



EPA/635/R-00/001

TOXICOLOGICAL REVIEW

OF

1,3-DICHLOROPROPENE

(CAS No. 542-75-6)

**In Support of Summary Information on the
Integrated Risk Information System (IRIS)**

May 2000

U.S. Environmental Protection Agency
Washington, DC

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to 1,3-dichloropropene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 1,3-dichloropropene.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 202-566-1676.

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The role of the chemical manager is to develop the draft document with input from internal and external reviewers. The final document reflects the Agency consensus position on the health effects of the chemical and does not necessarily reflect the opinion of the chemical manager.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agencywide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix B.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg/day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m³.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per µg/m³ air breathed. Another form in which risk is presented is drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for 1,3-dichloropropene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), and *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentration Issues and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, subject: Guidance on Risk Characterization.

Literature search strategies employed for this compound were based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

1,3-Dichloropropene is known as 3-chloroallyl chloride, alpha-chloroallyl chloride, gamma-chloroallyl chloride, 3-chloropropenyl chloride, 1,3-dichloropropylene, alpha, gamma-dichloropropylene, 1,3-dichloro-1-propene, DCP, and Telone II[®]. Some relevant physical and chemical properties of 1,3-dichloropropene are listed below (Hazardous Substances Data Base [HSDB], 1998).

CASRN: 542-75-6

Empirical formula: C₃H₄Cl₂

Molecular weight: 110.98

Vapor pressure: 3.7 Pa at 20°C

Density: 1.220 at 25°C

Boiling point: 108°C

Water solubility: 0.15%

Log K_{ow}: 1.36 (cis isomer); 1.41 (trans isomer)

Conversion factor: 1 ppm = 4.54 mg/m³ at 25°C; 1 mg/m³ = 0.22 ppm

At room temperature, 1,3-dichloropropene is a colorless to straw-colored liquid with a sharp, sweet, penetrating, chloroform-like odor (HSDB, 1998). It is miscible in most organic solvents and evaporates easily.

1,3-Dichloropropene is used extensively in agriculture as a preplanting fumigant, primarily for the control of nematodes affecting the roots of plants. It is one of the few remaining relatively inexpensive fumigants currently available; the registrations of similar fumigants, e.g., 1,2-dibromo-3-chloropropane (DBCP) and ethylene dibromide (EDB), have been suspended for most agricultural uses. Therefore, 1,3-dichloropropene is an agriculturally important pesticide, with a high annual volume of use throughout the United States and abroad. Commercial formulations of 1,3-dichloropropene (Telone II[®] soil fumigant) contain mixtures of cis (Z) and trans (E) isomers. The older formulations of technical-grade 1,3-dichloropropene called Telone II[®] contained approximately 89% cis- and trans-1,3-dichloropropene, 2.5% 1,2-dichloropropene, 1.5% of a trichloropropene isomer, and 1% epichlorohydrin (HSDB, 1998). Since about 1988, formulations of have replaced epichlorohydrin, the stabilizing agent, with epoxidized soybean oil. Commercial formulations that contain other dichloropropenes or dichloropropanes and other chemicals include D-D, Di-Trapex, and Vorlex.

The presence of other active ingredients and stabilizers in commercial formulations is a confounder in establishing the toxicity of 1,3-dichloropropene. Pure dichloropropene may

contain confounding chemicals as well, as it is unstable when exposed to heat and oxygen or heat and light (Watson et al., 1987). Degradation products will also form at room temperature if dichloropropene is stored for several weeks in the presence of oxygen (Watson et al., 1987).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

1,3-Dichloropropene toxicokinetics in humans appear to be similar to those observed in rodents. Inhalation studies with both humans and animals have shown that 1,3-dichloropropene vapors are readily absorbed, conjugated with glutathione (GSH) via glutathione S-transferase (GST), and rapidly excreted in the urine as N-acetyl-(S-3-chloroprop-2-enyl)cysteine (3CNAC), a mercapturic acid metabolite (see Figure 1). Thus, the major metabolic pathway for 1,3-dichloropropene leads to its detoxification and excretion. Ingestion studies in animals have demonstrated that the toxicokinetics of oral exposures are similar to those of inhalation exposures. 1,3-Dichloropropene is unlikely to accumulate in the body.

3.1. ABSORPTION

Stott and Kastl (1986) studied the inhalation pharmacokinetics of technical-grade 1,3-dichloropropene by exposing male Fischer 344 (F344) rats to mean vapor concentrations of 30, 90, 300, and 900 ppm (136, 409, 1,363, and 4,086 mg/m³, respectively) for 3 hours. These air concentrations produced vapor uptakes of 147, 307, 880, and 1,810 nanomoles per minute, and corresponding absorption fractions of 82%, 65%, 66%, and 62%, respectively. Based upon the uptake of dichloropropene vapors, average amounts of dichloropropene absorbed by rats over the 3-hour exposure period were approximately 14, 29, 85, and 171 mg/kg in the 136, 409, 1,363, and 4,086 mg/m³ exposure groups, respectively. Even though the rate of uptake increased with increasing exposure, the increase was not linear at higher concentrations. The decrease in vapor uptake at higher concentrations was associated with an exposure-related depression in ventilatory frequency, which was statistically significant at 409 mg/m³ and higher. Stott and Kastl (1986) indicate that Alarie (1973) observed exposure-related depression in ventilatory frequency with numerous respiratory irritants; they suggest that 1,3-dichloropropene is a respiratory irritant.

The major site of absorption of inhaled 1,3-dichloropropene in the rat is the lung rather than the nasal mucosa (Stott and Kastl, 1986). The localized uptake of vapors in rats exposed to 90 or 150 ppm (409 or 682 mg/m³, respectively) was examined by surgically isolating the upper and lower respiratory tract. The lower respiratory tract absorbed approximately 50% of inhaled dichloropropene vapors whereas the upper respiratory tract absorbed only 11%–16% of vapors. Total absorption rates were approximately 73% and 79% at 409 and 682 mg/m³, respectively.

In 1992, Waechter et al. showed that absorption of 1,3-dichloropropene from inhalation exposure in humans was similar to absorption in rats (Stott and Kastl, 1986). Six male volunteers were exposed to 1 ppm (4.54 mg/m³)¹ commercial Telone II[®] (50.6% cis isomer,

¹Calculated using conversion of 1 ppm = 4.54 mg/m³ at 25° C.

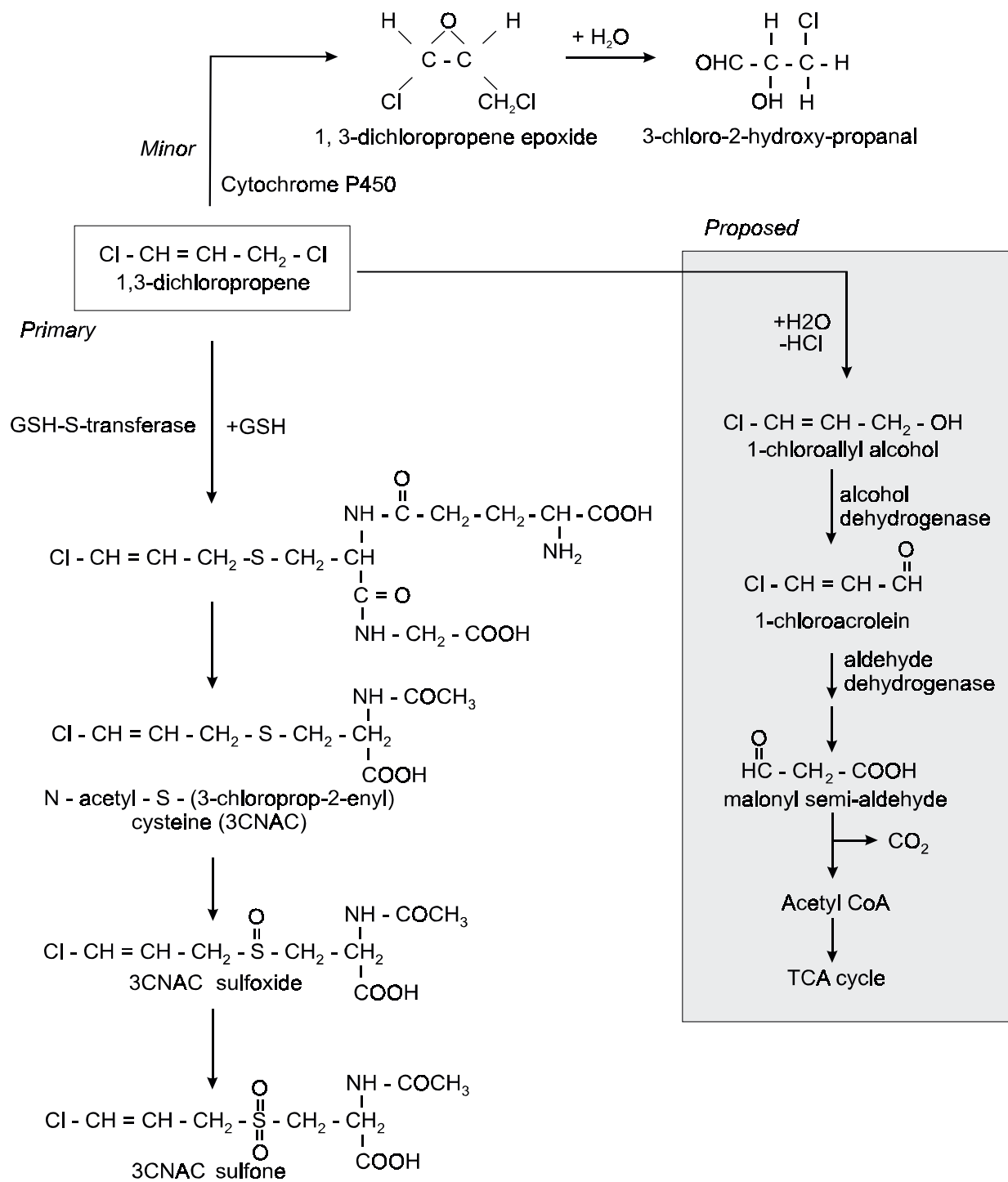


Figure 1. Metabolic pathways for 1,3-dichloropropene. Derived from Waechter and Kastl (1988) and Schneider et al. (1998a).

45.2% trans isomer) for 6 hours. The absorption of cis-1,3-dichloropropene was 72%–80% while the absorption of trans-1,3-dichloropropene was 77%–82%. A similar percentage of absorption (i.e., 82%) was found in rats exposed to 136 mg/m³ 1,3-dichloropropene for 3 hours (Stott and Kastl, 1986). Waechter et al. (1992) also found that the concentration of 1,3-dichloropropene in expired air plateaued in the first hour of exposure and declined rapidly postexposure to nondetectable levels in less than an hour.

Stott et al. (1998) showed that the pharmacokinetics of 1,3-dichloropropene microencapsulated in a starch-sucrose matrix were similar to those of neat 1,3-dichloropropene administered by gavage to F344 rats. Female rats were simultaneously gavaged with 25 mg/kg neat ¹³C-dichloropropene and 25 mg/kg microencapsulated ¹²C-dichloropropene in a corn oil suspension. Blood concentrations were measured at various intervals by gas chromatography/mass spectrometry (GC/MS). The absorption half-life of neat dichloropropene was 2.5 minutes for the cis isomer and 2.7 minutes for the trans isomer, while the absorption half-life for encapsulated dichloropropene was 1.3 minutes for the cis isomer and 2.3 minutes for the trans isomer. The elimination half-lives from blood were also similar. The cis/trans elimination half-lives for the alpha phase were 6.1 ± 0.9/6.2 ± 0.5 minutes for neat dichloropropene and 6.9 ± 1.6/6.6 ± 0.6 minutes for encapsulated dichloropropene. The cis/trans elimination half-lives for the beta phase were 32 ± 18/22 ± 5 minutes for neat dichloropropene and 20 ± 8/29 ± 5 minutes for encapsulated dichloropropene.

3.2. DISTRIBUTION

In the study by Stott and Kastl (1986) that examined the inhalation pharmacokinetics of technical-grade 1,3-dichloropropene in rats, the time required for blood concentrations of cis- and trans-dichloropropene to plateau was dependent on the exposure concentration. In the 136 and 409 mg/m³ groups, blood concentration plateaued after 1 hour of exposure. In the 1,363 mg/m³ group, 2–3 hours were required. Blood levels of dichloropropene in the 4,086 mg/m³ group did not reach a steady state during the 3-hour exposure period. In general, higher blood levels of trans-dichloropropene than cis-dichloropropene were observed in rats at all vapor concentrations, even though the cis:trans ratio of the technical-grade dichloropropene used in the study was 1.2:1. These findings are consistent with the faster metabolism of the cis isomer.

The human pharmacokinetic study by Waechter et al. (1992) also found that blood concentrations plateaued rapidly at low exposures for most subjects. In five of the six subjects, blood levels of 1,3-dichloropropene reached an apparent plateau within 1 hour of exposure to 4.54 mg/m³. Blood levels in the remaining subject increased throughout the exposure. Also, as in the rat study by Stott and Kastl (1986), trans-1,3-dichloropropene reached higher blood concentrations than did cis-1,3-dichloropropene, even though the cis:trans ratio of the technical-grade dichloropropene used in the study was 1.1 to 1.

Distribution studies in rats after gavage administration of 1,3-dichloropropene indicate that the forestomach, glandular stomach, kidney, liver, and bladder are primary organs of distribution compared with fat, skin, blood, and remaining carcass. Dietz et al. (1985) studied the amount of ¹⁴C-activity in these tissues of male F344 rats and B6C3F1 mice 48 hours after a

single oral dose of ^{14}C -1,3-dichloropropene (1 or 50 mg/kg to rats and 1 or 100 mg/kg to mice). At 1 mg/kg, forestomach and bladder had the highest ^{14}C -activities in both species and were followed by liver, kidney, and glandular stomach. ^{14}C -activity in the remaining tissues was much less. At the high dose, forestomach and kidney had the highest ^{14}C -activities in both species. In rats, these were followed by glandular stomach, liver, and bladder; in mice, they were followed by liver, fat, bladder, and glandular stomach. Because of the rapid metabolism and excretion of 1,3-dichloropropene, described in Sections 3.3 and 3.4, the ^{14}C -activities measured 48 hours after single doses actually represent metabolized dichloropropene rather than the parent compound.

3.3. METABOLISM

Climie et al. (1979) determined that GSH conjugation is the major metabolic pathway of cis-1,3-dichloro[^{14}C]propene after oral administration to female Wistar rats (see Figure 1). A hepatic GST catalyzes the conjugation of cis-1,3-dichloropropene with GSH. The conjugate is further metabolized to a mercapturic acid, cis-3CNAC, and is excreted in the urine. This metabolite accounted for 92% of the 0 to 24-hour cumulative urinary radioactivity. In vitro metabolic studies using rat liver preparations showed that the trans isomer is degraded similarly, but at a much slower rate (four to five times more slowly).

On the basis of pharmacokinetic studies, Dietz et al. (1985) concluded that the major pathway of metabolism and detoxification of both cis and trans isomers of 1,3-dichloropropene occurred via conjugation with GSH in male F344 rats and B6C3F1 mice after oral administration. Additionally, 3CNAC and its sulfone derivative were identified as the two major urinary metabolites (Figure 1). No parent compound was detected in the urine. 1,3-Dichloropropene undergoes substantial first-pass metabolism that follows linear pharmacokinetics over an oral gavage dose range of 1–100 mg/kg for mice and 1–50 mg/kg for rats (Dietz et al., 1985). Waechter et al. (1992) showed that the major metabolites after inhalation in humans, cis- and trans-3CNAC, were identical to those found in rats after oral exposure.

Although the major metabolic pathway of 1,3-dichloropropene is conjugation by GSH, Schneider et al. (1998a) found that epoxidation of 1,3-dichloropropene is a minor metabolic pathway in mouse liver at $\sim\text{LD}_{50}$ doses. Schneider et al. (1998a) administered either 350 mg/kg individual isomer or 700 mg/kg cis/trans-1,3-dichloropropene to male Swiss-Webster mice by intraperitoneal (ip) injection and then measured epoxide formation in the liver at various times up to 150 minutes later. GC/MS measurements showed that 1,3-dichloropropene concentrations in the liver peaked about 10 minutes after treatment and then decayed through apparent first-order kinetics, with half-lives of 36 minutes for the cis isomer and 50 minutes for the trans isomer. Epoxide concentrations were approximately two orders of magnitude lower than those of the parent compound. In in vitro experiments, Schneider et al. (1998a) demonstrated that conjugation of 1,3-dichloropropene with GSH decreases epoxide formation in mouse liver.

Dietz et al. (1985) showed that oral administration of 1,3-dichloropropene depletes tissue levels of nonprotein sulfhydryls (NPSH), an indication of GSH levels. A single gavage dose of 0, 1, 5, 25, 50, or 100 mg/kg ^{14}C -1,3-dichloropropene was administered to male F344 rats and

B6C3F1 mice. NPSH levels in selected tissues were measured 2 hours after administration. Significant depression of NPSH levels, to 51%–17% of control values, were observed in the forestomachs of rats and mice dosed with 25–100 mg/kg. Less severe, dose-dependent depression, to 70%–45% of control for the same doses, also occurred in the glandular stomach. Depression of NPSH levels in the liver was less than that in the forestomach and glandular stomach, with the rat liver about twice as sensitive as the mouse liver. There were no statistically significant changes in NPSH levels in the kidney or urinary bladder of either rats or mice. The no-observed-effect levels (NOELs) for NPSH depletion in forestomach were 1 mg/kg for rats and 5 mg/kg for mice. Covalent binding of 1,3-dichloropropene to macromolecules in the forestomach, glandular stomach, liver, kidney, and urinary bladder in male F344 rats and male B6C3F1 mice was also studied by Dietz et al. (1985). A single gavage dose of ¹⁴C-1,3-dichloropropene of 1, 50, or 100 mg/kg was administered, and binding was measured after 2 hours. Macromolecular covalent binding increased with increasing dose. Binding was highest in the forestomach and glandular stomach and lowest in the liver, kidney, and bladder, findings that correlated with the magnitude of tissue-specific decreases in NPSH.

3.4. EXCRETION

When Hutson et al. (1971) administered 2.53–2.70 mg of either cis- or trans-1,3-dichloro-[2-¹⁴C]propene by gavage to Carworth Farm E rats, 80%–90% of the radiolabel was eliminated in the feces, urine, or expired air during the first 24 hours of the experiment. Within 24 hours, 80.7% of the administered cis isomer and 56.5% of the trans isomer were eliminated in the urine. Approximately 3.9% of the cis isomer and 23.6% of the trans isomer were recovered in expired air as [¹⁴C]carbon dioxide (see proposed pathway in Figure 1). A small amount (1%–4%) of unmetabolized 1,3-dichloropropene was exhaled directly. After 4 days, about 1% of the administered dose of each isomer was found in the carcass. Thus, the rat retains little ingested 1,3-dichloropropene after oral administration.

Dietz et al. (1984a) administered by gavage 1 or 50 mg/kg ¹⁴C-cis, trans-1,3-dichloropropene to male rats and 1 or 100 mg/kg to male B6C3F1 mice. Elimination was measured by the amount of radioactivity present in expired air, urine, and feces. Urinary excretion was the predominant route of elimination during the 48 hours after dosing, accounting for 51%–61% of the administered dose in rats and 63%–79% in mice. Feces and expired carbon dioxide contained about 18% and 5%, respectively, of the administered radioactivity in rats, and 15% and 14%, respectively, of the administered radioactivity in mice. Only 2%–6% of the original dose remained in the carcasses at the end of 48 hours. The predominant urinary metabolite, identified as 3CNAC, confirmed the earlier findings by Climie et al. (1979). The sulfoxide or sulfone derivative of 3CNAC was tentatively identified as (an)other metabolite(s). Dietz et al. (1984a) calculated a urinary excretion half-life of approximately 5.5 hours for dichloropropene in rats and mice.

In the Stott and Kastl (1986) study in which F344 male rats were exposed to 136, 409, 1,363, and 4,086 mg/m³ 1,3-dichloropropene for 3 hours, a pronounced rapid elimination phase was observed in all rats exposed to 1,363 mg/m³ or less. In this initial phase, the half-life of cis-dichloropropene was calculated at 3–5 minutes for animals exposed to 136, 409, and 1,363

mg/m³ and increased to more than 14 minutes for animals exposed to 4,086 mg/m³. Rats exposed to trans-dichloropropene had a longer first-phase elimination half-life, averaging 6 minutes for the 136, 409, and 1,363 mg/m³ groups and 27 minutes for the 4,086 mg/m³ group. Following this first phase, both cis- and trans-dichloropropene exhibited a second, slower and longer phase of elimination in rats exposed to 1,363 or 4,086 mg/m³, roughly 25 to 43 minutes, independent of isomer or exposure concentrations. The initial phase of elimination primarily represents the redistribution of dichloropropene from blood to tissues, whereas the second phase of elimination is determined by the rate of metabolism. Disproportionately large increases in blood levels at the end of exposure occurred in rats exposed to 4,086 mg/m³ cis-dichloropropene and 1,363 and 4,086 mg/m³ trans-dichloropropene, which indicated nonlinear elimination at high exposures. The longer half-lives and disproportionately higher blood levels at the higher doses suggest that metabolism was saturated. The data also indicate that elimination of dichloropropene at lower exposure levels is mediated primarily via metabolism and not via simple exhalation of the parent compound, a result consistent with Hutson et al. (1971).

In the human study by Waechter et al. (1992), urinary excretion of 1,3-dichloropropene was an apparent first-order process at an inhalation exposure of 4.54 mg/m³ for 6 hours. The elimination half-lives for the initial phase were 4.2 ± 0.8 hours for the cis isomer and 3.2 ± 0.8 hours for the trans isomer, whereas the half-lives for the terminal phase were 12.3 ± 2.4 hours (cis isomer) and 17.1 ± 6 hours (trans isomer).

Fisher and Kilgore (1988a) conducted a series of experiments to assess the relationship among dichloropropene inhalation, GSH reduction in tissues, and serum lactate dehydrogenase (LDH) activity. These studies demonstrate that very high inhalation exposures of dichloropropene are required to produce significant decreases in GSH in all target organs except nasal tissue. It should be noted that the technical-grade dichloropropene used in this study and others by Fisher and Kilgore (1988b, 1989) contained epoxidized soybean oil as the stabilizing agent instead of epichlorohydrin. Male Sprague-Dawley rats were exposed to 1,3-dichloropropene for 1 hour at average concentrations of approximately 0, 2, 5, 33, 306, 771, 954, or 1,716 ppm (0, 8, 20, 150, 1,390, 3,504, 4,334, or 7,791 mg/m³, respectively)² for determination of GSH levels in the heart, kidney, liver, lung, and testes; 0, 5, 31, 70, or 222 ppm (0, 24, 143, 320, or 1,012 mg/m³, respectively)² for measurement of GSH levels in nasal tissue; and 0, 25, 660, or 2,277 ppm (0, 113, 2,995, or 10,336 mg/m³, respectively)² for assessment of lung dry weight/wet weight and serum LDH activity. The principal tissue affected by low exposure concentrations was nasal tissue. GSH in nasal tissue decreased in a concentration-dependent fashion to 27% at 24 mg/m³, 23% at 143 mg/m³, 18% at 320 mg/m³, and 12% at 1,012 mg/m³, compared to control values. Lung GSH also was decreased but remained relatively constant at approximately 70% of control values at all exposure concentrations up to 4,334 mg/m³ and showed no evidence of further depletion. In the heart and testis, no significant reductions were observed at concentrations up to 4,334 mg/m³. At the next highest dose of 7,791 mg/m³, however, lung, heart, and testis GSH levels were decreased significantly, whereas kidney GSH levels were not. Liver GSH content showed an exposure-related decrease only between 3,504 and 7,791 mg/m³. There were no changes in any lung weight parameters (wet weight,

²Calculated using conversion of 1 ppm = 4.54 mg/m³ at 25° C.

percent wet weight/body weight, relative dry weight/wet weight, or dry weight/body weight) for animals sacrificed either 2 or 6 hours postexposure. Serum LDH activity measured 6 hours after dichloropropene exposure did not exhibit any significant changes except for a decrease at 10,336 mg/m³.

