid scintillation counting and expressed as the number of DNA adducts/nucleotide. The S9 fraction was derived from Aroclor 1254-induced rat livers.

The selected dose was considered adequate. The positive controls induced the expected high level of DNA binding. **There was no evidence that ^{14}C-1,3-Dichloropropene induced a positive response in the presence of S9 activation with or without GSH. No conclusions could be reached for the nonactivated series of experiments because of the wide variability in the data. In contrast to these findings, the *in vitro* conversion of 1,3-D to the *cis* and *trans* epoxides as well as the formation of DNA adducts when these epoxides were reacted in vitro with 2'-deoxyguanosine raise concerns that conditions may not have been optimal to allow the epoxidation of 1,3-D to the DNA reactive epoxides. Since questions exist regarding the use of optimum conditions and because the nonactivated data were inconclusive, the study is considered unacceptable.**

The study is classified as Unacceptable (Nonguideline) because definitive conclusions could not be reached based on protocol issues.

CITATION: Stott, W.T., Gollapudi, B.B, Clements, C.M., Linscombe, V.A., Dittenber, D.A., Lick, S.J. and Johnson, K.A. (1997). 1,3-Dichloropropene: Mechanism of Tumorigenicity Studies in Male B6C3F1 Mice and Fischer 344 Rats; Health & Environmental Research Laboratories, The Dow Chemical Co., Midland, MI; Study No. 971121; Study Completion Date: December 12, 1997. (Unpublished) MRID NUMBER: 44446302

EXECUTIVE SUMMARY: A series of investigations were undertaken (MRID No.44446302) to determine the mechanism(s) by which 1,3-dichloropropene [96.5% (49.87% cis + 46.89% trans isomers)] induces liver tumors in rats via ingestion and lung and urinary bladder tumors in mice via inhalation. Accordingly, groups of six male Fischer 344 rats received oral gavage doses of 0, 5, 12.5, 25 or 100 mg/kg/day 1,3-D 5 days/week for 3, 12 or 26 days. In the inhalation phase of the study, groups of six male B6C3F1 mice were exposed (whole body) to vapor concentrations of 0, 10, 30, 60 or 150 ppm 1,3-D (equivalent to ≈45, 136, 272 or 682 mg/m^3) 1,3-D 6 hours/day, 5 days/week for 3, 12 or 26 days. Clinical signs were observed twice daily and body weights were recorded periodically throughout the exposure interval. On the appropriate study day, groups of six rats or mice per dose

were sacrificed and the following parameters were measured: 1) selected clinical chemistry (rats only); 2) gross necropsies and reduced glutathione (GSH) levels in liver (rats) and lungs (mice);and 3) cell proliferation and apoptosis in rat livers (0 and 100 mg/kg/day) and mouse lung and bladder epithelial cells (0 and 60 and 150 ppm). In addition, ancillary groups of rats and mice (6/dose/exposure regime) were included; these animals were sacrificed on study day 12 (9 exposures over 11 days and sacrifice 24 hours after the final dosing) and used to determine the rebound levels of GSH. DNA adduct formation was also determined in tissue recovered from the ancillary groups (4 animals/group) exposed to 0, 12.5 or 25 mg/kg/day (rat livers) or 0, 30 or 60 ppm (mouse lungs) using the ^{32}P-postlabeling assay.

Results were as follows:

RATS:

Treatment had no effect on mortality, clinical signs or body weight. GSH levels were significantly decreased in mid- and high dose rats (≥23%) at day 3 only. The hepatic GSH recovery, which was apparent by day 12 in the main group and in the rebound group, is indicative of an adaptive process and consistent with the detoxification of 1,3-D through GSH conjugation. However, the data from the 2-year bioassay in rats show that liver tumors were induced at 12.5 mg/kg, and at this level in the current study GSH was not significantly depleted. Thus, it is difficult to determine the role of GSH as a protective mechanism and how it relates to the tumorigenic response. Despite the protective action of GSH in the detoxification of 1,3-D, hepatotoxicity was observed in rats at 100 mg/kg/day as indicated by significant increases in serum ALT and AST which paralleled suggestive increases in cell proliferation. However, definitive conclusions can not be reached regarding effects on cell proliferation and apoptosis because of the variability in the data and the small sample size (6 animals/group). Similarly, while there was no evidence that 1,3-D at doses of 12.5 or 25 mg/kg/day induced detectable DNA adducts in rat livers, the small sample size (4 animals/group) does not provide confidence in the negative result.

