Listed "As Causing Cancer" under the Authoritative Body Mechanism and Under Review for Possible Delisting:

Allyl Chloride

1,1-Dichloroethane

p-Toluidine

October 30, 1998

California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
Reproductive and Cancer Hazard Assessment Section

Table of Contents

Preface

The California Environmental Protection Agency's (Cal/EPA) Office of Environmental Health Hazard Assessment (OEHHA), as lead agency for the implementation of the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), maintains the Proposition 65 list of chemicals that had been identified by the State to cause cancer, birth defects or other reproductive harm. One of the mechanisms by which a chemical can be put on the Proposition 65 list is when the chemical has been identified as causing cancer by an organization that has been designated as "authoritative" for purposes of Proposition 65. The authoritative bodies for identifying agents as causing cancer are: the US Environmental Protection Agency (US EPA), US Food and Drug Administration, National Institute Occupational Safety and Health, the National Toxicology Program, and the International Agency for Research on Cancer.

If the lead agency finds that a chemical is no longer identified by the authoritative body as causing cancer or reproductive toxicity, the listing under the Proposition can be reconsidered (Title 22, California Code of Regulation, Section 12306). Chemicals listed as causing cancer which are under reconsideration and which have been placed on the list by the authoritative bodies mechanism are referred to the Carcinogen Identification Committee, the state's qualified experts for carcinogenicity determinations under the Proposition. The Committee then makes a recommendation as to whether the chemical should remain on the list.

In a public meeting of the Committee held September 25, 1997, OEHHA informed the Committee of five candidate chemicals which may no longer be identified by the authoritative body as causing cancer: allyl chloride, 1,1-dichloroethane, *p*-toluidine, zineb, and chlorodibromomethane. This document provides background information on authoritative bodies reviews and carcinogenicity evidence for three of the five – allyl chloride, 1,1-dichloroethane, and *p*-toluidine. It is anticipated that a similar document on zineb and chlorodibromomethane will be released in the near future.

On December 10, 1998, a public meeting will be conducted and the Carcinogen Identification Committee will consider making a recommendation regarding whether allyl chloride, 1,1-dichloroethane, and *p*-toluidine should continue to be included on the Proposition 65 list. Comments on this document, or other issues related to the removal of these specific chemicals from the Proposition 65 list of chemicals known to the state to cause cancer should be directed by November 30, 1998 to: Cynthia Oshita, Proposition 65 Implementation Office, Office of Environmental Health Hazard Assessment, 301 Capitol Mall, 2nd Floor, Sacramento, CA 95814. Fax (916) 327-1097.

ALLYL CHLORIDE

Listing History

Allyl chloride (CAS No. 107-05-1; 3-chloropropene) was listed "as causing cancer" under Proposition 65 on January 1, 1990, based upon its classification by the US Environmental Protection Agency (US EPA) as a probable human carcinogen (Group B2) (US EPA, 1987). The classification as B2 was based on "the limited animal data, when combined with the supporting evidence for mutagenicity, metabolism to epichlorohydrin, and alkylation properties" (US EPA, 1986). In 1990, the US EPA revised the classification of allyl chloride to category C, possible human carcinogen. This re-classification was based on a lack of evidence in humans and "a low (but biologically important) incidence of forestomach tumors in female mice, and positive results in a variety of genetic toxicity tests" (US EPA, 1997). The alkylating properties and structural relationship to probable human carcinogens were also noted in the statement of the basis for this revised assessment.

Reviews by Other Authoritative Bodies

Other authoritative bodies have evaluated the evidence of carcinogenicity of allyl chloride. The International Agency for Research on Cancer (IARC, 1985b, 1987) concluded that allyl chloride was not classifiable as to its carcinogenicity (Group 3), based on inadequate evidence in experimental animals and the absence of data in humans. The National Cancer Institute (NCI, 1978) concluded there was suggestive evidence for carcinogenicity in both male and female $B6C3F_1$ mice based on the low incidence of rarely occurring neoplastic lesions of the forestomach, and no convincing evidence for carcinogenicity in rats of either sex. Neither National Institute of Occupational Safety and Health nor US Food and Drug Administration appears to have evaluated the evidence of carcinogenicity for allyl chloride.

Carcinogenicity Data Available

Epidemiological Studies

Between 1990 and 1996, three epidemiologic studies were carried out on factory workers who were primarily exposed to epichlorohydrin, but were also potentially exposed to allyl chloride (Enterline *et al.*, 1990; Olsen *et al*., 1994; Tsai *et al*., 1996). Enterline *et al.* (1990) concluded that a significant excess of leukemia occurred in workers with a latency of at least 20 years, but the data in the paper are difficult to interpret. In the Tsai *et al*. (1996) study, 95 percent confidence intervals on the standard mortality ratios always included 1.0. Olsen *et al*. (1994) suggested that the non-positive result in their study reflected the small cohort size and short follow-up. In the three studies, allyl chloride exposure was a side issue and where disease outcome was a concern, it was associated with non-cancer end-points. Therefore, none of these epidemiological studies are informative about the carcinogenic effects of allyl chloride.

Animal Data

Long-term oral studies in mice and rats of both sexes were reported by NCI (1978). They provided suggestive evidence of carcinogenicity of allyl chloride in mice, but the studies in rats were non-positive. Theiss *et al*. (1979a) reported a series of short-term injection studies using the lung adenoma model in male and female Strain A mice and Van Duuren *et al*. (1979) reported a skin-painting study. The injection studies had equivocal results, while the skin-painting study was negative. A skin-painting initiation-promotion study gave evidence of initiating activity. The studies performed and results obtained are described in detail below.

1. Mouse long-term oral studies (NCI, 1978): Male and female B6C3F_1 mice (50 animals per group) were exposed to allyl chloride by gavage in corn oil for 78 weeks and observed for 31-32 additional weeks. Males received 172 or 199 mg/kg_{bw}-day, while females received 129 or 258 mg/kg_{bw}-day. Control groups of 20 mice of each sex received corn oil alone, while further control groups of 20 animals of both sexes served as untreated controls. Survival in the high dose (HD) males was extremely poor due to severe toxicity; mortality was 50% by week 27, and only 10 members of this group survived past week 48. These were sacrificed in week 56. Survival was however adequate in the low dose (LD) males and both dose groups of females. Squamous cell carcinomas of the forestomach were observed in 2/46 of the low dose male mice, and in 2/48 of the low-dose female mice. Metastases were observed in the LD males. Additionally, in females squamous cell papillomas were observed at the same site (1/48 in the LD group and 3/45 in the HD group). No squamous cell carcinomas were observed in the HD female. A leiomyosarcoma of the forestomach was observed in 1/46 LD male mouse. No forestomach tumors were observed in either vehicle or untreated controls. The study authors described squamous cell carcinoma or papilloma as "infrequently observed in B6C3F_1 mice" and reported a historical control rate of 1/180 for both male and female mice at the laboratory where this study was conducted. Non-neoplastic proliferative lesions (acanthosis and hyperkeratosis) of the forestomach were observed in HD and LD mice of both sexes, whereas these lesions were not found among the untreated- or vehicle treated controls. (These lesions shared some histological features with squamous cell carcinomas). The incidence of hepatocellular carcinomas was increased in the LD male mice (8/46, compared to 2/20 in the vehicle controls), but this increase was within the historical range seen for this tumor in these mice. When compared to vehicle or untreated controls, the incidences of squamous cell carcinomas or papillomas of the forestomach were not statistically significant. However, assuming a binomial distribution with a probability of 1/180 for unexposed mice (based on the historical controls), the probabilities of the observed incidences of these tumors among the exposed mice were <0.029, <0.003, and <0.003, for LD males, LD and HD females, respectively. The power of the bioassay was limited by mortality, especially in the male mice. Based on these considerations, NCI (1978) concluded that the results are suggestive that allyl chloride is carcinogenic in male and female B6C3F_1 mice.