The dose-dependency of GSH metabolism was evaluated in another study by Fisher and Kilgore (1988b). Male Sprague-Dawley rats were exposed to technical-grade dichloropropene vapors, nose only, for 1 hour at concentrations up to 789 ppm (3,582 mg/m³)³ dichloropropene. Urine was collected for 24 hours postexposure for measurement of the urinary mercapturic acid metabolite of cis-dichloropropene, cis-3CNAC. The amount of urinary cis-3CNAC exhibited a concentration-dependent increase in rats exposed from 0 to 284 ppm (1,289 mg/m³)³. However, the amount of cis-3CNAC in the urine remained constant at exposures from 1,289 to 3,582 mg/m³, a finding consistent with saturation of metabolism at higher doses of dichloropropene as suggested by the Stott and Kastl (1986) study.

Fisher and Kilgore (1989) postulated that dichloropropene entering the rat via inhalation exposure is rapidly transformed into its GSH conjugate, (S-3-chloroprop-2-enyl)GSH (GSCP), which is the precursor to the mercapturic acid metabolite 3CNAC (Figure 1), and that GSCP can be measured in blood over time. In an initial range-finding study using male Sprague-Dawley rats, the blood level of GSCP did not significantly change when measured at 15, 30, 45, and 60 minutes during a 1-hour exposure to 610 ppm (2,769 mg/m³)³ technical-grade dichloropropene. In the main study, the concentrations of GSCP in blood following inhalation exposures to 78, 155, and 404 ppm (354, 704, and 1,834 mg/m³, respectively)³ dichloropropene were examined using equations for both monophasic and biphasic decay. GSCP was detected in the blood of rats at all concentrations. No significant differences were found between the regression lines of the mathematical equations expressed as either monophasic or biphasic decay at any exposure concentration. Thus, these results could fit either a one- or two-compartment model of elimination. Moreover, no significant differences were found between the regression lines for any exposure concentrations, which indicates that the elimination of GSCP was independent of exposure concentration. The apparent half-life of GSCP was 17 hours. These findings suggest that the formation of GSCP may occur by dose-independent mechanisms, or that the mechanisms responsible for formation of GSCP may be saturated at the exposure levels selected for the experiment. Alternatively, on the basis of the results of Stott and Kastl (1986), an initial rapid phase of GSCP elimination may have been present but not detected in the Fisher and Kilgore (1989) study because the first blood samples following exposure were collected after the initial, rapid elimination phase had already occurred. The significance of these findings is unclear.

Two biological monitoring studies in humans have demonstrated that there is a dose-dependent relationship between respiratory occupational exposure to 1,3-dichloropropene and excretion of the urinary mercapturic acids, cis- and trans-3CNAC (van Welie et al., 1991). In this study of 12 male workers in the flower bulb industry, exposure to cis- and trans-1,3-dichloropropene measured by personal air samplers ranged from 0.3 to 18.9 mg/m³ during 1- to 11-hour shifts. Urinary excretion of 3CNAC followed first-order elimination kinetics following

³Calculated using conversion of 1 ppm = 4.54 mg/m³ at 25° C.

exposure. Urinary elimination half-lives of 5.0 ± 1.2 hours for cis-3CNAC and 4.7 ± 1.3 hours for trans-3CNAC were not statistically different. The calculated coefficient of variation indicated that the elimination half-lives of cis- and trans-3CNAC were quite consistent among individuals. These human half-life values were similar to those reported by Waechter et al. (1992) for a clinical study (4.2 ± 8 hours for cis and 3.2 ± 0.8 hours for trans) and to those found by Dietz et al. (1984b) in rats and mice (i.e., 5.5 hours). van Welie et al. (1991) found high correlations ($r^2 = 0.93$) between respiratory 8-hour time-weighted average (TWA) exposures to cis- and trans-dichloropropene and cumulative urinary excretion of cis- and trans-3CNAC. cis-Dichloropropene yielded three times more 3CNAC than trans-dichloropropene, consistent with differences in the rate of metabolism between the isomers. Approximately 45% and 14%, respectively, of air levels of cis- and trans-dichloropropene were excreted as mercapturic acid metabolites (i.e., 3CNAC).

In a second study of 15 male applicators in the flower bulb industry (Osterloh et al., 1989), the relationship between air concentrations of dichloropropene and urinary levels of 3CNAC and N-acetylglucosaminidase (NAG) was assessed. The release of NAG, a renal tubular enzyme, may indicate low-level subclinical tissue injury. Breathing zone samples were analyzed for dichloropropene. Urine samples were collected before and at various times after application and analyzed for 3CNAC and NAG. Dichloropropene exposure concentrations were $0.26\text{--}9.39$ mg/m³ with a mean of 2.56 mg/m³. Exposure durations from 120 to 697 minutes yielded dichloropropene exposure rates of $62\text{--}3,700$ mg/m³/min. The 24-hour urinary excretion of 3CNAC was $0.50\text{--}9.17$ mg, with a mean of 2.57 mg. Twenty-four-hour 3CNAC and NAG urinary levels correlated well with dichloropropene exposure concentrations ($r^2 = 0.854$). The correlation increased when next-morning urine samples were used ($r^2 = 0.914$). There was also a correlation between next-morning 3CNAC levels and 24-hour urinary concentrations of NAG. The overall mean excretion of NAG was 2.63 milliunits/mg creatinine. Four subjects had NAG values in a clinically abnormal range, above 4 milliunits/mg. Nine subjects had increases in NAG that averaged 25% higher than their baseline values. The authors concluded that excretion of urinary 3CNAC is correlated with dichloropropene exposures. The investigators indicated that NAG levels above the normal range in four workers suggest that a subclinical nephrotoxicity may be associated with dichloropropene exposure. Alternatively, this increase may have been an adaptive response in the kinetics of detoxification and excretion of 1,3-dichloropropene. Follow-up urinary measurements were not conducted to evaluate whether levels returned to the normal clinical range following cessation of exposure for 24 hours or longer.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Bousema, MT; Wiemer, GR; Van Joost; TH. (1991) A classic case of sensitization to DD-95®. *Contact Derm* 24(2):132-133

DD-95® is a nematocide containing approximately 95% 1,3-dichloropropene. A case report is described in which a 44-year-old male process operator at a pesticide plant developed an

acute bullous dermatitis on the dorsae of both feet. This event, first reported in August 1988, recurred in September 1989. The operator was interviewed by medical personnel and recalled (a) soiling his shoes with DD-95[®] about 10 days before he developed dermatitis the first time, and (b) soiling his shoes again with the same compound 1 day before the dermatitis reappeared in September 1989. The patient was subsequently patch-tested with DD-95[®] at 2.0%, 1.0%, 0.5%, 0.1%, 0.03%, and 0.005% and responded positively to all concentrations up to 3 days following challenge. A control group of 20 volunteers were similarly tested at a concentration of 0.05% DD-95[®], but none showed any positive symptoms. The authors report that this is the third case study that has reported an association between contact dermatitis and occupational dermal exposure to DD-95[®]. The authors suggest that there is a small but distinct subgroup of individuals working with pesticides who develop an allergic reaction upon dermal contact with DD-95[®] and other pesticides containing mainly 1,3-dichloropropene.

4.1.2. Hernandez, AF; Martin-Rubi, JC; Ballesteros, JL; et al. (1994) Clinical and pathological findings in fatal 1,3-dichloropropene intoxication. Hum Exp Toxicol 13(5):303-306

A 27-year-old previously healthy male, working on a friend's farm, accidentally drank an unknown quantity of dichloropropene from a receptacle containing clear fluid because he thought it was water. After he realized it was not water, he vomited. He was taken to the hospital emergency department 2 hours later with acute gastrointestinal distress, sweating, tachypnoea, tachycardia, hypovolemic disturbance, and lividity on both legs. Over the next several hours, the patient developed respiratory and cardiac hypotension; metabolic acidosis; elevation of blood glucose and subsequent insulin-resistant hyperglycemia; abdominal wall distension and other gastrointestinal disturbances, including increased peritoneal fluid amylase levels, indicative of pancreatic disorder. These acutely toxic effects progressed to extensive bilateral interstitial and alveolar infiltration consistent with adult respiratory distress syndrome, as well as hemodynamic, gastrointestinal, liver, and kidney deterioration. Death from multiple organ failure occurred 38 hours following admission to the hospital. The autopsy revealed edematous brain and lungs, bloody-clear fluid in the abdominal cavity, congested spleen and lungs, and hemorrhagic exudate from the stomach mucosa. Histological examination of the stomach revealed congestion of the gastric mucosal vessels, autolysis, scattered mucosal erosions, and small foci of mononuclear inflammatory cells. Histological examination of the liver showed architectural anomalies, autolysis, sinusoidal congestion and small biliary thrombi.

Toxicological identification of the ingested compound by GC/MS confirmed that Telone II[®] (cis- and trans-1,3-dichloropropene) was the fatal agent. The initial body-burden fluid concentrations of the toxicant at the time of the emergency admission were 1.1 : mol/L in blood and 0.2 : mol/L in urine.

4.1.3. Kezic, S; Monster, AC; Verplanke, AJW; et al. (1996) Dermal absorption of cis-1,3-dichloropropene vapor: human experimental exposure. Hum Exp Toxicol 15(5):396-399

Because cis-1,3-dichloropropene-based pesticides account for a large majority of the total pesticides used in the Dutch flower bulb industry, the objective of this study was to estimate the significance of skin absorption of cis-1,3-dichloropropene vapor compared with inhalation uptake. Under controlled clinical exposure conditions, five adults (four males and one female) were dermally exposed on 1,277 cm² of the forearm and hand to 86 mg/m³ cis-1,3-dichloropropene for 45 minutes. Urine samples were collected before and after the exposure sessions and analyzed by GC/MS to determine levels of creatinine and cis-3CNAC. Urinary excretion of cis-3CNAC peaked during the first hour after exposure and declined thereafter, with an average half-life of 6 hours. The total amount of cis-3CNAC excreted over 24 hours averaged 48 µg. The mean total uptake of the cis-1,3-dichloropropene was estimated at 67 µg. The subject with the highest urinary excretion of cis-3CNAC (90.4 µg) complained of skin irritation experienced as tingling. The estimated permeability constant of 0.8 cm/hour for cis-1,3-dichloropropene is consistent with permeability constants reported for two other dihalomethane vapors in rats, dibromomethane and bromochloromethane.

To compare dermal absorption of cis-1,3-dichloropropene with absorption via inhalation, Kezic et al. (1996) used data from inhalation studies by Waechter et al. (1992) and van Welie et al. (1991). The data were normalized for identical exposure conditions: 8 hours exposure to 5 mg/m³ of cis-1,3-dichloropropene. A number of assumptions were made, including (a) dermal uptake from the forearm surface area can be linearly extrapolated to the whole-body surface area; (b) the permeability constant and metabolism of 1,3-dichloropropene are concentration-independent over the range of concentrations used in the three studies, and (c) co-exposure to trans-1,3-dichloropropene in the inhalation studies did not affect the human toxicokinetics of cis-1,3-dichloropropene.

The results of these analyses demonstrate that dermal absorption upon vapor exposure to dichloropropene does occur but is relatively minor in terms of total internal dose when compared with inhalation. Dermal exposure to cis-1,3-dichloropropene vapors was estimated to account for approximately 2%–4% of the total uptake under conditions of combined inhalation and whole-body dermal exposures. However, in certain acute high-exposure situations, such as accidental releases, dermal absorption as a route of entry may be more significant.

4.1.4. Markovitz, A; Crosby, WH. (1984) Chemical carcinogenesis. A soil fumigant, 1,3-dichloropropene, as possible cause of hematologic malignancies. Arch Intern Med 144:1409-1411

This study examined case reports of hematologic neoplasms following intoxicating exposure to 1,3-dichloropropene. In two firefighters, histiocytic (non-Hodgkin's) lymphomas appeared simultaneously several years after both men were exposed to 1,3-dichloropropene at the site of a chemical spill. In both cases, the cancers were refractory to standard regimens of treatment and the subjects died within a few months of one another. Six other firefighters, simultaneously exposed, did not develop any malignancies. Leukemia developed in a farmer a

few months after the right side of his head was exposed to 1,3-dichloropropene during soil application of the chemical (the hose he was using had a leak). This exposure occurred for 30 days. The farmer suffered from a smoldering leukemia until, after a second series of daily exposures 1 year later, the leukemia became extremely aggressive. He died of pneumonia in the hospital during treatment for leukemia.

4.1.5. Nater JP; Gooskens, VHJ. (1976) Occupational dermatosis due to a soil fumigant. Contact Dermatitis 2(4):227-229

The aim of this study was to determine whether occupational dermatitis resulting from direct contact with 1,3-dichloropropene was due to an allergic or a primary irritant reaction. Three cases of occupational skin contact with a common nematocide soil fumigant, D-D mixture, were examined. The mixture contained 1,3-dichloropropene, 1,2-dichloropropane, and epichlorohydrin. Patient 1 received two 1-week exposures 1 year apart and developed an itching erythematous rash. Patient 2 developed the rash after a single exposure. Patient 3 was employed spraying pesticides on a daily basis for 10 years between September and January. After 7 years he developed dermatitis on his arms, face, and ears, which subsided upon avoidance of the nematocide. Patch testing was performed with D-D, other preparations of 1,3-dichloropropene, and 1,2-dichloropropane at 1% in acetone (a concentration producing no reaction in five volunteers) and with the 20 standard allergens of the International Contact Dermatitis Research Group. Patch testing of all 1,3-dichloropropene preparations produced allergic reactions in patient 1 (with spongiosis, lymphocyte infiltration, and migration) but not in patients 2 or 3. No patients reacted positively to 1,2-dichloropropane. The results indicate that 1,3-dichloropropene is a primary irritant, as demonstrated by the occupational dermatitis in patients 2 and 3, but also that 1,3-dichloropropene can cause a contact allergic reaction, as demonstrated by the positive patch test in patient 1.

4.1.6. Brouwer, EJ; Evelo, CTA; Verplanke, AJW; et al. (1991) Biological effect monitoring of occupational exposure to 1,3-dichloropropene: effects on liver and renal function and on glutathione conjugation. Br J Ind Med 48(3):167-172

This study examined the liver and kidney effects of subchronic exposure to 1,3-dichloropropene in employees of the Dutch flower bulb industry. The cohort consisted of 14 commercial applicators who used 1,3-dichloropropene in soil fumigation operations in the Bollenstreek region of the Netherlands. Venous blood and spot urine samples were collected from the subjects at the start of the bulb culture season in July and after the season ended in October. Possible hepatotoxicity was assessed by determining serum activities of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, γ -glutamyltranspeptidase, alkaline phosphatase, and total serum bilirubin. Kidney function was evaluated by measuring serum β_2 -microglobulin and creatinine, urinary albumin, β_2 -microglobulin, retinol binding protein, β -galactosidase, and alanine aminopeptidase. Blood GSH concentration and erythrocyte GST activity were determined to evaluate the effect on blood GSH conjugation capacity.

Data from the environmental monitoring study indicated that the fumigators were exposed to TWA concentrations of 1.9–18.9 mg/m³ 1,3-dichloropropene. The Dutch standard of 5 mg/m³ was exceeded about 30% of the exposure time. A decrease in serum total bilirubin concentration was the only parameter of liver function to be significantly affected by 1,3-dichloropropene. Urine albumin and retinol binding protein concentration were significantly increased and serum creatinine concentration was significantly decreased by the end of the spraying season. Blood GSH concentration and erythrocyte GST activity were also significantly decreased. The authors felt that a subclinical nephrotoxic effect due to exposure to 1,3-dichloropropene over a spraying season could not be ruled out. Alternately, changes in serum chemistry and urine analysis parameters may have been adaptive responses to detoxification and elimination of 1,3-dichloropropene. The serum chemistry and urine analysis parameters of the exposed workers were not evaluated subsequently to assess whether the observed alterations returned to normal values. The decreases in GSH and GST values indicate that GSH conjugation is involved in 1,3-dichloropropene elimination and likely detoxification.

4.1.7. Hayes, WJ. (1982) Pesticides studied in man. Baltimore: Williams and Wilkins, pp. 139-171

In a collision between two trucks, a tank carried by one truck ruptured and spilled approximately 4,542 L 1,3-dichloropropene. An estimated 80 people were exposed to vapors. The most common signs and symptoms were headaches in six people, irritation of mucous membranes in five people, dizziness in five people, and chest discomfort in four people. Three persons became unconscious at the scene of the accident. Of 41 persons tested, 11 had slightly elevated serum glutamic oxaloacetic transaminase and/or glutamic pyruvic transaminase values. Within 48–72 hours, values reverted to normal in eight people, but five still had slightly elevated serum glutamic oxaloacetic transaminase.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Inhalation Studies

4.2.1.1. Parker, CM; Coaste, WB; Voelker, RW. (1982) Subchronic inhalation toxicity of 1,3-dichloropropene/1,2-dichloropropene (D-D) in mice and rats. *J Toxicol Environ Health* 9:899-910

Groups of F344 rats and CD-1 mice (28/sex/group) were exposed to vapors of D-D at nominal concentrations of 0, 5, 15, or 50 ppm (0, 22.7, 68.1, or 227 mg/m³)⁴ 6 hours/day, 5 days/week for either 6 (10/sex/group) or 12 weeks (19/sex/group). The D-D formulation contained 25% cis-1,3-dichloropropene; 27% trans-1,3-dichloropropene; 29% 1,2-dichloropropane; and minor amounts of 3,3-dichloropropene, 2,3-dichloropropene, and other related chlorinated hydrocarbons. Clinical symptoms, body and organ weights, hematology,

⁴Calculated using conversion of 1 ppm = 4.54 mg/m³ at 25° C.

serum chemistry, and urine analysis parameters were examined, and gross and histopathology were performed at sacrifice.

No clinical signs of toxicity were observed in either species. There were no statistically significant differences in mean body weights in either species at either 6-week or 12-week terminal sacrifices. White blood cell counts were significantly decreased at 12 weeks in the male mice exposed to 68.1 mg/m³ and in female mice exposed to 227 mg/m³ D-D. Glutamic pyruvic transaminase activity was significantly decreased in the 68.1 and 227 mg/m³ groups of female mice at 12 weeks. Other histologic, serum chemistry, and urinalysis parameters were either transiently altered or showed no changes that were dose-related or outside normal ranges. In rats, relative kidney weights in females and relative liver weights in males were significantly increased after 12 weeks of exposure to 227 mg/m³ D-D. In male mice, statistically significant decreases were observed in relative testis weight at 6 weeks, but not at 12 weeks, in the 227 mg/m³ group. Absolute and relative liver weights in male mice were statistically increased at 12 weeks in both the 22.7 and 227 mg/m³ groups, but not in the 68.1 mg/m³ group. The toxicological significance of the increased liver weights is questionable because control male mice had lower than normal liver weights. All other mean organ weights and relative weights were within normal ranges.

The only gross pathological change observed in any treated animals during the study was an increased incidence of enlarged peribronchial lymph nodes, with no accompanying histopathology, in all exposed mice after 6 weeks. The only treatment-related histopathology, which occurred at 12 weeks, was a slight to moderate diffuse hepatocyte enlargement in male mice exposed to 227 mg/m³ D-D (12/21 treated vs. 4/18 controls). In females, a similar but equivocal increase was also observed at 227 mg/m³ (6/18 treated vs. 1/18 controls). No treatment-related gross pathology or histopathology was observed in the respiratory tracts of either rats or mice.

Thus, the exposure-related effects in this study occurred after 12 weeks of exposure to 227 mg/m³ D-D. Female rats exhibited increased relative kidney weights whereas male rats and mice exhibited increased relative liver weights. In the absence of histopathologic changes such as degeneration or necrosis, or functional deficits as exhibited by abnormal serum or urine analyses, these changes in organ weights are considered adaptive rather than adverse. Therefore, the no-observed-adverse-effect-level (NOAEL) for both rats and mice is 227 mg/m³ D-D and 118 mg/m³ 1,3-dichloropropene (D-D was 52% 1,3-dichloropropene). There is no lowest-observed-adverse-effect-level (LOAEL).

4.2.1.2. Stott, WT; Young, JT; Calhoun, LL; et al. (1988) Subchronic toxicity of inhaled technical-grade 1,3-dichloropropene in rats and mice. *Fundam Appl Toxicol* 11:207-220

Male and female F344 rats and B6C3F1 mice (10/sex/group) were exposed to vapors of technical-grade dichloropropene for 6 hours/day, 5 days/week for 13 weeks at nominal concentrations of 0, 10, 30, 90, or 150 ppm (0, 45.4, 136, 409, or 681 mg/m³, respectively). The technical-grade formulation contained 90.9% 1,3-dichloropropene, 2.4% 1,2-dichloropropene, 1.2% epichlorohydrin, and an unnamed quantity of mixed isomers of chlorohexane, chlorohexene, and trichloropropene. The only treatment-related clinical effects observed during

the study were a transient brown discoloration of the muzzle fur of rats exposed to 681 mg/m³ immediately following exposure, and a strong mercaptan odor associated with the coats and urine of all rats and mice exposed to 409 or 681 mg/m³ of the formulation. There were no treatment-related differences in survival.

The body weights of male and female rats were depressed in an exposure-related manner, but were toxicologically significant (i.e., $\geq 10\%$) only at the 681 mg/m³ exposure level. Consistent with decreases in body weight, there were numerous changes in organ weight parameters. In males, the mean absolute weights of kidney, liver, brain, and heart were statistically decreased in the 681 mg/m³ group, but the mean relative weights of these organs were increased in the 409 and 681 mg/m³ groups. Relative testis weights of males in the 681 mg/m³ group were also increased. In females in the 681 mg/m³ group, absolute weights were decreased in the brain, heart, liver, and thymus gland, whereas relative weights were increased in the brain, heart, and kidney. The study authors attributed the decreased absolute organ weights and increased relative weights to the fact that rats were fasted prior to sacrifice, and stated that the findings were consistent with reduced body weights and proportionately lower body fat content and nonparenchymal cell mass in the high-dose animals, especially females.

A dose- and duration-related decrease in body weight was also observed in male and female mice. Again, the body weight decrease reached toxicological significance only at the 681 mg/m³ exposure. As with rats, the diminished growth rate and reduced body weights of mice in the 409 and 681 mg/m³ exposure groups were reflected in decreases in the absolute weights of the heart, kidney, liver, brain, and thymus glands. Because mice were not fasted prior to sacrifice, relative heart, kidney, and liver weights of male mice in the 409 and 681 mg/m³ groups were also decreased. Relative brain weights of male mice in the 681 mg/m³ group were increased. Female mice in the 681 mg/m³ group had decreased relative liver and thymus weights; those in the 409 and 681 mg/m³ groups had increased relative kidney weights. No pathological or histopathologic findings were observed in any of these organs in either rats or mice. The authors concluded that the observed organ weight changes were likely due to body weight decreases associated with nutritional changes and a general nonspecific effect of high-dose exposures to dichloropropene.

