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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 U.S. Environmental Protection Agency, U.S. Food and Drug Administration, National Institute Occupational Safety and Health, 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 (j)). 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 (CIC), 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, chlorodibromomethane, 1,1-dichloroethane, *p*-toluidine, and zineb. This document provides background information on authoritative bodies reviews and carcinogenicity evidence for all five chemicals.

Consideration of three of these chemicals was originally scheduled for the December 10, 1998, meeting of the CIC. At that meeting, the Committee deferred their consideration until the next meeting of the CIC.

On October 7, 1999, a public meeting will be conducted and the CIC will consider making a recommendation regarding whether allyl chloride, chlorodibromomethane, 1,1-dichloroethane, *p*-toluidine, and zineb should be removed from the Proposition 65 list. Written comments on this document, or other issues related to the reconsideration of the listing of these specific chemicals on the Proposition 65 list of chemicals known to the state to cause cancer should be directed by **September 27, 1999** 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 U.S. Environmental Protection Agency (U.S. EPA) as a probable human carcinogen (Group B2) (U.S. 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” (U.S. EPA, 1986). In 1990, the U.S. 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” (U.S. 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 B6C3F₁ mice based on the low incidence of rarely occurring neoplastic lesions of the forestomach. Neither the National Institute of Occupational Safety and Health nor the U.S. Food and Drug Administration appears to have evaluated the evidence of carcinogenicity for allyl chloride.

California-specific Use and Exposure

Allyl chloride is used as a chemical intermediate in the manufacture of pharmaceuticals, varnishes, epoxy resins, adhesives, plastics, glycerol, and insecticides. The primary stationary sources that have reported emissions of allyl chloride in California are automotive repair shops, educational services, and metal industries (ARB, 1997). The total emissions of allyl chloride from stationary sources in California are estimated to be at least 270 pounds per year, based on data reported under the Air Toxics “Hot Spots” Program (ARB, 1997). Possible sources of allyl chloride indoors include some varnishes and adhesives; in a sampling of indoor air in 125 households in Woodland, California in 1990, allyl chloride was not present in measurable concentrations in any of the samples (the method’s quantifiable limit was 0.6 micrograms per cubic meter) (ARB, 1997).

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. 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. Due to limitations in study design 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 B6C3F₁ 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 B6C3F₁ 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, and 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 B6C3F₁ 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 μ g 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), *Escherichia 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 three guanine and two 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. Metabolic activation to other reactive and mutagenic intermediates, including epichlorohydrin and acrolein, has been hypothesized based upon the appearance of their metabolic products (α -chlorohydrin, 3-chloro-2-hydroxypropyl mercapturic acid, and 3-hydroxypropyl mercapturic acid) in the urine of rats administered allyl chloride intraperitoneally (De Rooij *et al.*, 1996). It is possible that these metabolic products play a role in the observed genotoxicity under some circumstances. The earlier enzyme inhibition data of Neudecker and Henschler (1986), however, 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 for Allyl Chloride

Species, strain	End-point	Results	Reference
<i>Salmonella typhimurium</i> 100 (base substitution)	Reverse mutation	+ ^a	McCoy <i>et al.</i> , 1978; Eder <i>et al.</i> , 1982; Neudecker and Henschler, 1985; Neudecker and Henschler, 1986
1535 (base substitution)	"	+ ^a	McCoy <i>et al.</i> , 1978
	"	- ^b	Dean <i>et al.</i> , 1985
1538 (frame shift)	"	-	McCoy <i>et al.</i> , 1978 ^a , Dean <i>et al.</i> , 1985 ^b
<i>Escherichia coli</i> Pol ⁺ /Pol ⁻ WP ₂ , WP ₂ uvrA	DNA modification Reverse mutation	+ +	McCoy <i>et al.</i> , 1978 Dean <i>et al.</i> , 1985
<i>Saccharomyces cerevisiae</i> D4 JD1	Gene conversation "	+ +	McCoy <i>et al.</i> , 1978 Dean <i>et al.</i> , 1985
<i>Aspergillus nidulans</i>	Gene segregation	+	Crebelli <i>et al.</i> , 1984
Rat liver - epithelial type cells, <i>in vitro</i>	Clastogenicity	-	Dean <i>et al.</i> , 1985
HeLa cells, <i>in vitro</i>	Unscheduled DNA synthesis	+	Schiffmann <i>et al.</i> , 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 metabolite epichlorohydrin is listed under Proposition 65 as known to the State of California to cause cancer. U.S. EPA (1997) classifies this compound as a probable human carcinogen (B2) on the basis of sufficient evidence in animals. IARC

(1987) lists epichlorohydrin as a probable human carcinogen (2A) on the basis of sufficient evidence in animals and its activity in short-term genotoxicity tests.

Summary

Allyl chloride was listed “as causing cancer” under Proposition 65 based upon its classification by the U.S. EPA as a probable human carcinogen (Group B2). In 1990, the U.S. 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 potential formation of a known carcinogen as a metabolite.

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CHLORODIBROMOMETHANE

Listing History

Chlorodibromomethane (CAS No. 124-48-1, CDBM) was listed “as causing cancer” under Proposition 65 on January 1, 1990, based upon its classification by the U.S. Environmental Protection Agency (U.S. EPA) as a probable human carcinogen (Group B2) in its 1989 Health Effects Assessment Summary Tables (U.S. EPA, 1989). The classification as B2 was based on the induction of liver tumors in mice and positive mutagenicity data. Since that time, U.S. EPA has revised the classification of CDBM to category C (U.S. EPA, 1997), possible human carcinogen. According to the U.S. EPA’s Integrated Risk Information System (IRIS) (U.S. EPA, 1997), the category C classification is based on “inadequate human data and limited evidence of carcinogenicity in animals; namely, positive carcinogenic evidence in B6C3F1 mice (males and females), together with positive mutagenicity data, and structural similarity to other trihalomethanes, which are known animal carcinogens.” The reasoning for U.S. EPA’s reclassification of CDBM is not clear, but it does not appear to be based on significant new information.

Reviews by Other Authoritative Bodies

Other authoritative bodies have considered the carcinogenicity of CDBM. IARC (1991) concluded the chemical was not classifiable as to its carcinogenicity to humans (Group 3), based on limited evidence in experimental animals and absence of data in humans. NTP (1985) concluded that there was no evidence of carcinogenicity of CDBM in F344/N rats of either sex, equivocal evidence of carcinogenicity in male B6C3F₁ mice, and some evidence of carcinogenicity in female B6C3F₁ mice. Neither NIOSH nor FDA has evaluated the evidence of carcinogenicity of CDBM.

California-specific Use and Exposure

CDBM is a volatile organic compound that is found in marine macroalgae (IARC, 1991). It is a byproduct of water disinfection processes and is found in drinking water supplies (NTP, 1985; IARC, 1991). In California, CDBM has been detected in runoff from agricultural peat soils (Fujii et al., 1999) and in drinking water sources (CDHS, 1997).

Carcinogenicity Data Available

Epidemiological Studies

There are no epidemiological studies on chlorodibromomethane alone. Chlorodibromomethane is one of several trihalomethanes produced by the interaction of chlorine and organic material in water. Several studies have suggested a positive correlation between drinking chlorinated water and the incidence of several human cancers, particularly bladder, rectal and colon cancer (U.S. EPA, 1997; IARC, 1982; NAS, 1980). Trihalomethane levels in drinking water have also been correlated with increased frequency of brain, lymphoma and kidney cancer (IARC, 1982).