MICE:

No deaths or other clinical signs of toxicity were seen. Significantly decreased body weights (6-10%↓) were recorded for male mice at 150 ppm (main study) from study days 2-25; however, the only significant body weight effect (6%↓) on the corresponding mice in the ancillary group was recorded on study day 12 (after the final exposure of a total of 9 exposures). Decreases in lung GSH were dose-related and generally significant for all inhalation concentrations at all sample times. GSH decreases ranged from 5-50% following 3 uninterrupted days of treatment with 10-150 ppm 1,3-D. Comparable responses (15-55%↓) were sustained over 10 or 20 exposures. In contrast, mice in the rebound groups showed marked increases in GSH levels (range = 8-46% at 10-150 ppm, respectively); the 46% increase at the high concentration was significant. These data are indicative of conjugation of 1,3-D with GSH in pulmonary tissue. There was, however, no apparent dose- or time-related effect on cell proliferation in either the bronchiole epithelium or bladder transitional cells and no apparent effects on apoptosis. There was also no clear evidence that 1,3-D concentrations of 30 or 60 ppm induced detectable DNA adduct formation in mouse lungs. Nevertheless, the extreme variability in the cell growth and cell regulation

data and the small sample size for these endpoints (6 mice/group) in conjunction with the small sample size for the ^{32}P-postlabeling assay (4 mice/group) compromises the validity of the findings.

In addition to the above mentioned reservations regarding the sample size for both species, we have concerns as to whether a biological effective dose of the reactive *cis* and *trans* 1, 3-D metabolites was achieved in light of the evidence from the literature showing the *in vivo* mouse liver conversion of 1, 3-D to mutagenic *cis* and *trans* epoxides (Schneider et al., 1998a) and the formation of four DNA adducts when 1,3-D epoxides are reacted with 2'-deoxyguanosine (Schneider et al., 1998 b)[3]. Furthermore, there are data showing the *in vivo* formation of DNA lesions in the stomach, liver, kidneys, bladder, lungs, brain and bone marrow harvested 3 or 24 hours after mice received a single intraperitoneal (ip) dose of 150 mg/kg 1, 3-D (Sasaki et al., 1998)[4]. These inconsistencies in the ^{32}P-postlabeling assay make interpretation of the negative results uncertain (See Section D., reviewers' Discussion/Conclusions). In addition, the mutagenic potential of Telone must be viewed in the context of the weight-of-the-evidence considerations.

The study is classified as Unacceptable (Nonguideline) for a negative response and does not contribute to the MOA understanding for carcinogenesis.

CITATION: W.T. Stott and H.S. Stewart. (1996). Determination of glutathione transferase activities in several mammalian cell lines. The Toxicology Research Laboratory, Health and Environmental Sciences, The DOW Chemical Co.; Midland, MI. Laboratory Project Study ID: T2.06-001-014-001. August 27, 1996. MRID 44460503. Unpublished.

EXECUTIVE SUMMARY: The active ingredients of Telone II, an agricultural nematocide, are the mixed isomers of 1,3-dichloropropene (1,3-D). The interpretation of genotoxicity data for Telone has been confounded by the presence of contaminants and stabilizers in the test material. Generally, the genotoxic responses have been positive *in vitro* using standard bacterial and mammalian cell lines. However, the positive response has been reversed by the addition of glutathione (GSH) to the assay. It is postulated that the in vitro genotoxicity of Telone is related to the ability of the test system being used to conjugate with GSH. This report (MRID 44460503) measured Glutathione -S-transferase activity (GST) in the cytosol of male B6C3F1 mouse and F344 rat livers, cultured primary rat hepatocytes (HP), Chinese hamster ovary (CHO) cells and two Chinese hamster lung (CHL) cell lines. The substrates used were ^{13}C-1,3 D (>98% radiochemical purity), 4-chloro-1,3-dinitrobenzene(CBNB), para-nitro-phenylethylbromide

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[3] Schneider, M., Quistad, G.B., and Casida, J.E. (1998 b). N^2,7-Bis(1-hydroxy-2-oxopropyl)-2'-deoxyguanosine: Identical Noncyclic Adducts with 1,3-dichloropropene epoxides and methyglyoxal. Chem. Res. Toxicol. 11, 1536-1542.

[4] Sasaki, Y.F., Saga, A., Akasaka, M., Ishibashi, S. Yoshida, K., Quan Su, Y., Matsusaka, N., and Tsuda, S. (1998). Detection of in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs. Mutat. Res. 419;13-20.

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(NPEB) and trans-4-phenyl-3-buten-2-one (TPBO). The highest GST activity toward 1,3-D was found in rat and mouse liver cytosol. This was followed by HP, the two CHL cell lines, the CHO cell line and finally *Salmonella typhimurium*. For the most part CDNB was a better substrate than 1,3-D in all test systems while NPEB and TPBO were poorer substrates.

Overall, the data indicate that *S. typhimurium* has low level GST activity, which is consistent with the positive mutagenicity findings with this test system. However, conflicting results were obtained with the various cell lines investigated (i.e., high and low level GSH activity with cell lines producing negative mutagenicity data and relatively high GSH activity with two lines that were positive in standard mutagenicity assays (see Discussion). Based on these findings, no conclusions could be reached relative to the correlation between physiological levels of GSH and mitigation of mutagenic activity.

The study is classified as Unacceptable (Nonguideline) because definitive conclusions could not be reached; consequently, the findings do not contribute to the MOA understanding for carcinogenesis.