Clinical chemistry and hematologic changes in the 409 and 681 mg/m³ groups of both species were generally consistent with decreased body weights and associated nutritional status. There were no treatment-related changes in urinalysis parameters. The only observable gross pathology differences in the tissues of treated animals relative to controls were (a) a decrease in the amount of abdominal fat in female rats in the 681 mg/m³ group and (b) a decrease in the size of the thymus in male mice exposed to 681 mg/m³.

In rats, histopathologic changes were observed in the following organs or organ systems: (a) mild hyperplasia of the nasal respiratory epithelium in all male and female rats exposed to 409 or 681 mg/m³ and in 2/10 male rats exposed to 136 mg/m³; (b) slight to very slight degeneration of the olfactory epithelium in all male and female rats in the 681 mg/m³ group and 1 female in the 409 mg/m³ group; (c) incomplete development of the uteri and uterine tissue hypoplasia in 7/10 female rats in the 681 mg/m³ group, consisting of a significant decrease in the cross-sectional diameter of the uterine horns and a decrease in the number and stage of development of the uterine glands (examination of the ovaries revealed normal development in

graafian-follicles as well as corpora lutea); and (d) significant atrophy of mesenteric adipose tissue in females exposed to 681 mg/m³.

In mice, exposure-related histopathology of the nasal mucosa was similar to that observed in rats, consisting of slight to very slight degeneration of the olfactory neuroepithelium and hyperplasia of respiratory epithelium in all male mice exposed to 409 and 681 mg/m³ and in 9/10 female mice at these exposures. These animals also had small focal areas of respiratory metaplasia, a condition in which the damaged sensory olfactory epithelium is replaced by ciliated respiratory epithelium identical to that lining the remainder of the nasal cavity and respiratory tract. The urinary bladders of 7/10 and 6/10 female mice in the 409 and 681 mg/m³ groups, respectively, exhibited large confluent areas of moderate hyperplasia of the transitional epithelium. Mild aggregates of lymphoid cells in the subepithelial tissues were found to be associated with these areas of hyperplasia in about half the affected mice. Lymphoid aggregates without epithelial hyperplasia were also present in 9/10 female mice exposed to 136 mg/m³.

The organ weight, hematology, clinical chemistry, and gross pathology findings observed in this study in animals of both species appeared to be secondary to, or associated with, the decreases in body weight gain and terminal body weight. The most significant treatment-related histopathologic findings occurred in the nasal mucosa of both sexes of rats and mice and in the urinary bladders of female mice. The significant decrease in the size and development of the uterus of female rats in the 681 mg/m³ group appears to have resulted from the marked growth retardation in these animals (20% decrease in body weight by the end of the study), rather than from a direct effect of high-dose exposure to dichloropropene vapors.

The results of this study identify a subchronic NOAEL of 45.4 mg/m³ and a LOAEL of 136 mg/m³ technical-grade 1,3-dichloropropene based on degenerative changes in the nasal mucosa of both sexes of rats and mice. Because the technical-grade formulation was 90.9% 1,3-dichloropropene, the NOAEL is 41.3 mg/m³ and the LOAEL is 123.6 mg/m³ 1,3-dichloropropene.

4.2.1.3. Lomax, LG; Stott, WT; Johnson, KA; et al. (1989) The chronic toxicity and oncogenicity of inhaled technical-grade 1,3-dichloropropene in rats and mice. *Fundam Appl Toxicol* 12:418-431

Male and female F344 rats and B6C3F1 mice (50/sex/dose/level) were exposed via whole-body chamber inhalation to 0, 5, 20, or 60 ppm (0, 22.7, 90.8, or 272 mg/m³, respectively) technical-grade dichloropropene vapors for 6 hours/day, 5 days/week for 2 years. The technical-grade formulation consisted of 92.1% 1,3-dichloropropene, 0.7% 1,2-dichloropropane, mixtures of hexanes and hexadienes, and approximately 2% epoxidized soybean oil. Two satellite groups of rats and mice (10/sex/dose group) were also exposed to dichloropropene for 6 and 12 months, respectively. Standard protocols for chronic toxicity and carcinogenicity bioassays were followed.

No clinical signs indicative of toxicity were observed in treated animals throughout the study, and there were no significant differences in survival. Mean body weights of male and female rats exposed to 272 mg/m³ were decreased 3%–5% during the first 11–15 months of

treatment, but they reverted to normal during the remainder of the 2-year study. The body weights of male and female mice exposed to 272 mg/m³ dichloropropene were also depressed. In males, 3%–9% decreases were noted throughout the study. In females, decreases of 2%–11% in the 272 mg/m³ group occurred only during the first 5 months of the study. Body weight depression in rats and mice was not considered to be toxicologically significant.

There were no treatment-related changes in hematology, clinical chemistry, and urine analysis parameters in any of the treated groups in either rats or mice. Weights of the brain, heart, kidney, liver, and testis in treated rats did not differ significantly from control animals. The mean relative kidney and liver weights of male mice in the 272 mg/m³ exposure group were slightly lower (10%–15%) than mean control values at all exposure intervals (6, 12, and 24 months). At 90.8 mg/m³, male mice had statistically significant decreases in relative liver and kidney weights after 12 months of exposure, but not after 24 months. Small but statistically significant changes in relative heart, testis, and brain weights in male mice and in absolute heart and brain weights in female mice were observed in the 272 mg/m³ group following one or two of the three exposure periods. Organ weight changes in mice were sporadic and small and, with the exception of the liver and kidneys, were not associated with organ histopathology. Thus, most organ weight changes were considered to be due to decreased total body weights of the mice and/or normal biological variability, and without toxicological significance.

4.2.1.3.1. Nonneoplastic changes. In rats, gross pathological changes were not detected in either males or females. No significant increases in nasal histopathologic effects were observed in the 22.7 or 90.8 mg/m³ groups. Increased incidences were observed in both sexes exposed to 272 mg/m³ for 24 months, but not after exposure for 6 or 12 months. The incidences of these lesions at 24 months are shown in Table 1. The microscopic changes were located in the olfactory mucosa covering the upper portions of the nasal cavity, nasal septum, and turbinates and were characterized by. (a) unilateral or bilateral decreased thickness of the olfactory epithelium due to degenerative changes; (b) erosions of the olfactory epithelium; and (c) fibrosis beneath the affected olfactory epithelium, primarily in the ecto- and/or endoturbinates.

Gross pathological examination of mice showed an increase in lung masses in male mice exposed to 272 mg/m³ compared with controls. Statistically significant, treatment-related morphological changes in the urinary bladder were noted in females exposed to 90.8 and exposure concentration and duration. Changes occurred in one 272 mg/m³ and in males exposed to 272 mg/m³. The incidence of these changes increased with female mouse exposed to 90.8 mg/m³ for 12 months and in several females exposed to 90.8 mg/m³ for 24 months. Nearly half the female mice exposed to 272 mg/m³ were affected at 6 months, and nearly all were affected at 12 and 24 months. The incidence of this lesion at 24 months is shown in Table 2.

Microscopically, the urinary bladders of both sexes exhibited hyperplasia characterized by diffuse, uniform thickening of the transitional epithelium. In females, the hyperplasia was accompanied by inflammation in 6/48 and 8/45 cases in the 90.8 and 272 mg/m³ groups, respectively. For both sexes, the hyperplasia increased in frequency and severity with concentration, and in females exposed to 272 mg/m³, the hyperplasia increased with exposure duration. The incidence of hyperplasia was not statistically higher than in controls in the 22.7 mg/m³ groups exposed for 24 months.

Table 1. Incidence^a of selected noncancer effects in rats from Lomax et al. (1989)

Lesion	Males				Females			
	0 mg/m ³	22.7 mg/m ³	90.8 mg/m ³	272 mg/m ³	0 mg/m ³	22.7 mg/m ³	90.8 mg/m ³	272 mg/m ³
Decreased thickness of olfactory epithelium	0/50	1/50	1/50	20/50	0/50	0/50	0/50	15/50
Erosions of olfactory epithelium	0/50	0/50	1/50	15/50	0/50	0/50	0/50	6/50
Fibrosis of submucosa, olfactory epithelium	0/50	0/50	0/50	6/50	0/50	0/50	0/50	2/50

^a Compared with number examined.

Table 2. Incidence^a of selected cancer and noncancer effects in mice from Lomax et al. (1989)

Lesion	Males				Females			
	0 mg/m ³	22.7 mg/m ³	90.8 mg/m ³	272 mg/m ³	0 mg/m ³	22.7 mg/m ³	90.8 mg/m ³	272 mg/m ³
Hypertrophy/hyperplasia of respiratory epithelium (slight)	5/50	1/50	4/50	48/50	4/50	4/50	28/50	49/50
Degeneration of olfactory epithelium (slight)	1/50	0/50	1/50	48/50	0/50	0/50	1/50	45/50
Epithelial hyperplasia of urinary bladder	4/48	7/48	11/48	37/47	1/47	4/46	21/48	44/45
Bronchioalveolar adenoma	9/50	6/50	13/50	22/50	4/50	3/50	5/50	3/50

^a Compared to number examined. For tumor incidence, mice that died before the first tumor appeared were omitted from the number at risk.

Exposure-related histopathologic effects in nasal tissues in both sexes of mice consisted of hypertrophy and hyperplasia of the respiratory epithelium and/or degeneration of the olfactory epithelium. In all cases, these changes were graded as “slight,” involved approximately 10% or less of the total respective epithelium, and did not progress in severity or extent of distribution from one time period to the next. After 24 months of exposure, nearly all males and females in the 272 mg/m³ group exhibited nasal mucosa histopathology (Table 2), as did most female mice in the 90.8 mg/m³ group.

Additional microscopic changes noted in mice in the 272 mg/m³ group were (a) focal hyperplasia and hyperkeratosis in the forestomach of 8/50 males exposed for 24 months; (b) decreased vacuolation of renal proximal tubular epithelial cells in males exposed for 24 months; and (c) decreased hepatocyte vacuolation in males exposed for 6 and 12, but not 24, months, and in females exposed for 24 months.

Based on female mouse urinary bladder and nasal epithelial histopathology, this study identifies a NOAEL of 22.7 mg/m³ and a LOAEL of 90.8 mg/m³ technical-grade dichloropropene for mice. The NOAEL for nasal epithelial histopathology from exposure to technical-grade dichloropropene in rats is 90.8 mg/m³, while the LOAEL is 272 mg/m³. Because the technical-grade formulation was 92.1% 1,3-dichloropropene, the mouse NOAEL/LOAEL is 20.9 mg/m³/83.6 mg/m³ 1,3-dichloropropene and the rat NOAEL/LOAEL is 83.6 mg/m³/250.5 mg/m³ 1,3-dichloropropene.

4.2.1.3.2. Neoplastic lesions. In rats, there were no statistically significant increases in primary, benign, or malignant tumors in either males or females exposed to dichloropropene for 24 months compared with concurrent or historical controls. Despite degenerative changes in the nasal mucosa of rats and hyperplasia/hypertrophy of nasal epithelium in mice, no nasal tumors were noted in either species. In mice, a statistically significant increase in the incidence of benign lung tumors (i.e., bronchioalveolar adenomas) was observed in males in the 272 mg/m³ group after 24 months of exposure (see Table 2 for incidences). In spite of the prolonged hyperplastic response observed in the transitional epithelium of the urinary bladder, no dose-related tumorigenic responses were observed in either male or female mice. One adenoma and two carcinomas were diagnosed in female mice exposed to 90.8 mg/m³ technical-grade dichloropropene for 24 months; however, no bladder tumors were observed in male mice at any dose or in female mice in the 272 mg/m³ group.

A NOAEL of 90.8 mg/m³ and a LOAEL of 272 mg/m³ technical-grade dichloropropene was observed for neoplastic effects, i.e., bronchioalveolar adenomas in male mice. Since the technical-grade formulation was 92.1% 1,3-dichloropropene, the NOAEL was 83.6 mg/m³ and the LOAEL was 250.5 mg/m³ 1,3-dichloropropene. The NOAEL for tumorigenic effects in rats was 272 mg/m³ technical-grade dichloropropene, or 250.5 mg/m³ 1,3-dichloropropene. There was no LOAEL because tumors were not observed in rats.

4.2.2. Oral Studies

4.2.2.1. *Haut, KT; Stebbins, KE; Johnson, KA; et al. (1996) Subchronic toxicity of ingested 1,3-dichloropropene in rats and mice. Fundam Appl Toxicol 32:224-232*

Male and female F344 rats and B6C3F1 mice (10/sex/dose) were given 0, 5, 15, 50, or 100 mg/kg/day (rats) or 0, 15, 50, 100, or 175 mg/kg/day (mice) racemic 1,3-dichloropropene in their diets for 13 weeks. Satellite groups of rats (10/sex/dose) from the control and 100 mg/kg/day groups were retained for observation for 4 weeks following treatment in order to examine recovery.

1,3-Dichloropropene was administered in the diet by encapsulating it into a starch/sucrose microsphere matrix and mixing the microcapsules into rodent chow. The microcapsule technology provided a means by which oral toxicity data in rodents could be obtained using a contemporary Telone formulation (95.8% 1,3-dichloropropene) and a nonbolus oral dosing procedure. Although stability and mixability studies on the microcapsule formulation showed that (a) it was stable for several years at room temperature, (b) it was stable in rodent chow for at least 21 days postmixing, and (c) it provided a homogenous mixture with chow, no in-cage stability studies were performed to determine the stability of the microencapsulated formulation under actual feeding conditions. As discussed in Section 3.1, Stott et al. (1998) showed that the bioavailability of microencapsulated 1,3-dichloropropene is similar to that of neat 1,3-dichloropropene when both materials are given by gavage in corn oil. Analysis of the test diets during the study determined that all animals received approximately the targeted doses of 1,3-dichloropropene. Empty microspheres were mixed with rodent feed to serve as the appropriate control. Dietary administration of 1,3-dichloropropene is preferable over bolus dosing for the assessment of ingestion toxicity.

There were no treatment-related clinical signs of toxicity in rats or mice at any dose level over the course of the study. Food consumption in rats was consistently decreased for high-dose groups of males and females and occasionally depressed at lower doses relative to control values. Food consumption in treated mice was generally unchanged and only occasionally depressed at the higher dose levels. A dose-related statistically significant decrease in body weight, compared with controls, was observed in male rats at doses of 15 mg/kg/day and higher, in female rats at 50 mg/kg/day and higher, and in male and female mice at all doses. Decreases in body weight became toxicologically significant (i.e., $\geq 10\%$) in male rats at 50 mg/kg/day, in female rats at 100 mg/kg/day, and in mice at 100 mg/kg/day.

Although several organ weights (absolute and/or relative) in treated animals exhibited statistically significant differences compared with controls, the changes were consistent with decreases in body weight and were not considered toxicologically significant. Similarly, statistically significant differences in hematology, clinical chemistry, and urine analysis parameters, with the possible exception of alkaline phosphatase levels (in male rats), were either not dose-related or were consistent with the lower body weights and reduced nutritional status of affected animals. Thus, these differences were not considered to be toxicologically significant.

At necropsy, no gross pathology was observed in treated animals. Mild basal cell hyperplasia and a slight prominence of mononuclear cells (consisting of endothelial, fibroblast, and inflammatory cells) in the proximity of the basement membrane of the forestomach were noted in all treated male and female rats in dose groups of 15 mg/kg/day and higher. After 4 weeks of recovery, animals in the 100 mg/kg/day group (the only treated group continued through recovery) continued to exhibit basal cell hyperplasia; however, the severity and incidence were diminished compared with those observed immediately following cessation of treatment. These findings were attributed to localized portal-of-entry irritant effects on the forestomach. There were no histopathologic changes in the glandular stomach.

Histopathologic changes were noted in the livers of male mice at doses of 15 mg/kg/day and higher, and the changes consisted of a slight decrease in hepatocellular size, congruent with decreased liver weight and decreased body weight. Therefore, this finding was not considered to be toxicologically significant. Decreased vacuolation of tubular epithelial cells of the kidney was observed in male mice in the highest dose group (175 mg/kg/day). There were no histopathologic changes in the liver or the kidney in female mice.

On the basis of the results of this 13-week oral study, the NOAEL for rats is 5 mg/kg/day and the LOAEL is 15 mg/kg/day, based on the irritant effect manifested by mild basal cell hyperplasia of the nonglandular stomach in both sexes. For mice, the NOAEL is 50 mg/kg/day and the LOAEL is 100 mg/kg/day, based on a toxicologically significant decrease in body weight in both sexes.

4.2.2.2. *Stott, WT; Johnson, KA; Jeffries, TK; et al. (1995) Telone II® soil fumigant: two-year chronic toxicity/oncogenicity study in Fischer 344 rats. Prepared by Dow Chemical Company, Midland, MI. Study #M-003993-0311*

Male and female F344 rats (50/sex/dose) were administered a microencapsulated formulation of Telone II® (96% 1,3-dichloropropene and no epichlorohydrin) in the diet at doses of 0, 2.5, 12.5, or 25 mg/kg/day for 24 months. Satellite groups of rats (10/sex/dose) were administered Telone II® for 12 months. Standard bioassay data, including body weight, food consumption, clinical chemistry, hematology, urine analysis, organ weights, pathology, and histopathology, were collected. Ophthalmologic examinations were conducted at the start of the study and prior to necropsy. All animals were observed at least twice daily. Clinical examinations were conducted at least once weekly.

There is some concern about the bioavailability of the microencapsulated formulation of 1,3-dichloropropene used in this study. In the bioavailability study conducted by Stott et al. (1998), microencapsulated 1,3-dichloropropene in corn oil was coadministered with neat 1,3-dichloropropene in corn oil by gavage. Although conjecture may be made, the experiment did not delineate how the absorption of microencapsulated 1,3-dichloropropene in feed compares with gavage dosing. In addition, no in-cage stability tests were conducted so there is no assurance that the loaded microcapsules were stable during use.

No treatment-related effects were observed for either mortality or clinical signs in either sex. Body weights were significantly decreased in a dose-dependent manner in treated animals. Decreases were greater than 10% for both sexes at 25 mg/kg/day. In the 12.5 mg/kg/day group, average body weight decreases for males and females were 5% and 8%, respectively. Food consumption was statistically decreased and averaged 11% and 7% less than controls in males at 12.5 and 25 mg/kg/day, respectively, and 5% less than controls for females at 25 mg/kg/day. Thus, the observed decrement in body weights is at least partially attributable to reduced food consumption.

Decreased absolute adrenal, heart, and liver weights and higher relative brain and kidney weights were observed in males at 25 mg/kg/day. Females at 25 mg/kg/day had statistically significant decreases in adrenal and liver weights and increases in relative brain, heart, kidney, and liver weights compared with controls. No consistent treatment-related changes in hematologic, clinical chemistry, and urine analysis parameters were observed in any of the dosed groups.

4.2.2.2.1. Nonneoplastic changes. The only histopathology observed was in the forestomach, which exhibited mild basal cell hyperplasia of the mucosal lining characterized by increased cytoplasmic basophilia and increased number of cell layers in the basilar portion of the mucosa. Basal cell nuclei were oval in shape. There was a slight prominence of mononuclear cells at the basement membrane consisting of endothelial, fibroblast, and inflammatory cells. The forestomach hyperplasia is believed to be a manifestation of chronic irritation, which is consistent with the observation of primary dermal irritation (Nater and Gooskens, 1976) and other portal-of-entry effects from 1,3-dichloropropene exposure (Haut et al., 1996; Breslin et al., 1989; Lomax et al., 1989; Linnett et al., 1988; Stott et al., 1988). Forestomach lesions were noted in both sexes of rats fed microencapsulated dichloropropene at ≥ 15 mg/kg/day for 13 weeks (Haut et al., 1996). At 12 months of dietary exposure in the present study, forestomach lesions occurred in half the animals ingesting 12.5 mg/kg/day and in almost all the animals ingesting 25 mg/kg/day. Table 3 shows the incidence of forestomach hyperplasia at 24 months. The incidence of hyperplasia was statistically increased at 12.5 and 25 mg/kg/day. There were no indications of basal cell hyperplasia in the 2.5 mg/kg/day group. Other histopathologic changes in the liver and kidney were not considered to be treatment related, as they appeared to be age related and/or secondary to decreased body weight.

On the basis of results of this study, the NOAEL and LOAEL for noncancer effects are 2.5 and 12.5 mg/kg/day, respectively, based on chronic irritation manifested by an increased incidence of forestomach hyperplasia in rats of both sexes. The co-critical effect of body weight decrease, which was less sensitive, occurred at 25 mg/kg/day.

4.2.2.2.2. Neoplastic lesions. No statistically significant incidence of malignancies was observed in rats of either sex. A trend test identified an increased incidence of benign liver cell tumors, (i.e., hepatocellular adenomas), in both sexes of rats at 24 months but not at 12 months of exposure. At 24 months, the incidence of benign tumors was statistically increased by pairwise comparison only in males at 25 mg/kg/day (see Table 3 for incidences). A single nonfatal hepatocellular carcinoma was observed in a male rat in the 25 mg/kg/day group.

Table 3. Incidence^a of cancer and noncancer effects in rats from Stott et al. (1995)

Lesion	Males				Females			
	0 mg/kg/day	2.5 mg/kg/day	12.5 mg/kg/day	25 mg/kg/day	0 mg/kg/day	2.5 mg/kg/day	12.5 mg/kg/day	25 mg/kg/day
Basal cell hyperplasia in forestomach	3/50	3/50	20/50	30/50	0/50	1/50	20/50	37/50
Hepatocellular adenoma/carcinoma	2/49	1/50	6/50	10/49	0/49	0/50	0/50	4/50

^a 50 rats started in each group. For tumor incidence, rats that died before the first tumor appeared were omitted from the number at risk.

Females at 25 mg/kg/day showed a statistically significant decrease in the incidence of benign mammary gland fibroadenomas (0/50 vs. 8/50 for controls).

The NOAEL and LOAEL for tumors are 12.5 and 25 mg/kg/day, respectively, based on a statistically significant increase in benign hepatocellular adenomas. In this study, the suspicion that the rats may not have received adequate dosing of 1,3-dichloropropene (due to the lack of in-cage stability studies) is allayed by the presence of liver adenomas and forestomach hyperplasia seen in an earlier gavage study (NTP, 1985).

4.2.2.3. Redmond, JM; Stebbins, KE; Stott, WT. (1995) Telone II[®] soil fumigant: two-year dietary chronic toxicity/oncogenicity study in B6C3F1 mice—Final Report. Prepared by Dow Chemical Company, Midland, MI. Study #M-003993-032

Male and female B6C3F1 mice (50/sex/dose) were administered a microencapsulated formulation of Telone II[®] (95.8% 1,3-dichloropropene and no epichlorohydrin) in the diet at dose levels of 0, 2.5, 25, or 50 mg/kg/day for 24 months. Satellite groups of mice (10/sex/dose) were administered Telone II[®] for 12 months. Standard bioassay data, including body weights, food consumption, clinical chemistry, hematology, urine analysis, organ weights, pathology, and histopathology, were collected. Ophthalmologic examinations were conducted at the start of the study and prior to necropsy. All animals were observed at least twice daily. Clinical examinations were conducted at least weekly.

