

EVIDENCE ON THE CARCINOGENICITY OF

**1,3-Dichloro-2-Propanol
(1,3-DCP; α,γ -Dichlorohydrin)**

UPDATE

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**Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment**

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The Office of Environmental Health Hazard Assessment's (OEHHA) Reproductive and Cancer Hazard Assessment Branch was responsible for the preparation of this document.

Primary Authors

David W. Morry, Ph.D.
Staff Toxicologist
Cancer Toxicology and Epidemiology Section

Feng C. Tsai, Ph.D.
Staff Toxicologist
Cancer Toxicology and Epidemiology Section

Martha S. Sandy, Ph.D.
Chief, Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

Internal OEHHA Reviewers

George V. Alexeeff, Ph.D., D.A.B.T.
Deputy Director for Scientific Affairs

Lauren Zeise, Ph.D.
Chief, Reproductive and Cancer Hazard Assessment Branch

Robert Howd, Ph.D.
Chief, Water Toxicology Section
Pesticide and Environmental Toxicology Branch

PREFACE

Proposition 65¹ requires the publication of a list of chemicals “known to the state” to cause cancer or reproductive toxicity. It specifies that “a chemical is known to the state to cause cancer ... if in the opinion of the state’s qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer ...” The “state’s qualified experts” regarding findings of carcinogenicity are the members of the Carcinogen Identification Committee (CIC) of the OEHHA Science Advisory Board².

The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. After consultation with the CIC, OEHHA selected 1,3-dichloro-2-propanol (1,3-DCP) as a chemical for consideration for listing by the CIC. Upon selection, the public was given the opportunity to submit information relevant to the assessment of the evidence on the carcinogenicity of 1,3-DCP. OEHHA reviewed and considered those submissions in preparing this document.

OEHHA developed this document to provide the CIC with comprehensive information on 1,3-DCP carcinogenicity for use in its deliberations on whether or not the chemical should be listed under Proposition 65. This September 2010 update corrects three non-substantive errors on pages ii, 1, and 25.

At their September 21, 2010 meeting, the CIC, by a vote of seven in favor and zero against, found that 1,3-DCP had been “clearly shown through scientifically valid testing according to generally accepted principles to cause cancer.” Accordingly, “1,3-Dichloro-2-propanol (1,3-DCP)” was placed on the Proposition 65 list of chemicals known to the state to cause cancer.

¹ The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code 25249.5 *et seq.*)

² Title 27 Cal. Code of Regs. §25302

TABLE OF CONTENTS

1. EXECUTIVE SUMMARY.....	1
2. INTRODUCTION.....	3
2.1 Identity of 1,3-Dichloro-2-propanol (1,3-DCP; α,γ -Dichlorohydrin).....	3
2.2 Occurrence and Use.....	3
3. DATA ON CARCINOGENICITY.....	5
3.1 Carcinogenicity Studies in Humans.....	5
3.2 Carcinogenicity Studies in Animals.....	5
3.3 Other Relevant Data.....	12
3.3.1 <i>Genotoxicity</i>	12
3.3.2 <i>In Vitro Transformation Study</i>	16
3.3.3 <i>Pharmacokinetics and metabolism</i>	17
3.3.4 <i>Animal Tumor Pathology</i>	19
3.3.5 <i>Structure-Activity Comparisons</i>	20
4. MECHANISMS.....	26
5. REVIEWS BY OTHER AGENCIES.....	27
6. SUMMARY AND CONCLUSIONS.....	27
6.1 Summary of Evidence.....	27
6.2 Conclusion.....	29
7. REFERENCES.....	31

LIST OF TABLES

Table 1. Tumor Incidence in Male Wistar KFM-Han Rats Administered 1,3-DCP in Drinking Water for 104 weeks.....	p. 7
Table 2. Tumor Incidence in Female Wistar KFM-Han Rats Administered 1,3-DCP in Drinking Water for 104 Weeks.....	p. 9
Table 3. Tumor Incidence in Male Wistar KFM-Han Rats Administered 1,3-DCP in Drinking Water in the Chronic Toxicity 78-Week Study.....	p. 10
Table 4. Tumor Incidence in Female Wistar KFM-Han Rats Administered 1,3-DCP in Drinking Water in the Chronic Toxicity 78-Week Study.....	p. 11
Table 5. <i>In Vitro</i> Genotoxicity Studies on 1,3-DCP.....	p. 14
Table 6. <i>In Vivo</i> Genotoxicity Studies on 1,3-DCP.....	p. 16
Table 7. <i>In Vitro</i> Mouse Cell Transformation Assay	p. 17
Table 8. Structure-Activity Comparisons for 1,3-DCP.....	p. 22