CITATION: W.T. Stott, J.R. Gilbert, R.J. McGuirk, K.A. Brzak, M.D. Dryzga and M.J. Bartels. (1996) Bioavailability of microencapsulated Telone II soil fumigant in Fischer 344 rats. The Toxicology Research Laboratory, Health and Environmental Sciences, The DOW Chemical Co.; Midland, MI. Laboratory Project Study ID: M-003993-027. August 21, 1996, MRID 44460502. Unpublished

EXECUTIVE SUMMARY: To determine the relative bioavailability of microencapsulated Telone II (95.8%) following oral gavage, cis and trans (1:1 ratio) Telone II was administered by gavage to female rats as a mixture of neat and microencapsulated test article in corn oil (MRID 44460502). To differentiate the test article attributable to each formulation the neat Telone was isotopically labeled using ^{13}C in the 2 carbon position of the molecule. Pharmacokinetics studies were conducted in 2 phases using 6 female rats in each. In Phase I, animals were co-administered 25 mg/kg of ^{13}C-1,3-D (neat) and 25 mg/kg microencapsulated 1,3-D in a single corn oil solution/suspension. Samples were collected via an indwelling jugular canula over the first hour following dosing. The cis and trans isomers of Telone were quantitated separately in each sample. No sampling times were provided in the methods portion of the report. A table of mean blood concentration data provided collection times of 1, 3, 5, 10, 15, 20, 30, 40, 50 and 60 min. The only metabolite investigated was the mercapturic acid derivative of Telone, which was measured in urine. Metabolite characterization was not performed during these experiments.

In Phase II, 6 fasted female rats were fitted with indwelling hollow fiber membrane probes in their right jugular veins to monitor the blood levels of test article *in situ*. The probes were also connected directly to a mass spectrometer and the appropriate ions monitored continuously for 1,3-D and ^{13}C-1,3-D. Three of these animals were dosed with 25 or 50 mg/kg each of ^{13}C-1,3-D (neat) and microencapsulated 1,3-D. The remaining three rats were dosed with 25 or 50 mg/kg ^{13}C-1,3-D (neat) plus 7.5 and 15 mg/kg, respectively, microencapsulated 1,3-D. A total of 6 blood curves was obtained from 6 fasted rats. Subsequently, two of the 6 rats were provided feed and water and were used two more times each to generate blood curves. In each of these instances, the two rats were dosed with 25 or 50 mg/kg each of

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^{13}C-1,3-D (neat) and microencapsulated 1,3-D. Thus, a total of 10 blood curves was obtained from 6 animals.

In both Phases I and II, Telone II as either the neat or microencapsulated formulation was rapidly absorbed and excreted with an elimination half-life of between 27 and 43 min. In Phase I, 46% of the total area under curve (AUC) was contained under the curve for neat 1,3-D, and 54% under the microencapsulated blood curve. Based on these results the investigator postulates that the bioavailability of encapsulated Telone is equivalent, if not greater, to that of the neat chemical when administered by oral gavage. In general this postulation is supported by the data provided in this report, and the finding that urinary clearance to the mercapturate derivative is essentially the same for both formulation supports this conclusion (56 and 44% for the neat and microencapsulated formulations, respectively).

No concrete conclusions can be reached for this study for the following reasons: 1) the report is written in an unclear fashion. For instance, four different dosages were used during Phase II, it is stated that ≥2 dose levels were used; 2) some animal data were used more than once and the resulting data averaged with the other individual animals, thereby biasing the data; 3) no individual blood concentration results and numbers of animals used for each dosing group were provided; 4) no rationale is given for using female rats only (for more details see Discussion).

This study is classified as **unacceptable/nonguideline** as presently written. The authors concluded that the bioavailability of microencapsulated telone is equivalent, if not better, than that of the neat material after oral gavage. Although this postulation is generally supported by the data, the reporting deficiencies cited under **III. Discussion** prevent any conclusions to be drawn with certainty. This study can be re-reviewed by the Agency and may be upgraded to **acceptable/nonguideline** if the report is re-written with particular attention to clearly explaining the study design (group arrangements, animal numbers, dosing procedures and levels, sequence of data collection etc.), and to satisfactorily addressing the issues raised by the reviewers under **III. Discussion.**

IV. OTHER RELEVANT INFORMATION

A. SUMMARY OF CLASSIFICATION OF CARCINOGENIC POTENTIAL

i. Cancer Classification

On September 5, 1985, the Health Effects Division (HED) Cancer Peer Review Committee met to discuss and evaluate the database on Telone II with particular reference to the carcinogenic potential of the chemical. The Committee classified Telone II as a Group B2 carcinogen, probable human carcinogen. The weight-of-the-evidence judgement of potential human cancer risk is based on tumor findings at multiple sites in both sexes of two species and mutagenicity. There were increased tumors in both sexes of rats (Fischer 344) and mice (B6C3F1) after oral administration (MRID No. 00146469). Tumor types noted included forestomach, liver, mammary, thyroid, adrenal, urinary, and lung. The carcinogenic potency factor (Q_1*) for humans via the oral route was calculated to be 1.75 x 10^{-1} using the Multistage Model to plot the incidence of combined forestomach, liver, adrenal, and

thyroid tumors in the male rats. The oral Q_1* was later revised to incorporate the 3/4 interspecies scaling factor. The revised oral Q_1* is 1.22×10^{-1} $(mg/kg/day)^{-1}$ in human equivalents (Fisher 1997). Under the "Guidelines for Carcinogen Risk Assessment" (EPA, 1996)[5], Telone would be regarded as "likely" to be carcinogenic in humans by the oral and inhalation routes.