No treatment-related clinical signs were observed in any treatment groups. Body weights and body weight gains were significantly decreased in a dose-dependent manner in 25 and 50 mg/kg/day groups. Male body weights in those groups were more than 10% less than controls (11%–23%), while female body weights were 7%–9% lower than controls. The body weight decreases may be at least partially explained by the 6% lower food consumption in those groups. There were no statistically or biologically significant weight changes for either sex at 2.5 mg/kg/day.

Decreased absolute heart, liver, and kidney weights and higher relative brain, heart, kidney, and testes weights were observed in 25 and 50 mg/kg/day males. Females in those groups had statistically significant decreases in heart, kidney, and liver weights. Higher relative brain weight, compared with concurrent controls, was observed in females treated with 50 mg/kg/day. Changes in organ weights were considered to be secondary to decreased body weights. No consistent treatment-related changes in hematologic, clinical chemistry, and urine analysis parameters were observed in any of the treated groups.

4.2.2.3.1. Nonneoplastic changes. Treatment-related pathology and histopathology were not observed in any of the groups except for a decrease in hepatocyte size in 50 mg/kg/day males at 12 months but not at 24 months of treatment. These effects were considered to be secondary to decreased body weights.

On the basis of the results of this study, the NOAEL and LOAEL for noncancer effects are 2.5 and 25 mg/kg/day, respectively, based on statistically and toxicologically significant decreases in body weight for male mice.

4.2.2.3.2. Neoplastic lesions. No increase in tumor incidence was observed in any of the dose groups. Thus, the NOAEL for cancer effects is 50 mg/kg/day. There is no LOAEL because no tumors were detected.

The NOAEL/LOAEL for noncancer and cancer in this study may be uncertain. Because in-cage stability tests were not conducted, there is no assurance that the loaded microcapsules were stable during use. In addition, the mice did not exhibit the cancer (urinary bladder tumors) and noncancer effects (urinary bladder hyperplasia, forestomach hyperplasia, and hydronephrosis) seen in an earlier gavage study (NTP, 1985; see Section 4.2.2.4). The incidence of lung tumors (combined bronchioalveolar adenoma and carcinoma) in the two studies are similar for similar doses, however. Control rates for females were 2/50 in NTP (1985) and 3/50 in Redmond et al. (1995), whereas those for males were 1/50 in NTP (1985) and 6/50 in Redmond et al. (1995). Half of the control male mice in NTP (1985) died at 1 year from events unrelated to chemical exposure. Incidence rates for the 50 mg/kg groups were 13/50 in NTP (1985) and 11/50 in Redmond et al. (1995) for males and 4/50 in NTP (1985) and 5/50 in Redmond et al. (1995) for females.

4.2.2.4. National Toxicology Program (NTP). (1985) Toxicology and carcinogenesis studies of Telone II® (technical-grade 1,3-dichloropropene containing 1% epichlorohydrin as a stabilizer) in F344/N rats and B6C3F1 mice (gavage studies). U.S. Department of Health and Human Services Technical Report Series No. 269
Yang, RSH; Huff, JE; Boorman, GA; et al. (1986) Chronic toxicology and carcinogenesis studies of Telone II® by gavage in Fischer-344 rats and B6C3F1 mice. J Toxicol Environ Health 18:377-392

Toxicology and carcinogenesis ingestion studies of Telone II® (88%–90% 1,3-dichloropropene, 2.5% 1,2-dichloropropane, 1.5% trichloropropene isomer, and 1% epichlorohydrin) were conducted by administering the commercial-grade formulation in corn oil

by gavage to groups of 52 male and 52 female F344/N rats at doses of 0, 25, or 50 mg/kg and to groups of 50 male and 50 female B6C3F1 mice at doses of 0, 50, or 100 mg/kg, three times weekly for 104 weeks. Ancillary studies were conducted in which additional dose groups containing five male and five female rats were killed after receiving Telone II® for 9, 16, 21, 24, or 27 months. At study termination, there were no toxicologically significant changes in body weight in either species. However, 25 vehicle control mice died from myocarditis during weeks 48–51. Survival in treated rats was comparable to that in vehicle controls. Survival of female mice was statistically lower in the 100 mg/kg group.

4.2.2.4.1. Nonneoplastic lesions. In rats, increases in hyperplastic lesions of the basal layer of the squamous epithelium in the forestomach were observed in both sexes at 25 and 50 mg/kg. These lesions were duration dependent and were seen as early as 9–16 months after treatment began. The incidence for males was significantly increased in the 2-year and ancillary studies combined at both doses, whereas the incidence for females was significant only at 50 mg/kg. Table 4 shows the incidence for these effects in the 2-year study. Stott et al. (1995) also observed these forestomach lesions in both sexes of rats receiving ≥ 12.5 mg/kg/day 1,3-dichloropropene in the diet.

Three types of nonneoplastic changes were observed in mice. Epithelial hyperplasia of the forestomach was statistically significant for females at 100 mg/kg but not for males in any treated group (see Table 5 for incidences). The incidence of transitional epithelial hyperplasia of the urinary bladder (see Table 5) was also observed with statistical significance in both sexes at 50 mg/kg and 100 mg/kg. Such lesions in the urinary bladder have also been noted in inhalation studies. Hyperplasia of the transitional epithelium of the urinary bladder was observed in female mice exposed to ≥ 409 mg/m³ technical-grade dichloropropene for 13 weeks (Stott et al., 1988) and to 90.8 mg/m³ for 2 years (Lomax et al., 1989). In the chronic study (Lomax et al., 1989), male mice were affected at 272 mg/m³ technical-grade dichloropropene. The third nonneoplastic change found in mice (NTP, 1985) was an increased incidence of hydronephrosis exhibited by female mice in the 100 mg/kg group.

The LOAEL for rats, based on hyperplastic lesions of the forestomach, is 25 mg/kg. Averaging the exposure over 7 days yields a LOAEL of 10.7 mg/kg/day. This study does not identify a NOAEL. For mice, the LOAEL is 50 mg/kg, based on epithelial hyperplasia in the urinary bladder. Averaging the exposure over 7 days yields a LOAEL of 21.4 mg/kg/day. There is no NOAEL for mice.

4.2.2.4.2. Neoplastic lesions. In rats, an increase in the incidence of forestomach tumors, mainly benign tumors, was observed (see Table 4 for incidences). In males there was a statistically significant increase in the 50 mg/kg group for squamous cell papilloma and squamous cell papillomas and carcinomas combined. In female rats, a statistically significant increase was only observed for squamous cell papillomas at 50 mg/kg when the 2-year and ancillary studies were combined. Although the nonneoplastic lesions of the forestomach developed within 1 year of exposure, the neoplastic lesions did not appear until 24 months after exposure began. The incidence of forestomach tumors at 25 mg/kg was similar to controls for both sexes.

Table 4. Incidence^a of selected cancer and noncancer effects in rats from NTP (1985) 2-year study

Lesion	Males			Females		
	0 mg/kg/day	25 mg/kg/day	50 mg/kg/day	0 mg/kg/day	25 mg/kg/day	50 mg/kg/day
Basal cell or epithelial hyperplasia of forestomach	2/52	5/52	13/52	1/52	0/52	16/52
Squamous cell papilloma/carcinoma of forestomach	1/49	1/48	13/50	0/47	2/45	3/48
Liver neoplastic nodule	1/49	6/48	7/50	6/46	6/42	10/49
Hepatocellular carcinoma	0/49	0/48	1/50	0/46	0/42	0/49

^a 52 rats started in each group. For tumor incidence, rats that died before the first tumor appeared were omitted from the number at risk.

Table 5. Incidence^a of selected cancer and noncancer effects in mice from NTP (1985) 2-year study

Lesion	Males			Females		
	0 mg/kg/day	50 mg/kg/day	100 mg/kg/day	0 mg/kg/day	50 mg/kg/day	100 mg/kg/day
Basal cell or epithelial hyperplasia of forestomach	0/52	0/50	4/50	1/50	1/50	21/50
Squamous cell papilloma/carcinoma of forestomach	0/37	2/47	3/49	0/50	1/50	4/47
Bronchioalveolar adenoma/carcinoma	1/22	13/40	12/44	2/50	4/50	8/47
Urinary bladder epithelial hyperplasia	0/50	9/50	18/50	2/50	15/50	19/47
Urinary bladder transitional cell carcinoma	0/50	0/50	2/50	0/50	8/50	21/47

^a 50 mice started in each group. For tumor incidence, mice that died before the first tumor appeared were omitted from the number at risk.

Neoplastic nodules, classified as nodular hyperplasia and described as “small focal lesions causing only minimal compression, with little or no cytologic atypia in livers, or with toxic or anoxic hepatic changes,” (NTP, 1985) were noted in the livers of male rats. In the current classification scheme, neoplastic nodules are classified as adenomas (Maronpot et al., 1986). The increased incidence was statistically significant for the 25 and 50 mg/kg doses. One male rat in the 50 mg/kg group exhibited a hepatocellular carcinoma after 2 years of exposure. In light of the occurrence of a liver carcinoma, the biological significance of the neoplastic liver nodules is increased. There were no statistically significant increases in liver tumors in female rats. Stott et al. (1995) also observed liver adenomas in male rats receiving 25 mg/kg/day 1,3-dichloropropene in the diet.

In mice, the most toxicologically significant neoplastic finding was a dose-related statistically significant increase in the incidence of transitional cell carcinoma of the urinary bladder in females in both 50 and 100 mg/kg dose groups (see Table 5 for incidences). Two males in the 100 mg/kg group also developed transitional cell carcinoma of the bladder, but the incidence was not statistically significant. Urinary bladder carcinoma was not observed in the 2-year feeding study of Redmond et al. (1995).

An increase in the incidence of bronchioalveolar adenomas in the lung was statistically significant in female mice at 100 mg/kg (see Table 5 for incidences). One carcinoma was found in the 50 mg/kg group but not in the 100 mg/kg group. In male mice, a statistically significant increase in the incidence of bronchioalveolar adenomas was observed at 50 mg/kg but not at 100 mg/kg. Two additional males in the 50 mg/kg group and three additional males in the 100 mg/kg group were diagnosed with bronchioalveolar carcinoma. Thus, the combined incidences of lung adenomas and carcinomas in male mice reached statistical significance for both 50 and 100 mg/kg groups. A significant increase in the incidence of bronchioalveolar adenomas was also observed in male mice exposed to 272 mg/m³ technical-grade dichloropropene by inhalation for 24 months (Lomax et al., 1989).

Forestomach tumors were also observed in mice. The incidences for both males and females were statistically significant at 100 mg/kg (see Table 5 for incidences).

From the rat study, NTP (1985) concluded that there was clear evidence of carcinogenicity in male rats, based on the combined incidences of squamous cell papillomas and carcinomas of the forestomach and the increased incidence of liver adenoma. In female rats, there was some evidence of carcinogenicity, based on the increased incidence of squamous cell papillomas of the forestomach. However, NTP (1985) recognized that epichlorohydrin, a stabilizer present in Telone II[®], may be partially responsible for the hyperplasia and squamous cell papilloma/carcinoma, at least in rat forestomach. NTP states this is plausible because the same types of lesions were found by Konishi et al. (1980) in a drinking water study with Wistar rats, and because the local exposure of the stomach to epichlorohydrin may have reached a concentration similar to that administered by Konishi et al. (1980). Subsequent to the NTP (1985) study, a 2-year gavage study with epichlorohydrin was published (Wester et al., 1985). Wester et al. (1985) observed a 28% incidence of forestomach papilloma/carcinoma in male Wistar rats and a 15% incidence in females given 1.4 mg/kg/day epichlorohydrin. In comparison, NTP (1985) found a 22% incidence in males and a 10% incidence in females at 2.1

mg/kg/day (50 mg/kg/day Telone II® × 0.1 epichlorohydrin × 3 days/7 days). The chronic feeding study by Stott et al. (1995), which did not include epichlorohydrin, reported forestomach hyperplasia in rats but no carcinomas or papillomas.

With regard to the mouse studies, NTP concluded that the male mouse study was inadequate for investigation of carcinogenicity because of the greatly reduced survival in the vehicle control group. In females, however, there was clear evidence of carcinogenicity, based on the increased incidence of transitional cell carcinoma of the urinary bladder, a very rare form of rodent cancer. Supporting evidence for carcinogenicity of Telone II® in female mice included the increased incidences of alveolar/bronchiolar adenomas of the lung and combined squamous cell papillomas and/or carcinomas of the forestomach (not statistically significant) at the highest dose, 100 mg/kg. Chronic toxicity of Telone II® was evidenced by hyperplasia of the forestomach in both sexes of rats and mice, and epithelial hyperplasia of the urinary bladder in male and female mice. Based on the serial-sacrifice (ancillary) study, development of both hyperplasia and carcinogenicity of the forestomach in rats was dependent on exposure duration.

On the basis of forestomach and liver neoplasms in rats, and urinary bladder and lung neoplasms in mice, the LOAEL for cancer in the NTP (1985) study is 21.4 mg/kg (50 mg/kg/day × 3 days/7 days). The NOAEL for rats is 10.7 mg/kg (25 mg/kg/day × 3 days/7 days). There is no NOAEL for mice.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Breslin, WJ; Kirk, HO; Streeter, CM; et al. (1989) 1,3-Dichloropropene: two-generation inhalation reproduction study in Fischer 344 rats. *Fundam Appl Toxicol* 12:129-143

The reproductive and developmental effects of inhaled technical-grade 1,3-dichloropropene were studied using F344 rats. The formulation was 92% 1,3-dichloropropene, 2% epoxidized soybean oil, and unknown amounts of chlorinated and unchlorinated alkanes and alkenes. The F₀ generation animals (30/sex/group) were exposed via whole-body inhalation to 0, 10, 30, or 90 ppm (0, 45.4, 136, or 409 mg/m³, respectively)⁵ 1,3-dichloropropene for 6 hours/day, 5 days/week for 10 weeks before mating and for 6 hours/day, 7 days/week during mating, gestation, and lactation. Weaned F₁ rats were subjected to the same dosing regimen. The animals were evaluated for fertility, pup survival, length of gestation, litter size, pup body weight, pup sex ratio, gross pathology, and histologic alterations.

No effects in any animals were noted at 45.4 or 136 mg/m³. At 409 mg/m³, males in the F₀ and F₁ generations exhibited a statistically significant decrease in body weight compared to controls, but the decrease was less than 10% and is not considered to be toxicologically significant. Gross and histologic examinations were conducted on all F₀ and F₁ adults and on randomly selected F_{1b} and F_{2b} weanlings. 1,3-Dichloropropene exposure had no significant effect

⁵Calculated using conversion of 1 ppm = 4.54 mg/m³ at 25° C.

on either behavior or clinical appearance of the animals. In both adults and litters, no toxicologically significant changes in mating and fertility indices, including cohabitation time required for mating, gestation length, litter size, pup survival, and pup body weights, were observed. There were no increases in either physical or behavioral abnormalities of the pups. Parental toxicity was observed only at 409 mg/m³ and consisted of histopathological changes of the nasal mucosa of the adult male and female rats. The alterations consisted of slight focal hyperplasia of the respiratory epithelium and/or focal degenerative changes of the olfactory epithelium. The nasal mucosa histopathology resulting from inhalation exposure to 1,3-dichloropropene has been observed in other high-dose inhalation exposure studies and is most likely due to a localized irritant effect (Linnett et al., 1988; Stott et al., 1988).

These results demonstrate that 1,3-dichloropropene is not a reproductive or developmental toxicant via inhalation in a two-generation reproduction study with F344 rats at exposures as high as 409 mg/m³. The NOAEL for reproductive/developmental toxicity is 376 mg/m³ because the formulation was 92% 1,3-dichloropropene. There is no LOAEL for reproductive/developmental toxicity. The NOAEL and LOAEL for parental toxicity are 125 and 376 mg/m³, respectively, based on nasal histopathology.

4.3.2. Linnett, SL; Clark, DG; Blair, D; et al. (1988) Effects of subchronic inhalation of D-D (1,3-dichloropropene/1,2-dichloropropene) on reproduction in male and female rats. *Fundam Appl Toxicol* 10:214-223

The reproductive toxicity of D-D was determined in a single-generation study with Wistar rats. Groups of 30 male rats of proven fertility and 24 virgin females were exposed by inhalation to nominal concentrations of 0, 10, 30, or 90 ppm (0, 45.4, 136, or 409 mg/m³, respectively)⁶ D-D for 6 hours/day, 5 days/week for 10 weeks. The major components of D-D are cis-1,3-dichloropropene (28.1% weight/weight), trans-1,3-dichloropropene (25.6% weight/weight), and 1,2-dichloropropene (25.6% weight/weight). Minor components include 2,3-dichloropropene, 3,3-dichloropropene, 1,2,3-trichloropropane, trichloropropene, and allyl chloride. The fertility of 20 males per exposure level (male fertility subgroup) was evaluated at intervals during and after treatment by mating them with unexposed females. On the 12th day following confirmed mating, each female was killed and examined according to standard indices of mating and fertility, including numbers of corpora lutea, uterine implantation and uterine resorption sites, and percent preimplantation and postimplantation losses. Males were sacrificed 5 weeks postexposure and given standard toxicological evaluations, including semen analysis.

The fertility of 15 females per exposure level (female fertility subgroup) was assessed by mating them with unexposed proven males at the end of the 10-week treatment period and allowing them to deliver a litter. Exposure was not continued during gestation because this study was designed to evaluate the effects of D-D on female libido, estrus cycling, and indices of mating, conception, gestation, and fertility, rather than effects on fetal development. All females

⁶Calculated using conversion of 1 ppm = 4.54 mg/m³ at 25° C.

in this subgroup were sacrificed 7 weeks postexposure and evaluated according to standard toxicological guidelines. The remaining 9 females and 10 males from each treatment group (toxicological subgroup) were not used in fertility assessment but were sacrificed immediately postexposure for standard toxicological evaluation, including semen analysis for males.

No treatment-related effects were observed in any of the mating, fertility, fecundity, and reproductive pathology/histopathology endpoints, including sperm morphology and estrus cycling. Male rats exposed to 409 mg/m³ exhibited a statistically significant decrease in body weight, but the decrease was < 10% and is not considered to be toxicologically significant. Males also exhibited a statistically significant increases in relative kidney and liver weights; however, there were no associated changes in clinical chemistry or urine analysis parameters, or in organ pathology or histopathology. Unlike other studies of repeated inhalation exposure to high doses of 1,3-dichloropropene (i.e., Breslin et al., 1989; Stott et al., 1988), no histopathology of the nasal turbinates was found at 409 mg/m³. This may be due to the fact that D-D contained only 57% (w/w) 1,3-dichloropropene, whereas other 1,3-dichloropropene-based fumigants (such as Telone II[®]) contain about 90% (w/w) dichloropropene.

Under the conditions of this well-conducted rat study, there was no evidence of reproductive toxicity associated with inhalation exposure to D-D at doses up to 409 mg/m³. The NOAEL for reproductive toxicity was 233 mg/m³ 1,3-dichloropropene (409 mg/m³ D-D with 57% 1,3-dichloropropene). There was no LOAEL.

4.3.3. Hanley, TR, Jr; John-Greene, JA; Young, JT; et al. (1988) Evaluation of the effects of inhalation exposure to 1,3-dichloropropene on fetal development in rats and rabbits. *Fundam Appl Toxicol* 8:562-570

Technical-grade 1,3-dichloropropene (90.1% w/w) was evaluated for its potential effects on embryonal and fetal development in F344 rats and New Zealand white rabbits. Groups of 30 bred rats and 25–31 inseminated rabbits were exposed via inhalation to 0, 20, 60, or 120 ppm (0, 91, 272, or 545 mg/m³, respectively) 1,3-dichloropropene for 6 hours/day during gestation days 6–15 (rats) or 6–18 (rabbits). Control groups of 30 rats and 29 rabbits were exposed to filtered room air in a manner similar to treated groups.

In rats, maternal body weight gain was depressed in all exposed groups. Significant depression in food consumption was observed in all exposed groups, along with a significant decrease in water consumption in rats exposed to 545 mg/m³. However, there were no consistent or dose-related effects on any of the following reproductive parameters: implantations, resorptions, litter size, fetal body weight, and fetal length. Although pregnancy rates in the 91 and 272 mg/m³ groups were lower than the control rate, with a statistically significant decrease at 272 mg/m³, these findings were not considered to be toxicologically significant because (a) there was no consistent dose-effect (the pregnancy rate at 545 mg/m³ was higher than the control group rate), and (b) pregnancy rates in the 91 and 272 mg/m³ groups were within the historical control range of the laboratory.

External, visceral, and skeletal examination of the pups revealed no major abnormalities or malformations. A slight but statistically significant increase in delayed ossification of the vertebra central was observed among fetuses in the 545 mg/m³ exposure group compared with controls. This increase was considered to have little toxicological significance because it was judged to be secondary to the significant maternal toxicity observed among females in the 545 mg/m³ group.

Because no significant developmental effects were detected, the NOAEL for developmental toxicity in rats was 490 mg/m³ (90% of 545 mg/m³ formulation) and there was no LOAEL. Based on decreased body weight, the NOAEL for maternal toxicity in rats was 245 mg/m³ and the LOAEL was 490 mg/m³ 1,3-dichloropropene (both corrected for 90% 1,3-dichloropropene).

In rabbits, statistically significant exposure-related decreases in maternal weight gain were observed at 272 and 545 mg/m³. There were no treatment-related adverse effects on pregnancy rate, implantation and resorption rates, preimplantation losses, litter size, or fetal measurements among any of the exposed groups. External, visceral, and skeletal examination of the pups did not show evidence of treatment-related abnormalities or malformations. Statistically significant decreases in the incidence of two minor skeletal variants among the exposed groups (delayed ossification of the hyoid in the high-dose group, and the presence of cervical spurs in the low- and high-dose groups) were considered to be indicative of the normal variability among rabbit pups and thus not toxicologically significant.

The NOAEL for developmental toxicity in rabbits was 490 mg/m³ (90% of 545 mg/m³) as no effects were detected. There was no LOAEL. Based on decreased body weight, the NOAEL for maternal toxicity in rabbits was 82 mg/m³ (90% of 91 mg/m³), with a LOAEL of 245 mg/m³ (90% of 272 mg/m³).

The weight and strength of evidence from three well-conducted reproductive/developmental toxicity studies in two species indicates that 1,3-dichloropropene is not a reproductive or developmental toxicant.

4.4. OTHER STUDIES

4.4.1. Acute Toxicity

Oral LD₅₀s range from 140 to 710 mg/kg 1,3-dichloropropene for rats and 300–640 mg/kg for mice (U.S. EPA, 1998c). The LC₅₀ for Telone II[®] for a 4-hour exposure in rats was 904 ppm (4,104 mg/m³)⁷ for females and between 846 and 990 ppm (3,841 and 4,495 mg/m³)⁷ for males (Streeter et al., 1987).

⁷Calculated using conversion of 1 ppm = 4.54 mg/m³ at 25° C.

4.4.2. Neurotoxicity

A single oral dose of 3,500 mg/kg in dogs caused staggering, partial central nervous system (CNS) depression, and death within 24 hours (U.S. EPA, 1980).