Animal Data

1. Mouse chronic gavage studies (NTP, 1985): Male and female B6C3F₁ mice (50 animals per group) were given CDBM in corn oil by gavage at dosages of 0, 50, or 100 mg/kg_{bw}, five days per week for 105 weeks. An accidental overdose caused the death of 35 low-dose male mice at weeks 58-59; thus tumor incidence data for this group were considered inadequate for analysis. Low-dose female mice received the same dose preparation at week 58, but no adverse effects were noted. Nine high-dose male mice died at week 82; no explanation for these deaths was provided in the report. The percent survival in male mice was 88, 14 and 58% in the control, low- and high-dose groups, respectively. The survival rate in female mice was comparable for all groups, approximately 63%.

In male mice, the incidence of hepatocellular carcinomas in the high-dose group (19/50) was significantly greater ($p = 0.03$) than that in the vehicle control group (10/50); the combined incidence of hepatocellular adenomas or carcinomas in high-dose males (27/50) was significantly increased above that in controls by the life table test ($p = 0.007$) but not by the incidental tumor test ($p = 0.06$). In female mice, hepatocellular adenomas and hepatocellular adenomas or carcinomas (combined) occurred with significant positive trends ($p = 0.004$ and $p = 0.002$, respectively), and the incidence of combined tumors in the high-dose group (19/50) was significantly greater than that of the vehicle control group (6/50; $p = 0.01$). NTP (1985) concluded that there was some evidence of carcinogenicity of CDBM in female mice and equivocal evidence of carcinogenicity of CDBM in male mice.

2. Mouse chronic oral studies (Voronin *et al.*, 1987): Male and female CBA x C57B1/6 mice were given CDBM in drinking water for 104 weeks at concentrations of 0.04 mg/l (50 animals/group), 4.0 mg/l (50 animal/group), or 400 mg/l (55 animals/group) equivalent to doses of 0.008, 0.76, or 76 mg/kg-day. Seventy-five males and 50 females served as controls. In male mice, the number of animals surviving to the appearance of the first tumor was as follows: control, 63; low-dose, 13; medium-dose, 33; and high-dose, 39. In female mice, the numbers were: control, 34; low-dose, 27; medium-dose 40; and high-dose, 32. Average survival time/group or number of terminal survivors/group was not reported. An increase in tumor incidence was not observed in CDBM-treated animals. It is not clear whether necessary precautions were taken to minimize volatilization of CDBM from the drinking water in these studies.
3. Rat chronic gavage studies (NTP, 1985): Male and female F344/N rats (50 animals/group) were given CDBM in corn oil by gavage at 0, 40, or 80 mg/kg_{bw} on five days per week for 104 weeks. The final survival rates of all groups were comparable and no evidence for carcinogenicity was seen in rats under the study conditions.
4. Rat chronic dietary studies (Tobe *et al.*, 1982; as cited in U.S. EPA, 1997). In the preliminary results of an unpublished two-year feeding study using groups of 40 SPF Wistar rats, the authors observed no increase in gross tumors in male rats treated with

CDBM at doses of 10, 39, or 210 mg/kg-day, or in female rats treated at doses of 17, 66, or 350 mg/kg-day. Control groups consisted of 70 rats/sex. However, only five or seven rats/sex/dose group were examined following 18 or 24 months of exposure.

Other Relevant Data

Genotoxicity

The results of genotoxicity studies of CDBM are summarized in Table 2. CDBM has been shown to be genotoxic in a number of *in vitro* tests. CDBM was mutagenic to *Salmonella typhimurium* TA 98 and TA100 when tested in desiccators, a procedure used to decrease the loss of volatile compounds (Simmon *et al.*, 1977; Khudolei *et al.*, 1989). Results were negative when plate incorporation (Simmon *et al.*, 1977) or preincubation (NTP, 1985; Zeiger *et al.*, 1987) methods were used. Nestmann and Lee (1985) reported positive results for gene conversion in *Saccharomyces cerevisiae* strain D4 without, but not with, metabolic activation. Morimoto and Koizumi (1983) reported that CDBM produced sister chromatid exchanges in cultured human lymphocytes and Fujie *et al.* (1993) reported that CDBM induced sister chromatid exchange in a dose-dependent manner in rat erythroblastic leukemia cells. Matsuoka *et al.* (1996) observed that CDBM produced polyploidy in the mouse lymphoma assay.

CDBM has also been shown to produce positive results in several short-term *in vivo* tests. Morimoto and Koizumi (1983) demonstrated that CDBM is capable of causing sister chromatid exchange in bone marrow cells of mice. Fujie *et al.* (1990) reported that CDBM produced bone marrow chromosome aberrations in rats after intraperitoneal injection or oral administration. However, Stocker *et al.* (1997) reported that CDBM did not cause unscheduled DNA synthesis in rat liver, and CDBM was not observed to increase the production of renal DNA strand breaks in male F344 rats (Potter *et al.*, 1996). Shelby and Witt (1995) reported that CDBM was negative in the mouse bone marrow micronucleus and chromosomal aberration tests.

Table 2. Summary of Genotoxicity Test Results for Chlorodibromomethane.

Test system	End-point	Results	Reference
<i>Salmonella typhimurium</i>			
TA100 (base substitution)	Reverse mutation	+ ^{a, b}	Simmon <i>et al.</i> (1977), Varma <i>et al.</i> (1988), Khudolei <i>et al.</i> (1989)
		-	NTP (1985), Khudoley <i>et al.</i> (1987), Mersch-Sunderman (1989)
TA102 (base substitution)	Reverse mutation	-	Mersch-Sunderman (1989)
TA1535 (base substitution)	Reverse mutation	+ ^c	Varma <i>et al.</i> (1988)
		-	NTP (1985)
TA1537 (frame shift)	Reverse mutation	+ ^c	Varma <i>et al.</i> (1988)
		-	NTP (1985)
TA98 (frame shift)	Reverse mutation	+ ^d	Varma <i>et al.</i> (1988), Khudolei <i>et al.</i> (1989)
		-	NTP (1985), Khudoley <i>et al.</i> (1987), Mersch-Sunderman (1989)
TA97 (frame shift)	Reverse mutation	-	Mersch-Sunderman (1989)
<i>Saccharomyces cerevisiae</i> D7	Gene conversion	+	Nestmann & Lee (1985), Callen <i>et al.</i> (1980)
<i>Saccharomyces cerevisiae</i> XV185-14C	Reverse mutation	-	Nestmann & Lee (1985)
Chromosomal aberration, mouse lymphoma cells	Clastogenicity	+	Matsuoka <i>et al.</i> (1996)
Chromosomal aberration in Chinese hamster cells	Clastogenicity	+ ^d	Ishidate (1987)
Sister chromatid exchange, rat erythroblastic leukemia cells	Clastogenicity	+	Fujie <i>et al.</i> (1993)
Sister chromatid exchange, human lymphocytes <i>in vitro</i>	Clastogenicity	+	Morimoto & Koizumi (1983)
Sister chromatid exchange, ICR/SJ mouse bone-marrow cells	Clastogenicity	+	Morimoto & Koizumi (1983)
Micronucleus test, mouse bone marrow cells <i>in vivo</i>	Clastogenicity	-	Hayashi <i>et al.</i> (1988), Shelby & Witt (1995)
Chromosome aberration test, mouse bone marrow cells <i>in vivo</i>	Clastogenicity	-	Shelby & Witt (1995)
Chromosome aberration test, rat bone marrow cells <i>in vivo</i>	Clastogenicity	+	Fujie <i>et al.</i> (1990)
Rat liver unscheduled DNA synthesis test, <i>in vivo</i>	Unscheduled DNA synthesis	-	Stocker <i>et al.</i> (1997)
DNA strand break in rat kidney cells <i>in vivo</i>	DNA damage	-	Potter <i>et al.</i> (1996)