2. Mouse 24-week intraperitoneal injection studies (Theiss *et al*., 1979): Groups of 10 male and 10 female Strain A mice were administered allyl chloride by

intraperitoneal injection (0.65, 1.6, or 3.2 mg/kg_{bw}) three times a week for a total of 24 injections. Twenty-four weeks after the first injection, the lungs (only) were examined superficially for adenomas. Although both male and female mice were used, the tumor data were reported for the two sexes combined. The HD mice exhibited an increase ($p<0.05$) in the average number of lung adenomas per mouse compared to vehicle controls (combined sexes, number of tumors per mouse: HD 0.60 ± 0.15, middle dose 0.5 ± 0.27, LD 0.6 ± 0.20, vehicle control 0.19 ± 0.10). The increase in the HD group was significant by only one of the two tests (t and χ^2) used by the authors, who rated allyl chloride tumorigenicity in this experiment as intermediate.

3. Rat long-term oral studies (NCI, 1978): Male and female Osborne-Mendel rats (50 animals per group) were exposed to allyl chloride by gavage in corn oil for 78 weeks and observed for 31-32 additional weeks. Males received 57 or 77 mg/kg_{bw}-day, while females received 55 or 73 mg/kg_{bw}-day. Control groups of 20 rats of each sex received corn oil alone, while further control groups of 20 animals of both sexes served as untreated controls. There was extremely poor survival among the HD rats of both sexes; 50% survival times were 14 weeks for the males and 38 weeks for the females. These early deaths were not associated with tumors. No differences in the frequencies of tumors between treated and untreated or vehicle treated control rats were found at either dose level, although the data from the HD groups are clearly not suitable for analysis of late-occurring tumors. The authors concluded that there was no evidence for the carcinogenicity of allyl chloride in rats, but also noted the low power of the study due to high mortality, especially in the HD groups of both sexes.

4. Mouse long-term skin painting study (Van Duuren *et al*., 1979): Groups of 30 female Ha:ICR Swiss mice received 94 mg or 31 mg allyl chloride in 0.2 ml acetone per mouse, 3 times a week. A vehicle control group of 30 mice (acetone only) and an untreated group of 100 mice were also included in the study, along with groups exposed similarly to a number of other compounds. The study duration was not stated exactly, but was between 440 and 594 days, and mean survival time of the mice was between 317 and 589 days. No skin papillomas or carcinomas were observed. The incidence of other tumors (lung and stomach being noted as sites examined) was not significantly different from no-treatment or vehicle controls.

5. Mouse skin painting initiation/promotion study (Van Duuren *et al*., 1979): 30 female Ha:ICR Swiss mice received a single application of 94 mg allyl chloride in 0.2 ml acetone per mouse. Starting 14 days later, animals received 2.5 µg of the promoter phorbol myristate acetate (PMA) in 0.1 ml acetone, three times a week. A vehicle control group of 30 mice (acetone only), an untreated group of 100 mice, and two groups, of 120 and 90 mice, receiving PMA treatment only, were also included in the study. (This study of allyl chloride, along with a number of other compounds, was performed concurrently with the repeated skin painting study noted above.) Skin papillomas were observed in 7/30 mice (10 papillomas in total), a significant ($p<0.025$) increase compared to the animals receiving PMA alone (9/120, 6/90). The

authors concluded that allyl chloride showed significant initiation activity in this assay.

Other Relevant Data

Genotoxicity
A summary of the genotoxicity results appears in Table 1. Allyl chloride was genotoxic in *Salmonella typhimurium*, strains 100 and 1535 (McCoy *et al*., 1978; Neudecker *et al*., 1980; Eder et al., 1982; Neudecker and Henschler, 1985), *Escherischia coli* strains WP2 and SP2uvrA (Dean *et al*., 1985), *Saccharomyces cerevisiae* (Dean *et al*., 1985), and *Aspergillus nidulans* (Crebelli et al., 1984). Dean *et al*. (1985) obtained negative genotoxicity test results in *Salmonella typhimurium* (strains 1535 and 1538) using a plate incorporation protocol. McCoy *et al*. (1978) and Dean *et al*. (1985) suggest that these negative results should be interpreted with care, because of the volatility of allyl chloride, and recommend filter disc or preincubation procedures for use with volatile compounds. Using the filter disc method, allyl chloride was positive in *Salmonella typhimurium* strain 1535 (a base substitution mutant), but still negative in the frame-shift mutant strain 1538 (McCoy *et al*., 1978).

Allyl chloride caused *in vitro* unscheduled DNA synthesis in HeLa cells (Schiffmann *et al*., 1983). Allyl chloride binds to DNA, *in vitro*, with a half-life of 360 hours (Eder *et al*., 1987). Among the reaction products identified were 3 guanine and 2 adenine adducts.

Allyl chloride mutagenicity does not require the presence of metabolic activation, and is generally not enhanced by it. However, extended incubation times or higher concentrations of allyl chloride can result in increased mutagenicity in the presence of metabolic activation (Simmon, 1978; Neudecker and Henschler, 1985). Halo-allyl compounds generally are direct alkylating agents and with few exceptions, biological activity follows chemical reactivity (for a more detailed discussion, see Neudecker *et al*, 1980; Eder *et al*, 1982). It is therefore probable that the unchanged compound is one of the genotoxic species. However, metabolic activation to other reactive and mutagenic intermediates, including epichlorohydrin, acrolein and glycidaldehyde, occurs (De Rooij *et al.*, 1996), and it is possible that these metabolic products also play a role in the observed genotoxicity under some circumstances. The enzyme inhibition data of Neudecker and Henschler (1986) suggest a role for a pathway that is initiated by the formation of allyl alcohol, and continues with the formation of acrolein and glycidaldehyde, but not for direct epoxidation to epichlorhydrin.

Table 1. Summary of Genotoxicity Test Results of Allyl Chloride

Species, strain	End-point	Results	Reference
Salmonella typhimurium			
100 (base substitution)	Reverse mutation	+[a]	McCoy et al, 1978; Eder et al, 1982; Neudecker and Henschler, 1985; Neudecker and Henschler, 1986
1535 (base substitution)	"	+[a]	McCoy et al, 1978
	"	-[b]	Dean et al, 1985
1538 (frame shift)	"	-	McCoy et al, 1978[a], Dean et al, 1985[b]
Escherischia coli			
Pol^+/Pol^-	DNA modification	+	McCoy et al, 1978
WP_2, WP_2uvrA	Reverse mutation	+	Dean et al, 1985
Saccharomyces cerevisiae			
D4	Gene conversation	+	McCoy et al, 1978
JD1	"	+	Dean et al, 1985
Aspergillus nidulans	Gene segregation	+	Crebelli et al, 1984
Rat liver - epithelial type cells, *in vitro*	Clastogenicity	-	Dean et al, 1985
HeLa cells, *in vitro*	Unscheduled DNA synthesis	+	Schiffmann et al, 1983

[a] Filter disc or preincubation procedures used to minimize evaporation.

[b] Mutagenicity testing occurred under conditions of plate incorporation.

Structure Activity Comparisons

Several allyl compounds are known mutagens and/or carcinogens. Allyl chloride is among the less chemically reactive of the allyl compounds, and exhibits lower mutagenic potencies than those of the more reactive allyl compounds such as 1,3-dichloropropene or 1-chloro-2-butene (Neudecker *et al*., 1980; Eder *et al*., 1982; Henschler, 1985). For one structurally related compound, 3-chloro-2-methylpropene, the presence of the methyl group in the latter appears to enhance the carcinogenic, as well as the mutagenic activity (Eder *et al*., 1982; Chan *et al.*, 1986).

The proposed metabolites epichlorohydrin and glycidaldehyde are listed under Proposition 65 as known to the State of California to cause cancer. These compounds are classified by US EPA (1997) as probable human carcinogens (B2) on the basis of sufficient evidence in animals. IARC (1987) lists epichlorohydrin as a probable human carcinogen (2A), and glycidaldehyde as a possible human carcinogen (2B) on the basis of sufficient evidence in animals and, in the case of epichlorohydrin, its activity in short-term genotoxicity tests.

Summary

Allyl chloride was listed "as causing cancer" under Proposition 65 based upon its classification by the US EPA as a probable human carcinogen (Group B2). In 1990, the US EPA revised the classification of allyl chloride to category C, possible human carcinogen. This re-classification was based on a lack of evidence in humans and "a low (but biologically important) incidence of forestomach tumors in female mice, and positive results in a variety of genetic toxicity tests". The alkylating properties and structural relationship to probable human carcinogens were also noted in the statement of the basis for this revised assessment.