4.4.3. Mutagenicity

Early in vitro mutagenicity testing of 1,3-dichloropropene using the Ames *Salmonella* test usually elicited positive results for mutagenicity (e.g., Vithayathil et al., 1983; Stolzenberg and Hine, 1980; Haworth et al., 1983). However, in 1984, Talcott and King demonstrated that preparations of 1,3-dichloropropene assayed in vitro for mutagenic activity contained direct-acting mutagenic polar impurities. Four commercial preparations of 1,3-dichloropropene were tested for mutagenic activity before and after silicic acid chromatography. All samples were positive before purification and negative afterwards. Polar impurities were isolated from one preparation and tested positive for mutagenicity in the Ames *Salmonella* test. Talcott and King (1984) regenerated a mixture of mutagenic polar impurities by refluxing a purified preparation for 6 hours and then analyzed the mixture using GC/MS. Although the mixture was too complex to be characterized completely, two known mutagens, epichlorohydrin and 1,3-dichloro-2-propanol, were tentatively identified. NTP (1985) and Watson et al. (1987) confirmed the findings that purified 1,3-dichloropropene is not mutagenic in the Ames *Salmonella* test. Watson et al. (1987) also identified two additional trace impurities: cis- and trans-2-chloro-3-(chloromethyl)oxiranes. In addition, Watson et al. (1987) reported that purification by gas chromatography can produce mutagenic trace impurities. Thus, the weight of evidence of these data suggests that the mutagenic activity of 1,3-dichloropropene preparations in earlier bacterial tests was likely due to mutagenic polar impurities and not to 1,3-dichloropropene.

Although purified 1,3-dichloropropene was not directly mutagenic, Watson et al. (1987) observed mutagenic activity after the addition of washed microsomes from rat liver. Mutagenicity was abolished, however, when GSH at normal physiological concentrations (5 mM) was added to the bacterial culture. Watson et al. (1987) have suggested that cis-1,3-dichloropropene undergoes mono-oxygenase-dependent bioactivation to mutagenic metabolites only in the absence of GSH. These findings are consistent with the results of Creedy et al. (1984), which showed that GSH eradicated the microbial mutagenicity of both isomers of 1,3-dichloropropene. Thus, microbial assays show that physiological concentrations of GSH provide efficient protection against the mutagenic activity of 1,3-dichloropropene and associated trace impurities.

Neudecker and Henschler (1986) used enzyme inhibitors to determine whether rat liver enzymes (i.e., S9) metabolize allylic chloropropenes, such as 1,3-dichloropropene, via epoxidation or via cleavage of the allylic chlorine, which subsequently forms the allylic chloroalcohol, the aldehyde, and then acrylic acid. The investigators distinguished these pathways by measuring mutagenicity in *Salmonella* TA100. Addition of SKF525, an inhibitor of microsomal oxygenase that prevents formation of 1,3-dichloropropene epoxide, or 1,1,1-trichloropropene-2,3-oxide, an inhibitor of epoxide hydrolase that prevents metabolism of the epoxide, had no effect on mutagenicity. However, addition of cyanamide, an inhibitor of

aldehyde dehydrogenase that prevents metabolism of the aldehyde formed from 1,3-dichloropropene, clearly increased mutagenic activity. The addition of GSH markedly reduced mutagenicity. The authors hypothesized that in the absence of GSH, S9 metabolically activates 1,3-dichloropropene by hydrolysis to chloroalcohols that subsequently oxidize to 3-chloroacrolein (hydrolytic-oxidative pathway) and then to the respective acrylic acid.

In contrast to the results of Neudecker and Henschler (1986), Schneider et al. (1998a) found that epoxidation of 1,3-dichloropropene is a minor metabolic pathway in mouse liver. Relatively stable 1,3-dichloropropene epoxides were measured by GC/MS in mouse liver after in vitro addition of 1,3-dichloropropene to microsomes and after in vivo administration of LD₅₀ doses (i.e., 350 or 700 mg/kg) of 1,3-dichloropropene. 3-Chloroacrolein, a metabolite postulated by Neudecker and Henschler (1986), was not observed in studies by Schneider et al. (1998a). Schneider et al. (1998a) also showed that conjugation of 1,3-dichloropropene with GSH decreases epoxide formation. The authors showed that cis and trans epoxides are mutagenic in the *Salmonella* TA100 assay. The addition of GSH to the assay, with or without GST, diminished the mutagenicity of cis-1,3-dichloropropene epoxide, the most potent isomer, and obliterated the mutagenicity of trans-1,3-dichloropropene epoxide. The investigators postulated that the epoxides or their decomposition products (i.e., 3-chloro-2-hydroxypropanal) are responsible for the mutagenicity of 1,3-dichloropropene in the presence of liver enzymes.

Martelli et al. (1993) investigated the cytotoxicity and genotoxicity of 1,3-dichloropropene in cultured Chinese hamster lung, i.e., V79 cells, and in hepatocytes from male Sprague-Dawley rats. DNA fragmentation was significantly increased in a dose-dependent manner in V79 cells, which cannot metabolize 1,3-dichloropropene, after 1 hour incubation with subtoxic concentrations (1.8–5.6 mM) of 1,3-dichloropropene. This result is inconsistent with the *Salmonella* assays that showed no genotoxic activity without metabolic activation (Talcott and King, 1984; NTP, 1985; Watson et al., 1987). During an experiment to determine the time course for DNA repair, DNA lesions in V79 cells were only partially repaired 24 hours after removal of 1,3-dichloropropene. Subtoxic concentrations (0.18–0.56 mM) did not produce DNA fragmentation after 20 hours' incubation. Thus, in V79 cells, it appears that DNA fragmentation due to subtoxic concentrations of 1,3-dichloropropene was successfully repaired. However, rat hepatocytes, which have an intact metabolizing system, were more sensitive to DNA fragmentation. DNA fragmentation produced by 0.18–1 mM 1,3-dichloropropene in rat hepatocytes was reduced by both GSH and inhibition of cytochrome P450 activity with metapyrone. This experiment showed that the protective effect of GSH in bacterial assays (Watson et al., 1987; Creedy et al., 1984; Neudecker and Henschler, 1986) also applies to mammalian cells, and contradicts the finding of Neudecker and Henschler (1986) that metabolism by cytochrome P450 has no role in the mutagenicity of 1,3-dichloropropene.

Ghia et al. (1993) examined the genotoxic activity of 1,3-dichloropropene using a battery of short-term in vivo tests. Male Sprague-Dawley rats were administered doses of 1,3-dichloropropene ranging from 62.5 mg/kg to 250 mg/kg by either a single oral gavage or a single ip injection. Animals were pretreated with either buthionine-sulfoximine (BSO) or diethylmaleate (DEM) to reduce GSH levels, or with methoxsalen (MS) to inhibit cytochrome P450. DNA fragmentation, unscheduled DNA synthesis (UDS), and micronucleus (MN) frequency were quantitated. A dose-dependent increase in DNA fragmentation was most pronounced in the

liver (site for tumors at 25 mg/kg/day in Stott et al. [1995] and NTP [1985] at 50 mg/kg) and stomach mucosa (site for tumors at 50 mg/kg in NTP [1985]) and occurred to a lesser extent in the kidney. No DNA fragmentation occurred in the lung, bone marrow, or brain, which are sites where no tumors were detected in Stott et al. (1998) or NTP (1985). Partial repair was observed after 24 hours. Reduction of GSH levels with BSO or DEM pretreatment did not affect DNA fragmentation in the liver, but that was explained by the fact that neither BSO nor DEM increased depletion of liver GSH over that caused by dichloropropene alone. The inhibition of cytochrome P450 with MS reduced the frequency of DNA fragmentation in the liver as shown by Martelli et al. (1993) in rat hepatocytes. Despite the fact that the 125 mg/kg dose administered was 5 times higher than that of Stott et al. (1995) and 2.5 times higher than that of NTP (1985), there was no evidence of DNA repair induction in UDS assays. In addition, no statistically significant increases in micronucleated polychromatic erythrocytes (PCE) in bone marrow (consistent with the absence of DNA fragmentation) and spleen or in micronucleated hepatocytes were observed at the same dose.

The authors concluded that DNA fragmentation *in vivo* correlated well with 1,3-dichloropropene carcinogenic activity in the rat liver and stomach mucosa observed by Stott et al. (1995) and NTP (1985), respectively; however, the doses used by Ghia et al. (1993) were at least 2.5 times those producing liver tumors in Stott et al. (1995) and 1.25 times those producing forestomach tumors in NTP (1985). In addition, even at the high doses used by Ghia et al. (1993), the genotoxicity results of the rat hepatocyte DNA repair assay and the MN assay of bone marrow, spleen, and liver cells were negative.

Von der Hude et al. (1987) assessed the genotoxicity of several halogenated short-chain hydrocarbons, including *cis*- and *trans*-1,3-dichloropropene, using the *in vitro* sister chromatid exchange (SCE) test in the Chinese hamster V79 cell line. Without S9 activation, 0.1–0.4 mM 1,3-dichloropropene showed a dose-dependent increase in the frequency of SCE. Higher concentrations were required to induce significant SCE frequencies with 1,3-dichloropropene compared with other short-chain chlorinated hydrocarbons tested. The observed increase in SCE was abolished by the addition of rat liver S9 mix. These results are inconsistent with those of Watson et al. (1987), which showed mutagenic activity of purified 1,3-dichloropropene after the addition of S9, but not without S9. Moreover, Von der Hude et al. (1987) used a formulation purified by gas chromatography, and as established by Watson et al. (1987), impurities due to such “purification” have mutagenic activity. Thus, the positive response to 1,3-dichloropropene in this assay was probably caused by mutagenic impurities rather than dichloropropene.

Kevekordes et al. (1996) tested a number of pesticides for clastogenic and aneugenic properties in (a) an *in vivo* mouse bone marrow MN test and (b) an *in vitro* SCE assay using human lymphocytes in the presence or absence of rat liver S9. 1,3-Dichloropropene by gavage significantly increased the frequency of micronucleated PCE in the bone marrow cells of female mice at the two highest doses tested (187 and 234 mg/kg), whereas no increase in PCE was observed in male mice at doses up to 280 mg/kg/day.

With and without S9 activation, the frequency of SCE in cultured human lymphocytes was statistically increased compared with controls, but only at the highest dose tested (100 μ M). In the discussion of these findings, the authors point out that 1,3-dichloropropene formulations

are likely to contain a number of mutagenic impurities (Kevekordes et al., 1996). Therefore, the mutagenic activity cannot necessarily be attributed to 1,3-dichloropropene.

1,3-Dichloropropene does not produce dominant lethal mutations in Wistar or F344 rats or New Zealand white rabbits, as evidenced by the absence of embryonic or fetal deaths in inhalation studies by Hanley et al. (1988) and Linnett et al. (1988).

Valencia et al. (1985) evaluated 1,3-dichloropropene for its potential to induce sex-linked recessive lethal mutations in *Drosophila melanogaster*, using a standard NTP protocol. Canton-S wild-type males were treated with concentrations of 1,3-dichloropropene that resulted in approximately 30% mortality. Following treatment, males were mated individually to three harems of virgin females to produce three broods for analysis. 1,3-Dichloropropene produced sex-linked recessive lethal mutations in males at 5,570 ppm administered by feeding. However, it appears that this dose was cytotoxic, so the results are of questionable validity for assessing genotoxic potential.

Stott et al. (1997a) studied the in vitro binding potential of 11 mM ^{14}C -1,3-dichloropropene to calf thymus DNA in the presence or absence of rat liver S9 and in the presence of S9 + GSH. The measurement of DNA adducts exhibited a significant amount of variation and showed no significant difference in control and treated groups.

4.4.4. Mechanistic Studies

Stott et al. (1997b) conducted a series of studies to elucidate the potential mechanisms of tumorigenicity of 1,3-dichloropropene in male B6C3F1 mice and F344 rats. The selection of dose, sex, species, and route of administration was based on the tumors seen in 2-year oral and inhalation bioassays with rats and mice. In the oral study, hepatocellular adenomas were observed in male rats fed 25 mg/kg/day 1,3-dichloropropene (Stott et al., 1995). In the inhalation study, bronchioalveolar adenomas were observed in male mice at 272 mg/m³ (Lomax et al., 1989). Urinary bladder tumors were noted in female mice gavaged with 50 mg/kg 1,3-dichloropropene 3 times/week and in male mice at 100 mg/kg (NTP, 1985). Nonneoplastic bladder effects in mice were observed at 25 mg/kg thrice weekly by gavage (NTP, 1985) and at 90.8 mg/m³ by inhalation (Lomax et al., 1989).

Stott et al. (1997b) gavaged male rats with 0, 5, 12.5, 25, or 100 mg/kg/day 1,3-dichloropropene, 5 days/week for 3, 12, or 26 days. Male mice were exposed to whole-body inhalation concentrations of 0, 10, 30, 60, or 150 ppm for 6 hours/day, 5 days/week for 3, 12, or 26 days. The following mechanistic endpoints were evaluated:

1. GSH levels in rat liver and mouse lung: GSH protects against tissue injury, cytotoxicity, and mutagenicity.
2. Levels of DNA replication as determined by increased regenerative cell proliferation in rat liver and mouse epithelia from urinary bladder and bronchiole: Increases in this measure indicate cytotoxicity, cytolethality, and compensatory

cell division and thus provide evidence to support a nongenotoxic mode of tumorigenic action.

3. Rates of apoptosis in rat liver and mouse epithelia from urinary bladder and bronchiole: Changes in apoptosis may be associated with compensatory cell regeneration and/or a disruption of normal cellular functioning and would support a nongenotoxic mode of action.
4. Adduct formation in rat liver and mouse lung measured by the ³²P-Post-Labeling assay: The formation of DNA adducts usually demonstrates that the test compound is interacting directly with genetic material and thus indicates genotoxicity.

Results from this study (Stott et al., 1997b) included a dose-dependent decrease in tissue GSH levels. Although liver GSH levels increased back to control levels by the end of the exposure period (26 days), GSH levels in mouse lung did not. Both tissues showed a rebound (greater than control levels) in GSH levels when animals exposed for 11 days were tested 24 hours after dosing was terminated. No changes were noted in either cell proliferation or apoptosis rates in rat liver, mouse lung, or urinary bladder epithelia. In addition, no unique DNA adduct formation or increase in the incidence of normally occurring adducts was found in rat liver or mouse lung.

The authors concluded that these studies provide scientific support to a weight-of-evidence conclusion that tumorigenesis associated with high-dose ingestion or inhalation of 1,3-dichloropropene is nongenotoxic in etiology and is not dependent on (a) enhanced cell proliferation, (b) depressed rates of apoptosis, or (c) increased or unique DNA adduct formation. However, these mechanistic studies did not identify a mechanism of action for tumor formation. Neither the genotoxic nor the nongenotoxic mechanisms tested elicited positive results. The GSH studies, which showed that 1,3-dichloropropene at doses used in chronic bioassays depletes GSH in target organs, were consistent with GSH protection against cytotoxicity and tumorigenicity by conjugating with 1,3-dichloropropene. Bacterial assays (Watson et al., 1987; Creedy et al., 1984; Neudecker and Henschler, 1986) and in vitro mammalian assays (Martelli et al., 1993) have also shown that GSH protects against genotoxic effects.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)—ORAL AND INHALATION

Despite the high potential for occupational exposures of agricultural workers, reports of adverse health effects reflect only relatively mild effects. Dermatitis is the only effect noted in humans after repeated occupational exposures, and only two case studies have been published. Exposure to high concentrations in cases of chemical spills, however, can produce severe toxicity manifested by a dose-related range of acute neurotoxic symptoms. Accidental ingestion of large quantities of 1,3-dichloropropene has been fatal.

No epidemiologic data on the chronic health effects of 1,3-dichloropropene could be found. In chronic and subchronic high-dose animal studies, histopathologic changes have been noted in target organs along the portals of entry (e.g., forestomach for oral administration; nasal

mucosa and lung for inhalation) and/or in organs involved in the metabolism (liver) and excretion of conjugated metabolites (e.g., urinary bladder and kidney). It should be noted that early studies used technical-grade mixtures of 1,3-dichloropropene that contained 2%–10% impurities and stabilizing agents. Epichlorohydrin, a stabilizer in older formulations, has produced forestomach lesions in rodent studies, and it is likely that epichlorohydrin contributed to these effects in the NTP (1985) study. More recent formulations of commercial 1,3-dichloropropene have replaced epichlorohydrin with epoxidized soybean oil (Lomax et al., 1989; Breslin et al., 1989). Nonetheless, commercial formulations may still contain a number of potentially toxic impurities whose presence may contribute to toxic effects.

Neither reproductive nor developmental toxicity has been observed in a well-conducted two-generation study in rats or in developmental studies in rats and rabbits at maternal inhalation concentrations up to 376 mg/m³ 1,3-dichloropropene (Breslin et al., 1989; Linnett et al., 1988; Hanley et al., 1988). Even concentrations that produced parental toxicity (i.e., decreased body weight and/or nasal histopathology) did not produce reproductive or developmental effects (Hanley et al., 1988; Breslin et al., 1989).

The toxicokinetics of 1,3-dichloropropene are reasonably well understood. 1,3-Dichloropropene is rapidly absorbed and quickly conjugated with GSH into mercapturic acids (Climie et al., 1979; Dietz et al., 1985; Waechter and Kastl, 1988; Waechter et al., 1992), which are rapidly excreted in the urine. The extent of epoxidation, a minor metabolic pathway identified at ~LD₅₀ doses in mice, is reduced by conjugation of 1,3-dichloropropene with GSH. 1,3-Dichloropropene does not bioaccumulate in target tissue to any significant degree (Hutson et al., 1971; Dietz et al., 1984a). Relatively high repeated exposures to 1,3-dichloropropene are required to significantly deplete GSH in target organs, with the exception of nasal tissue. Nonlinear kinetics consistent with saturation of GSH-mediated conjugation systems have been reported at exposure levels of 1,363–4,086 mg/m³ in rats (Fisher and Kilgore, 1988a,b). Pharmacokinetic studies have demonstrated that reductions in GSH due to repeated administration of 1,3-dichloropropene occur over a range of doses (22.7–7,786 mg/m³ by inhalation and 12.5–100 mg/kg orally), that significant depletion occurs in most tissues only at high doses, and that GSH levels rebound upon cessation of exposure (Stott et al., 1997b). Thus, it appears likely that toxicity is associated with depletion of GSH. Based on in vitro studies and biological monitoring of workers exposed to 1,3-dichloropropene vapors, human toxicokinetics and metabolism by GSH conjugation appear to be similar to those in rodents.

4.5.1. Inhalation Studies

The olfactory and/or nasal epithelium is a primary target organ for both rats and mice inhaling vaporized formulations of technical-grade 1,3-dichloropropene.

Histopathology of the nasal mucosa was observed in male and female rats (Lomax et al., 1989) exposed to 272 mg/m³ 1,3-dichloropropene (with epoxidized soybean oil as the stabilizing agent) for 24 months, but not after exposure for 6 or 12 months, or to lower doses. Although similar histopathology is produced in the mouse nasal mucosa at 90.8 mg/m³, the severity of the response is less (Lomax et al., 1989). The other target organ for inhalation exposure is the mouse

urinary bladder (Lomax et al., 1989). Morphological changes in the urinary bladder occurred in females exposed to 90.8 or 272 mg/m³ and in males exposed to 272 mg/m³. Microscopically, the urinary bladders exhibited extensive hyperplasia and some inflammation. No effects were observed in rats or mice at 22.7 mg/m³ 1,3-dichloropropene.

4.5.2. Oral Studies

In early studies (NTP, 1985; Yang et al., 1986), oral gavage was employed as the means of administration, and a formulation of 1,3-dichloropropene containing epichlorohydrin was used as the test substance. 1,3-Dichloropropene produced forestomach hyperplasia in rats and mice. Other target organs included the mouse urinary bladder (epithelial hyperplasia), rat liver (neoplastic nodule formation), and mouse kidney (hydronephrosis).

When the method of oral administration of 1,3-dichloropropene was changed to feeding (Haut et al., 1996; Stott et al., 1995; Redmond et al., 1995), forestomach lesions still occurred in rats at 12.5 mg/kg/day and higher, but compared with the NTP (1985) study, the severity of hyperplasia was reduced. Other targets identified in the NTP gavage study—mouse forestomach, urinary bladder, kidney, and rat liver—exhibited no histopathology in the feeding studies (Stott et al., 1995; Redmond et al., 1995). Differences in histopathology between the NTP (1985) and the feeding studies may be due to the method of compound administration (daily dietary exposure vs. concentrated bolus dosing). Other investigators have shown that oral gavage increases blood levels of toxicant and toxicity compared with the same dose administered by gastric infusion over 2 hours (Sanzgiri et al., 1995). The decrease in the number of target organs in the feeding studies may also be because of the absence of epichlorohydrin in the feeding formulation. In the mouse dietary study (Redmond et al., 1995), there is some uncertainty as to whether the mice received the intended dose because of the absence of cancer (urinary bladder tumors) and noncancer effects (urinary bladder hyperplasia, forestomach hyperplasia, and hydronephrosis) seen in the NTP (1985) study. However, the incidences of lung tumors (combined bronchioalveolar adenoma and carcinoma) in the two studies are similar for similar doses. The other major toxic effect in the feeding studies was reduced body weight at the higher doses in both rats and mice.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CLASSIFICATION— SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

The only evidence associating carcinogenicity in humans to 1,3-dichloropropene exposures is three case studies in which two firemen and one farmer were accidentally exposed to acute high doses and subsequently developed blood cancers (non-Hodgkin's lymphoma and leukemia). Case reports are often anecdotal or highly selective, but they may identify an association when there are unique features such as uncommon tumors (U.S. EPA, 1996a). Non-Hodgkin's lymphoma and leukemia, however, are not rare cancers. In 1979, the same year the reported lymphomas were diagnosed, the age-adjusted incidence of non-Hodgkin's lymphoma in

the United States for males, 11.8 per 100,000 population, was between that of skin cancer, 8.9/100,000, and stomach cancer, 13.6/100,000 (Ries et al., 1998). In 1975, the year the reported leukemia was diagnosed, 13.6/100,000 cases were reported in males. These case studies do not provide a basis for inferring a causal association between exposure to 1,3-dichloropropene and blood cancers because the possibility of confounding factors has not been considered or ruled out (U.S. EPA, 1987). Additionally, animal bioassays do not suggest that the hematopoietic system is a target organ of 1,3-dichloropropene carcinogenicity.

Two-year animal bioassays indicate that 1,3-dichloropropene is carcinogenic at relatively high doses. Feeding studies in rodents by Stott et al. (1995) found a late-onset increase in the incidence of benign hepatocellular adenomas (with one hepatocarcinoma) in male rats at the highest dose tested, 25 mg/kg/day. No treatment-related tumors were observed in female rats or in male or female mice fed up to 50 mg/kg/day. The thrice weekly gavage study by NTP (1985) found significant incidences of bronchioalveolar, forestomach, and urinary bladder tumors in mice at 50 mg/kg and forestomach and liver tumors in rats at 25 mg/kg. With the exception of the urinary bladder tumors in mice, most tumors were benign. In rats at 50 mg/kg, four carcinomas were observed in the forestomach and one was observed in the liver. In mice, eight carcinomas in urinary bladder and three in bronchioalveolar areas were observed at 50 mg/kg while two were observed in the forestomach at 100 mg/kg. Although the NTP study was rejected for RfD development by EPA (IRIS, online 10/1/90), because the thrice-weekly high-dose gavage regime was not well designed to study chronic toxicity, the data do show that 1,3-dichloropropene is a carcinogen at relatively high bolus doses. Current test guidelines recommend seven times weekly gavage, but indicate that five times/week is acceptable (U.S. EPA, 1998d). NTP acknowledged that the epichlorohydrin used as a stabilizer in Telone II® may be partially responsible for the squamous cell papillomas and carcinomas, at least in the rat forestomach, as hyperplasia, papilloma, and carcinoma were found in the forestomachs of rats in an epichlorohydrin drinking water study (Konishi et al., 1980). The chronic feeding study by Stott et al. (1995), which did not include epichlorohydrin, found forestomach hyperplasia in rats but no carcinomas or papillomas.