On August 23, 1989, the HED Cancer Peer Review Committee met to assess the inhalation studies in rats and mice (MRID Nos. 40312201 and 40312300). The Committee reaffirmed the Group B2 classification with the additional information from the inhalation studies (increased bronchioloalveolar adenomas in male B6C3F1 mice). The Q_1* for humans via the inhalation route was calculated to be 9.66×10^{-2}.

On December 19, 1994, the inhalation Q_1* was revised to 5.33×10^{-2} and was based on lung bronchioloalveolar adenoma tumor rates in male mice. The Q_1* was revised to incorporate the 3/4 interspecies scaling factor (Fisher 1994) .

HED determined that it is appropriate to calculate cancer risk estimates for Telone II using the Q_1* derived from the linearized low dose extrapolation model. The Q_1* used in this assessment was based on the incidence of bronchioloalveolar adenomas in males in a mouse inhalation carcinogenicity study (MRID No. 40312300).

On January 30, 1997, The HED RfD Peer Review Committee met to discuss and evaluate dietary admix (microcapsules) rat and mouse studies (MRID Nos. 43763501, 43757901) as well as to review previously submitted studies upon which the RfD and the carcinogenicity classification were based. The Committee concluded that the results from the newly received studies did not necessitate having the Carcinogenicity Peer Review Committee reevaluate the carcinogenicity classification or the Q_1*.

ii. Weight-of-the-Evidence

The weight-of-the-evidence for the carcinogenicity of Telone II was based on the increased incidence of tumors in both sexes of rats ad mice after oral administration (tumor types noted included forestomach, liver, mammary, thyroid, adrenal, urinary and lung), on lung bronchioloalveolar adenoma tumor rates in male mice and on the inherent mutagenicity of Telone II in bacteria, Drosophila and mammalian cell lines.

iii. Registrant's Rebuttal

In response to the conclusions presented above, the registrant requested that the Agency consider the re-classification of 1,3-D as likely to be carcinogenic to humans only at conditions of high

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[5]EPA (1996). Proposed guidelines for carcinogen risk assessment. National Center for Environmental Assessment, Office of Research and Development. EPA/600/P-92/003C. April 1996.

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exposure by either the oral or inhalation routes but is unlikely to pose a carcinogenic risk under normal conditions of use (see Attachment 2). They further stated that based on the lack of mutagenicity in mammalian systems coupled with the rapid detoxification at non-saturating doses as well as the general growth-promoting effects on high background tumor rates suggest a nonmutagenic MOA for this material. Finally, they recommended that a nonlinear-response approach would be appropriate for Telone II.

B. SUMMARY OF PRE-EXISTING MUTAGENICITY STUDIES (PRIOR TO THE NEW SUBMISSIONS)

Central to the Agency's position regarding the carcinogenicity of Telone II is the evidence of mutagenicity in both procaryote and eukaryotes. These issues have been reviewed and discussed at length (see Attachment 3, Memorandum from K.L Dearfield to A.C. Levy, No. 25, 1987) with the conclusion that "based on the available evidence in bacterial, Drosophila and mammalian cell studies, Telone II presents a mutagenicity concern." Presented below is a brief summary, extracted from the Dearfield memo, on the mutagenicity findings of studies submitted to the Agency prior to the current submission:

There was a positive effect in the Salmonella assay in strains G46, TA98, TA100 and TA1535 with and without S9 activation and in strains TA1538 and TA1537 with S9 activation. Responses approximately 100x and 10x over background in strains TA1535 and TA100, respectively, were seen (MRID No. 00039688). Telone II in the absence of metabolic activation was positive in the *Bacillus subtilis* rec-assay only at 1250 μg/plate (MRID No. 00039688). Up to a cytotoxic concentration of 1000 μg/plate, no positive results were reported in the *Escherichia coli* reversion test with or without S9 activation (MRID No. 00039688). A mouse host-mediated assay with *Salmonella typhimurium* strain G46 was negative. However, the oral gavage dosing of the mice up to 60 mg/kg may not have been high enough as adequate toxicity was not reported (MRID No. 00039680). Non-reproducible increases (just at 2 background) were reported in the nonactivated phase of the Chinese hamster ovary (CHO/HGPRT) gene mutation assay at 100, 150, 200 and 250 μM (MRID No. 00159679). Telone II was also positive for the induction of sex-linked recessive lethal mutations and reciprocal translocations in *Drosophila melanogaster* (MRID No. 00146469). Telone II was, however, negative in an unscheduled DNA synthesis (UDS) assay with primary rat hepatocytes up to consistently cytotoxic doses (>4 μM) (MRID No. 00146467).