^a Experiments performed in closed containers generally gave positive results

^b Exogenous metabolic activation was required for some test systems but not others

^c Positive results were obtained with and without exogenous metabolic activation

^d Positive results were obtained with exogenous metabolic activation

Structure Activity Comparisons

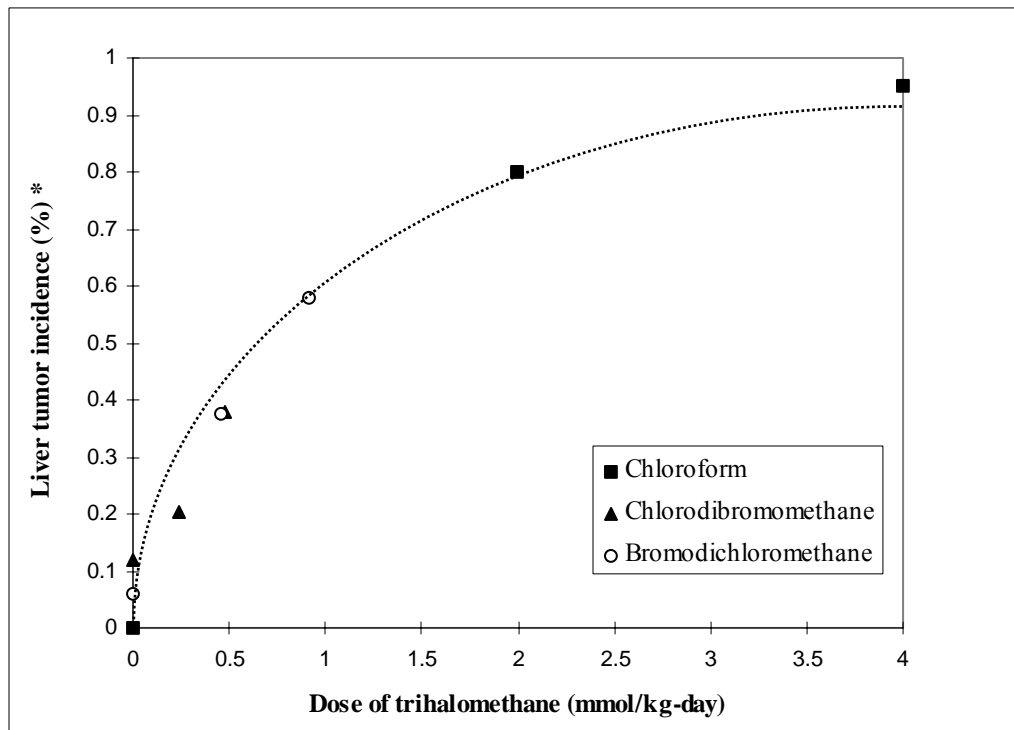
There are four chemicals in the trihalomethane family: bromoform, chloroform, CDBM, and dichlorobromomethane. With the exception of CDBM, all are classified by U.S. EPA (1997) as B2 carcinogens. Furthermore, chloroform, CDBM, and dichlorobromomethane all cause hepatic tumors in B6C3F₁ mice but not in rats (Dunnick *et al.*, 1993). Though the mechanism for the hepatic carcinogenicity of CDBM is not known, the similarities among the structure and toxicity of chloroform, CDBM, and dichlorobromomethane suggest that similar mechanisms may be involved. Dunnick *et al.* (1993) showed that the potencies of these three chemicals in inducing liver tumors in mice are similar (Figure 1). It has been hypothesized that the carcinogenicity of chloroform may be due to one of its metabolites, phosgene, which is known to cross-link DNA (U.S. EPA, 1997).

Recently, DeMarini *et al.* (1997) showed that the mutagenicities of CDBM and other trihalomethanes are mediated by theta-class glutathione S-transferase. Using a special strain of *Salmonella* (RSJ100), a derivative of the base-substitution strain TA1535 (hisG46, rfa, delta uvrB), they determined the mutation spectra of bromoform, chloroform, CDBM, and dichlorobromomethane at the hisG46 allele. The four trihalomethanes produced nearly identical mutation spectra at this target site. These investigators found that the majority (96-100%) of the mutations caused by these trihalomethanes were GC→AT transitions, with 87-100% of these mutations occurring at the second position of the CCC/GGG target. The ability of theta-class glutathione S-transferase to mediate the mutagenicity of these trihalomethanes and the almost exclusive induction of GC→AT transitions suggest that these compounds are activated by a similar pathway in RSJ100, possibly through similar reactive intermediates.

Liver is not the only target organ of the trihalomethanes. Chloroform has also been shown to cause renal neoplasms in male Osborne-Mendel rats, and dichlorobromomethane has also been shown to cause neoplasms of the kidney and large intestine in F344/N rats of both sexes and kidney tumors in male B6C3F₁ mice. Bromoform, the only trihalomethane which does not induce liver tumors in mice, has been shown by NTP (1989) to induce tumors of the large intestine in male and female rats. NTP (1989) concluded from its studies of bromoform that there was clear evidence of carcinogenicity in female rats and some evidence of carcinogenicity in male rats.

Many trihalomethanes have also been shown to be genotoxic in some test systems. Bromodichloromethane and bromoform were mutagenic to *Salmonella typhimurium*. They induced mutations in mouse lymphoma L5178Y cells, and sister chromatid exchanges in Chinese hamster cells and human lymphocytes *in vitro* (IARC, 1991). Chloroform caused mitotic recombination in *Saccharomyces* (Callen *et al.*, 1980) and induced sister chromatid exchange in cultured human lymphocytes and in mouse bone marrow cells exposed *in vivo* (Morimoto and Koizumi, 1983).

Figure 1. Dose-response relationship for induction of liver neoplasms in female B6C3F₁ mice exposed to trihalomethanes. (Adapted from Dunnick *et al.*, 1993).



* For chloroform, incidence of liver carcinoma; chlorodibromomethane and bromodichloromethane, incidence of liver adenoma or carcinoma.

Summary

CDBM was listed “as causing cancer” under Proposition 65, based on its classification by the U.S. EPA as a probable human carcinogen (Group B2) in the Agency’s 1989 Health Effects Assessment Summary Tables (U.S. EPA, 1989). In 1992, U.S. EPA revised the classification of CDBM to Group C, a possible human carcinogen. This re-classification was based on a lack of evidence in humans and limited evidence in mice (U.S. EPA, 1997). The reclassification to Group C appears not to be based on significant new information but to reflect a change in professional judgment regarding the strength of the data.

CDBM is a byproduct of water chlorination. Some studies have suggested a positive correlation between drinking chlorinated water and the incidence of certain cancers. However, there have been no epidemiological studies of CDBM alone.