In chronic inhalation bioassays, a statistically significant increase in the incidence of benign lung adenomas was observed in male mice only at the highest exposure of 272 mg/m³ dichloropropene, but no malignancies were observed (Lomax et al., 1989). The tumors occurred with late onset as they were observed after 24 months of exposure, but not after 6 or 12 months. No tumors were reported for female mice or for male or female rats. Despite the dose-dependent hypertrophy and hyperplasia of the nasal respiratory epithelium and/or degeneration of the olfactory epithelium in rats at the highest exposure of 272 mg/m³, no animals developed tumors in the nasal mucosa. Mice also exhibited these effects, but all cases were graded as “slight” histopathologic changes, involved approximately 10% or less of the total respective epithelium, and did not progress in severity or distribution from one exposure duration to the next. Most animals exposed to 272 mg/m³ for 6, 12, or 24 months exhibited nasal histopathology. For the 24-month exposures, the incidence of nasal histopathology was significant in female mice at 90.8 mg/m³ and in male mice at 272 mg/m³ dichloropropene.

The lack of tumorigenesis in the rat nasal mucosa may be due to the relatively low uptake of toxic vapors in this tissue (Stott and Kastl, 1986) and the protective action of GSH. Uptake is

much higher in the rat lung than in the nasal mucosa. Additionally, whereas GSH is depleted in a dose-dependent manner in the nasal mucosa, depletion appears to be dose-independent in the lung. Decreases of up to 70% of control values are maintained across a wide range of dose levels (Fisher and Kilgore, 1988a). The relatively low uptake and rapid detoxification of inhaled 1,3-dichloropropene by GSH in the nasal mucosa appear to be sufficient to protect against carcinogenicity, but not toxicity, along the primary portal of entry. In the rat lung, neither toxicity nor carcinogenicity was observed.

The mutagenicity and genotoxicity of 1,3-dichloropropene have been extensively studied in both in vitro and in vivo assays. Early bacterial studies demonstrated that 1,3-dichloropropene was mutagenic in a variety of test systems in the absence of metabolic activation. Although later studies showed that these findings were due to mutagenic impurities in the 1,3-dichloropropene formulation, even purified 1,3-dichloropropene produced mutations in the presence of S9. Bacterial reversions were prevented, however, by the addition of physiological concentrations of GSH.

In the absence (verified or assumed) of mutagenic impurities, 1,3-dichloropropene has produced mixed results in mammalian in vitro and in vivo genotoxicity studies. Although the positive studies indicate that 1,3-dichloropropene can be mutagenic, the relevance of these studies to mammalian tumor formation is uncertain owing to the high concentrations or doses used. The lowest concentration used in in vitro studies, ~ 0.1 mM, is still two orders of magnitude higher than that found in rat blood after high acute doses of 1,3-dichloropropene. The peak blood level detected after a 3-hour exposure of rats to 409 mg/m³ 1,3-dichloropropene (the highest concentration in the 2-year chronic bioassay by Lomax et al. [1989] was 227 mg/m³) was 0.004 mM 1,3-dichloropropene (Stott and Kastl, 1986). The peak blood level detected in rats after a 25 mg/kg gavage with 1,3-dichloropropene (highest dietary dose administered by Stott et al., 1995) was approximately 0.0027 mM 1,3-dichloropropene (Stott et al., 1998). Even the lowest doses used in in vivo genotoxicity tests (62.5 mg/kg in rats by Ghia et al., 1993) were more than twice those used in the chronic bioassays (Stott et al., 1995). Although several high-concentration and high-dose genotoxicity studies have shown that 1,3-dichloropropene is mutagenic, the relevance of these studies to tumor formation in chronic rodent bioassays is uncertain because of the lack of information about the relative sensitivity of the test systems. However, the weight of the evidence in the short-term studies suggests that 1,3-dichloropropene is mutagenic.

Although the major metabolic pathway of 1,3-dichloropropene is conjugation by GSH and subsequent excretion in the urine, Schneider et al. (1998a) found that epoxidation of 1,3-dichloropropene is a minor metabolic pathway in mouse liver at ~LD₅₀ doses. The doses administered were 3.5–7 times the maximum dose given to mice in the NTP (1985) study and 7–14 times those given to mice in the feeding study of Redmond et al. (1995). Schneider et al. (1998a) showed that the epoxides were mutagenic in bacterial assays and that the mutagenicity was decreased (cis-epoxide) or abolished (trans-epoxide) by the addition of GSH. The investigators also demonstrated that conjugation of 1,3-dichloropropene with GSH decreases epoxide formation in mouse liver. The authors postulated that the epoxides or their decomposition products are responsible for the mutagenicity of 1,3-dichloropropene in the presence of liver enzymes and showed that the epoxides bind to deoxyguanosine in vitro

(Schneider et al., 1998b). Stott et al. (1997a,b), however, found no evidence of DNA adduct formation in vivo after subchronic exposures to tumorigenic doses of 1,3-dichloropropene. It is possible that GSH effectively scavenged 1,3-dichloropropene in the subchronic studies and that lifetime exposures to high doses of 1,3-dichloropropene eventually lead to significant GSH depletion and lack of protection from the genotoxic metabolites. 1,3-Dichloropropene may be nongenotoxic at low-dose exposures that do not interfere significantly with normal function of GSH, but bioassay data showing the protective effect of GSH against tumor formation are lacking.

Although the available human data are inadequate, under EPA's proposed cancer risk assessment guidelines (1996), the weight of evidence indicates that 1,3-dichloropropene is clearly a rodent carcinogen and is "likely to be carcinogenic to humans." This characterization is based on tumors observed in chronic animal bioassays for both inhalation and oral routes of exposure. Although the chronic dietary and inhalation bioassays suggest that tumors may not occur at low doses, a nonlinear mechanism of tumor formation is not supported by mechanistic data. In fact, the mutagenic properties of 1,3-dichloropropene suggest a genotoxic mechanism of action. The mutagenic properties and the absence of data to support a nonlinear mechanism of tumor formation require the quantitative assessment to default to a linear model. Under current EPA (1987) cancer risk assessment guidelines, 1,3-dichloropropene is characterized as a class "B2," probable human carcinogen, with little or no evidence for carcinogenicity in humans and sufficient evidence in animals. This classification is based on observations of tumors in F344 rats (forestomach, liver) and B6C3F1 mice (forestomach, urinary bladder, and lung) at high bolus doses, observations of benign liver tumors in F344 rats at lower dietary doses, and the formation of mutagenic epoxide metabolites at high, \sim LD₅₀, doses.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

There are no human studies that indicate the relative sensitivity of children and adults to the toxic effects of 1,3-dichloropropene. Although no animal studies have examined the effect of 1,3-dichloropropene exposure on juvenile animals, well-conducted studies in rats and rabbits show no evidence of developmental toxicity (Hanley et al., 1988; Linnett et al., 1988; Breslin et al., 1989) even at doses that caused maternal toxicity. On the basis of these results, it is unlikely that 1,3-dichloropropene causes developmental toxicity in humans, but its effects on children are unknown.

4.7.2. Possible Gender Differences

There are no human data that suggest that gender differences in toxicity or tumorigenicity might occur as a result of exposure to 1,3-dichloropropene. In chronic animal studies, the female mouse was more sensitive to the urinary bladder toxicity induced by inhalation exposure to 1,3-dichloropropene, but male mice exhibited bronchioalveolar adenomas while female mice did not (Lomax et al., 1989). Inhalation exposure also produced mild kidney histopathology in female

mice and mild kidney and liver histopathology in male mice (Lomax et al., 1989). In a feeding study, male mice also exhibited a decrease in body weight when females did not (Redmond et al., 1995). In a rat feeding study, only males exhibited liver adenomas (Stott et al., 1995), but both sexes had neoplastic liver nodules in a gavage study (NTP, 1985). However, the relevance of gender differences in rodents to those in humans is unknown.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

There are no chronic human studies suitable for dose-response assessment, but there are four chronic studies for orally administered 1,3-dichloropropene in rats and mice: two gavage bioassays by NTP (1985) and two dietary studies (Stott et al., 1995; Redmond et al., 1995).

Limitations of the gavage studies include the thrice-weekly dosing regime. Current test guidelines recommend seven times weekly, but indicate that five times/week is acceptable (U.S. EPA, 1998d). The NTP (1985) study was rejected by EPA for RfD development (IRIS, online 10/1/90) because the dosing regimen (high doses by gavage three times/week) was not well designed to study chronic toxicity. Another problem with the NTP (1985) study is that the dichloropropene formulation contained epichlorohydrin, which NTP acknowledged as a possible contributor to tumorigenic effects in the forestomach. In addition, gavage administration is much less relevant to human exposure than dietary administration. Gavage administration delivered a single bolus dose, but human exposure would be similar to dietary intake, which occurs at intervals throughout the course of a day. The dietary studies, however, lack information about the in-cage stability of the food mixture. The absence of such information leaves doubt as to the actual dose received by the animals.

The rat dietary study of Stott et al. (1995) is the most appropriate choice of a principal study for derivation of toxicity values for nonneoplastic effects because adverse effects were seen at lower doses than in the mouse dietary study (Redmond et al., 1995). Although statistically and toxicologically significant decrements in body weight were reported in rats (Stott et al., 1995) and in male mice (Redmond et al., 1995) at 25 mg/kg/day, no significant pathology or histopathology was observed in mice of either sex at any dose. In rats, a statistically significant increase in the incidence of forestomach histopathology was observed at 12.5 and 25 mg/kg/day for both sexes (see Table 6 for incidences). The histopathology consisted of mild basal cell hyperplasia of the mucosal lining and was characterized by (a) a prominence of the basal layers of the mucosa due to increased cytoplasmic basophilia and (b) an increased number of cell layers in the basal portion of the mucosa. The hyperplasia was graded as very slight or slight.

Of the two major effects, body weight decrease and forestomach hyperplasia, data from the most sensitive effect, forestomach hyperplasia, were used to develop the RfD, as hyperplasia occurred at lower doses. The forestomach hyperplasia is a manifestation of chronic irritation and is consistent with the observation of primary dermal irritation (Nater and Gooskens, 1976) and

Table 6. Incidence of forestomach histopathology in male F344 rats

Administered dose (mg/kg/day)	Forestomach histopathology (animal incidence)
0	3/100
2.5	4/100
12.5	40/100
25	67/100

other portal-of-entry effects from 1,3-dichloropropene exposure (Haut et al., 1996; Breslin et al., 1989; Lomax et al., 1989; Linnett et al., 1988; Stott et al., 1988). The irritant effects of 1,3-dichloropropene on the stomach in humans are verified by a case report of gastric mucosal erosion produced by a human poisoning incident (Hernandez et al., 1994; see Section 4.1.2). The lack of chronic irritation (i.e., forestomach hyperplasia) or body weight decrease at 2.5 mg/kg/day defines the study NOAEL. The LOAEL is 12.5 mg/kg/day. No adjustment for exposure duration is necessary because 1,3-dichloropropene was administered daily in the diet for 2 years.

5.1.2. Methods of Analysis—Benchmark Dose Analysis

The incidence of treated animals with forestomach histopathology is a quantitative measure of toxicity amenable to benchmark dose (BMD) analysis. BMD analysis was chosen because it uses the entire dose-response curve to identify the point of departure, it does not depend upon dose spacing, and it is sensitive to the number of animals used in the study. The data available met the suggested criteria (U.S. EPA, 1995) of at least three dose levels, with two doses eliciting a greater than minimum and less than maximum response.

The seven statistical models for dichotomous data from U.S. EPA's Benchmark Dose Software Version 1.1b were used to identify the model that best fit the dose-response curve (Appendix A). The best model was chosen by eliminating all models that did not have a statistically significant goodness-of-fit ($p > 0.05$). The remaining models were then ranked by best visual fit of the data, especially for the lower doses, as observed in the graphical output of the Benchmark Dose Software. The model with the best visual fit and a statistically significant goodness-of-fit was used to estimate the BMD_{10} (maximum likelihood estimate at 10% risk) and the $BMDL_{10}$ (95% lower confidence limit on the BMD_{10}).

The results for gamma, multistage, and Weibull models were statistically significant for goodness-of-fit. The gamma model was chosen because the visual fit at low doses was the best of the three models. The gamma model yielded a BMD_{10} of 5.07 mg/kg/day and a $BMDL_{10}$ of 3.38 mg/kg/day (Appendix A).

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UF) and Modifying Factors (MF)

Uncertainty factors (UFs) are applied to account for uncertainties in extrapolation from rodent bioassay data to human exposure conditions, for unknown variability in human sensitivities, for data deficiencies, and for other factors. The default uncertainty factor of 10 for interspecies extrapolation is applied because there are no data on the relative sensitivity of rats and humans to stomach irritation. Because there are no data documenting the nature and extent of variability in human susceptibilities to 1,3-dichloropropene, the default uncertainty factor of 10 is also applied to protect sensitive human subpopulations. The database for 1,3-dichloropropene is substantial and includes studies of genotoxicity, mode of action, pharmacokinetics, reproductive and developmental toxicity, systemic toxicity, and cancer. Therefore, no additional UFs or MFs are needed.

The BMD_{10} and $BMDL_{10}$ are divided by a total UF of 100 to yield the RfD.

$$BMD_{10} = 5.07 \div 100 = 0.05 \text{ mg/kg/day}$$

$$BMDL_{10} = 3.38 \div 100 = 0.03 \text{ mg/kg/day}$$

Thus, the RfD derived from the $BMDL_{10}$ is 0.03 mg/kg/day.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

Lomax et al. (1989), the only chronic inhalation bioassay for 1,3-dichloropropene, was chosen as the principal study because it was well designed and well conducted and used both rats and mice. The two potential critical effects in this study are histopathology of the respiratory epithelium in the nasal tract in rats and mice and hyperplasia and inflammation in the urinary bladder in mice. Although nasal tract histopathology was observed in both genders of rats exposed to 272 mg/m³, the female mouse was more sensitive with increased incidences of histopathology at 90.8 mg/m³. The nasal histopathology was characterized by hypertrophy and hyperplasia of the respiratory epithelium and/or degeneration of the olfactory epithelium. Urinary bladder hyperplasia also occurred at 90.8 mg/m³ in female mice and at 272 mg/m³ in males. Microscopically, the urinary bladders of both sexes exhibited hyperplasia characterized by diffuse, uniform thickening of the transitional epithelium. In females, the hyperplasia was accompanied by inflammation in 20%–30% of affected animals. Generally, the hyperplasia increased in severity with increasing concentration, and in the high-dose female group, with increasing exposure duration.

Nasal histopathology was chosen as the most relevant critical effect because it was also found in subchronic studies of rats or mice (Stott et al., 1988; Breslin et al., 1989) and because it was reported in humans exposed to 1,3-dichloropropene (Markovitz and Crosby, 1984). Table 7 shows the incidences for nasal histopathology in female mice. The lack of any such effect at 3.7

Table 7. Incidence of nasal histopathology in female B6C3F1 mice

Administered dose (mg/m ³)	Adjusted administered dose (mg/m ³) ^a	Nasal hypertrophy/hyperplasia
0	0	4/50
22.7	3.7	4/50
90.8	14.9	28/50
272	44.7	49/50

^a Correction for purity of formulation concentration (92%) and correction for intermittent exposure to continuous exposure: $22.7 \text{ mg/m}^3 \times 0.92 \times 6/24 \text{ hrs} \times 5/7 \text{ days} = 3.7 \text{ mg/m}^3$.

mg/m³, adjusted for purity and continuous exposure duration, defines the NOAEL. The LOAEL, adjusted for purity and continuous exposure duration, is 14.9 mg/m³ 1,3-dichloropropene.

5.2.2. Methods of Analysis—Benchmark Concentration Analysis

Benchmark concentration (BMC) analysis was chosen because it uses the entire dose-response curve to identify the point of departure, it does not depend upon dose spacing, and it is sensitive to the number of animals used in the study. The data available met the suggested criteria of at least three dose levels with two doses eliciting a greater than minimum and less than maximum response (U.S. EPA, 1995).

The seven statistical models for dichotomous data from U.S. EPA's Benchmark Dose Software Version 1.1b were applied to the incidence data for the adjusted administered doses (see Appendix A). The best model fit was determined by eliminating all models that did not have a statistically significant goodness-of-fit ($p > 0.05$). The remaining models were then ranked by best visual fit of the data, especially for the lower doses, as observed in the graphical output of the Benchmark Dose Software. The model with statistically significant goodness-of-fit and best visual fit was used to estimate the BMC at 10% risk and the 95% lower confidence limit of the BMC, the BMCL₁₀.

The gamma, logistic, multistage, Weibull, and quantal-quadratic models provided statistically significant fits (see Appendix A). The gamma model was the best fit overall because it provided the best visual fit. This model yielded a BMC₁₀ of 5.91 mg/m³ and a BMCL₁₀ of 3.66 mg/m³ (Appendix A).

1,3-Dichloropropene is a Category 2 gas (U.S. EPA, 1994b) because it is not highly reactive or water soluble and it produces both respiratory (nasal histopathology) and remote effects (urinary bladder histopathology). For Category 2 gases, adjustment of animal exposure to human equivalent concentrations (HECs) is based on algorithms for Category 1 or Category 3 gases, depending upon whether the major effect is respiratory or systemic. Because the critical target was the nasal mucosa, algorithms for extrathoracic effects for Category 1 gases are used to

adjust animal exposure concentrations of 1,3-dichloropropene to HECs (U.S. EPA, 1994b). The HEC for a Category 1 gas is derived by multiplying the animal BMC_{10} and $BMCL_{10}$ by an interspecies dosimetric adjustment for gas:respiratory effects in the extrathoracic area of the respiratory tract, according to the following calculation (U.S. EPA, 1994b):

$$RGDR(ET) = (MV_a/S_a)/(MV_h/S_h)$$

where:

RGDR(ET) = regional gas dose ratio for the extrathoracic area of the respiratory tract
 MV_a = animal minute volume (mouse = 0.041 L/min)
 MV_h = human minute volume (13.8 L/min)
 S_a = surface area of the extrathoracic region in the animal (mouse = 3 cm²)
 S_h = surface area of the extrathoracic region in the human (200 cm²).

Using default values, the $RGDR(ET) = (0.041/3)/(13.8/200) = 0.014/0.069 = 0.198$. The animal BMC_{10} and $BMCL_{10}$ are then multiplied by 0.198 to yield the HECs for these values:

$$BMC_{10\ HEC} = BMC_{10} \times 0.198 = 5.91 \times 0.198 = 1.17\ \text{mg/m}^3$$

$$BMCL_{10\ HEC} = BMCL_{10} \times 0.198 = 3.66 \times 0.198 = 0.725\ \text{mg/m}^3.$$

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UFs) and Modifying Factors (MFs)

UFs are applied to account for uncertainties in extrapolation from rodent bioassay data to human exposure conditions, for unknown variability in human sensitivities, for data deficiencies, and for other factors. Historically, UFs were applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent EPA practice, however, also includes use of a partial UF such as $10^{1/2}$ (U.S. EPA, 1994) under conditions where toxicokinetics and mechanistic information are available, or data are available on the nature and extent of human variability, or prior interspecific adjustment has already been conducted (e.g., using pharmacokinetic or dosimetric scaling).

For long-term rodent bioassays, the default UFs for interspecies extrapolation and within-species variability are each 10. Half of that factor, $10^{1/2}$, or 3, reflects the pharmacokinetic component of uncertainty and half represents the pharmacodynamic component of uncertainty. The toxicokinetics of 1,3-dichloropropene are reasonably well understood and do not involve bioaccumulation. Instead, 1,3-dichloropropene is rapidly conjugated via GSH-mediated systems to mercapturic acids and excreted in the urine. The toxicokinetics in rats and humans are similar. The calculation of an HEC adjustment reduces the uncertainty associated with interspecies variation. Therefore, the use of a UF of 3, instead of the default UF of 10, is more than justified for interspecies extrapolation. There are no data documenting the nature and extent of variability in human susceptibility; therefore, the default UF of 10 is used for within-species variation. The database is substantial and includes studies of pharmacokinetics, reproductive and developmental toxicity, systemic toxicity, mechanism of action and mutagenicity/genotoxicity. Therefore, no additional UFs or MFs are needed.

The BMC_{10} and $BMCL_{10}$ are divided by a total uncertainty factor of 30 to yield the RfC for noncancer effects:

$$\begin{aligned} BMC_{10\text{HEC}} &= 1.17 \text{ mg/m}^3 \div 30 = 0.039 \text{ mg/m}^3 \\ BMCL_{10\text{HEC}} &= 0.725 \text{ mg/m}^3 \div 30 = 0.024 \text{ mg/m}^3 \end{aligned}$$

Thus, the RfC derived from the $BMCL_{10\text{HEC}}$ is 0.02 mg/m^3 .

5.3. CANCER ASSESSMENT

As discussed in Section 4.6, human data are inadequate for assessment of the potential human carcinogenicity of 1,3-dichloropropene. The human data on 1,3-dichloropropene, which consist of anecdotal reports of three cases of cancer, cannot be used to infer a causal association with 1,3-dichloropropene exposure because the possibility of confounding factors has not been considered or ruled out (U.S. EPA, 1987).

Animal carcinogenicity data are sufficient to provide a quantitative assessment of the potential human carcinogenicity of 1,3-dichloropropene. The weight of evidence for both the oral and inhalation carcinogenicity of 1,3-dichloropropene indicates that this compound is carcinogenic in animals. A gavage study in rodents (NTP, 1985) indicates that 1,3-dichloropropene at relatively high bolus doses is carcinogenic at multiple sites (forestomach and liver in rats, and forestomach, urinary bladder, and lung in mice). On the other hand, dietary or inhalation administration only produced tumors in target organs with extensive geriatric changes (rat liver adenomas in Stott et al. [1995]) and/or high background incidences of benign tumors (mouse lung adenomas in Lomax et al. [1989]), and only at the highest doses tested.

EPA cancer risk assessment guidelines (U.S. EPA, 1996a and 1987) recommend a linear quantitative cancer assessment for 1,3-dichloropropene because there is evidence that 1,3-dichloropropene is a mutagen. To support a nonlinear assessment, the guidelines require the identification of a nonlinear mode of tumor formation. Although GSH is hypothesized to protect against tumor formation, which would result in a nonlinear dose-response, this hypothesis is not supported by the available mechanistic data. Thus, in the absence of definitive data for a nonlinear mechanism of tumor formation, a linear approach is taken for the cancer dose-response assessment. The linear approach assumes that a straight line best represents the shape of the dose response from the point of departure to the origin.