No epidemiological studies that are informative about the carcinogenic effects of allyl chloride are available. Suggestive evidence of carcinogenicity of allyl chloride has been observed in male and female mice in long-term oral (gavage) studies. Small increases in incidence of squamous cell carcinomas and papillomas of the forestomach were observed in both sexes. These were not statistically significant relative to the concurrent vehicle controls or untreated animals, but were considered significant relative to historical control data from the same laboratory. Similar studies in male and female rats were non-positive. The power of both the rat and mouse studies to detect a carcinogenic effect was compromised by severe toxicity resulting in early mortality, especially in the high-dose groups of male mice, and male and female rats. Allyl chloride has demonstrated genotoxicity *in vitro* in a number of test systems, including reverse mutation assays and tests for clastogenicity and unscheduled DNA synthesis. It appears likely that allyl chloride is a direct-acting mutagen, although additional mutagenic activity is associated with the formation of metabolites under some conditions. Additional supporting evidence is provided by allyl chloride's structural relationship to other known mutagens and carcinogens, and by the identification of known carcinogens as its metabolites.

References

Chan PC, Haseman JK, Boorman GA, Huff J, Manus AG, and Cardy RH (1986). Forestomach lesions in rats and mice administered 3-chloro-2-methylpropene by gavage for two years. *Cancer Res* **46**:6349-6352.

Crebelli R, Conti G, Conti L, and Carere A (1984). Induction of somatic segregation by halogenated aliphatic hydrocarbons in *Aspergillus nidulans*. *Mut Res* **138**:33-38.

De Rooij BM, Commandeur JMM, Groot EJ, Boogaard PJ, Vermeulen NPE (1996). Biotransformation of allyl chloride in the rat. *Drug Metab Dispos* **24**(7):765-772.

Dean BJ, Brooks TM, Hodson-Walker G, and Hutson DH (1985). Genetic toxicology testing of 41 industrial chemicals. *Mut Res* **153**:57-77.

Eder E, Neudecker T, Lutz D, and Henschler D (1982). Correlation of alkylating and mutagenic activities of allyl and allylic compounds: standard alkylation test vs. kinetic investigation. *Chem-Bio Interactions* **38**:303-315.

Eder E, Lutz D, and Jorns M (1987). Allylic compounds bind directly to DNA: investigation of the binding mechanisms *in vitro*. *Chem-biol Interactions* **61**:97-108.

Enterline PE, Henderson V, and Marsh G (1990). Mortality of workers potentially exposed to epichlorohydrin. *Br J Ind Med* **47**:269-276.

Henschler D (1985). Halogenated alkenes and alkenes. In: *Bioactivation of foreign compounds.* MW Anders (Ed.), Academic Press, Inc., NY. pp. 317-347.

International Agency for Research on Cancer (IARC, 1985a). *IARC Monographs on the evaluation of cancer risks to humans. General remarks on the substances considered.* Volume 36, pp. 31-34. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1985b). Allyl chloride. In: *IARC monographs on the evaluation of cancer risks to humans. Volume 36,* pp. 39-54. IARC, Lyon, France.

International Agency for Research on Cancer (IARC, 1987). *IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluation of carcinogenicity: An updating of IARC monographs Volumes 1 to 42.* Supplement 7, pp. 56 and 64. IARC, Lyon, France.

National Cancer Institute (NCI, 1978). *Bioassay of allyl chloride for possible carcinogenicity*. NCI-CG-TR-73. US Department of Health, Education, and Welfare, Public Health Service, Bethesda, MD.

Neudecker T and Henscher D (1985). Mutagenicity of chloro-olefins in the Salmonella/mammalian microsome test. I. Allyl chloride mutagenicity re-examined. *Mut Res* **157**:145-148.

Neudecker T and Henschler D (1986). Mutagenicity of chloroolefins in the Salmonella/mammalian microsome test. III. Metabolic activation of the allylic chloropropenes allyl chloride, 1,3-dichloropropene, 2,3-dichloro-1-propene, 1,2,3-trichloropropene, 1,1,2,3-tetrachloro-2-propene and hexachloropropene by S9 mix via two different metabolic pathways. *Mut Res* **170**:1-9.

Neudecker T, Lutz D, Eder E, and Henschler D (1980). Structure-activity relationship in halogen and alkyl substituted allyl and allylic compounds: Correlation of alkylating and mutagenic properties. *Biochem Pharmacol* **29**:2611-2617.

Olsen GW, Lacy SE, Chamberlin SR, Albert DL, Arceneaux TG, Bullard LF, Stafford BA, and Boswell JM (1994). Retrospective cohort mortality study of workers with potential exposure to epichlorohydrin and allyl chloride. *Am J Ind Med* **25**:205-218.

Schiffmann D, Eder E, Neudecker T, and Henscher D (1983). Induction of unscheduled DNA synthesis in HeLa cells by allylic compounds. *Cancer Letters* **20**:263-269.

Simmon VF (1978). Structural Correlations of Carcinogenic and Mutagenic Alkyl Halides. In: *Structural correlates of carcinogenesis and mutagenesis*. Proceedings of the Second FDA Office of Science Summer Symposium, 31 August - 2 September 1977, Asher IM and Zervos (Eds.). DHEW/PHS/FDA, DHEW Publ No 79-1046, Rockville MD. pp. 163-171.

Tsai SP, Gilstrap EL, Ross CE (1996). Mortality study of employees with potential exposure to epichlorohydrin: a 10 year update. *Occup Environ Med* **53**:299-304.

Theiss JC, Shimkin MB, and Piorier LA (1979). Induction of pulmonary adenomas in strain A mice by substituted organohalides. *Cancer Research* **39**:391-395.

United States Environmental Protection Agency (US EPA, 1986). *Health and environmental effects profile for allyl chloride*. Environmental Criteria and Assessment Office, US EPA, Cincinnati, OH. ECAO-CIN-P186, Final Draft.

United States Environmental Protection Agency (US EPA, 1987). *Health Assessment document for beryllium*. Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, US EPA, Research Triangle Park, NC. EPA/600/8-84/026F, Table 7-18, pp. 7-82 – 7-85.

United States Environmental Protection Agency (US EPA, 1997). *Integrated Risk Information Service* (*IRIS)*. Environmental Criteria and Assessment Office, US EPA, Cincinnati OH.

Van Duuren BL, Goldschmidt BM, Loewengart G, Smith AC, Melchionne S, Seldman I, and Roth D (1979). Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *J Natl Can Inst* **63**:1433-1439.

1,1-DICHLOROETHANE (1,1-DCA)

Listing History

1,1-Dichloroethane (1,1-DCA, CAS No. 75-34-3, ethylidene dichloride) was listed "as causing cancer" under Proposition 65 on January 1, 1990, based upon its classification by the US EPA as a probable human carcinogen (Group B2) in its 1989 Health Effects Assessment Summary Tables (HEAST) (US EPA, 1989). US EPA (1989) noted hemangiosarcomas in the rat as the tumor of concern and referenced two earlier US EPA assessments (US EPA 1984, 1985). In December 1996, US EPA revised the classification of 1,1-DCA to category C, possible human carcinogen. This re-classification was based on a lack of evidence in humans and limited evidence in rats and mice (US EPA, 1997). Although the rationale for the reclassification to Group C was not described by US EPA (1990), the changes appear to reflect differences in professional judgment rather than on significant new information.

Reviews by Other Authoritative Bodies

The National Institute for Occupational Safety and Health (NIOSH, 1978) issued a Current Intelligence Bulletin which reviewed the toxicity of nine chloroethane compounds, including 1,1-DCA. NIOSH did not make any conclusions regarding 1,1-DCA carcinogenicity since the NCI bioassay on 1,1-DCA was undergoing "retesting ... because the previous tests were inconclusive; low survival rates complicated the interpretation of the bioassay results" (NIOSH, 1978). However, NIOSH (1978) stated that 1,1-DCA should be treated in the workplace with caution because of its structural similarity to 4 chloroethanes that were shown to be carcinogenic in animals.