LIST OF FIGURES

Figure 1. Chemical Structure of 1,3-DCP.....	p. 3
Figure 2. Metabolic pathways for 1,3-DCP	p. 19

1. EXECUTIVE SUMMARY

1,3-Dichloro-2-propanol (1,3-DCP) is a high production volume industrial chemical. It is used in the manufacture of epichlorohydrin, a key industrial chemical used in the synthesis of a wide variety of chemical products. It is also used in the manufacture of 1,2,3-trichloropropane, a chemical intermediate used for polymer manufacture, and in the manufacture of 1,3-dichloropropene, a soil fumigant.

1,3-DCP is also one of several chloropropanols that can be formed in foods during processing, cooking and storage as a result of chloride ions reacting with glycerol and other lipids present in the food. 1,3-DCP has been detected in acid-hydrolyzed vegetable protein (acid-HVP), foods containing acid-HVP, such as soy and oyster sauces, and in some foods that do not contain acid-HVP, such as malt products, sausage, minced beef, ham, and battered and fried fish.

1,3-DCP and other chloropropanols also may be present as impurities in epichlorohydrin-containing products, such as water flocculants and “wet-strength” resins used in food-contact materials and some paper products.

1,3-DCP has been tested for carcinogenicity in two-year drinking water studies in male and female rats. 1,3-DCP significantly increased the incidence of malignant tumors of the liver and tongue in male and female Wistar KFM-Han rats, and the combined incidence of benign and malignant tumors of the liver, tongue, and thyroid in both sexes, as well as the kidney in males. Squamous cell tumors of the tongue are rare in untreated rats of this type. In addition, many of the liver tumors observed in treated females metastasized to the lungs, indicating the highly malignant nature of these tumors.

1,3-DCP has been found to be genotoxic in a variety of assays involving prokaryotic and eukaryotic cells, and to induce malignant transformation in mouse cells in culture.

Additional evidence of carcinogenicity comes from ten compounds that are either structurally similar to 1,3-DCP, are metabolized to 1,3-DCP or a structurally similar compound, or share common metabolites with 1,3-DCP. All of these compounds have positive results in genotoxicity assays performed *in vitro*, and all but 1,3-DCP, 3-MCPD, 2,3-dibromo-1-propanol, and 1,3-dichloropropene also have some positive genotoxicity results *in vivo*. All have some positive carcinogenicity data in rodent studies, and seven are listed under Proposition 65 as causing cancer and classified by IARC as either Group 2A or Group 2B carcinogens (*i.e.*, epichlorohydrin, glycidol, 2,3-dibromo-1-propanol, 1,2,3-trichloropropane, 1,3-dichloropropene, 1,2-dibromo-3-chloropropane (DBCP), and tris(2,3-dibromopropyl)phosphate (TDPP)). Many of these compounds induce tumors at multiple sites, and in most cases in more than one sex/species. Certain tumor sites such as liver, kidney, tongue, mouth or oral cavity, and mammary gland are common target sites shared by many of these compounds.

In summary, the evidence for carcinogenicity of 1,3-DCP comes from:

- Studies in rats
 - Tumors at multiple sites in males
 - Liver
 - Tongue
 - Thyroid
 - Kidney
 - Tumors at multiple sites in females
 - Liver
 - Tongue
 - Thyroid
- Positive findings in a variety of *in vitro* genotoxicity test systems
- Malignant transformation of mammalian cells in culture
- Metabolism of 1,3-DCP to multiple genotoxic compounds, including two genotoxic carcinogens
 - Epichlorohydrin (carcinogen identified by IARC, listed under Proposition 65)
 - Glycidol (carcinogen identified by IARC, listed under Proposition 65)
 - 1,3-Dichloroacetone
 - 3-MCPD
- Structure-activity considerations with seven carcinogens identified by IARC and listed under Proposition 65.