In a series of rodent chronic gavage studies, NTP (1985) reported a significant increase in liver tumors (combined hepatocellular adenomas and carcinomas) in female B6C3F₁ mice exposed to CDBM and a significant increase in hepatocellular carcinoma in male mice. The incidence of combined hepatocellular adenomas and carcinomas in male mice was not significant due, in part, to a much higher incidence of liver tumors in corn oil gavage control male mice. (NTP, 1985).

Adding to the weight-of-evidence are observations that CDBM was genotoxic in a number of test systems, inducing mutations in bacteria and yeast, and chromosomal aberrations and sister chromatid exchanges in mammalian cells both *in vitro* and *in vivo*.

Additional supporting evidence is provided by comparisons with structurally related compounds. CDBM is one of four trihalomethanes (bromoform, chloroform, CDBM, and dichlorobromomethane). The other trihalomethanes have also been shown to be genotoxic. Recent studies in *Salmonella typhimurium* strain RSJ100 show that the mutagenicities of CDBM and other trihalomethanes are mediated by theta-class glutathione S-transferase and that the mutation spectra produced by each of the trihalomethanes are nearly identical, suggesting a common reactive intermediate or class of intermediates.

All four trihalomethanes have also been shown to cause cancer in animals. CDBM, chloroform, and dichlorobromomethane all cause hepatic tumors in B6C3F₁ mice but not in rats. Chloroform has also been shown to cause renal neoplasms in male rats and dichlorobromomethane has also been shown to cause neoplasms of the kidney and large intestine in male and female rats, and kidney tumors in male B6C3F₁ mice. Bromoform has been shown to cause tumors of the large intestine in male rats.

Chloroform, dichlorobromomethane, and bromoform are classified by U.S. EPA as probable human carcinogens (B2). The dose-response relationships of CDBM, chloroform, and dichlorobromomethane in inducing liver tumors in mice appear to be

similar (Figure 1) and suggest that similar mechanisms of carcinogenesis may be involved.

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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 U.S. Environmental Protection Agency (U.S. EPA) as a probable human carcinogen (Group B2) in its 1989 Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1989a). U.S. EPA (1989a) noted hemangiosarcomas in the rat as the tumor of concern and referenced two earlier U.S. EPA assessments (U.S. EPA, 1984; 1985). U.S. EPA revised the classification of 1,1-DCA to Group C, possible human carcinogen, which was posted in its Integrated Risk Information System (IRIS) database on October 1990 (personal communication, U.S. EPA, 1999a). The Group C classification was based on a lack of evidence in humans and evidence in animals U.S. EPA identified as limited (U.S. EPA, 1999b). Although the rationale for the reclassification to Group C was not described on IRIS (U.S. EPA, 1999b), the reclassification appears to be due to a change in professional judgment, rather than being based on significant new information. U.S. EPA felt that a Group C classification better reflected an overall weakness in the database (personal communication, U.S. EPA, 1999c).

This is documented in the summary notes from the Carcinogen Risk Assessment Verification Endeavor (CRAVE) Work Group on 1,1-DCA (U.S. EPA, 1989b) which state:

“The statistical significance of the findings will be reported and an explanation added as to why they are discounted [in the IRIS record]. It will also be noted that other statistical tests are now considered more appropriate (Life Table tests)¹. The Supporting Data section will be rewritten to emphasize positive findings since the primary animal study has many limitations and to include SAR with 1,2-dichloroethane (re: tumor types and survival).” (U.S. EPA, 1989b).

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 in 1978 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 four chloroethanes that were shown to be carcinogenic in animals.

U.S. EPA (1989a) listed 1,1-DCA as a Group B2, probable carcinogen, and referenced two earlier assessments conducted by its Office of Health and Environmental Assessment for the Office of Solid Waste and Emergency Response (U.S. EPA, 1984; 1985). The U.S. EPA (1984) assessment, which was intended to be a brief quantitative assessment, concluded that the evidence was insufficient to develop a cancer potency value. The U.S. EPA (1985) assessment classified 1,1-DCA as a Group B2, probable human

¹ Gold and Zeiger (1997) conducted survival analysis (e.g., similar to life table tests) which indicated multiple positive associations with exposure to 1,1-DCA and various cancers. See text below.

carcinogen, based on sufficient evidence in animals. While the document describes separately the toxicological evidence for both 1,1-DCA and 1,2-DCA, the evidence cited in support of the cancer classification for 1,1-DCA includes some of the cancer studies pertaining to 1,2-DCA. It is not clear if this was intended (e.g., based on structure-activity considerations) or unintended.

No additional reviews by other authoritative bodies designated for the Proposition 65 program (i.e., the National Toxicology Program, the International Agency for Research on Cancer, U.S. Food and Drug Administration) were located regarding the evidence of carcinogenicity of 1,1-DCA.

California-specific Use and Exposure

1,1-DCA is used primarily as a chemical intermediate in the production of vinyl chloride and 1,1,1-trichloroethane, and to a lesser extent, high vacuum rubber (ATSDR, 1990; HSDB, 1997). 1,1-DCA is also used as a solvent for plastics, oils and fats; as a cleaning agent for its degreasing properties, and as an extraction solvent for heat sensitive substances (HSDB, 1997). Other uses include fabric spreading, varnish and finish removers, and ore flotation (ATSDR, 1990). Consumer products that may contain 1,1-DCA include lubricating oils, other industrial chemical specialty products and specialty cleaning and sanitation products (EDF, 1999). Primary stationary sources of 1,1-DCA in California are sanitary services, combination utility services, and national security facilities (ARB, 1997).

One hundred million pounds were reported produced in the U.S. in 1977 (ATSDR, 1990); no accurate information on production was found after this date (ATSDR, 1990). According to 1986 figures compiled from federal reporting of releases of 1,1-DCA for the Toxic Release Inventory (TRI), 21,961 pounds of off-site releases and 3,891,561 pounds of total production-related waste were reported (U.S. EPA, 1998a). Most of the manufacturing plants and associated environmental releases are in Louisiana and Texas (ATSDR, 1990; EDF, 1999). TRI reporting of 1,1-DCA has been required since 1994; however, no company has filed a TRI use report for 1,1-DCA in California from 1994 through 1998 (personal communication, DTSC, 1999). Based on data reported under the California Air Toxics "Hot Spots" Program (Assembly Bill 2588), total emissions of 1,1-DCA from stationary sources were estimated to be less than 30 pounds per year (ARB, 1997). No information on imports of 1,1-DCA was located. There are no known natural sources of 1,1-DCA.

Ambient air concentrations were measured at low levels in an industrial area of Louisiana and near a waste disposal site in New Jersey (ATSDR, 1990). ARB does not have ambient air measurements of 1,1-DCA for California (ARB, 1997); however, since most production is conducted outside of California, air concentrations in California are expected to be low. Groundwater contamination of 11 chlorinated chemicals, including 1,1-DCA, has been reported around aerospace manufacturing facilities in Sacramento, California (HSDB, 1997).

Carcinogenicity Data Available

Epidemiological studies

No human studies of the long-term health effects of exposure to 1,1-DCA were identified by ATSDR (1990) or more recently by OEHHA.