US EPA (1989) listed 1,1-DCA as a group B2, probable carcinogen, and referenced two earlier assessments conduced by the Office of Health and Environmental Assessment for the Office of Solid Waste and Emergency Response (US EPA 1984, 1985). The US EPA (1984) assessment concluded that based on Carcinogen Identification Group listing criteria at the time 1,1-DCA would be listed as Group D -- unclassifiable as to carcinogenicity. The US EPA (1985) assessment provided an interim designation of Group C, possible carcinogen, based on limited evidence in animals.

No additional reviews by other authoritative bodies designated for the Proposition 65 program (i.e., NTP, IARC, US FDA) were located regarding the evidence of carcinogenicity of 1,1-DCA.

Carcinogenicity Data Available

Epidemiological studies
No human studies of the long-term health effects of exposure to 1,1-DCA were identified by US EPA (1997) or more recently by OEHHA.

Animal Data

Rat chronic gavage studies (NCI, 1978a): Technical grade 1,1-DCA (purity 99 %) was administered in corn oil by gavage, 5 days/week, for 9 weeks to male rats (50/dose group) at dosages of 350 and 700 mg/kg-day, and to female rats (50/dose group) at doses of 750 and 1,500 mg/kg-day. After 9 weeks, the doses were increased to 450 and 900 mg/kg-day (males) and 900 and 1,800 mg/kg-day (females), respectively. At week 18 the dosages for the female rats were reduced to 450 and 900 mg/kg-day. At week 32 of the experiment, dosing was stopped for 1 week and was resumed at the previous doses for 4 weeks. This cyclical pattern of "1 week off -- 4 weeks on" was continued until week 78, at which point the dosing was stopped. Animals were then observed for an additional 33 weeks prior to sacrifice. NCI (1978a) reported time-weighted average doses over the 78-week dosing period of 382 and 764 mg/kg-day for males and 475 and 950 mg/kg-day for females. An untreated control group (20/sex) and a vehicle control group (20/sex) were also studied. Vehicle control rats from a concurrent experiment were used for a pooled vehicle control group (40/sex).

Survival was poor for both male and female rats; 80 percent of all rats in these studies were observed to have pneumonia. For the untreated control, vehicle control, low- and high-dose groups, respectively, percent survival at the end of the study was 30, 5, 4 and 8 for male rats, and 40, 20, 16 and 18 for female rats. No treatment-related tumors were observed in male rats. In female rats, for the pooled vehicle controls, low- and high-dose groups, the incidences of circulatory system hemangiosarcoma were 0/39, 0/50 and 4/50, and for mammary gland adenoma was 1/39, 1/50 and 5/50, respectively. These increases were not statistically significant ($p>0.05$) using Fischer exact test; however, the hemangiosarcomas were statistically significant ($p=0.02$) using a Cochran-Armitage trend test.

Mouse chronic gavage studies (NCI, 1978a): Male B6C3F_1 mice (50/dose group) were administered 1,1-DCA in corn oil via gavage, 5 days/week, for 6 weeks at doses of 900 and 1,800 mg/kg-day. Doses were increased to 1,200 and 2,400 mg/kg-day for 3 weeks, then increased to 1,500 and 3,000 mg/kg-day for 69 weeks, at which point dosing was ceased. Male mice were observed for an additional 13 weeks before sacrifice. Female B6C3F_1 mice (50/dose group) were administered 1,1-DCA in corn oil via gavage, 5 days/week for 6 weeks at doses of 900 and 1,800 mg/kg-day. Doses were increased to 1,200 and 2,400 mg/kg-day for 3 weeks, then increased again to 1,500 and 3,000 mg/kg-day for 11 weeks. The doses were increased a final time to 1,800 and 3,600 mg/kg-day for 58 weeks. Female mice were observed for an additional 13 weeks (untreated) before sacrifice. NCI (1978a) estimated time-weighted averages for the 78-week dosing period to be 1,442 and 2,885 mg/kg-day for males and 1,665 and 3,331 mg/kg-day for females. An untreated control group (20/sex) and a vehicle control group (20/sex) were also studied. Vehicle control rats from a concurrent experiment were used for a pooled vehicle control group (40/sex).

Survival was poor for all groups of male mice and for the high-dose female mice. For the untreated control, vehicle control, low- and high-dose groups, percent survival at the end of the study was 35, 55, 62 and 32 for male mice and 80, 80, 80 and 50 for female mice, respectively. In male mice living past 52 weeks, incidences of hepatocellular carcinoma

were increased over controls. For the pooled vehicle controls, low- and high-dose groups, the incidence of liver tumors was 6/72, 8/48 and 8/32, respectively. Incidence in the high-dose group was statistically significantly increased ($p=0.03$) above that of control mice. If one includes the male mice administered 1,1-DCA that did not survive past 52 weeks, the increased incidence in hepatocellular carcinoma was not statistically significant (NCI, 1978a). Significantly increased incidences ($p<0.05$) of endometrial stromal polyps were also observed in the high-dose group of 1,1-DCA-treated female mice. For the pooled vehicle controls, low- and high-dose groups, the incidences of benign uterine polyps in female mice were 0/79, 0/47 and 4/46, respectively.

NCI (1978a) stated in its technical report that ". . . under the conditions of this bioassay there was no conclusive evidence for carcinogenicity of 1,1-dichloroethane in Osborne-Mendel rats or B6C3F_1 mice."

Other Relevant Data

Initiation/promotion studies
Klaunig *et al*. (1986) evaluated the tumor promoting potential of 1,1-DCA in male C3H mice and female C57BL mice initiated with diethylnitrosamine. Male C3H mice (35/group) and female C57BL mice (35/group), each 32 days of age, were administered diethylnitrosamine in the drinking water at a dosage of 0 (non-initiated) or 10 mg/L (initiated) for 4 weeks. After 4 weeks, initiated groups were administered 1,1-DCA in drinking water for 52 weeks at doses of 0 (non-promoted), 835 mg/L or 2,500 mg/L. Animals were sacrificed at 56 weeks of age. No increases in liver or lung tumors were observed in uninitiated mice administered 1,1-DCA compared to that of controls. The short study duration severely limits the utility of this finding. In mice initiated with diethylnitrosamine, both control and 1,1-DCA-treated mice showed 100% tumor incidence at 52 weeks. Thus, information on the tumor promoting potential of 1,1-DCA is limited. However, the incidences of liver tumors at 26 weeks (interim sacrifice) were not different in 1,1-DCA-treated mice to that of controls, indicating that 1,1-DCA did not appear to shorten the time to appearance of tumor.

Using a rat liver foci assay, Story *et al*. (1986) and Milman (1988) studied the tumor initiating and promoting potential of 1,1-DCA and other chlorinated hydrocarbons. In the promotion study, 10 male rats were given partial hepatectomies, followed by a single dose of diethylnitrosamine, 24 hour after surgery, and then administered 1,1-DCA for 5 weeks and sacrificed a week later. Promotion with 1,1-DCA greater than doubled the number of foci compared to that of corn oil. In the initiation study, a similar regimen was followed except that 1,1-DCA was used instead of diethylnitrosamine and promotion was accomplished through administration of phenobarbital. Initiation by 1,1-DCA did not increase number of liver foci relative to controls.

Also employing a rat liver foci assay, Herren-Freund and Pereira (1986) examined the initiating potential of 1,1-DCA and other by-products of water disinfection. One day after male F344 rats were given a two-thirds partial hepatectomy, 1,1-DCA was administered at a dose of 7.33 mmol/kg. Seven days after dosing, rats were administered

phenobarbital until day 56, then sacrificed on day 63. Liver foci, as measured by GGT activity, were counted. 1,1-DCA did not significantly increase the number of liver foci.