Data from the open literature also indicate that Telone II is mutagenic in Salmonella and cultured mouse lymphoma cells and induces chromosomal aberrations, sister chromatid exchanges and DNA strand breaks in several mammalian cell lines *in vitro*. Telone II did not cause micronuclei induction in rat or mouse bone marrow cells but was positive for DNA strand breaks in rat liver. Overall, the data from somatic cell assays are indicative of a mutagenic concern for Telone II and support the weight-of-the-evidence evaluation for carcinogenicity.

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In addition to the above studies, an inhalation dominant lethal assay (MRID No. 44302801) was submitted to confirm the results of the positive *Drosophila melanogaster* sex-linked recessive lethal assay. Telone II tested negative in this assay. Results show that Telone II, administered by inhalation at concentrations up to 150 ppm (≈682 mg/m^3) 6 hours/day, 7 days/week for 10 weeks did not induce a dominant lethal effect in male rat germinal cells. The negative findings of this study lessen the concern for germ cell effects; they do not, however, rule out the mutagenic potential seen in somatic cells.

C. NEW MUTAGENICITY DATA SUBMITTED BY THE REGISTRANT

i. Introduction

To counter the Agency's position regarding the mutagenicity of Telone II, the registrant submitted new mutagenicity studies (see Executive Summaries in Section III) in support of their argument that there was no convincing evidence that "unequivocally" identified Telone II as a mutagen. They further indicated that the bacterial mutagenicity was due to impurities, stabilizers and/or autooxidative products. The new data submitted by the registrant to support their position that Telone II is not mutagenic and that the earlier findings of mutagenicity were associated with impurities, stabilizers and autooxidation products included the *Salmonella typhimurium* mammalian microsome mouse lung S9 mutagenicity (MRID No. 44460501) performed with an assumed 100% test material purified through a silica acid column and did not contain an epoxide soybean stabilizer. The test material was also stored at 5±3 °C under nitrogen. Additional studies in which no special precautions were taken to ensure that Telone II [96.0% (49.3% *cis* + 46.7% *trans* isomers)] would not autooxidize to a mutagenic form included a Transgenic Big Blue Mouse in vivo gene mutation assay (MRID No. 44470501), an *in vitro* DNA binding study in calf thymus DNA (MRID No. 44446301), an *in vitro* glutathione transferase activity in mammalian cell lines study (MRID No. 44460503), a mechanistic tumorigenicity study in mice and rats (MRID No. 44446302), and bioavailability (MRID No. 44460502). Citations and Executive Summaries from the review of these studies are presented in Section C, full DERs are attached (See Attachment 1).

ii. Comments Regarding the Newly Submitted Mutagenicity Studies

1. *Salmonella typhimurium* mammalian microsome (mouse lung S9) mutagenicity assay; MRID No. 44460501

The negative results from this study illustrate the point that handling the test sample to prevent the generation of polar impurities and/or products of autooxidation eliminated the mutagenicity. Nevertheless, the handling procedures are unrealistic and do not simulate the normal environmental conditions of use or *in vivo* physiological conditions. Furthermore, the lack of mutagenicity in the presence of mouse lung S9 activation with or without glutathione was likely associated with the low level of microsomal/oxidative proteins in the lung preparations and likely represents a "false negative" response. We based this conclusion on the absence of direct mutagenic activity of silicic acid purified

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1,3-D, stored under N2 to prevent autoxidation to mutagenic impurities, which was previously demonstrated by Watson et al. (1987). Similarly, the ability of mono-oxygenases in rat liver microsomes to convert purified 1,3-D to a mutagen for *S. typhimurium* TA100 has been shown by Watson et al. (1987). We further noted that in the series of experiments by Watson et al. (1987), the mutagenic response was enhanced in the presence of increasing levels of washed microsomes with the maximum effect attained in the presence of 0.9 mg microsomal protein/plate. These data support the investigators' conclusion that an oxidative metabolite was responsible for the mutagenic activity seen with TA100. Independent confirmation that 1,3-D is oxidized to mutagenic epoxides via a minor metabolic pathway *in vivo* in the livers of mice treated with 1,3-D and also *in vitro* by the mouse liver cytochrome P450-dependent microsome-NADPH system has been presented by Schneider et al. (1998a). In this study, equal caution was taken to prevent and/or identify the formation of reactive impurities and/or autoxidation products. Findings indicate the *in vivo* and *in vitro* generation of *both cis* and *trans* 1,3-D-epoxides with the *cis* form generated at a higher yield than the *trans* epoxide both *in vitro* and *in vivo* and that the *cis* isomer reacts slower than the *trans* epoxide with GSH alone or catalyzed by GSH S-transferase. Similarly, the *cis* epoxide was more potent in *S. typhimurium* TA100 than the *trans* isomer (≈1850 revertants at 50 nmol *cis* isomer versus ≈850 revertants at 50 nmol for the *trans* isomer). Overall the results of Watson et al. (1987) and Schneider et al. (1998a) dispute the data presented in the current submission.

Based on the above considerations, this study is judged not to provide acceptable evidence of a negative response in this preincubation bacterial gene mutation assay under the specified assay and storage conditions of the purified test substance.