2. INTRODUCTION

2.1 Identity of 1,3-Dichloro-2-propanol (1,3-DCP; α,γ -Dichlorohydrin)

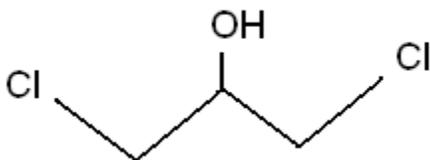


Figure 1. Chemical Structure of 1,3-DCP

Molecular Formula: $C_3H_6Cl_2O$

Molecular Weight: 128.98

CAS Registry Number: 96-23-1

IUPAC Systematic Name: 1,3-Dichloropropan-2-ol

Synonyms: 1,3-Dichloro-2-hydroxypropane, 1,3-Dichlorohydrin, 1,3-Dichloroisopropanol, 1,3-Dichloroisopropyl alcohol, 1,3-Dichloropropanol, Dichlorohydrin, Glycerol 1,3-dichlorohydrin, Propylene dichlorohydrin

Chemical Class: Chloropropanols / glycerol chlorohydrins

Chemical Appearance: Colorless liquid

Melting Point: $-4^{\circ}C$

Boiling point: $174.3^{\circ}C$ (at 760 mmHg)

Water Solubility: 15.2 g per 100 g water (at $20^{\circ}C$)

Vapor pressure: 0.75 mmHg (at $0^{\circ}C$)

Octanol-water coefficient: 54.6 (at pH 1-10)

2.2 Occurrence and Use

1,3-DCP is a semi-volatile organic liquid with a sweet ethereal odor that is soluble in water and organic solvents. It is a high production volume chemical. Its primary use is as an intermediate in the production of epichlorohydrin, an epoxide that is used in the production of glycerol (glycerin), plastics, epoxy glues and resins, and elastomers. 1,3-DCP is also used as an intermediate in the production of 1,3-dichloropropene (a soil fumigant, Telone® II) and 1,2,3-trichloropropane (a chemical intermediate used for polymer manufacture) (ILS, 2005; Kim *et al.*, 2007). 1,3-DCP may be an impurity

present in these three chemicals, as well as in bis(2-chloro-1-methylethyl) ether, all of which are carcinogens listed under Proposition 65. It may also be present as an impurity of acrylic paints containing glycidyl esters, and of a quaternary ammonium compound (i.e., (3-chloro-2-hydroxypropyl)trimethylammonium chloride) used in paper and textile manufacturing (ILS, 2005).

1,3-DCP and several other chloropropanols, including 2,3-DCP, 2-monochloropropane-1,3-diol (2-MCPD), and 3-monochloropropane-1,2-diol (3-MCPD), can be formed in foods during processing, cooking and storage as a result of the reaction of chloride ions with glycerol and other lipids present in the food (WHO, 2007). 3-MCPD and 2-MCPD are formed first. Further chlorination of MCPDs may form dichloropropanols. 3-MCPD and 1,3-DCP are among the major chloropropanols identified in foods (Baer *et al.*, 2010). 1,3-DCP has been detected in acid-hydrolyzed vegetable protein (acid-HVP), foods containing acid-HVP, and some foods that do not contain acid-HVP, such as malt products, sausage (raw, cooked or dry-fried), minced beef (raw or cooked), ham, and fish fillets (battered and fried) (WHO, 2007).

1,3-DCP and other chloropropanols are formed during the acid hydrolysis of vegetable protein. If the acid hydrolysis step is followed by an alkaline hydrolysis step, levels of 1,3-DCP and other chloropropanols in acid-HVP can be reduced. Acid-HVP is a widely used ingredient added to prepared and processed foods, including many soy sauces, oyster sauces, other sauces, instant soups, bouillon cubes, gravy mixes, savory snacks, spreads, stuffings, ready-to-eat meals, instant noodles, frozen dinners, and other frozen prepared foods (ILS, 2005). Soy sauces prepared using traditional fermentation processes, without the addition of acid-HVP, generally do not contain 1,3-DCP or other chloropropanols (Crews *et al.*, 2003; Nyman *et al.*, 2003; WHO, 2007). Most of the available data on the presence of 1,3-DCP in foods come from studies that focused primarily on acid-HVP-containing foods, thus the extent to which 1,3-DCP may be present in foods that do not contain acid-HVP is unknown. A recent study found that pyrolysis of the synthetic sweetener sucralose, a polychlorinated compound, in the presence of glycerol, can also result in the formation of chloropropanols (>75% 3-MCPD, 15%-23% 1,3-DCP, <5% 1,2-DCP) (Rahn and Yaylayan, 2010). This finding suggests that the use of sucralose in baked goods may lead to chloropropanol formation.