Animal Data

1. Rat chronic gavage studies (NCI, 1978a): Technical grade 1,1-DCA (purity 99 %) was administered in corn oil by gavage, five days/week, for nine 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 nine 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 one week and was resumed at the previous doses for four weeks. This cyclical pattern of “one week off -- four 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 concurrent experiments (chronic bioassays for trichloroethylene and 1,1-DCA) comprised 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 mammary gland adenoma were 1/39, 1/50 and 5/50, respectively. These increases were not statistically significant for pairwise comparisons between treated and control groups ($p > 0.05$), Fisher exact test; however, the hemangiosarcomas incidence increased with increasing dose ($p = 0.02$ Cochran-Armitage trend test). Because of the early mortality in the rats, NCI (1978a) performed additional analyses on rats surviving past 52 weeks. In female rats, incidences of mammary gland adenocarcinoma were 0/16, 1/28 and 5/31 in the matched vehicle controls, low- and high-dose groups, respectively. These incidences significantly increased with increasing dose ($p = 0.034$ Cochran-Armitage trend test). Dr. Lois Swirsky Gold and colleagues used the individual animal survival data to conduct survival analyses (Gold and Zeiger, 1997). In female rats, they reported statistically significant associations of exposure and hemangiosarcomas of the circulatory system with matched controls ($p < 0.05$) or pooled controls ($p < 0.02$) and adenocarcinomas of the mammary gland ($p < 0.04$) with matched controls.

2. Mouse chronic gavage studies (NCI, 1978a): Male B6C3F₁ mice (50/dose group) were administered 1,1-DCA in corn oil via gavage, five days/week, for six weeks at

doses of 900 and 1,800 mg/kg-day. Doses were increased to 1,200 and 2,400 mg/kg-day for three 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 B6C3F₁ mice (50/dose group) were administered 1,1-DCA in corn oil via gavage, five days/week for six weeks at doses of 900 and 1,800 mg/kg-day. Doses were increased to 1,200 and 2,400 mg/kg-day for three 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 four concurrent experiments (chronic bioassays for 1,1,2-trichloroethane, trichloroethylene, allyl chloride and 1,1-DCA) comprised a pooled vehicle control group (80/sex). These control mice were of the same strain, tested by the same laboratory no more than six months apart, and examined by the same pathologist.

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, significant increases in tumors were not observed for any site, when survival was not taken into account. However, in male mice living past 52 weeks, incidences of hepatocellular carcinoma were increased in the low- and high-dose groups over controls. For the pooled vehicle controls, low- and high-dose groups, the incidences of liver tumors were 6/72, 8/48 and 8/32, respectively. Incidence in the high-dose group was significantly increased ($p=0.03$) above that of control mice (NCI, 1978a). In female mice the incidences of benign uterine polyps were 0/79, 0/47 and 4/46 for the pooled controls, low- and high-dose groups, respectively. Trend tests indicated a significantly increased association of endometrial stromal polyps and dosage relative to matched ($p=0.036$) or to pooled controls ($p=0.017$). The Fisher exact test was positive for endometrial polyps in the high-dose group ($p=0.017$) relative to the pooled vehicle controls. None of the 180 laboratory historical vehicle control B6C3F₁ mice used in studies by the NCI Bioassay Program had ever exhibited an endometrial stromal polyp. Survival analysis conducted by Gold and colleagues (Gold and Zeiger, 1997) reported a significant association with exposure and uterine endometrial polyps ($p<0.004$) compared to pooled controls in female mice. Additional positive findings included liver tumors ($p<0.05$) and lung tumors ($p<0.04$) in male mice compared to matched controls (Gold and Zeiger, 1997).

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 B6C3F₁ 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 four weeks. After four 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 tumors.

Using a rat liver foci assay, Story *et al.* (1986) and Milman *et al.* (1988) studied the tumor initiating and promoting potential of 1,1-DCA and other chlorinated hydrocarbons. In the promotion study, ten male rats per group were given partial hepatectomies, followed by a single dose of diethylnitrosamine 24 hours after surgery, and then administered 1,1-DCA or corn oil for five weeks and sacrificed a week later. Treatment with 1,1-DCA as a promoter more than doubled the number of foci compared to treatment with corn oil. In the initiation study, a similar regimen was followed except that 1,1-DCA was used as the initiator instead of diethylnitrosamine and phenobarbital was administered as the promoter. Initiation with 1,1-DCA did not increase the 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 3. 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.

Table 3. Summary of Genotoxicity Test Results for 1,1-DCA.

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

¹ Without exogenous activation

² With exogenous activation

Mixed results for genotoxicity were obtained in mammalian cells. 1,1-DCA was negative in a 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 radiolabelled 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) were 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 two mice were pooled for each experiment) were administered a single intraperitoneal injection of 900 mg 1,1-DCA/kg body weight and sacrificed four hours later. Livers were harvested and analyzed using a DNA unwinding assay. The fraction of double-stranded DNA in treated liver was not significantly different from that in controls. The authors noted that for the eight polychloroethanes tested, the assay did not correlate well with the reported carcinogenic findings.

Colacci *et al.* (1985) administered radiolabelled 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 and calf thymus DNA were also formed *in vitro* with activation by liver or lung microsomal fractions, but not kidney or stomach microsomal fractions (Colacci *et al.*, 1985).

Structure-Activity Comparisons

Examination of the toxicological evidence for other haloethanes, especially the isomer 1,2-DCA, provides important information relevant to assessing the overall weight of evidence of the carcinogenic potential of 1,1-DCA. NCI (1978b) studied the carcinogenicity of 1,2-DCA via gavage 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 found significantly increased in pairwise comparisons ($p < 0.05$, Fisher exact test) but were significant in trend or lifetable analyses (i.e., mammary gland adenomas and circulatory system hemangiosarcomas), statistically significant 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). 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. U.S. EPA (1998b) lists 1,2-DCA as a Group B2, probable human carcinogen, based primarily on these studies, e.g., on the induction of several tumor types in rats and mice treated by gavage (NCI, 1978b) and lung papillomas in mice after topical application (van Duuren *et al.*, 1979).

Three additional series of cancer studies of 1,2-DCA reported negative findings, two were long-term inhalation studies (Maltoni *et al.*, 1980; Cheever *et al.*, 1990), and the third was a one-year 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, seven hours/day, five 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, seven hours/day, five 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 (an inhibitor of cytochrome P450). 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 had a higher incidence of mammary adenocarcinomas relative to controls. 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 the generation of 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 P450 enzymes is saturated at high doses (or from a bolus dose as expected by gavage dosing (e.g., NCI, 1978b)). Thus, the administration of high or bolus doses of 1,2-DCA would result in an enhancement of the agent's carcinogenic activity. 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 disulfiram (an inhibitor of cytochrome P450 enzymes) produced high incidences of tumors at multiple sites whereas 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 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 ³⁶Cl-labeled 1,1-DCA was removed after 30 minutes of incubation with microsomes and less than 0.5% of the ³⁶Cl-labeled 1,2-DCA was removed.