5.3.1. Oral Exposure—Choice of Study/Data With Rationale and Justification

All the chronic studies (NTP, 1985; Stott et al., 1995; Redmond et al., 1995) for orally administered 1,3-dichloropropene were relatively well conducted, but each study has distinct limitations. Limitations of the NTP gavage studies (1985) include the bolus dosing and the thrice-weekly rate of administration. Current test guidelines recommend seven times weekly, but indicate that five times/week is acceptable (U.S. EPA, 1998d). The NTP (1985) study was rejected by EPA for RfD development (IRIS, online 10/1/90) because the dosing regimen (high doses by gavage three times/week) was not well designed to study chronic toxicity. Another

problem with the NTP (1985) study is that the dichloropropene formulation contained epichlorohydrin, which NTP acknowledged as a possible contributor to tumorigenic effects in the forestomach. In addition, gavage administration is less relevant to human exposure than is dietary administration. However, the dietary studies, which used a microencapsulation technique, did not provide data on the in-cage stability of the food mixture. The absence of such information leaves uncertainty as to the actual dose received by the animals. In the absence of a single best study, both the NTP (1985) and Stott et al. (1995) studies will be used for the quantitative cancer assessment.

NTP (1985) reported forestomach squamous cell papilloma and carcinoma in male F344 rats at 50 mg/kg technical-grade 1,3-dichloropropene thrice weekly. Rats exhibited a significant incidence of liver adenomas at 25 mg/kg, and one carcinoma was observed at 50 mg/kg. Incidences of liver tumors and forestomach tumors were not statistically significant in female rats. At 50 mg/kg, female B6C3F1 mice gavaged thrice weekly exhibited statistically significant transitional cell carcinoma of the urinary bladder, while male mice displayed bronchioalveolar adenoma/carcinoma. Both sexes of mice exhibited significant incidences of forestomach papilloma/carcinoma and bronchioalveolar adenoma/carcinoma at 100 mg/kg.

In the dietary study, Stott et al. (1995) showed that F344 rats exposed to up to 25 mg/kg/day 1,3-dichloropropene developed late-onset benign liver tumors (see Table 8). One nonfatal hepatocellular carcinoma was also observed. A small, nonsignificant increase in

Table 8. Incidence of tumors in chronic bioassays

Administered dose (mg/kg/event) ^a	Human equivalent dose (mg/kg/day) ^b	Hepatocellular adenoma/carcinoma: male rats (NTP, 1985)	Urinary bladder carcinoma: female mice (NTP, 1985)	Hepatocellular adenoma/carcinomas: male rats (Stott et al., 1995)
0	0	1/49	0/50	2/49
2.5	0.65	—	—	1/50
12.5	3.22	—	—	6/50
25	2.75	6/48	—	—
25	6.31	—	—	10/49
50	2.88	—	8/50	—
50	5.4	8/50	—	—
100	5.81	—	21/47	—

^aDaily doses for dietary study (Stott et al., 1995); dose per gavage for NTP (1985) study.

^bAdministered doses averaged over 7 days/week (if necessary) and adjusted to human equivalent doses by multiplying by (animal body weight/human body weight)^{1/4} and the % 1,3-dichloropropene in the formulation (92% for NTP [1985] and 96% for Stott et al. [1995]).

— Dose not used.

hepatocellular adenomas was also observed in female rats, but the increased incidence was within the historical control range. No tumors were observed in a 2-year dietary study with mice exposed to up to 50 mg/kg/day 1,3-dichloropropene (Redmond et al., 1995) in the diet, but as explained earlier, dosing may have been inadequate.

The tumor data chosen for the quantitative assessment are shown in Table 8. Since there was concordance in rat liver tumors in both the gavage (NTP, 1985) and feeding studies (Stott et al., 1995), these tumors were chosen for quantitative assessment. The forestomach tumor data in rats and mice in the NTP (1985) study were not chosen due to the confounding effects of epichlorohydrin in the formulation and because the tumors did not appear in the feeding studies (Stott et al., 1995; Redmond et al., 1995). The male mouse tumor data (bronchioalveolar adenoma/carcinoma) are unacceptable for quantitative assessment because the control group survival was inadequate owing to early deaths attributed to myocarditis. Although the urinary bladder tumors in female mice in the gavage study (NTP, 1985) were not observed in the feeding study (Redmond et al., 1995), these data were chosen for quantitative assessment because transitional cell carcinoma of the bladder is a rare tumor and because the dosing for mice in the feeding study may have been inadequate, as it was not verified by in-cage stability measurements.

5.3.1.1. Dose Conversion and Dose-Response Analysis

Thrice-weekly gavage doses (NTP, 1985) were converted to an average daily dose by multiplying by 3 times/week and dividing by 7 days/week. In accordance with cancer risk assessment guidelines (U.S. EPA, 1996a), daily doses from both NTP (1985) and Stott et al. (1995) were adjusted to human equivalent doses by dividing by (human body weight/animal body weight)^{1/4} using 70 kg as the human body weight and the final body weights for the animal weights. Doses were also adjusted for the purity of the formulation (see Appendix A, II.).

Oral cancer potency factors were calculated from each set of tumor data in Table 8 using recommendations from both the proposed cancer risk assessment guidelines (U.S. EPA, 1996a) and the existing cancer risk assessment guidelines (U.S. EPA, 1987). The multistage model for extra risk from EPA's Benchmark Dose Software, Version 1.1b, was used for analysis in accordance with the proposed guidelines. Human equivalent doses and tumor incidences in Table 8 were used to calculate the point of departure, the 95% lower confidence limit of the ED₁₀ (LED₁₀) (U.S. EPA, 1996a). The cancer slope factor (i.e., risk at 1 mg/kg/day) was estimated by drawing a straight line from the point of departure to the origin, thus, the cancer slope = 0.1/LED₁₀ (see Table 9). For analysis by the existing guidelines, the GLOBAL86 linearized multistage model for extra risk was applied to the same data to determine the slope at 1 mg/kg/day. For both analyses, the unit risk for drinking water was calculated by multiplying the cancer slope factor by 1/70 kg, 2 L/day and 0.001 (for conversion of mg to µg). Risk-specific concentrations corresponding to 10⁻⁴, 10⁻⁵, and 10⁻⁶ risk were calculated by dividing risk level by unit risk. Table 9 shows the oral cancer potency results from the multistage model (proposed guidelines) and Table 10 shows the results for the linearized multistage model (existing guidelines).

Table 9. Multistage cancer potency calculations

Parameter	Hepatocellular adenoma/ carcinoma: male rats (NTP, 1985)	Urinary bladder carcinoma: female mice (NTP, 1985)	Hepatocellular adenoma/ carcinoma: male rats (Stott et al., 1995)
LED ₁₀	2 mg/kg/day	1 mg/kg/day	2 mg/kg/day
Slope factor (mg/kg/day) ⁻¹	5E-2	1E-1	4E-2
Drinking water unit risk (risk per : g/L)	1E-6	3E-6	1E-6
10 ⁻⁴ risk	7E+1 : g/L	4E+1 : g/L	8E+1 : g/L
10 ⁻⁵ risk	7E0 : g/L	4E0 : g/L	8E0 : g/L
10 ⁻⁶ risk	7E-1 : g/L	4E-1 : g/L	8E-1 : g/L

Table 10. Linearized multistage cancer potency calculations

Parameter	Hepatocellular adenoma/ carcinoma: male rats (NTP, 1985)	Urinary bladder carcinoma: female mice (NTP, 1985)	Hepatocellular adenoma/ carcinoma: male rats (Stott et al., 1995)
Slope factor (mg/kg/day) ⁻¹	5E-2	1E-1	5E-2
Drinking water unit risk (risk per : g/L)	2E-6	3E-6	1E-6
10 ⁻⁴ risk	7E+1 : g/L	4E+1 : g/L	8E+1 : g/L
10 ⁻⁵ risk	7E0 : g/L	4E0 : g/L	8E0 : g/L
10 ⁻⁶ risk	7E+1 : g/L	4E-1 : g/L	8E-1 : g/L

The cancer slope factors calculated by the linearized multistage model ranged from 5E-2 to 1E-1 (mg/kg/day)⁻¹ and the cancer slope factors from the multistage model ranged from 4E-2 to 1E-1 (mg/kg/day)⁻¹. Although there was little difference in the results between the two models, the cancer slope factors calculated from the linearized multistage model are recommended because the proposed cancer guidelines have not been finalized. Because there is less uncertainty in the delivered dose for the NTP (1985) study, the slope factor of 1E-1 (mg/kg/day)⁻¹ from the mouse bladder tumor data is recommended.

5.3.2. Inhalation Exposure—Choice of Study/Data With Rationale and Justification

The critical study for assessment of inhalation cancer potency is the study by Lomax et al. (1989) in which rats and mice were exposed to up to 272 mg/m³ of 1,3-dichloropropene for 2 years. This was a well-designed and well-conducted bioassay that followed standard guidelines. Epoxidized soybean oil replaced epichlorohydrin as the stabilizing agent in this formulation of 1,3-dichloropropene, which eliminated a potentially confounding effect of epichlorohydrin. The study by Lomax et al. (1989) is the only 2-year inhalation bioassay available. Confidence in this

The only neoplastic response observed in any species and sex was an increased incidence of benign lung tumors (bronchioalveolar adenomas), with late onset, in male mice at 272 mg/m³ (see Table 11). The incidence in the high-dose group exceeded the range of historical control rates among mice in the same laboratory. No statistically significant incidence of tumors was found in male or female rats at any exposure level.

5.3.2.1. Dose Conversion and Dose-Response Analysis

The administered dose was adjusted for purity and for continuous exposure duration as shown in the notes for Table 11. The adjustment to convert animal exposure concentrations to HECs for a Category 2 gas depends upon the critical target. For the critical effect of bronchioalveolar adenoma, algorithms for the thoracic effects of Category 1 gases are used to adjust animal exposure concentrations of 1,3-dichloropropene to HECs (U.S. EPA, 1994b). The HEC for a Category 1 gas is derived by multiplying the duration-adjusted concentrations by an interspecies dosimetric adjustment for gas:respiratory effects in the tracheobronchial and

Table 11. Incidence of bronchioalveolar adenomas in male mice exposed to 1,3-dichloropropene via inhalation

Administered dose (mg/m³)	Purity and duration adjusted dose^a (mg/m³)	Human equivalent concentration^b (mg/m³)	Tumor incidence
0	0	0	9/50
22.7	3.7	11.9	6/50
90.8	15	48.2	13/50
272	45	144.4	22/50

^aCorrection for purity of formulation concentration (92%) and correction for intermittent exposure to continuous exposure: $22.7 \text{ mg/m}^3 \times 0.92 \times 6/24 \text{ hours} \times 5/7 \text{ days} = 3.7 \text{ mg/m}^3$.

^bCorrection for thoracic effects using RGDR(TH) of 3.21 as described in the text.

pulmonary (i.e., thoracic) regions of the lung, according to the following calculation (U.S. EPA, 1994b):

$$RGDR(TH) = (MV_a/S_a)/(MV_h/S_h)$$

where

RGDR(TH) = regional gas dose ratio for the thoracic area of the lung

MV_a = animal minute volume (mouse = 0.041 L/min)

MV_h = human minute volume (13.8 L/min)

S_a = surface area of the thoracic region of the animal lung (mouse = 503.5 cm²)

S_h = surface area of the thoracic region of the human lung (543,200 cm²).

Using default values, $RGDR(TH) = (0.041/503.5)/(13.8/543,200) = 3.21$.

Purity- and duration- adjusted animal concentration $\times 3.21 =$ HEC value:

$$3.7 \text{ mg/m}^3 \times 3.21 = 11.9 \text{ mg/m}^3.$$

Inhalation unit risk factors were calculated from the tumor data in Table 11 using recommendations from both the proposed cancer risk assessment guidelines (U.S. EPA, 1996a) and the existing cancer risk assessment guidelines (U.S. EPA, 1987). The multistage model for extra risk from EPA's Benchmark Dose Software, Version 1.1b, was used for analysis in accordance with the proposed guidelines. HECs and tumor incidences in Table 11 were used to calculate the point of departure, the 95% lower confidence limit of the EC₁₀ (LEC₁₀) (U.S. EPA, 1987). The cancer slope factor, or unit risk (i.e., risk at 1 : g/m³), was estimated by multiplying the LEC₁₀ by 1,000 to convert mg to : g, and then drawing a straight line from the point of departure to the origin. Thus, the unit risk = 0.1/(LEC₁₀ \times 1,000). Concentrations corresponding to doses yielding 10⁻⁴, 10⁻⁵, and 10⁻⁶ risk levels were calculated by dividing risk level by unit risk. Table 12 shows the inhalation cancer potency results for both the multistage (proposed guidelines) and linearized multistage (existing guidelines) analyses. The air unit risks for both the multistage and linearized multistage model were 4E-6 (: g/m³)⁻¹.

Table 12. Inhalation cancer potency results

Parameter	Multistage
Point of departure, 95% LCL of ED ₁₀	24 mg/m ³
Air unit risk (95% UCL on risk at 1 : g/m ³)	4E-6 (: g/m ³) ⁻¹
Concentration at 10 ⁻⁴ risk	2E+1 : g/m ³
Concentration at 10 ⁻⁵ risk	2E0 : g/m ³
Concentration at 10 ⁻⁶ risk	2E-1 : g/m ³

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

1,3-Dichloropropene is a colorless to straw-colored liquid with a sharp, sweet, penetrating, chloroform-like odor (HSDB, 1998). It is miscible in organic solvents and evaporates easily. 1,3-Dichloropropene is used extensively in agriculture as a preplanting fumigant for the control of nematodes. Commercial formulations, including Telone, D-D, Di-Trapex, and Vorlex, contain mixtures of cis and trans isomers, other chloropropenes, chloropropanes, and stabilizers.

In both humans and animals, 1,3-dichloropropene is rapidly absorbed, conjugated with GSH to form water-soluble mercapturic acids, and quickly excreted in the urine. GSH reduces the formation of mutagenic epoxides, which are produced through a minor metabolic pathway at high (~LD₅₀) doses of dichloropropene. 1,3-Dichloropropene does not bioaccumulate in target tissues. In vitro and in vivo mutagenicity and genotoxicity tests have yielded mixed results.

The only repeated exposure human toxicity data for 1,3-dichloropropene are case study data showing that direct contact produced dermatitis. Accidental high-dose poisoning following chemical spills or accidental releases has caused a dose-related range of acute neurotoxic symptoms. Accidental ingestion of large quantities of 1,3-dichloropropene has also been reported to be fatal. In chronic studies with animals, 1,3-dichloropropene produces histopathology at the portal of entry or in organs involved in excretion of metabolites. Specifically, inhalation exposure produces nasal histopathology in mice and rats and urinary bladder hyperplasia in mice (Lomax et al., 1989). Mild hyperplasia of the forestomach was observed in rats ingesting 1,3-dichloropropene with their feed (Stott et al., 1995). No toxicologically significant effects were noted in reproductive (Breslin et al., 1989) or developmental toxicity studies with rats and rabbits (Hanley et al., 1988).

There is no evidence associating carcinogenicity in humans to 1,3-dichloropropene exposures. Three case studies in which men accidentally exposed to acute high doses subsequently developed blood cancers cannot be used to infer a causal association with 1,3-dichloropropene exposure because the possibility of confounding factors has not been considered or ruled out (U.S. EPA, 1987).

Chronic gavage studies in animals (NTP, 1985) yielded forestomach squamous cell papilloma and carcinoma in rats and mice at bolus doses of 50 mg/kg, but no tumors were observed in the glandular stomach, which is more relevant to humans. Liver tumors were observed in male rats, and urinary bladder tumors were observed in female mice. In chronic dietary studies with rats, 1,3-dichloropropene produced an increased incidence of benign hepatocellular adenomas and one nonfatal hepatocellular carcinoma in male rats at the highest dose tested, 25 mg/kg/day (Stott et al., 1995). No tumors were observed in mice (Redmond et al., 1995). In chronic inhalation studies with rats and mice, 1,3-dichloropropene significantly increased the incidence of benign bronchioalveolar tumors in the lungs of male mice at the highest dose tested, 272 mg/m³, but produced no tumors in rats (Lomax et al., 1989).

Several high-concentration and high-dose genotoxicity studies have shown that 1,3-dichloropropene is mutagenic in the presence of metabolizing enzymes and that GSH protects against the mutagenic effects (Creedy et al., 1984; Watson et al., 1987; and Schneider et al., 1998a,b). Because there is no evidence for a nongenotoxic mode of action, the mode of action of 1,3-dichloropropene is assumed to be DNA toxicity.

Under the proposed cancer risk assessment guidelines (U.S. EPA, 1996a), the weight of evidence for evaluation of cancer hazard strongly suggests that 1,3-dichloropropene is likely to be carcinogenic in humans. Although its tumorigenic action is dose-dependent in chronic animal bioassays, positive evidence of mutagenicity and the lack of mode-of-action data to support a nonlinear mechanism requires the quantitative cancer assessment to assume a linear dose response.

Under EPA's (1987) cancer risk assessment guidelines, 1,3-dichloropropene would be characterized as a class B2, probable human carcinogen, i.e., inadequate data in humans, sufficient data in animals. This characterization is supported by observations of tumors in F344 rats (forestomach, liver) and B6C3F1 mice (forestomach, urinary bladder, and lung) at high bolus doses, observations of liver tumors in F344 rats at lower dietary doses, and the formation of mutagenic epoxide metabolites at high doses.

A recent International Agency for Research on Cancer Working Group (IARC, 1999) report also concluded that there was sufficient evidence in animals to determine that 1,3-dichloropropene is possibly carcinogenic to humans. In addition, 1,3-dichloropropene is listed under California's Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) as a chemical known to the State to cause cancer (OEHHA, 1998).

6.2. DOSE RESPONSE

The quantitative estimates of human risk as a result of low-level chronic exposure to 1,3-dichloropropene are based on high-dose animal experiments because no human data exist.

6.2.1. Noncancer Dose-Response Assessment

Noncancer ingestion potency estimates were derived from a 2-year chronic bioassay (Stott et al., 1995) in which chronic irritation, exhibited by mild histopathology of the rat forestomach, was the critical effect. Significantly reduced body weight, which occurred in both rats and mice at higher doses, was the co-critical effect. The RfD of 0.03 mg/kg/day was calculated using BMD analysis and the application of UF = 100 to extrapolate from rats to humans and to account for within species variability among humans.

The overall confidence in the oral RfD is high. The confidence in the principal study is high. The study was well designed and well conducted and followed standard guidelines for chronic bioassays. Results from a subchronic ingestion study by Haut et al. (1996) are consistent with the findings in the 2-year bioassay. The confidence in the database, judged here as high, is

much improved from the earlier version on IRIS (10/1/90) because of the availability of three new dietary bioassay studies (Stott et al., 1995; Haut et al., 1996; Redmond et al., 1995) as well as new studies on metabolism and genotoxicity. Well-conducted studies on reproductive and developmental toxicity used inhalation as the route of administration. However, sufficient toxicokinetics are available to show that 1,3-dichloropropene is well absorbed by all routes.

The RfC, the daily inhalation exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime, is 0.02 mg/m³. The RfC was derived from the 2-year inhalation bioassay by Lomax et al. (1989). Histopathology was observed in the nasal epithelium of both rats and mice. The female mouse was identified as the most sensitive sex and species because statistically significant increases in the incidence of nasal histopathology were observed at lower doses than in the male mouse or in either gender of rat.

The overall confidence in the RfC is high. The inhalation toxicity potency values are based on the findings of a well-conducted 2-year bioassay (Lomax et al. 1989). The results of the 2-year bioassay are supported by the findings from a 90-day subchronic inhalation study (Stott et al., 1988). The overall confidence in the database is high because of the availability of 90-day and 2-year bioassays, as well as studies on reproductive and developmental toxicity, toxicokinetics, ingestion toxicity, and genotoxicity.

The UF used to extrapolate from rat to human and to account for within-species variability among humans is 30. Justification for the use of a partial UF for extrapolation from rats to humans is threefold: (a) toxicokinetics between humans and rats (see Section 3), based on absorption, metabolism, and excretion studies, are similar; (b) 1,3-dichloropropene does not bioaccumulate; and (c) interspecies dosimetric adjustments for pharmacokinetic differences were made when the HEC was calculated.

6.2.2. Cancer Dose-Response Assessment

Human data are inadequate for assessment of the potential human carcinogenicity of 1,3-dichloropropene. Animal data from exposures indicate that 1,3-dichloropropene is carcinogenic. A chronic gavage study in rodents indicates that 1,3-dichloropropene is carcinogenic when high bolus doses are administered. Although chronic feeding bioassays indicated that dichloropropene's tumorigenic action is dose-dependent, the lack of mode-of-action data to support a nonlinear mechanism requires the quantitative cancer assessment to assume a linear dose response to derive oral and inhalation cancer potency values. The linear approach assumes that a straight line best represents the shape of the dose response from the point of departure, at the lower end of the range of experimental observation, to lower doses.

Cancer potencies were calculated from chronic dietary, gavage, and inhalation data using both proposed and existing guidelines, with similar results. The oral cancer slope factors were 5×10^{-2} to 1×10^{-1} (mg/kg/day)⁻¹. The slope factor of 1×10^{-1} (mg/kg/day)⁻¹ from the NTP (1985) study is recommended because there is less uncertainty in the delivered dose in that study. The California Environmental Protection Agency included forestomach tumors and calculated similar cancer slope factors, 3.4×10^{-2} to 9.1×10^{-2} (mg/kg/day)⁻¹, from the same studies (OHHEA,

1999). The inhalation unit risk for the current assessment is $4 \times 10^{-6} (\mu\text{g}/\text{m}^3)^{-1}$. An inhalation unit risk of $1.6 \times 10^{-5} (\mu\text{g}/\text{m}^3)^{-1}$ is derived from the inhalation slope factor reported by the California Environmental Protection Agency (Department of Pesticide Regulation, 1994). The different interspecies dosimetry adjustment used by the two agencies is responsible for the difference in unit risks.

Confidence in the database is medium to high. Major database uncertainties are the importance of 1,3-dichloropropene's mutagenic potential in a whole-animal system and the precise mechanism of tumorigenic action. The results from short-term mutagenicity assays of the parent compound are mixed, and although 1,3-dichloropropene is metabolized to mutagenic epoxides at $\sim\text{LD}_{50}$ doses, the extent of epoxide formation in vivo at the low doses characteristic of chronic exposure is unknown. In vitro assays indicated that the presence of GSH decreases epoxide formation and abolishes or greatly reduces the mutagenic response. Thus, the linear quantitative assessment provides a very conservative estimate of cancer potency.

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**APPENDIX A
DOSE-RESPONSE CALCULATIONS**

I. NONCANCER DATA

A. Oral Exposure/RfD (Stott et al., 1995)

Forestomach histopathology in F344 rats

Administered dose (mg/kg/day)	Incidence of (forestomach histopathology)
0	3/100
2.5	4/100
12.5	40/100
25	67/100

1. Determined BMDL₁₀ (95% lower confidence limit [LCL] of BMD₁₀) using U.S. EPA Benchmark Dose Software Version 1.1b, 1998. Selected models had statistically significant goodness-of-fit statistics (p -value>0.05) and best visual fit, particularly at low doses. The gamma model was chosen because of better visual fit than the multistage or Weibull models.

BMD results: forestomach histopathology in F344 rats

Model	Chi-square goodness-of-fit p -value	p -value > 0.05	Visual rank	BMD ₁₀ (mg/kg/day)	BMDL ₁₀ (mg/kg/day)
Gamma	0.2435	X	1	5.07	3.38
Logistic	0.0028			7.25	6.31
Multistage	0.0677	X	3	4.6	2.87
Probit	0.0100			6.75	5.9
Quantal-linear	0.0439			2.71	2.3
Quantal-quadratic	0.0306			7.17	6.57
Weibull	0.1589	X	2	4.82	3.22

2. Derived RfD by applying necessary UFs to BMDL₁₀. UF = 10 for interspecies extrapolation × 10 for intraspecies extrapolation = 100:

$$\begin{aligned} \text{BMD}_{10} &= 5.07 \div 100 = 0.05 \text{ mg/kg/day} \\ \text{BMDL}_{10} &= 3.38 \div 100 = 0.03 \text{ mg/kg/day} \end{aligned}$$

The RfD derived from the BMDL₁₀ is 0.03 mg/kg/day.