1,3-DCP and other chloropropanols can be formed from the degradation of epichlorohydrin in aqueous media as a result of epichlorohydrin's slow hydrolysis in the presence of chloride ions (ILS, 2005). 1,3-DCP and other chloropropanols also may be present as impurities in epichlorohydrin compounds and products derived from them, such as epoxy resins and dimethylamine-epichlorohydrin copolymers (DECs) (ILS, 2005). DECs are used in flocculants and coagulants for water purification, and 1,3-DCP has been detected in finished drinking water as a result of such use (COC, 2001). Epichlorohydrin copolymers containing low concentrations of 1,3-DCP and 3-MCPD are also used as "wet-strength" resins for products such as tea bags, coffee filters, sausage casings, absorbents packaged with meats, and paper towels and tissues (Tritscher, 2004; ILS, 2005). The U.S. Food and Drug Administration (U.S. FDA) has set limits for 1,3-DCP in these products. DECs are also used as decolorizing agents and flocculants

in the clarification of refinery sugar liquors and juices, and in the production of high-fructose corn syrup (ILS, 2005).

Epichlorohydrin emitted into air or water may undergo hydrolysis, resulting in the formation of 1,3-DCP. 1,3-DCP has been detected in pulp mill effluents (ILS, 2005).

1,3-DCP has been detected in emissions from polyurethane carpet cushions/pads (Schaeffer *et al.*, 1996; CPSC, 1996). 1,3-DCP is a thermal degradation product of the flame retardant TDCPP [tris(1,3-dichloro-2-propyl) phosphate; Fyrol FR-2], which is a 3:1 ester of 1,3-DCP with phosphoric acid (ILS, 2005). 1,3-DCP may also be formed as a result of metabolism or hydrolysis of TDCPP (ILS, 2005). TDCPP is used to treat fabrics, upholstery, and polyurethane foam.

3. DATA ON CARCINOGENICITY

3.1 Carcinogenicity Studies in Humans

No carcinogenicity studies in humans were found in the published literature.

3.2 Carcinogenicity Studies in Animals

A review of the scientific literature regarding carcinogenicity studies of 1,3-DCP in experimental animals identified long-term drinking water studies in male and female Wistar rats.

Male and female Wistar KFM-Han rats were given 1,3-DCP in drinking water [Research and Consulting Company (1986), as reported in WHO (2007) and Hercules (1989)]. The studies in each sex included two portions, a carcinogenicity portion and a chronic toxicity portion. Fifty rats per sex per group were assigned to the 104-week carcinogenicity portions of the studies. Thirty additional rats per sex per group were assigned to the chronic toxicity portions of the studies, in which 10 animals per sex per group were sacrificed after 26, 52 and 78 weeks of treatment. 1,3-DCP was administered in drinking water at concentrations of 0, 27, 80, or 240 milligrams/liter (mg/L) for up to 104 weeks. This resulted in average daily doses of 1,3-DCP of 0, 2.1, 6.3, and 19 milligrams/kilogram body weight per day (mg/kg bw/day) in males, and 0, 3.4, 9.6, and 30 mg/kg bw/day in females.

In the 104-week carcinogenicity studies, no treatment-related differences in survival were observed in low- or mid-dose rats, as compared to controls. Survival at 104 weeks was reduced in high-dose males and females, as compared to controls (males: 36% vs. 64%, females: 46% vs. 74%). Information on survival at other time points was not available. Body weight gains were significantly reduced in high-dose males and females after 74 and 78 weeks, respectively.