Genotoxicity

The results of genotoxicity studies of 1,1-DCA are summarized in Table 2. In bacterial mutagenesis assays, experiments conducted in a closed system (desiccator) were positive for mutagenicity. However, tests using a standard, open test system were negative, presumably because of loss of 1,1-DCA to volatilization. Zeiger *et al.* (1992) investigated the genotoxicity of 1,1-DCA and numerous other compounds in a standard *Salmonella in vitro* assay (Ames test) employing different combinations of strains (TA97, TA98, TA100, TA1535) and activation systems (+/- hamster, rat, or mouse S9). 1,1-DCA was negative in all permutations studied. Simmon *et al.* (1977) likewise reported that 1,1-DCA was negative for mutagenicity in Salmonella test strains TA1535, TA100, TA1537, TA1538, and TA98. However, Riccio *et al.* (1983) and Mitoma *et al.* (1984) tested the mutagenicity of 1,1-DCA in a desiccator in the presence or absence of exogenous activation and obtained positive results for strains TA98, TA100, and TA1535 but not TA1537.

Mixed results for genotoxicity were obtained in mammalian cells. 1,1-DCA was negative in BALB/c-3T3 cell transformation assay, conducted in a sealed incubation chamber, without exogenous activation (Arthur D. Little, Inc., 1983; Tu *et al.*, 1985). However, 1,1-DCA was positive in rat and mouse hepatocyte DNA-repair tests, as measured by radiolabeled thymidine incorporation (Williams, 1983; Williams et al., 1989). Positive results were reported from a viral transformation assay in which Syrian Hamster Embryo cells were incubated with 1,1-DCA prior to treatment with adenovirus SA7 (Hatch *et al.*, 1983).

1,1-DCA was tested for the potential to induce mitotic segregation in the fungus, *Aspergillus nidulans* (diploid strain P1). Significant increases in the frequency of abnormal colonies which produced euploid segregants (haploids and non-disjunctional haploids) was observed for 1,1-DCA-treated cells versus controls (Crebelli *et al.*, 1988; 1995).

Taningher *et al.* (1991) examined the DNA-damaging activity of 1,1-DCA and other polychloroethanes in mouse liver by a fluorometric assay of DNA unwinding. Male BALB/c mice (11 experiments, livers of 2 mice were pooled for each experiment) were administered a single intraperitoneal injection of 900 mg 1,1-DCA/kg body weight and sacrificed 4 hours later. Livers were harvested and analyzed using DNA unwinding assay. The fraction of double-stranded DNA was not significantly different from that of control mice. The authors noted that for the eight polychloroethanes tested, the assay did not correlate well with the reported carcinogenic findings.

Table 2. Genotoxicity of 1,1-DCA

Test System	Response -S9[1] +S9[2]	References
Bacteria		
Reverse Mutation (*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538)	- -	Simmon *et al*., 1977; Zeiger *et al*., 1992
Reverse Mutation (*S. typhimurium* TA98, TA100, and TA1535) (tests conducted in a closed system)	+ +	Riccio *et al*., 1983; Mitoma *et al*., 1984
Mammalian Cells		
Cell transformation assay (BALB/c-3T3)	-	Arthur D. Little, Inc., 1983; Tu *et al*., 1985
DNA-repair test (rat and mouse hepatocytes)	+	Williams, 1983; Williams *et al*., 1989
Viral transformation assay (Syrian Hamster Embryo cells)	+	Hatch *et al*., 1983
Fungus and Yeast		
Induction of mitotic segregation, haploids and non-disjuctional haploids; mitotic arrest (*Aspergillus nidulans,* diploid strain P1).	+	Crebelli *et al*., 1988; 1995
"Genetic Activity" (*Saccaromyces Cerevisiae*, D7 strain)	- -	Bronzetti *et al*., 1987 (abstract)
Mouse (in vivo)		
Fluorometric assay of alkaline DNA unwinding	-	Taningher *et al*., 1991

[1] Without exogenous activation
[2] With exogenous activation

Collaci *et al*. (1985) administered radiolabeled 1,1-DCA to both rats and mice, resulting in covalent binding to DNA, RNA and protein in the liver, kidney, lung and stomach of both species. The binding index was similar to that of other weak carcinogens. Covalent adducts of 1,1-DCA were similarly produced *in vitro* with activation by liver or lung microsomal fractions (Collaci *et al*., 1985).

Structure-Activity Comparisons
Examination of the toxicological evidence of other haloethanes, especially the isomer 1,2-DCA, provides important information towards the overall weight of evidence of the carcinogenic potential of 1,1-DCA. NCI (1978b) studied the carcinogenicity of 1,2-DCA in rats and mice in two-year bioassays. Significant increases in the incidences of forestomach squamous cell carcinomas and circulatory system hemangiosarcomas were

observed in male rats. Increased incidences of mammary adenocarcinomas were observed in female rats and mice, hepatocellular carcinomas in male mice, and endometrial stromal polyps and sarcomas in female mice. Thus, the same tumor types observed in rodents treated with 1,1-DCA (i.e., hepatocellular carcinomas in male mice and endometrial polyps in female mice) were also observed in rodents treated with 1,2-DCA. Also, for those carcinogenic endpoints in the 1,1-DCA bioassay which were not statistically significant ($p<0.05$) by Fisher exact test (i.e., mammary gland adenomas and circulatory system hemangiosarcomas), positive findings were observed for 1,2-DCA. Dosages used in the NCI (1978b) studies of 1,2-DCA were much lower than those employed for 1,1-DCA. The time-weighted average doses of 1,2-DCA were 0, 47 and 95 mg/kg-day (male and female rats), 0, 97 and 195 mg/kg-day (male mice), and 0, 195, 299 mg/kg-day (female mice), although actual doses of 1,2-DCA were higher. Thus, 1,2-DCA appears to be more potent than 1,1-DCA but produces a similar pattern of tumors. In a separate study conducted by van Duuren *et al.* (1979), ICR/Ha Swiss mice treated topically with 1,2-DCA exhibited significant increases in benign lung papillomas, but not skin carcinomas. US EPA (1998) lists 1,2-DCA as group B2, probable human carcinogen, based primarily on these two studies, e.g., on the induction of several tumor types in rats and mice treated by gavage (NCI, 1978a) and lung papillomas in mice after topical application (van Duuren *et al.* 1979).

Three additional cancer studies of 1,2- DCA reported negative findings, 2 inhalation studies (Maltoni *et al.* 1980; Cheever *et al.* 1990) and 1 drinking water study (Klaunig *et al.* 1986). Maltoni *et al.* (1980) exposed rats and mice (90/sex/group) to 1,2-DCA via inhalation at doses of 0, 5, 10, 50, or 150-250 ppm, 7 hours/day, 5 days/week for 78 weeks and did not observe any increase in tumor rates at the end of 148 weeks. Cheever *et al.* (1990) exposed rats (100/sex/group) to 1,2-DCA via inhalation at doses of 0 or 50 ppm, 7 hours/day, 5 days/week for two years. Half of the exposed rats were fed with normal diet and the other half were fed with a diet containing 0.05% disulfiram. Cheever *et al*., (1990) observed no increase in tumor rates among rats exposed to 1,2-DCA alone. However, they found a significant increase in the incidence of intrahepatic bile duct cholangiomas in both male and female rats exposed to 1,2-DCA and disulfiram. Male rats exposed to both chemicals also had an increased incidence of subcutaneous fibromas, neoplastic nodules, and interstitial cell tumors of the testes. Female rats exposed to both chemicals also had a higher incidence of mammary adenocarcinomas. Klaunig *et al.* (1986) exposed male mice (35/group) to 1,2-DCA in drinking water at concentrations of 0, 835 or 2,500 mg/L for one year and reported no increase in tumor incidences.

There is evidence to suggest that the reason for the differences between the positive and non-positive bioassays of 1,2-DCA may be due to a dose-rate effect. 1,2-DCA appears to have two metabolic pathways leading to genotoxic species. One pathway involves oxidation of 1,2-DCA by cytochrome P450 which leads to the formation of gem-chlorohydrin and 2-chloroacetaldehyde. The other pathway involves conjugation of 1,2-DCA with glutathione which can lead to the formation of a reactive episulfonium ion. It appears that the normal detoxification pathway mediated through the cytochrome P-450 enzymes is saturated at high doses (or from a bolus dose as expected by gavage dosing (e.g., NCI 1978b)) and consequently enhanced the carcinogenic potency of

1,2 DCA. Evidence for this assertion comes primarily from studies of metabolism and distribution of 1,2-DCA at different doses and from mechanistic cancer studies in which 1,2-DCA when administered with disulfuram (an inhibitor of cytochrome P-450 enzymes) produced high incidences of tumors at multiple sites where as 1,2-DCA administered alone was negative (Cheever *et al.* 1990). However, since the metabolism of 1,1-DCA has not been well studied, it is not known to what extent the information on 1,2-DCA's mode of action is applicable to 1,1-DCA.