Mitoma *et al.* (1985) administered a number of chlorinated hydrocarbons, including 1,1-DCA and 1,2-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 were metabolized, recovered mostly via exhaled CO₂ 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 were 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 CO₂ 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) found 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 LD₅₀. 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 U.S. EPA as a probable human carcinogen (Group B2) in the Agency's 1989 Health Effects Assessment Summary Tables (U.S. EPA, 1989a). U.S. EPA (1989a) noted hemangiosarcomas in the rat as the tumor of concern and referenced two earlier U.S. EPA assessments (U.S. EPA, 1984; 1985). In 1990, U.S. EPA revised the classification of 1,1-DCA to Group C, a possible human carcinogen. This re-classification was based on a lack of evidence in humans and limited evidence in rats and mice (U.S. EPA, 1999b). The reclassification to Group C reflected a change in professional judgment regarding the strength of the data rather than being based 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 rodent 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. An additional positive association between exposure and lung tumors in male mice was observed using survival analysis. In female rats surviving past one year, significant associations with dose of 1,1-DCA and mammary gland tumors and hemangiosarcomas of the circulatory system were reported. These associations were also positive using survival analysis. 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 the incidences of tumors at the same sites as observed for 1,1-DCA. 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 and 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 induce P450 enzymes and was positive in certain tumor promotion studies.

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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 U.S. Environmental Protection Agency (U.S. 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 (U.S. EPA, 1986). Since that time, U.S. EPA has reclassified *p*-toluidine as a Group C carcinogen (U.S. EPA, 1988).

In 1992, The National Institute for Occupational Safety and Health (NIOSH), another authoritative body for the purpose of identifying chemicals as causing cancer under Proposition 65, 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). In 1998, the U.S. Food and Drug Administration (U.S. FDA), another Proposition 65 authoritative body, also classified *p*-toluidine as a carcinogen in a cancer risk assessment of *p*-toluidine in surgical sutures made with D&C Violet No. 2, a color additive synthesized with *p*-toluidine as a starting material and thought to contain residual amounts of the starting materials. A similar cancer risk assessment was also made for poly(L-lactic acid) meniscal tacks used in knee surgery also containing D&C Violet No. 2 (U.S. FDA, 1999). In these risk assessments, U.S. FDA concluded that no harm would result from exposure to *p*-toluidine from these uses of D&C Violet No. 2.

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). 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.

The U.S. FDA also appears to have identified *p*-toluidine as a carcinogen (U.S. FDA, 1998). In an amendment to food additive regulations, U.S. FDA identified *p*-toluidine as an impurity present in D&C Violet No. 2, a color additive used in glycolide/dioxanone/trimethylene carbonate tripolymer absorbable sutures for general surgery. In this final rule, U.S. FDA stated that the "...commercial product will contain residual amounts of the starting materials, including *p*-toluidine. This fact is significant because Weisburger et al., have demonstrated that *p*-toluidine is a carcinogen in the mouse." U.S. FDA also describes *p*-toluidine as "the carcinogenic chemical that may be present as an impurity in the additive." Supporting the calculation of cancer risk to humans:

"The agency used data from a long-term rodent bioassay on *p*-toluidine conducted by Weisburger et al. (Ref. 1), to estimate the upper-bound limit of lifetime human risk from exposure to this chemical resulting from the proposed use of the additive. The authors reported that the rodent bioassay showed that the test material caused an increased incidence of hepatomas (liver tumors)."

It appears that the 1998 U.S. FDA document meets the criteria for formality and identification in Sections 12306(d)(1) and 12306(d)(2). Specifically, U.S. FDA identifies *p*-toluidine as causing cancer in a document that indicates that such identification is a final action and concludes that the chemical causes cancer. This conclusion is set forth in an official document utilized by the authoritative body for regulatory purposes. In a second ruling in 1999, U.S. FDA used the same information regarding the carcinogenicity of *p*-toluidine that was cited in the U.S. FDA (1998) document to a second exposure scenario involving the use of D&C Violet No. 2 in meniscal tacks used in knee surgery. It thus appears that this second U.S. FDA document (U.S. FDA, 1999) also meets the criteria for formality and identification in Sections 12306(d)(1) and 12306(d)(2).

California-Specific Use and Exposure

No information has been identified indicating that *p*-toluidine is manufactured in California. However, as noted by U.S. FDA (1998), the use of *p*-toluidine as a chemical intermediate in the production of certain dyes and color additives may result in exposures from the use of products containing residual amounts of the starting materials, such as the products used in medical procedures (sutures, tacks).

Carcinogenicity Data Available

Epidemiological studies

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

Animal Data

1. 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."
2. 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 following oral treatment with *p*-toluidine (Seiler, 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 U.S. EPA as a probable human carcinogen (Group B2) with sufficient evidence in animals and no data as evidence in humans (U.S. EPA, 1986). Since that time, U.S. EPA has reclassified *p*-toluidine as a Group C carcinogen (U.S. EPA, 1988). Two other authoritative bodies for the purpose of formally identifying chemicals as causing cancer under Proposition 65, NIOSH and U.S. FDA, have addressed the carcinogenicity of *p*-toluidine. In 1992, NIOSH classified *p*-toluidine as a potential carcinogen and recommended that OSHA label this substance a potential occupational carcinogen. In 1998 and 1999, U.S. FDA developed rulings regarding color additives to medical devices which identified *p*-toluidine as a 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*, inducing unscheduled DNA synthesis in rat hepatocytes, and forming DNA adducts with rat liver DNA, but was negative in bacterial mutagenicity tests.

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ZINEB

Listing History

Zineb (CAS No. 12122-67-7) was listed “as causing cancer” under Proposition 65 on January 1, 1990, based upon its classification by the U.S. EPA as a probable human carcinogen (Group B2) in a Federal Register Notice (U.S. EPA, 1988). According to a U.S. EPA (1990) publication, the cancer classification for zineb is based on the toxicity information for ethylene thiourea (ETU), a metabolite, contaminant, and degradation product of zineb.

Current U.S. EPA lists and summary documents no longer provide the U.S. EPA cancer classification for zineb. This has prompted OEHHA’s review for the possible removal of zineb from the Proposition 65 list of chemicals known to cause cancer. It appears that the U.S. EPA cancer classification for zineb ceased to be reported after a Special Review of zineb and related pesticides was initiated. As described below in more detail, all registrations for the use of zineb were cancelled during the Special Review, and thus zineb was dropped from the Special Review process, and a cancer classification was never re-introduced as part of the U.S. EPA’s lists and other documents (*e.g.*, IRIS). Inquiries of U.S. EPA staff have failed to provide any insight as to U.S. EPA’s current classification of zineb. OEHHA has found no evidence to indicate that U.S. EPA has changed its conclusion that zineb causes cancer.

U.S. EPA Regulatory History of Zineb

Regulatory History

U.S. EPA has conducted two Special Reviews on the ethylene bisdithiocarbamate (EBDC) fungicides zineb, mancozeb, maneb, metiram, and nabam (U.S. EPA, 1989a). The Special Review process, formerly called the Rebuttable Presumption Against Registration, is a mechanism by which U.S. EPA collects information on the risks and benefits associated with the uses of pesticides to determine whether any use causes unreasonable adverse effects to human health or the environment. In 1977, U.S. EPA initiated a Rebuttable Presumption Against Registration for the EBDC fungicides zineb, mancozeb, maneb, metiram and nabam, based on the presumption that the EBDCs and their shared metabolite and degradation product ethylene thiourea (ETU) posed three potential risks to humans and/or the environment: carcinogenicity, developmental toxicity, and acute toxicity to aquatic organisms. In 1982, U.S. EPA completed this first Special Review by issuing a Final Determination to continue registration of all uses of EBDC pesticides, based on the finding that there were insufficient exposure data to reach any regulatory conclusions regarding potential cancer risk to humans (U.S. EPA, 1982). The 1982 Final Determination also adopted risk reduction measures to preclude unreasonable adverse effects.