Histopathology was performed on all major organs and tissues from all control and high-dose group males and females, and from those low- and mid-dose animals that died prior to week 104. Histopathology on organs and tissues from low- and mid-dose animals that survived to week 104 was limited to microscopic examination of the adrenal gland, esophagus, kidney, lungs, thyroid gland, and tongue.

Neoplastic findings

1,3-DCP induced tumors of the kidney, liver, thyroid, and tongue in male rats (Table 1), and tumors of the liver, thyroid and tongue in female rats (Table 2).

Specifically, among male rats the incidences of:

- renal tubular adenomas and renal tubular adenomas and carcinomas (combined) were significantly increased ($p < 0.05$) in the high-dose group, and dose-related trends ($p < 0.001$) were observed for renal tubular adenoma and renal tubular adenomas and carcinomas (combined).
- hepatocellular carcinomas ($p < 0.05$) and hepatocellular adenomas and carcinomas (combined) ($p < 0.05$) were significantly increased in the high-dose group, and dose-related trends ($p < 0.001$) were observed for hepatocellular carcinomas and hepatocellular adenomas and carcinomas (combined).
- thyroid follicular cell adenomas and carcinomas (combined) were marginally significantly increased in the high-dose group ($p = 0.052$), and dose-related trends ($p < 0.05$) were observed for thyroid follicular cell adenomas and thyroid follicular cell adenomas and carcinomas (combined).
- squamous cell papillomas ($p < 0.05$), squamous cell carcinomas ($p < 0.05$), and squamous cell papillomas and carcinomas (combined) ($p < 0.001$) of the tongue were significantly increased in the high-dose group, and dose-related trends ($p < 0.001$) were observed for squamous cell papillomas, squamous cell carcinomas, and squamous cell papillomas and carcinomas (combined) of the tongue.

In addition, a single hemangiosarcoma was observed in the liver of one high-dose male rat, and papillary carcinomas of the oral cavity were observed in two high-dose males (Table 1).

Table 1. Tumor Incidence¹ in Male Wistar KFM-Han Rats Administered 1,3-DCP in Drinking Water for 104 Weeks.

Tumor Site/Type	Dose (mg/kg bw/day)				Trend test ²
	0	2.1	6.3	19	p-value
KIDNEY					
Renal tubular adenomas	0/50	0/50	3/50	9/50*	p<0.001
Renal tubular carcinomas	0/50	0/50	0/50	1/50	NS
Combined adenomas and carcinomas	0/50	0/50	3/50	9/50*	p<0.001
LIVER³					
Hepatocellular adenomas	1/50	0/50	0/50	0/50	NS
Hepatocellular carcinomas	0/50	0/50	2/50	8/50*	p<0.001
Combined adenomas and carcinomas	1/50	0/50	2/50	8/50*	p<0.001
Hemangiosarcoma	0/50	0/50	0/50	1/50	NS
THYROID					
Thyroid follicular adenomas	0/50	0/50	2/50	3/48	p<0.05
Thyroid follicular carcinomas	0/50	0/50	2/50	1/48	NS
Combined adenomas and carcinomas	0/50	0/50	4/50	4/48 ⁴	p<0.05
TONGUE					
Squamous cell papillomas	0/50	1/50	0/49	6/50*	p<0.001
Squamous cell carcinomas	0/50	0/50	0/49	6/50*	p<0.001
Combined papillomas and carcinomas	0/50	1/50	0/49	12/50**	p<0.001
Oral cavity (non-tongue)					
Papillary carcinomas ⁵	0/50	0/50	0/50	2/50	NS

* p< 0.05; ** p<0.001, pairwise comparison with controls by Fisher exact test (performed by OEHHA)

¹ As reported in WHO (2007), unless otherwise specified.

² Exact trend test (by OEHHA)

³ Histopathology was not performed on the livers of low- and mid-dose animals that survived to the end of the study.

⁴ p = 0.052, marginally significant by pairwise comparison, Fisher exact test (performed by OEHHA)

⁵ As reported in Hercules (1989).