Weisberger (1977) compared the results of carcinogenicity studies conducted on a number of halogenated alkanes. The only consistent findings across the class of chemicals tested (beyond the comparison of 1,1-DCA to 1,2-DCA described above) were those of hepatocellular carcinomas in mice. Increased incidence of hepatocellular carcinoma among short-chain halogenated hydrocarbons was observed in male and female mice for trichloroethylene, chloroform, 1,1,2-trichloroethane, hexachloroethane, tetrachloroethylene and carbon tetrachloride, and in males only for 1,1,1-trichloroethane, iodoform, 1,2-DCA, and 1,1-DCA.

Metabolism

The metabolism of 1,1-DCA has not been well characterized. McCall *et al*. (1983) studied the formation of metabolites in liver microsomes from rats induced by phenobarbital. Of the metabolites measured, acetic acid was the primary metabolite, with smaller amounts of chloroacetic acid and trace amounts of dichloroacetic acid and chloroacetaldehyde.

Chloroethanes have been shown to undergo oxidative dechlorination by liver microsomes (Van Dyke and Wineman, 1971). In this test system, 13.5% of the ^{36}Cl-labeled 1,1-DCA was removed after 30 minutes of incubation with microsomes and less than 0.5% of the ^{36}Cl-labeled 1,2-DCA was removed.

Mitoma *et al.* (1985) administered a number of chlorinated hydrocarbons, including 1,1-DCA to male rats and male mice at the highest dosage used in the respective NCI cancer bioassays. Doses of 1,1-DCA were 700 mg/kg in rats and 1800 mg/kg in mice; doses of 1,2-DCA were 100 mg/kg in rats and 150 mg/kg in mice. Different metabolic profiles were observed for 1,1-DCA and 1,2-DCA. For 1,1-DCA, an estimated 86% of the dose in rats and 70% of the dose in mice were expired unchanged in the exhaled air. Only 7.5% of the dose of 1,1-DCA given to rats and 29% of the dose of 1,1-DCA given to mice was metabolized, recovered mostly via exhaled CO_2 with smaller percentages recovered in the excreta and in the carcass. For rats and mice administered 1,2-DCA, an estimated 11% of the dose to rats and 8% of the dose to mice was expired in the exhaled air. An estimated 85% of the dose of 1,2-DCA given to rats and 102% of the dose of 1,1-DCA given to mice were metabolized and recovered mostly in the excreta with smaller percentages recovered as exhaled CO_2 and in the carcass. In metabolism studies of 1,2-DCA in mice employing doses ranging from 50 to 170 mg/kg, Yllner (1971) observed a clear positive trend between the dose of 1,2-DCA and the proportion of the total dose exhaled unchanged (11% at 50 mg/kg versus 42% at 170 mg/kg). Likewise the proportion of dose of 1,2-DCA excreted in the urine followed an inverse trend (71% at

50-mg/kg versus 48% at 170 mg/kg). It is not known if dose-related metabolism of 1,1-DCA would follow a similar trend to that of 1,2-DCA.

The possibility that 1,1-DCA might be bioactivated to free radical intermediates through reductive metabolism (dechlorination) has been investigated by a number of investigators. Thompson *et al*. (1984) compared the reductive metabolism of tetra-, tri-, and di-chloroethanes by rat liver microsomes. Unlike the more highly chlorinated ethanes, no reductive metabolism was detected for 1,1-DCA and 1,2-DCA. Similarly, Klecka and Gonsior (1984) demonstrated that 1,1-DCA, unlike other chlorinated methanes, did not appear to be reduced by an iron (II) porphyrin reaction system. However, Tomasi *et al*. (1984), using electron-spin resonance spectroscopy, reported formation of free radicals from 1,1-DCA bioactivated by rat hepatocytes under hypoxic, but not normoxic, conditions.

Paolini *et al.* (1992; 1994) studied the *in vivo* induction of mouse liver P450IIB1 by administering 13 halogenated hydrocarbons via intraperitoneal injections at doses of 50, 25, 12.5 and 6.25% of each compounds' respective LD_{50}. 1,1-DCA and 1,1,2,2-tetrachloroethane ranked highest of the compounds tested in induction potency.

Summary

1,1-Dichloroethane was listed "as causing cancer" under Proposition 65 based upon its classification by the US EPA as a probable human carcinogen (Group B2) in its 1989 Health Effects Assessment Summary Tables. US EPA (1989) noted hemangiosarcomas in the rat as the tumor of concern and referenced two earlier US EPA assessments (US EPA 1984, 1985). In December 1996, US EPA revised the classification of 1,1-DCA to category C, possible human carcinogen. This re-classification was based on a lack of evidence in humans and limited evidence in rats and mice (US EPA, 1997). Although the rationale for the reclassification to Group C was not described by US EPA, the changes appear to reflect differences in professional judgment rather than on significant new information.

No human studies of the long-term health effects of exposure to 1,1-DCA were identified by OEHHA. In a series of rat chronic gavage studies, NCI (1978a) reported that 1,1-DCA caused significant increases in hepatocellular carcinoma in male mice and endometrial polyps in female mice. Issues of study quality such as widespread infection in the rats, use of high doses, and poor survival of the animals limit the strength of these findings. The structurally related compound, 1,2-DCA, also induced significant increases in hepatocellular carcinoma in male mice and endometrial polyps in female mice were also observed. Adding to the weight-of-evidence are the observations that 1,1-DCA was mutagenic to bacteria in closed test systems, and was mutagenic and clastogenic in mammalian cell, fungus or yeast test systems. Also, 1,1-DCA administered to rats or mice, or in test systems *in vitro*, resulted in covalent binding to DNA, RNA and other cellular macromolecules. 1,1-DCA also exhibited the capacity to induced P450 enzymes and was positive in certain tumor promotion studies.

References

Agency for Toxic Substances and Disease Registry (ATSDR, 1990). *Toxicological profile for 1,1-dichloroethane*. US Department of Health and Human Services, TP-90-12.

Arthur D. Little, Inc. (1983). Cell transformation assays of 11 chlorinated hydrocarbon analogs. Microfiche No. OTS0509392, Document No. 40-8324457 (as reported by US EPA, 1997).

Bronzetti G, Galli A, Vellosi R, Rossi F, Morichetti E, Del Carratore R (1987). Genetic activity of chlorinated ethanes. *Eur J Cancer Clin Oncol* **23**(11):1737-1738. (abstract).

Cheever KL, Cholakis JM, El-Hawari AM, Kovatch RM, Weisburger EK. (1990). Ethylene dichloride: The influence of disulfiram or ethanol on oncogenicity, metabolism, and DNA covalent binding in rats. Fundam Appl Toxicol **14**:243-261.

Colacci A, Arfellini G, Mazzullo, M, Prodi G, Grilli S (1985). Genotoxicity of 1,1-dichloroethane, *Res Commun Chem Pathol Pharmacol* **49**(2):243-254.

Crebelli R, Benigni R, Franekic J, Conti G, Conti L, Carere A (1988). Induction of chromosome malsegregation by halogenated organic solvents in *Aspergillus midulans*: unspecific or specific mechanism? *Mutat Res* **201**:401-411.

Crebelli R, Andreoli C, Carere A, Conti L, Crochi B, Cotta-Ramusino M, Benigni R (1995). Toxicology of halogenated aliphatic hydrocarbons: Structural and molecular determinants for the disturbance of chromosome segregation and the induction of lipid peroxidation. *Chem Biol Interact* **98**(2):113-129.