On July 17, 1987, U.S. EPA initiated a second Special Review of the EBDC pesticides (*i.e.*, mancozeb, maneb, metiram, nabam and zineb) (U.S. EPA, 1987). This action was prompted by the Agency’s concern over the possible adverse effects of ETU, a common

contaminant, metabolite and degradation product of EBDCs, which U.S. EPA has classified as a B2 carcinogen. In the course of this second Special Review, U.S. EPA examined the available toxicological and human exposure data and concluded that the risks from continued use of the EBDCs outweighed the benefits. Accordingly, U.S. EPA proposed to cancel many of the uses of the EBDC pesticides (U.S. EPA, 1989a).

In 1986, the zineb technical registrants cancelled registration of their products in the U.S. In July 1988, U.S. EPA suspended all zineb product uses when formulators failed to submit data required under Federal Insecticide, Fungicide and Rodenticide Act section 3(C)(2)(B). Between 1989 and 1990, U.S. EPA accepted a number of voluntary cancellation requests from other affected zineb registrants. In 1991, U.S. EPA cancelled all remaining registrations for zineb since no registrants came forward to support continuing registration of the pesticide. U.S. EPA estimated at that time that about two years would be required for zineb-treated commodities to clear the market. Accordingly, U.S. EPA announced December 31, 1994 as the tolerance revocation date for all commodities (except grapes for wine use). To accommodate the longer shelf-life of bottled wines and the longer wine-processing time required before bottling and shipping, the tolerance expiration date for grapes designated for wine use was set as December 31, 1997 (U.S. EPA, 1992).

In the absence of zineb tolerances, any agricultural commodity or processed food imported into the U.S. found to contain EBDC residues traceable to a previous zineb use would be a violation of the Federal Food, Drug, and Cosmetic Act.

Reviews by Other Authoritative Bodies

Among the authoritative bodies of Proposition 65 for purposes of formally identifying chemicals as causing cancer, only IARC has evaluated the carcinogenic potential of zineb and classified it as a Group 3 carcinogen (IARC, 1987). IARC found that there was insufficient evidence from animal data and no human data regarding the carcinogenic potential of zineb (IARC, 1976; 1987).

California-Specific Use and Exposure

Registration for all pesticide products containing zineb is currently “inactive” in California. Strict current tolerance for zineb residues on food produced outside of the U.S. also suggests it is unlikely to be present in products brought into California. Therefore, the risk of exposure to zineb in California appears to be small.

Carcinogenicity Data Available

Human Data

No human studies of the long-term health effects of exposure to zineb were identified. However, one survey study was identified which found a correlation between the presumed use of dithiocarbamate-containing pesticides, of which zineb is one, and increased cancer risk. Specifically, this health survey reported a correlation between data

on sales of dithiocarbamate-containing pesticides and production statistics for crops commonly treated with dithiocarbamate-containing pesticides and the incidence of liver and thyroid cancer in New York, Florida, Maine, and Pennsylvania (von Meyer, 1977; as cited in Houeto *et al.*, 1995). While useful for the purpose of generating hypotheses, this survey is non-informative with respect to human cancer risk from exposure to zineb.

Animal Data

A number of animal studies are available to assess the carcinogenic potential of zineb and are described briefly below. The power of these studies to detect a carcinogenic effect is generally limited by study design (*e.g.*, short treatment and study duration) and the small number of surviving animals in the experiments.

1. Mouse sub-chronic oral studies (Chernov and Khitsenko, 1969; as cited in IARC, 1976): C57BL mice (n=79) and Strain A mice (n=101) were given six weekly oral doses of 3,500 mg/kg_{bw} zineb and killed three months after the beginning of the experiment. Another 29 C57BL mice were given 11 weekly oral doses of 1,750 mg/kg_{bw} and killed six months after the beginning of the experiment. Control groups for each strain were also included. Among C57BL mice in the high-dose group there was a statistically significant increase in the incidence of lung adenomas relative to controls (6/79 vs. 0/87; p=0.01). Among C57BL mice in the low-dose group, the incidence of lung adenomas was also increased relative to controls (2/29 vs. 0/59), however the increase was not statistically significant. Among Strain A mice, the incidence of lung adenomas in the zineb-dosed group was not significantly increased relative to the control group (35/101 vs. 30/97).
2. Mouse 76-week oral studies (Innes *et al.*, 1969): (C57BL/6xC3H/Anf)F₁ and (C57BL/6xAKR/Anf)F₁ strain mice (18/sex/group) were given zineb daily at 464 mg/kg_{bw} in gelatin by stomach tube, from seven days of age through four weeks of age, then fed zineb in the diet 1,298 mg zineb/kg_{feed} for approximately 18 months. The selected dose given was the maximum tolerated dose for infant and young mice. Untreated or vehicle control groups (n=79-90) of each sex and strain were also included. No statistically significant treatment-related increases in tumors (p<0.05) were observed in any sex-strain subgroup or in the combined sexes of either strain.
3. Mouse single dose subcutaneous injection studies (NTIS, 1968; as referenced in IARC, 1976): Twenty-eight-day-old (C57BL/6xC3H/Anf)F₁ and (C57BL/6xAKR/Anf)F₁ strain mice (18/sex/strain) were given single subcutaneous injections of 1,000 mg/kg_{bw} commercial zineb (97% pure) in gelatin and were observed until they were about 78 weeks of age. Groups of untreated and vehicle-injected control animals of each sex and strain were also included (original numbers of each not specified by IARC; number of necropsied controls ranged from 141 to 161). Among (C57BL/6xC3H/Anf)F₁ males, 5/18 animals in the treated group developed systemic reticulum-cell sarcomas, compared with 8/141 animals in a pooled control group (p<0.01). No other treatment-related increases in tumor incidence were observed. No increases in tumor incidence over that in controls were observed in (C57BL/6xAKR/Anf)F₁ strain mice.

4. Rat 130-week oral studies (Mitsumori *et al.*, 1979): JCL-Wistar SPF rats (80/sex/group) were fed diets containing 0, 40, 200, 1,000, or 5,000 ppm zineb for 130 weeks. In addition to the terminal sacrifice, interim sacrifices of eight animals were performed at 26, 52, and 104 weeks. Hematological, biochemical, and pathological evaluations were performed following sacrifice and on dead or moribund animals. A significantly higher incidence of thyroid tumors was observed in the 5,000 ppm male group (37.5%) as compared with that in control males (11.3%), predominantly from the occurrence of cystic adenomas after 104 weeks. An increased incidence of subcutaneous fibromas was also observed in this group of animals. No other treatment related tumors were observed.
5. Rat 22-month gavage study (Andrianova and Alekseev, 1970 as cited in IARC, 1976): Random-bred rats (n=60) were treated by stomach tube with weekly doses of 285 mg/kg_{bw} commercial zineb (89.6% pure) in water for up to 22 months. Two of 10 zineb-treated rats surviving to 22 months developed tumors (one adenocarcinoma and one lymphosarcoma of the intestine). One of 46 untreated survivors developed a fibrosarcoma.
6. Rat chronic oral studies (Blackwell-Smith *et al.*, 1953; as cited in IARC, 1976): Rats (10/sex/group) were fed diet containing 0, 500, 1,000, 2,500, 5,000 or 10,000 mg zineb per kg diet for two years. Among the male rats, there were 1, 2, 3, 2, 1, and 1 tumor-bearing animals in these increasing dose-groups, respectively, and among female rats, 1, 0, 1, 1, 0, and 0 tumor-bearing animals, respectively. With the exception of one malignant tumor of the small bowel (male at 10,000 mg/kg), one mammary carcinoma (female at 1,000 mg/kg), and one papillary adenocarcinoma of the thyroid (female at 2,500 mg/kg), all other tumors observed were malignant tumors of the lung.
7. Rat subcutaneous implant study (Andrianova and Alekseev, 1970; as cited in IARC, 1976): Random-bred rats (n=48) were subcutaneously implanted with 20 mg/kg_{bw} commercial zineb (89.6% pure) contained in a 250 mg paraffin pellet. Four of six zineb-treated rats surviving to 22 months developed tumors (one each malignant hepatoma, fibrosarcoma, spindle-cell sarcoma, and rhabdomyosarcoma). One of 46 untreated no-implant survivors developed a fibrosarcoma.