2. *In vivo* assay for gene mutations using Transgenic Big Blue mice; MRID No. 44470501

Telone II tested negative in this study up to a high dose of 150 ppm (equivalent to≈682 mg/m^3) Telone II 6 hours/day, 5 days/week for 2 weeks, which represents ≈ 2.5-fold the carcinogenic dose. However, whether the dose that evaded metabolic detoxification and penetrated to the biologically significant site (i.e., DNA) was sufficient to provoke a mutagenic effect is problematic because there are data showing the *in vivo* mouse liver conversion of Telone II (10 min. after ip treatment with 700 mg/kg *cis/ trans* 1,3-D) to mutagenic *cis* and *trans* epoxides (Schneider et al., 1998a). There are also data showing the *in vivo* formation of DNA lesions in the stomach, colon, liver, kidneys, bladder, lungs, brain and bone marrow harvested 3 or 24 hours after mice received a single intraperitoneal (ip) dose of 150 mg/kg 1,3-D (Sasaki et al., 1998). Hence, there is no assurance that a biologically effective dose was reached within the short exposure (5 days/week for 2 weeks).

There is also uncertainty regarding the length of mutation expression (17 days). The expression time (54 weeks) for the positive control, DEN, a powerful liver carcinogen illustrates this concern regarding length of expression time for the Telone II-treated tissue. Without some assurance that 17 days is sufficient for mutation induction, if any, by Telone II to be fixed and detected, the negative findings from this study are uncertain.

Furthermore, there is an issue regarding the reliability of the lacI gene as an appropriate surrogate gene for Telone II induced mutations. The findings of Monroe et al. (1998)[6] showing that a concentration of N-methyl-N-nitrosourea (MNU) considered to be "negative" in splenic T cells of Big Blue mice treated with MNU by three transgenes (lacI, cII, or cI) despite the powerful increase in hprt$^-$ mutations in cultured splenic T cells emphasizes the growing concern that transgenic models are not sufficiently sensitive (Skopek 1998)[7].

The Agency welcomes the use of an innovative molecular based approach such as the Big Blue Transgenic system for the detection of *in vivo* gene mutations and acknowledges that the method has promise. It is of further note that this transgene system is under consideration by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)[8] as a new test procedure. It is, however, not without limitations as an assay system, and, therefore, results from this model should be interpreted in the context of the weight-of-the-evidence.

Based on the above considerations, we assess that the study does not provide sufficient evidence to support a conclusion on nonmutagenicity in the context of all the available data.

3. *In vitro* DNA binding assay (MRID No. 44446301)

^{14}C-1,3-D tested negative up to an acceptable high dose (final concentration = 11 mM, equivalent to ≈1220 mg/mL). However, no conclusions could be reached for the nonactivated 1,3-D preparation because of the wide variability in the data. In the presence of S9 activation with or without GSH, 1,3-D failed to bind to calf thymus DNA. Nevertheless, the lack of a clear positive effect either with or without S9 activation in this *in vitro* study is unexpected since 1,3-D handled under conventional laboratory conditions and not subjected to special purification procedures, as was the case in the currently reviewed study, is mutagenic in *Salmonella typhimurium*. Furthermore, there are data showing the *in vitro* mouse liver microsome-NADPH activation of 1, 3-D (0.1 mM *cis* or *trans* or 0.2 mM *cis/trans* mixture) as well as the *in vivo* mouse liver conversion of 1, 3-D to mutagenic *cis* and

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[6] Monroe, J.J., Kort, K., and Skopek, T. (1998). Mnu-induced lambda cII/cI mutations in Big Blue (B6C3F1)mice. Environ. Mol. Mutagen 31(Suppl. 29):15.

[7] Skopek, T. (1998). Transgenic mutation models: research, testing, and reality checks. Environ. Mol. Mutagen 32:104-105.

[8] Validation and Regulatory Acceptance of Toxicological Test Methods Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (1997). NIH Publication No: 97-3981.

trans epoxides (Schneider et al. 1997)[9]; Schneider et al., 1998a) and the formation of four DNA adducts when 1,3-D epoxides (400 μmol) are reacted with 2'-deoxyguanosine *in vitro* for 48 hours (Schneider et al., 1998b). Additionally, there are data showing the *in vivo* formation of DNA lesions in the stomach, liver, kidneys, bladder, lungs, brain and bone marrow harvested 3 or 24 hours after mice received a single intraperitoneal (ip) dose of 150 mg/kg 1,3-D (Sasaki et al., 1998). It is, therefore, questionable whether conditions were optimal to allow P450-dependent epoxidation of 1,3-D to the *cis* and *trans* 1,3-D epoxides. Based on these considerations, no definitive conclusions can be reached, therefore, the assay does not provide acceptable evidence of a negative response in this *in vitro* test system.