Hatch GG, Mamay PD, Ayer ML, Casto BC, Nesnow S (1983). Chemical enhancement of viral transformation in Syrian hamster embryo cells by gaseous and volatile chlorinated methanes and ethanes. *Cancer Res* **43**(5):1945-1950.

Herren-Freund SL, Pereira MA (1986). Carcinogenicity of by-products of disinfection in mouse and rat liver. *Environ Hlth Perspect* **69:**59-65.

Klaunig JE, Ruch RJ, Pereira, MA (1986). Carcinogenicity of chlorinated methane and ethane compounds administered in drinking water to mice. *Environ Hlth Perspect* **69**:89-95.

Klecka GM, Gonsior SJ (1984). Reductive dechlorination of chlorinated methanes and ethanes by reduced iron (II) porphyrins. *Chemosphere* **13**(3):391-402.

Infante PF, Tsongas TA (1982). Mutagenic and oncogenic effects of chloromethanes, chloroethanes and halogenated analogues of vinyl chloride. *Environ Sci Res* **25**:301-327.

Maltoni C, Valgimigli L, Scarnato C. (1980). Long-term carcinogenic bioassays on ethylene dichloride administered by inhalation to rats and mice. In Ames B, Infante P, Reitz R (eds.) *Banbury Report 5 Ethylene dichloride: A potential health risk?* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 3-33.

McCall SN, Jurgens P, Ivanetich KM (1983). Hepatic microsomal metabolism of the dichloroethanes. *Biochem Pharmacol* **32**:207-213.

Milman HA, Story DL, Riccio ES (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes. *Ann NY Acad Sci* **534**:521-530.

Mitoma C, Tyson CA, Riccio, ES (1984). Investigations of the species sensitivities and mechanism of carcinogenicity of halogenated hydrocarbons. Microfiche No. OTS0509408, Document No. 40-8424225 (as reported by US EPA 1997).

Mitoma C, Steeger T, Jackson SE, Wheeler KP, Rogers JH, Milman HA (1985). Metabolic disposition study of chlorinated hydrocarbons in rats and mice. *Drug Chem Toxicol* **8**(3):183-194.

National Cancer Institute (NCI, 1978a). *Bioassay of 1,1-Dichloroethane for Possible Carcinogenicity.* CAS No. 78-34-3. NCI/NTP Technical Report No. 066. DHEW Publ. No. (NIH) 78-1316, Washington, DC.

National Cancer Institute (NCI, 1978b). *Bioassay of 1,2-Dichloroethane for Possible Carcinogenicity*. NCI Carcinogenesis Technical Report Series No. 55. DHEW Publ. No. (NIH) 78-1361, Washington, DC.

National Institute for Occupational Safety and Health (NIOSH, 1978). *Current Intelligence Bulletin 27. Chloroethanes: review of toxicity.* US Department of Health, Education, and Welfare, Center for Disease Control, August 21, 1978.

Office of Environmental Health Hazard Assessment (OEHHA, 1998 draft). *Public health goal for 1,2-dichloroethane in drinking water.* Pesticide and Environmental Toxicology Section, OEHHA, California Environmental Protection Agency, Sacramento, California, August 1998.

Paolini M, Mesirca R, Pozzetti L, Biagi GL, Cantelli-Forti G (1992). Selective induction of murine liver cytochrome P450 IIB1 by halogenated hydrocarbons. *Toxicol Environ Chem* **36**(3-4):235-49.

Paolini M, Mesirca R, Pozzetti L, Sapone A, Biagi GL, Trieff NM, Cantelli-Forti G (1994). Correlation between murine liver cytochrome P450 2B1 induction by halogenated hydrocarbons and toxicity. *Toxicol Environ Chem* **44**(1-2):55-64.

Riccio E, Griffin A, Mortelmans K, Milman ME (1983). A comparative mutagenicity study of volatile halogenated hydrocarbons using different metabolic activation systems. *Environ Mutagen*, **5**: 472. (Abstract)

Simmon VF, Kauhanen K, Tardiff RG (1977). Mutagenetic activity of chemicals identified in drinking water. In: Scott D, Bridges BA, Sobels FH (eds.) *Progress in Genetic Toxicology*, Elsevier/North Holland Inc., NY, pp. 249-258.

Story DL, Meierhenry EF, Tyson CA, Milman HA (1986). Differences in rat liver enzyme-altered foci produced by chlorinated aliphatics and phenobarbital. *Toxicol Ind Hlth* **2**(4):351-362.

Taningher M, Parodi S, Grilli S, Colacci A, Mazzullo M, Bordone R, Santi L (1991). Lack of correlation between alkaline DNA framentation and DNA covalent binding induced by polychloroethanes after in vivo administration. Problems to the assessment of a carcinogenic hazard. *Cancer Detection and Prevention* **15**(1):35-39.

Thompson JA, Ho B, Mastovich SL Reductive Metabolism (1984). Of 1,1,1,2-tetrachloroethane and related chloroethanes by rat liver microsomes. *Chemi-Bio Interact* **51**(3):321-333.

Tomasi A, Albano E, Bini A, Botti B, Slater TF, Vannini V (1984). Free radical intermediates under hypoxic conditions in the metabolism of halogenated carcinogens. *Toxicol Pathol* **12**(3):240-246.

Tu, AS, Murray, TA, Hatch KM, Sivak A, Milman, HA (1985). In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes. *Cancer Lett* **28**(1):85-92.

US Environmental Protection Agency (US EPA, 1984). *Health Effects Assessment for 1,1-dichloroethane.* Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

US Environmental Protection Agency (US EPA, 1985). *Health and Environmental Effects Profile for Dichloroethanes.* Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

US Environmental Protection Agency (US EPA, 1989). *Health Effects Assessment Summary Tables. Annual Update.* Prepared by the Office Research and Development and the Office of Emergency and Remedial Response, Washington, DC.

US Environmental Protection Agency (US EPA, 1997). 1,1-Dichlorethane. Integrated Risk Information System (IRIS). Office of Health and Environmental Assessment,

Environmental Criteria and Assessment Office, Cincinnati, OH, last revised 12/96.

US Environmental Protection Agency (US EPA, 1998). 1,2-Dichlorethane. Integrated Risk Information System (IRIS). Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, last revised 1/91.

van Duuren BB, Goldschmidt G, Loewengart, Smith AC, Mechionne S, Seldman I, Roth D (1979). Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *JNCI* **63**:1433-1439.

Van Dyke RA, Wineman CG (1971). Enzymatic dechlorination: dechlorination of chloroethanes and propanes *in vitro*. *Biochem Pharmacol* **20**:463-470.

Weisburger EK (1977). Carcinogenicity studies on halogenated hydrocarbons. *Environ Hlth Perspect* **21**:7-16.

Williams GM (1983). DNA repair tests for 11 chlorinated hydrocarbon analogs to determine potential carcinogenicity. Naylor Dana Institute, final report, TR-507-18A. Microfiche No. OTS0509403, Document No. 40+8324292.

Williams GM, Mori H, McQueen CA (1989). Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals. *Mutat Res* **221**:263-286.

Yllner S. (1971). Metabolism of 1,2-dichloroethane-^{14}C in the mouse. *Acta Pharmacol et Toxicol* **30**:257-265.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K (1992). Salmonella mutagenicity tests. V. Results from the testing of 311 chemicals. *Environ Mol Mutagen* **19**(Suppl 21):2-141.

P-TOLUIDINE

Listing History

Para-toluidine (CAS No. 106-49-0) was listed "as causing cancer" under Proposition 65 on January 1, 1990, based upon its classification by the US Environmental Protection Agency (US EPA) in the document "Methodology for Evaluating Potential Carcinogenicity in Support of Reportable Quantity Adjustments Pursuant to CERCLA Section 102" as a probable human carcinogen (Group B2) with sufficient evidence in animals and no data as evidence in humans (US EPA, 1986). Since that time, US EPA has reclassified *p*-toluidine as a Group C carcinogen (US EPA, 1988). In 1992, NIOSH, another authoritative body of Proposition 65 on carcinogens, classified *p*-toluidine as a potential carcinogen and recommended that OSHA label this substance a potential occupational carcinogen (a more detailed account of NIOSH's evaluation on *p*-toluidine is given below).