Other Relevant Data

Genotoxicity

The results of genotoxicity studies of zineb are summarized in Table 4. Zineb has been shown to increase gene mutation and mitotic chromosome malsegregation in *Saccharomyces cerevisiae* (Franekic *et al.*, 1994; Croce *et al.*, 1995). Shiau *et al.* (1980) and Felkner *et al.* (1981) showed zineb caused DNA-damage and mutation in the repair-deficient strains of *Bacillus subtilis*. Tripathy *et al.* (1988) showed that zineb induced genetic damage to *Drosophila*, affecting both somatic and germ cells. However, Benes and Sram (1969) observed no mutagenic activity in *Drosophila* in an earlier study. Croce

et al. (1995) and Franekic *et al.* (1994) reported that zineb was ineffective in inducing mutation in *Salmonella typhimurium*, with or without metabolic activation. Pilinskaya (1974; as cited in IARC, 1976) observed an increase in the number of chromosome aberrations in peripheral blood lymphocytes of workers occupationally exposed to zineb.

Table 4. Summary of Genotoxicity Test Results for Zineb.

Species, strain	End-point	Results	Reference
<i>Salmonella typhimurium</i>	Reverse mutation	–	Croce <i>et al.</i> , 1995
		–	Franekic <i>et al.</i> , 1994
Bacillus subtilis	DNA damage Mutation	+	Shiau <i>et al.</i> , 1980
		+	Felkner <i>et al.</i> , 1981
Saccharomyces cerevisiae	Gene mutation Mitotic chromosome malsegregation	+	Franekic <i>et al.</i> , 1994
		+	Croce <i>et al.</i> , 1995
Drosophila melanogaster	Genetic damage to somatic and germ cells Mutagenicity	+	Tripathy <i>et al.</i> , 1988
		–	Benes and Sram, 1969
Human peripheral blood lymphocytes	Increased chromosome aberrations	+	Pilinskaya, 1974 (cited in IARC, 1976)

Metabolism / Structure-Activity Comparisons

The EBDC fungicides are compounds formed with an ethylene bisdithiocarbamate anion and various cations. Among the EBDCs produced are those with salts of zinc (zineb), manganous zinc (mancozeb), disodium (nabam), and ammonia complex of zinc (metiram). EBDCs identified as causing cancer under Proposition 65 are zineb, mancozeb, maneb, and metiram. IARC (1987) has evaluated two of the EBDC fungicides, zineb and maneb, and classified both as Group 3 chemicals, not classifiable as to carcinogenicity to humans.

An essential component of the carcinogenicity concern regarding the EBDC fungicides is their known metabolism and degradation to ETU. Zineb, mancozeb, maneb, metiram, and nabam are known to be metabolized to ETU. Engst *et al.* (1971; as cited in IARC, 1976) reported that in rats, zineb is transformed to ethylene bistiuram monosulphide and subsequently to ETU. In occupationally and non-occupationally exposed humans, urinary ETU has been shown to be an indicator of EBDC exposure (Sciarra *et al.*, 1994; Kurttio *et al.*, 1990).

IARC (1987) identified ETU as a Group 2B carcinogen based upon inadequate evidence in humans and sufficient evidence in animals. According to IARC (1987): “In three studies, ethylene thiourea produced high incidences of follicular carcinomas of the thyroid in rats after oral administration; animals of each sex were affected, although male rats had a higher incidence. Lower doses produced thyroid follicular hyperplasia. In mice, oral administration of ethylene thiourea produced liver tumors; the thyroids of these animals were not examined.” As discussed above, zineb induced thyroid tumors in

male rats (Mitsumori *et al.*, 1979). Evidence for the development of thyroid tumors has also been observed for both mancozeb and metiram (U.S. EPA, 1997). U.S. EPA (1989b) stated that ETU is a strong animal carcinogen and has the potential to cause cancer in humans. ETU is also identified as known to cause cancer under Proposition 65. U.S. EPA's carcinogenicity concern from exposure to nabam is based upon its metabolism to ETU (U.S. EPA, 1996).

Summary

Zineb was listed "as causing cancer" under Proposition 65 in 1990 based upon its classification by the U.S. EPA as a probable human carcinogen (Group B2) in the *Federal Register* (U.S. EPA, 1988). There is no evidence to indicate that U.S. EPA has changed its conclusion that zineb causes cancer; however, current U.S. EPA lists and summary documents no longer provide the U.S. EPA cancer classification for zineb. It appears that the U.S. EPA cancer classification for zineb ceased to be reported after a Special Review of zineb and related pesticides was initiated. All registrations for the use of zineb were cancelled during the Special Review, and thus zineb was dropped from the Special Review process, and a cancer classification was never re-introduced.

No epidemiological studies that are informative about the carcinogenic effects of zineb are available. There are a number of animal studies investigating the carcinogenic potential of zineb; however, many are limited by deficiencies in study design and the small number of surviving animals in the experiments. Evidence of carcinogenicity is provided by one sub-chronic, three-month study, in which zineb significantly increased the incidence of lung adenomas in mice receiving zineb for six weeks. Evidence of carcinogenicity also comes from a long-term feeding study, in which zineb significantly increased the incidence of thyroid tumors in male rats. Interpretation of positive tumor findings in a single subcutaneous injection study in the mouse and in a subcutaneous implant study in the rat is complicated by multiple limitations in study design. In other studies in rats and mice, treatment with zineb was not associated with increases in tumor incidence. Zineb has demonstrated genotoxicity in a number of test systems, inducing genotoxicity and mutagenicity in *Bacillus subtilis* and *Saccharomyces cerevisiae* and causing genetic damage to *Drosophila*. However, zineb was not mutagenic in *Salmonella typhimurium*, with or without metabolic activation. Zineb also produced increased chromosomal aberrations in peripheral blood lymphocytes of workers exposed to zineb. Additional supporting evidence is provided by zineb's structural similarity to other EBDC fungicides that have shown carcinogenic activity, with the thyroid as a common site of tumor development. As is the case for all the EBDC fungicides, ETU is a metabolite, degradation product and contaminant of zineb. There is evidence from animal studies indicating that ETU is a carcinogen. ETU induces liver tumors in mice and thyroid tumors in rats and has been identified as a Group 2B carcinogen by IARC and an animal carcinogen by U.S. EPA (1989b).

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