B. Inhalation Exposure/RfC (Lomax et al., 1989)

1. Converted formulation doses to 1,3-dichloropropene doses. Duration adjusted to 24 hours/day, 7 days/week.

Duration and purity-adjusted concentration = (% 1,3-dichloropropene in commercial formulation) × 6/24 hrs × 5/7 days = 22.7 × 0.92 × 6/24 hrs × 5/7 = 3.7 mg/m³.

Incidence of nasal histopathology in female B6C3F1 mice

Administered dose (mg/m ³)	Adjusted dose ^a (mg/m ³)	Incidence of nasal hypertrophy/hyperplasia
0	0	4/50
22.7	3.7	4/50
90.8	14.9	28/50
272	44.7	49/50

^aExposure duration and purity adjustments.

2. Determined BMCL₁₀ (95% LCL of BMC₁₀) using U.S. EPA Benchmark Dose Software Version 1.1b, 1998. Selected model with statistically significant goodness-of-fit and best visual fit, particularly at low doses. The gamma, logistic, multistage, quantal-quadratic and Weibull models provided statistically significant fits. Of these, the gamma model was chosen because it provided the best visual fit at low doses.

BMC results: nasal hypertrophy/hyperplasia in female B6C3F1 mice

Model	Chi-square goodness-of-fit <i>p</i> -value	<i>p</i> -value > 0.05	Visual rank	BMC ₁₀ (mg/m ³)	BMCL ₁₀ (mg/m ³)
Gamma	0.4654	X	1	5.91	3.66
Logistic	0.0614	X	4	5.43	4.38
Multistage	0.1066	X	5	5.01	2.63
Probit	0.0219			5.23	4.36
Quantal-linear	0.0085			1.92	1.54
Quantal-quadratic	0.1312	X	2	6.34	5.43
Weibull	0.2202	X	3	4.97	3.14

3. Applied adjustment for HEC for a Category 1 gas to BMC₁₀ and BMCL₁₀. The HEC for a Category 1 gas is derived by multiplying the animal BMC₁₀ and BMCL₁₀ by an interspecies dosimetric adjustment for extrathoracic effects according to the following calculation (U.S. EPA, 1994b):

$$RGDR(ET) = (MV_a/S_a)/(MV_h/S_h)$$

where

RGDR(ET) = regional gas dose ratio for the extrathoracic area of the respiratory tract

MV_a = animal minute volume (mouse = 0.041 L/min)

MV_h = human minute volume (13.8 L/min)

S_a = surface area of the extrathoracic region in the animal (mouse = 3 cm²)

S_h = surface area of the extrathoracic region in the human (200 cm²).

Using default values, the RGDR(ET) = (0.041/3)/(13.8/200) = 0.014/0.069 = 0.198.

The animal BMC₁₀ and BMCL₁₀ are then multiplied by 0.198 to yield the HECs of these values:

$$BMC_{10\text{HEC}} = BMC_{10} \times 0.198 = 5.91 \times 0.198 = 1.17 \text{ mg/m}^3$$

$$BMCL_{10\text{HEC}} = BMCL_{10} \times 0.198 = 3.66 \times 0.198 = 0.725 \text{ mg/m}^3$$

4. Derived RfC by applying necessary UFs to BMC_{10HEC} and BMCL_{10HEC}.

UF = 3 for interspecies extrapolation × 10 for intraspecies extrapolation = 30

$$BMC_{10\text{HEC}} = 1.17 \text{ mg/m}^3 \div 30 = 0.039 \text{ mg/m}^3$$

$$BMCL_{10\text{HEC}} = 0.725 \text{ mg/m}^3 \div 30 = 0.024 \text{ mg/m}^3$$

The RfC is 0.02 mg/m³.

II. CANCER DATA

A. Oral exposure (Stott et al., 1995; ad libitum in feed; male rats, hepatocellular adenoma/carcinoma in male rats)(NTP, 1985; gavage; hepatocellular adenoma/carcinoma in male rats and urinary bladder carcinoma in female mice)

1. Scaled administered doses to human equivalent doses by multiplying administered dose \times (animal BW/human BW)^{1/4} \times 0.96 (purity). Human weight = 70 kg.

Incidence of hepatocellular adenoma/carcinoma in F344 rats

Administered dose (mg/kg/day)	Final mean animal weight (kg)	Human equivalent dose (mg/kg/day)	Incidence of hepatocellular adenoma ^a
0	0.384	0	2/49
2.5	0.374	0.65	1/50
12.5	0.364	3.22	6/50
25	0.335	6.31	10/49

^a Fifty rats started in each group. Because hepatocellular adenomas and carcinomas were not observed until study termination, rats who died before day 365 were excluded from the analysis: one control rat (day 195) and one high-dose rat (day 187).

2. Fit data to multistage model from U.S. EPA's Benchmark Dose Software version 1.1b.

Multistage model results for hepatocellular adenoma/carcinoma (Stott et al., 1995)

Model	Chi-square goodness-of-fit <i>p</i> -value	ED ₁₀ (mg/kg/day)	LED ₁₀ (mg/kg/day)
Multistage	0.3341 ^a	4.02	2.25

^a Statistically significant fit.

3. For linear assessment per proposed cancer risk assessment guidelines (U.S. EPA, 1996a), used 95% lower confidence level of ED₁₀ (same as BMD₁₀) from the multistage model for extra risk as the point of departure. Cancer slope factor (risk at 1 mg/kg/day) was estimated by drawing a straight line from point of departure to the origin, thus cancer slope = 0.1/BMD₁₀. Also used GLOBAL86 linearized multistage model per existing cancer risk assessment guidelines (U. S. EPA, 1987). Unit risk for drinking water = cancer slope factor \times 1/70 kg \times 2 L/day \times 1E-3. Concentrations corresponding to 10⁻⁴, 10⁻⁵, and 10⁻⁶ risk levels were calculated by dividing risk level by unit risk.

Linear assessment results for cancer via oral route hepatocellular adenoma/carcinoma (Stott et al., 1995)

Parameter	BMDS value	GLOBAL86 value
Point of departure, 95% LCL of ED ₁₀	2.3 mg/kg/day	2.3 mg/kg/day
Cancer slope factor (95% UCL on risk at 1 mg/kg/day)	4.4E-2 (mg/kg/day) ⁻¹	4.6E-2 (mg/kg/day) ⁻¹
Drinking water unit risk	1.3E-6 per (: g/L)	1.3E-6 per (: g/L)
Concentration at 10 ⁻⁴ risk	7.7E+1 : g/L	7.7E+1 : g/L
Concentration at 10 ⁻⁵ risk	7.7E0 : g/L	7.7E0 : g/L
Concentration at 10 ⁻⁶ risk	7.7E-1 : g/L	7.7E-1 : g/L

B. Oral exposure, by gavage three times per week NTP (1985); male rats, hepatocellular adenoma/carcinoma

1. Scaled administered doses to continuous human equivalent doses by administered dose × (3 gavages/week ÷ 7 days/week) × (animal BW/human BW)^{1/4} × 0.92 (purity). Human weight = 70 kg.

Incidence of hepatocellular adenoma/carcinoma in F344 rats (NTP, 1985)

Administered dose (mg/kg/day)	Mean final animal weight (kg)	Human equivalent (continuous) dose (mg/kg/day)	Incidence of hepatocellular adenoma/carcinoma^a
0	0.418	0	1/49
25	0.423	2.75	6/48
50	0.393	5.40	8/50

^a 52 rats started in each group. Since hepatocellular adenomas and carcinomas were not observed until weeks 106-108, rats who died during the first 52 weeks of the study were excluded from the analysis: three control rats (weeks 1, 8, and 49), four low dose rats (weeks 2 and 3) and two high dose rats (weeks 12 and 28).

2. Fit data to linear multistage model from U.S. EPA's Benchmark Dose Software version 1.1b.

**Model results for hepatocellular adenoma/carcinoma:
hepatocellular adenoma/carcinoma (NTP, 1985)**

Model	Chi-square goodness-of-fit <i>p</i>-value	ED₁₀ (mg/kg/day)	LED₁₀ (mg/kg/day)
Multistage	0.5412 ^a	3.38	2.05

^a Statistically significant fit.

3. For linear assessment per proposed cancer risk assessment guidelines (U.S. EPA, 1996a), used 95% LCL of ED₁₀ (LED₁₀) from the linear multistage model for extra risk as the point of departure. Cancer slope factor (risk at 1 mg/kg/day) was estimated by drawing a straight line from point of departure to the origin, thus cancer slope = 0.1/LED₁₀. Also used GLOBAL86 linearized multistage model per existing cancer risk assessment guidelines (U.S. EPA, 1987). Unit risk for drinking water = cancer slope factor × 1/70 kg × 2 L/day × 1E-3. Concentrations corresponding to calculating doses yielding 10⁻⁴, 10⁻⁵, and 10⁻⁶ risk levels were calculated by dividing risk level by unit risk.

**Linear assessment results for cancer via oral route: Hepatocellular
adenoma/carcinoma (NTP, 1985)**

Parameter	BMDS value	GLOBAL86 value
Point of departure, 95% LCL of ED ₁₀	2.0 mg/kg/day	2.0 mg/kg/day
Cancer slope factor (95% UCL on risk at 1 mg/kg-day)	4.9E-2 (mg/kg/day) ⁻¹	5.1E-2 (mg/kg/day) ⁻¹
Drinking water unit risk	1.4E-6 per (: g/L)	1.5E-6 per (: g/L)
Concentration at 10 ⁻⁴ risk	7.1E+1 : g/L	6.7E+1 : g/L
Concentration at 10 ⁻⁵ risk	7.1E0 : g/L	6.7E0 : g/L
Concentration at 10 ⁻⁶ risk	7.1E-1 : g/L	6.7E-1 : g/L

C. Oral exposure, by gavage three times per week NTP (1985); female mice, urinary bladder transitional cell carcinomas

1. Scaled administered doses to continuous human equivalent doses by administered dose × (3 gavages/week ÷ 7 days/week) × (animal BW/human BW)^{1/4} × 0.92 (purity). Human weight = 70 kg.

Incidence of urinary bladder carcinoma in B6C3F1 mice

Administered dose (mg/kg/day)	Mean final animal weight (kg)	Human equivalent dose (mg/kg/day)	Incidence of urinary bladder carcinomas^a
0	0.035	0	0/50
50	0.032	2.88	8/50
100	0.033	5.81	21/47

^a 50 mice started in each group. Because these carcinomas were first observed in week 75, mice who died before week 52 were excluded from the analysis: three high-dose mice (weeks 1, 25 and 30).

2. Fit data to linear multistage model from U.S. EPA's Benchmark Dose Software version 1.1b.

Model results for urinary bladder carcinoma in B6C3F1 mice (NTP, 1985)

Model	Chi-square goodness-of-fit <i>p</i>-value	ED₁₀ (mg/kg/day)	LED₁₀ (mg/kg/day)
Multistage	1.0 ^a	2.12	1.02

^a Statistically significant fit.

3. For linear assessment per proposed cancer risk assessment guidelines (U.S. EPA, 1996a), used 95% LCL of ED₁₀ (LED₁₀) from the linear multistage model for extra risk as the point of departure. Cancer slope factor (risk at 1 mg/kg/day) was estimated by drawing a straight line from point of departure to the origin, thus cancer slope = 0.1/LED₁₀. Also used GLOBAL86 linearized multistage model per existing cancer risk assessment guidelines (U. S. EPA, 1987). Unit risk for drinking water = cancer slope factor × 1/70 kg × 2 L/day × 1E-3. Concentrations corresponding to 10⁻⁴, 10⁻⁵, and 10⁻⁶ risk levels were calculated by dividing risk level by unit risk.

Linear assessment results for cancer via oral route urinary bladder carcinoma (NTP, 1985)

Parameter	BMDS value	GLOBAL86 value
Point of departure, 95% LCL of ED ₁₀	1.0 mg/kg/day	1.0 mg/kg/day
Cancer slope factor (95% UCL on risk at 1 mg/kg/day)	9.8E-2 (mg/kg/day) ⁻¹	9.8E-2 (mg/kg/day) ⁻¹
Drinking water unit risk	2.8E-6 per (: g/L)	2.8E-6 per (: g/L)
Concentration at 10 ⁻⁴ risk	3.6E+1 : g/L	3.6E+1 : g/L
Concentration at 10 ⁻⁵ risk	3.6E0 : g/L	3.6E0 : g/L
Concentration at 10 ⁻⁶ risk	3.6E-1 : g/L	3.6E-1 : g/L

D. Inhalation exposure: Lomax et al., 1989

1. Purity, duration and HEC adjustments.

Purity- and duration-adjusted concentration = exposure concentration × % 1,3-dichloropropene in commercial formulation × 6/24 hrs × 5/7 days = 22.7 mg/m³ × 0.92 × 6/24 hrs × 5/7 = 3.7 mg/m³.

HEC for a category 1 gas is derived by multiplying the animal exposure concentrations by dosimetric adjustment for thoracic (tracheobronchial + pulmonary) effects, because tumors were found in the bronchioalveolar region, according to the following calculation (U.S. EPA, 1994b):

$$RGDR(TH) = (MV_a/S_a) / (MV_h/S_h)$$

where

RGDR(TH) = regional gas dose ratio for the thoracic area of the lung

MV_a = animal minute volume (mouse = 0.041 L/min)

MV_h = human minute volume (13.8 L/min)

S_a = surface area of the thoracic region in animal (mouse = 503.5 cm²)

S_h = surface area of the thoracic region in human (543,200 cm²).

Using default values, the RGDR(TH) = (0.041/503.5)(13.8/543200) = 3.21.

Purity and duration animal concentration × 3.21 = HEC value:

$$3.7 \text{ mg/m}^3 \times 3.21 = 11.9 \text{ mg/m}^3.$$

Incidence of bronchioalveolar adenomas in male mice exposed to 1,3- dichloropropene via inhalation (Lomax et al., 1989)

Administered dose (mg/m³)	Adjusted dose^a (mg/m³)	Incidence of bronchioalveolar adenomas
0	0	9/50
22.7	11.9	6/50
90.8	48.2	13/50
272	144.4	22/50

^aCorrection for purity, duration, HEC.

2. Fit data to linear multistage model from U.S. EPA's Benchmark Dose Software, version 1.1b.

Model results for bronchioalveolar adenomas in male mice

Model	Chi-square goodness-of-fit <i>p</i>-value	MLE₁₀ (mg/m³)	BMC₁₀ (mg/m³)
Multistage	0.2397 ^a	47.33	24.13

^a Statistically significant fit.

3. For linear assessment per proposed cancer risk assessment guidelines (U.S. EPA, 1996a), used point of departure, 95% LCL of the EC₁₀ (LEC₁₀) from the linear multistage model for extra risk. Unit risk (risk at 1 µg/m³) was estimated by drawing a straight line from point of departure to the origin and converting mg to µg. Thus, unit risk = 0.1/(LEC₁₀ × 1,000). Also used GLOBAL86 linearized multistage model per existing cancer risk assessment guidelines (U. S. EPA, 1987). Concentrations corresponding to 10⁻⁴, 10⁻⁵, and 10⁻⁶ risk levels were calculated by dividing risk level by unit risk.

Linear assessment results for cancer via inhalation route

Parameter	BMDS value
Point of departure, 95% LCL of ED ₁₀	24.1 mg/m ³
Air unit risk (95% UCL on risk at 1 : g/m ³)	4.1E-6 (: g/m ³) ⁻¹
Concentration at 10 ⁻⁴ risk	2.4E+1 : g/m ³
Concentration at 10 ⁻⁵ risk	2.4E0 : g/m ³
Concentration at 10 ⁻⁶ risk	2.4E-1 : g/m ³

APPENDIX B. EXTERNAL PEER REVIEW— SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for 1,3-dichloropropene have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists outside EPA in accordance with EPA guidance on peer review (U.S. EPA, 1994). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

(1) General Comments

A. Appropriateness of the critical studies and critical effects for the RfD and RfC

All three reviewers agreed with the critical study (Lomax et al., 1989) and the critical effect, nasal hyperplasia, for RfC derivation. All reviewers agreed with the selection of the feeding study of Stott et al. (1995) as the critical study for the RfD; however, one reviewer questioned the use of forestomach hyperplasia as the critical effect on the basis that the relevance to humans was marginal. This reviewer requested that more supporting information be added, e.g., data for chemicals that cause serious lesions in the forestomach of rats and that also cause adverse effects in the glandular stomach of animals that do not possess a forestomach.

Response to Comment: Even though humans do not have a forestomach, the hyperplasia is important as a manifestation of chronic irritation and is consistent with other portal-of-entry effects of dichloropropene (Nater and Gooskens, 1976; Haut et al., 1996; Breslin et al., 1989; Lomax et al., 1989; Linnett et al., 1988; Stott et al., 1988). Thus, Sections 4.2.2 and 5.1.1 have been altered to characterize forestomach hyperplasia as a manifestation of chronic irritation. Evidence of relevance to humans, a case report of gastric mucosal erosion produced by a human poisoning incident (Hernandez et al., 1994), has also been added to Sections 4.1.2 and 5.1.1.

B. Appropriateness of the uncertainty and modifying factors applied to the RfD and RfC

Two reviewers agreed with the uncertainty and modifying factors applied to both the RfC and RfD. The dissenting reviewer indicated that an uncertainty factor of < 10 should be used to protect sensitive subpopulations in the RfD derivation because the document had previously concluded that children and women were not subpopulations at risk. This reviewer also stated that the 10-fold uncertainty factor applied to the RfC to protect sensitive subpopulations was not warranted because human variability to a contact irritant such as 1,3-dichloropropene should be no more than two- or threefold.

Response to Comment: The intraspecies UF is intended to protect the sick and elderly in addition to protecting children. In the absence of data to show the range of variability in human

response, the UF to protect sensitive subpopulations in the derivation of the RfD remains at 10. Regarding the analogous UF in the derivation of the RfC, there are no data to support less than 10-fold variability in the responses of humans to inhaled irritants. Thus, the UF to protect sensitive subpopulations remains at 10.

C. Use of benchmark dose models

One reviewer had reservations about the routine use of benchmark dose models and recommended also presenting the RfD and RfC as derived by the classical NOAEL method.

Response to Comment: Because the NOAEL method limits the quantitative assessment to the experimental doses used in the critical study, modeling techniques that use the entire dose-response curve are preferred for adequate data sets. For 1,3-dichloropropene, the method used would have made little difference because the points of departure for both methods were almost identical. The NOAEL for the oral study was 2.5 mg/kg/day whereas the BMDL₁₀ was 3.4 mg/kg/day. The NOAEL for the inhalation study was 3.7 mg/m³ and the BMCL₁₀ was 3.66 mg/m³. The NOAEL for oral exposure was identified in Section 5.1.1 and the NOAEL for inhalation exposure has been added to Section 5.2.1.

D. Interspecies scaling factor for oral quantitative cancer assessment

One reviewer remarked that the basis for calculating the HED (human body weight/animal body weight)^{1/4} was not apparent. The reviewer indicated that major interspecies determinants would include the levels of reduced GSH and GST activity in the liver and the ability to regenerate GSH.

Response to Comment: The scaling factor used to determine the HED is recommended by the proposed cancer risk assessment guidelines (U.S. EPA, 1996a). The reference for the scaling factor has been added to Section 5.3.1.1. The scaling factor, which represents an adjustment for metabolic rate across animals of different sizes, is used in the absence of chemical-specific data on interspecies sensitivity. There are several isoforms of GST that confer target organ and species sensitivity. Because the literature search for 1,3-dichloropropene did not find information on the specific GST isoform that catalyzes its metabolism, a more specific interspecies extrapolation cannot be performed.

(2) Comments on Chemical-Specific Questions

A. Cancer classification

All reviewers agreed with the B2 carcinogen classification.

B. Choice of data for the oral quantitative cancer assessment

One reviewer stated that because the NTP (1985) study had serious experimental design problems, the feeding study (Stott et al., 1995) should be used. This reviewer indicated that the gavage administration (NTP, 1985) was a serious problem because it was not relevant to human exposures, which occur intermittently over the course of a day. The reviewer cited a study by Sanzgiri et al. (1995) to show that gavage administration of carbon tetrachloride produces higher blood levels and toxicity than a 2-hour gastric infusion of the same dose. Another reviewer agreed with using both gavage and feeding studies for liver tumors in rats, but remarked that the use of the mouse bladder cancer data from the NTP (1985) study was questionable because of the decreased survival in control animals and the presence of epichlorohydrin, a known carcinogen, in the dichloropropene formulation. The third reviewer remarked that the use of a single data set alone would not increase the reliability of the oral cancer estimate as long as a linear dose-response assessment was performed. If a single study were to be used, this reviewer preferred the study by Stott et al. (1995).

Response to Comment: The variety of opinions on this topic reflect the Agency's difficulty in choosing the appropriate data set between the feeding study in rats (Stott et al., 1995) and the gavage study in mice and rats (NTP, 1985). The liver tumors in rats were important because they were observed in both feeding and gavage studies, but neither study was clearly better than the other (see Section 5.3.1), so both were used. Transitional cell carcinoma of the urinary bladder was observed in the mouse gavage study (NTP, 1985), but it did not appear in the feeding study (Redmond et al., 1995). The mouse gavage data was used because it was a rare tumor type. The lack of effects, other than decreased body weight, in the mouse feeding study (Redmond et al., 1995) and the lack of in-cage stability studies of the food mixture cast doubt that the mice in the feeding study received the intended dose. All three data sets were used because no single data set was clearly the best.

C. Appropriateness of linear vs. nonlinear quantitative cancer assessment

All reviewers agreed that there was evidence that the dose-response for cancer was nonlinear; however, two reviewers agreed that the linear assessment was appropriate in the absence of more definitive mode-of-action data for tumor formation. The other reviewer opined that cancer formation was clearly a threshold phenomenon and that a nonlinear assessment is entirely appropriate. The third reviewer noted that two apparent modes of action had been identified and that both were threshold phenomena. The modes of action cited by this reviewer were epoxide formation with subsequent reactive metabolites that bind to DNA, and chronic irritation and cell killing, which results in degenerative changes and regenerative hyperplasia.

Response to Comment: The dose-response for mouse urinary bladder tumors seemed to be rather linear, but only two doses were used in that study (NTP, 1985). The dose-responses for rat liver tumors (NTP, 1985; Stott et al., 1995) and for mouse bronchioalveolar adenomas were nonlinear. Regardless of the shape of the dose-response, both current (U.S. EPA, 1987) and proposed (U.S. EPA, 1996a) cancer guidelines require defaulting to a linear dose-response assessment in the absence of definitive mode-of-action data supporting a nonlinear mechanism of

tumor formation. Because there was evidence of mutagenicity (Watson et al., 1987; Martelli et al., 1993; Ghia et al., 1993; Kevekordes et al., 1996, Schneider et al., 1998a) in short-term in vitro and in vivo tests, the cancer guidelines (U.S. EPA, 1996a; U.S. EPA, 1987) call for a linear dose-response assessment.