4. GSH activities in several mammalian cell lines (MRID No. 44460503)

The data from this study clearly show that *S. typhimurium* has low level GST activity. This finding is consistent with the investigators' statements regarding the positive results in the *in vitro* bacterial mutagenicity assays. However, mixed results were obtained with the mammalian cell lines tested. For example, rat hepatocytes, for which the mutagenicity data were negative, had the highest level of GSH activity with 1,3-D (21.1 nmoles product/min/mg protein). However, CHO cells, also negative for mutagenicity, had the lowest level of GSH activity (3.22 nmoles product/min/mg protein). In contrast, both the CHL (DEDE) and CHL (DON) cells, which were positive for mutagenicity, had relatively high net GSH activity with 1,3-D (13.4 and 9.43 nmoles product/min/mg protein, respectively). We also noted that using CDNB as the substrate produced conflicting results relative to the correlation between GSH activity and protection against mutagenic events. Therefore, the investigators' statement, "The results of this study are consistent with the hypothesis that any potential genotoxicity of 1,3-D is largely mitigated by the presence of physiological levels of GSH and GST activity." is not supported by the presented data. Based on these considerations, we conclude that the study is unacceptable. While the study is unacceptable, the data showing major differences in net activities of mixed GSH isoenzymes of different mammalian cell lines do suggest that similar variations may exist in whole animals and humans.

5. Mechanism of tumorigenicity studies in male rats and mice (MRID No. 44446302)

Telone II was negative in this study for DNA adduct formation. The results from the analysis of GSH levels do, nevertheless, indicate that the target tissues of both species were exposed to 1, 3-D (≥25 mg/kg/day in rat liver and ≥10 ppm in mouse lung). However, the data from the 2-year bioassay in rats show that liver tumors were induced at 12.5 mg/kg, and at this level in the current study GSH was not significantly depleted. Thus, it is difficult to determine the role of GSH as a protective mechanism and how it relates to the tumorigenic response. Furthermore, whether the dose of the reactive metabolites (*cis* and *trans* 1, 3-D epoxides) that evaded metabolic detoxification and penetrated to the

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[9] Schneider, M., Quistad, G.B., and Casida, J.E. (1997). Sterospecific epoxidation of *cis*- and *trans*-1,3-dichloropropene in mice and mouse liver microsomes and mutagenic activity of the epoxides. Abstract presented at the American Chemical Society Meeting, August 1997.

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biologically significant site (i.e., DNA) was sufficient to provoke DNA adduct formation is also unclear since there are data showing the *in vivo* mouse liver conversion of 1, 3-D to mutagenic *cis* and *trans* epoxides (Schneider et al., 1998a) and the formation of four DNA adducts when 1, 3-D epoxides are reacted with 2'-deoxyguanosine(Schneider et al., 1998b). Additionally, there are data showing the *in vivo* formation of DNA lesions in the stomach, liver, kidneys, bladder, lungs, brain and bone marrow harvested 3 or 24 hours after mice received a single intraperitoneal (ip) dose of 150 mg/kg 1,3-D (Sasaki et al., 1998). Hence, there is no assurance that a biologically effective dose was reached using either the oral or inhalation routes within the exposure time (5 days/week for 2 weeks).

Despite the protective action of GSH in the detoxification of 1,3-D, hepatotoxicity was observed in rats at 100 mg/kg/day as indicated by increases in serum alanine aminotransferase(ALT) and aspartate aminotransferase (AST). It was also of note that in the study of Sasaki et al. (1998) mice receiving a single ip dose of 150 mg/kg 1, 3-D showed no overt toxicity or organ necrosis. Thus, the significant ($p<0.01$-0.001) migration of nuclear DNA in all sampled organs was not associated with toxic death to the cells. Additional information provided by Dr. Sasaki indicated that the purity of the test sample of 1,3-D used in the comet assay was >80%; whether the sample contained epichlorohyrin was not available (Sasaki, 1999)[10]. However, the profile of DNA damage induced by ip 140 mg/kg epichlorohyrin was distinct from 1,3-D (i.e, positive only in two tissues, stomach and lung versus positive in nine tissues for 1,3-D).

There are reservations with the cell proliferation and apoptosis data. With the exception of a suggestive increase in cell proliferation for high-dose rat livers, which paralleled increased liver enzyme levels, there were no apparent effects on cell proliferation or apoptosis in either strain. A definitive conclusion can not be reached regarding the potential of the test material to alter cell proliferation or apoptosis rates in rat liver and/or mouse lung or urinary bladder following short term exposure to 1, 3-D because of the wide variability in the data in conjunction with the small sample sizes. Similarly, it is not clear whether the suggestive increase in cell proliferation, which occurred in 50% of the high-dose rats is biologically relevant or is related to the variability in the data. Since a high variation in cell proliferation rates is expected, the use of a larger sample size may have resolved the issue. The same assessment (i.e, variability in the results and small sample size) can be made for the data on apoptosis. In addition, the lack of a positive control for the *in situ* end-labeling method (i.e., ApopTag® Plus Kit) used to identify apoptosis is of concern since more conventional H&E staining has been shown to be more sensitive for the detection of apoptosis [11]. Overall, the findings for both the rat and mouse cell growth and cell regulation phase of the study illustrate the difficulties that have been encountered by other

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[10]Sasaki, Y.F. (1999). Personal communications between Y. F. Sasaki and N.E. McCarroll, USEPA. 1/18/99.