Reviews by Other Authoritative Bodies

No information regarding the carcinogenicity of *p*-toluidine has been located from the International Agency for Research on Cancer (IARC) and the U.S. Food and Drug Administration (US FDA). The National Toxicology Program (NTP) has not tested *p*-toluidine in a carcinogenicity bioassay.

The National Institute of Occupational Safety and Health (NIOSH) has reviewed the carcinogenicity of *p*-toluidine. In its 1992 *Recommendations for Occupational Safety and Health, Compendium of Policy Documents and Statements* (NIOSH, 1992), NIOSH cites health effects for *p*-toluidine as "potential for cancer; tumors of the liver in animals" using the testimony on the OSHA's proposed rule on air contaminants in support of this finding (NIOSH, 1988). In this testimony (29 CFR Part 1910), *p*-toluidine is included on a list of 53 chemicals which NIOSH states "should be designated as potential occupational carcinogens" and "not only should be designated as carcinogens, but for which there remains a substantial level of risk at the proposed [OSHA] PEL". In support of this finding, NIOSH submitted the following evidence:

> "*Para*-toluidine is an aromatic amine which has been shown to cause cancer in 30% of mice when fed at a monthly average concentration of 1,000 ppm in the diet (Weisburger *et al*., 1978). The existing TLV and OSHA's proposed PEL is 2 ppm. OSHA's risk assessment predicts that 1.2-1.9% of workers (maximum likelihood estimate of risk of 12/1,000 workers with an upper bound of 19/1,000 workers) will develop cancer when exposed to that level for a working lifetime. That degree of risk is highly significant, and the proposed PEL is, therefore, considered non-protective. These data indicate that *p*-toluidine meets the OSHA definition of a potential occupational carcinogen as defined in 29CFR1990. Therefore, NIOSH recommends that OSHA label this substance a potential occupational carcinogen" (NIOSH, 1988).

It appears that the NIOSH 1992 document meets the criteria for formality and identification in Sections 12306(d)(1) and 12306(d)(2). Specifically, NIOSH includes *p*-toluidine on a list of chemicals causing cancer and concludes that the chemical causes cancer. This conclusion is set forth in an official document utilized by the authoritative body for regulatory purposes.

Carcinogenicity Data Available

Epidemiological studies
No human studies of the long-term effects of exposure to *p*-toluidine were identified by OEHHA.

Animal Data
Mouse chronic oral studies (Weisburger *et al.*, 1978). Groups of male and female HaM/ICR-derived CD-1 mice (25/sex/dose) were initially fed diets containing 1,000 or 2,000 mg *p*-toluidine/kg diet for 6 months, then 500 or 1,000 mg *p*-toluidine/kg diet for 12 months, respectively. Mice were then held on control diet for 3 months, before termination. Among male mice, hepatomas were significantly increased in the high-dose group (9/18 vs. 3/18 simultaneous control mice; $p = 0.038$) and the low-dose group (8/17 vs. 7/99 pooled control mice; $p = 0.0014$). Among female mice, hepatomas were significantly increased in the high-dose group (3/17 vs. 1/102 pooled control mice; $p = 0.009$; 0/20, simultaneous controls). Weisburger *et al.* concluded: "Male mice at both dose levels exhibited a significant increase in hepatomas. Female mice at the high dose level also showed an increase in liver tumors."

Rat chronic oral study (Weisburger *et al.*, 1978). Groups of male Charles River rats (25/dose) were fed diet containing 1,000 or 2,000 mg *p*-toluidine/kg diet for 18 months, and then held on control diet for 6 months before termination of the study. No significant increases in tumor incidences were observed between treated and control rats (Weisburger *et al.*, 1978).

Other Relevant Data

Genotoxicity
Assays of *p*-toluidine in *Salmonella typhimurium* and *Escherichia coli* have shown no evidence of mutagenicity (Pai, *et al.*, 1978; Thompson *et al.*, 1983; Jung *et al.*, 1992; Mueller *et al.*, 1993). In cultured rat hepatocytes, unscheduled DNA synthesis was stimulated by *p*-toluidine (Thompson *et al.*, 1983), but testicular DNA synthesis was inhibited in mice followed oral treatment with *p*-toluidine (Seiler *et al.*, 1977). An investigation of the ability of *p*-toluidine to bind to hepatic macromolecules showed that DNA binding occurred and peaked approximately 24 hours following oral dosing (Brock *et al.*, 1990).

Summary

p-Toluidine was listed "as causing cancer" under Proposition 65 based upon its classification by the US EPA as a probable human carcinogen (Group B2) with sufficient evidence in animals and no data as evidence in humans (US EPA, 1986). Since that time, US EPA has reclassified *p*-toluidine as a Group C carcinogen (US EPA, 1988). In 1992, NIOSH, another authoritative body of Proposition 65 on carcinogens, classified *p*-toluidine as a potential carcinogen and recommended that OSHA label this substance a potential occupational carcinogen.

In a series of mouse chronic oral studies, Weisburger *et al.* (1978) reported that *p*-toluidine induced significant increases of liver tumors in the treated male and female mice. *p*-Toluidine has demonstrated genotoxicity *in vitro* in a number of test systems, including induction of unscheduled DNA synthesis and formation of DNA adducts, but was negative in some bacterial mutagenicity tests.

References

Brock WJ, Hundley SG, Lieder PH (1990). Hepatic macromolecular binding and tissue distribution of ortho- and para-toluidine in rats. Toxicol Lett **54**(2-3):317-25.

Jung R, Engelhart G, Herbolt B, Jaeckh R, Mueller W (1992). Collaborative study of mutagenicity with *Salmonella typhimurium* TA102. Mutat Res **278**(4):265-70.

Mueller W, Engelhart G, Herbold B, Jaeckh R, Jung R (1993). Evaluation of mutagenicity testing with *Salmonella typhimurium* TA102 in three different laboratories. Environ Health Perspect **101**(Suppl 3):33-6.

NIOSH (1988). Testimony of the National Institute for Occupational Safety and Health on the Occupational Safety and Health Administration's Proposed Rule on Air Contaminants. 29 CFR Part 1910, Docket No. H-020. Presented at the OSHA Informal Public Hearing, August 1, 1988. Washington, DC.

NIOSH (1992). National Institute for Occupational Safety and Health. Recommendations for Occupational Safety and Health. Compendium of Policy Documents and Statements. US Department of Health and Human Services. Public Health Service. Centers for Disease Control. Publ. No. 92-100.

Pai V, Bloomfield SF, Jones J, Gorrod JW (1978). Mutagenicity testing of nitrogenous compounds and their N-oxidized products using trp^+ reversion in *E. coli*. Biol Oxid Nitrogen, Proc Int Symp 2nd (1977), pp. 375-82.

Seiler JP (1977). Inhibition of testicular DNA synthesis by chemical mutagens and carcinogens. Preliminary results in the validation of a novel short term test. Mutat Res **46**:305-10.

Thompson CZ, Hill LE, Epp JK, Probst GS (1983). The induction of bacterial mutation and hepatocyte unscheduled DNA synthesis by monosubstituted anilines. Environ Mutagen **5**(6):803-11.

US EPA (1986). US Environmental Protection Agency. Office of Research and Development. Methodology for Evaluating Potential Carcinogenicity in Support of Reportable Quantity Adjustments Pursuant to CERCLA Section 102. Prepared for the Office of Emergency and Remedial Response by the Carcinogen Assessment Group, Office of Health and Environmental Assessment, Washington, DC. External Review Draft. OHEA-C-073. December, 1986.

US EPA (1988). US Environmental Protection Agency. Evaluation of the Potential Carcinogenicity of *p*-Toluidine (CAS No. 106-49-0) in Support of Reportable Quantity Adjustments Pursuant to CERCLA Section 102. Prepared for the Office of Emergency and Remedial Response by the Carcinogen Assessment Group, Office of Health and Environmental Assessment, Washington, DC. PB93-201192. Report No. EPA/600/8-91/188. Final, June 1988.

Weisburger EK, Russfield AB, Homburger F, Boger E, Van Dongen CG, Chu KC (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J Environ Pathol Toxicol **2**:325-356.