[11] Roberts, R.A., Soames, A.R., James, N. H., Gill, J.H., Wheeldon, E.B. (1995). Dosing-induced stress causes hepatocyte apoptosis in rats primed by the rodent hepatocarcinogen cyproterone acetate. Toxicol. Appl. Pharmacol. 135, 192-199.

investigators in interpreting data from cell proliferation and apoptosis studies because of the variability in the data (Goldsworth, et al., 1996)[12].

While the findings from the ^{32}P-postlabeling assay suggest that 1,3-D did not cause detectable DNA adducts in rat liver or mouse lung tissue exposed to the tumorigenic dose, there are concerns regarding the previously cited evidence of DNA adduct formation (Schneider et al., 1998 b) and the positive results in the *in vivo* comet test (Sasaki et al., 1998). Although the route of compound administration in the comet test differed from the currently reviewed study, the findings indicate that under the appropriate conditions, 1,3-D can be detected as DNA damaging in whole animals. Similarly, the previously stated comments of Dr. Sasaki regarding the possible presence of epichlorohyrin in the test sample did not explain all of the organ sites that were positive with the formulation in the comet assay. In addition, there are concerns regarding the small sample size (4 animals/group) and the impact on the power of the test to detect a biological meaningful response. Greater confidence in the data would have been assured if all phases of the study had been conducted with a minimum of 10 animals/group.

As stated earlier, the Agency applauds the use of innovative new approaches such as the ^{32}P-postlabeling assay for the detection of DNA adducts. Because of the divergent techniques used by different investigators, interlaboratory comparisons are ongoing. Nevertheless, it has become a consensus approach that identified DNA adducts formed by the test material under study be included as standards. Inclusion of the *cis* and *trans* 1, 3-D epoxide DNA adducts identified by Schneider et al. (1998 b) is critical to insure that the test system was capable of detecting the appropriate adducts.

Based on the above considerations, the study does not provide convincing evidence of a negative response in this whole animal test system, and the study authors' claims are not sufficiently compelling to conclude that 1,3-D does not induce tumors through DNA adduct formation, particularly in light of the weight-of-the-evidence.

D. MODE OF ACTION

The registrant presented studies to support their claim that the lack of positive mutagenicity findings from *in vivo* assays and the absence of evidence for the metabolic activation to an epoxide *in vivo* indicates that a mutagenic MOA does not account for the lung adenomas in mice and liver adenomas in rats in the inhalation and dietary bioassay, respectively. However, based on our review of the new studies, the in-house mutagenicity data and findings from the open literature, we disagree with this claim. There is convincing evidence from the open literature of mutagenicity in bacteria as well as demonstration of both the *in vitro* and *in vivo* formation of mutagenic epoxides and the induction *in vivo* of DNA strand breaks. Therefore, the registrant has not presented compelling information to conclude that Telone II does not induce tumors through a mutagenic MOA. In agreement with the Dearfield memo, we conclude

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[12]Goldsworth, T.L., Conolly, R.B. and Fransson-Steen, R. (1996). Apoptosis and cancer risk assessment. Mutat. Res 365:71-90.

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that Telone II presents a potential mutagenicity concern, and this is a plausible MOA for both the oral and inhalation exposures.

The registrants also claimed that "Telone II is not likely to present a carcinogenic hazard to humans when protective mechanisms are present, such as sufficient GSH to completely conjugate and detoxify the administered dose." We also disagree with this claim because liver adenomas were observed at levels of Telone II that do not depleted GSH. Furthermore, the findings from the *in vitro* GSH activity in mammalian cell lines study (MRID No. 44460503) demonstrated that negative mutagenicity was obtained in two mammalian cell lines that had either the highest or the lowest level of GSH activity while cell lines clearly vulnerable to mutagenic attack had relatively high levels of GSH activity. These data emphasize the point that varying levels of physiological defense mechanisms may also exist in the intact animal and conceivably in humans and therefore, raise the issue of possible Telone II effects on animals with lower than expected GSH levels, compromised GSH systems (i.e, geriatric animals) and animals or tissues deficient in GSH.

Finally, as part of the *Proposed Guidelines for Carcinogen Risk Assessment* (1996), criteria are being considered to evaluate a postulated carcinogenic MOA for an agent. The only key influence on the carcinogenic process that has been identified for Telone II is mutagenicity, which has been reproduced for both *in vitro* and *in vivo* studies by independent investigators. From the available Telone II data, we assess that sufficient information has not been presented by the registrant to identify a MOA for tumor formation, other than mutagenicity.

E. CONCLUSIONS

Based on all of the above considerations, we conclude that the registrant has not provided a compelling argument to rule out a mutagenic MOA for Telone II and the protective action of GSH has not been clearly demonstrated. Furthermore, the proposed criteria for evaluation of a postulated MOA tend to support mutagenicity as the most plausible event in the carcinogenesis of Telone II. We conclude, therefore, that the registrant's proposal to use the nonlinear Margin of Exposure (MOE) approach is not justified. Based on all considerations, the results from the newly submitted studies do not necessitate having the Cancer Assessment Review Committee reevaluate the carcinogenicity or the need for low-dose extrapolation.