NS: not significant

As shown in Table 2, among female rats the incidences of:

- hepatocellular carcinomas and hepatocellular adenomas and carcinomas (combined) ($p < 0.001$) were significantly increased in the high-dose group, and dose-related trends were observed for hepatocellular adenomas ($p < 0.05$), hepatocellular carcinomas ($p < 0.001$), and hepatocellular adenomas and carcinomas (combined) ($p < 0.001$).
- a dose-related trend was observed for thyroid follicular cell adenomas and carcinomas (combined) ($p < 0.05$).
- squamous cell papillomas ($p < 0.01$) and squamous cell papillomas and carcinomas (combined) ($p < 0.001$) of the tongue were significantly increased in the high-dose group, and dose-related trends ($p < 0.001$) were observed for squamous cell papillomas, squamous cell carcinomas, and squamous cell papillomas and carcinomas (combined) of the tongue.

In addition, a single hemangiosarcoma was observed in the liver of one high-dose female rat and a single papillary carcinoma of the oral cavity was observed in one mid-dose female (Table 2). The predominant tumor type observed in female rats was hepatocellular carcinoma, which occurred in 72% of the high-dose females. In addition, 25% of the hepatocellular carcinomas metastasized to the lungs of the affected animals.

Table 2. Tumor Incidence¹ in Female Wistar KFM-Han Rats Administered 1,3-DCP in Drinking Water for 104 Weeks.

Tumor Site/Type	Dose (mg/kg bw/day)				Trend test ² p-value
	0	3.4	9.6	30	
LIVER³					
Hepatocellular adenomas	1/50	1/50	1/50	5/50	p<0.05
Hepatocellular carcinomas	0/50	0/50	1/50	36/50**	p<0.001
Combined adenomas and carcinomas	1/50	1/50	2/50	41/50**	p<0.001
Hemangiosarcoma	0/50	0/50	0/50	1/50	NS
THYROID					
Thyroid follicular adenomas	1/50	0/50	3/50	3/49	NS
Thyroid follicular carcinomas	0/50	0/50	0/50	2/49	NS
Combined adenomas and carcinomas	1/50	0/50	3/50	5/49	p<0.05
TONGUE					
Squamous cell papillomas	0/50	0/50	0/50	7/49*	p<0.001
Squamous cell carcinomas	0/50	1/50	1/50	4/49 ⁴	p<0.001
Combined papillomas and carcinomas	0/50	1/50	1/50	11/49**	p<0.001
Oral cavity (non-tongue)					
Papillary carcinomas ⁵	0/50	0/50	1/50	0/50	NS

* p< 0.01; ** p<0.001 pairwise comparison with controls by Fisher exact test (performed by OEHHA)

¹ As reported in WHO (2007), unless otherwise specified.

² Exact trend test (by OEHHA)

³ Histopathology was not performed on the livers of low- and mid-dose animals that survived to the end of the study.

⁴ p = 0.056, marginally significant by pairwise comparison with controls by Fisher exact test

⁵ As reported in Hercules (1989)

NS: not significant

Tumors of the liver, kidney, and tongue were also observed in the chronic toxicity studies, in which 10 rats per sex per dose group were sacrificed after 26-, 52-, and 78-weeks of exposure to 1,3-DCP in drinking water. At the end of 26 weeks, one

hepatocellular adenoma was observed in a mid-dose male rat. At the end of 52 weeks, one hepatocellular adenoma and one hepatocellular carcinoma were observed in high-dose female rats. It is not clear from the summary whether these two tumors appeared in the same rat or in two different animals (Hercules, 1989). At the end of 78 weeks, liver, kidney, thyroid and tongue tumors were observed in male rats (Table 3), and liver and tongue tumors were observed in female rats (Table 4).

Table 3. Tumor Incidence¹ in Male Wistar KFM-Han Rats Administered 1,3-DCP in Drinking Water in the Chronic Toxicity 78-Week Study.

Tumor Site/Type	Dose (mg/kg bw/day)				Trend test ² p-value
	0	2.1	6.3	19	
KIDNEY					
Renal tubular adenoma	0/10	0/10	0/10	1/10	NS
LIVER					
Hepatocellular carcinoma	0/10	0/10	0/10	3/10	p<0.05
THYROID					
Thyroid follicular adenoma	0/10	0/10	1/10	0/10	NS
TONGUE					
Squamous cell carcinoma	0/10	0/10	1/10	0/10	NS

¹ As reported in Hercules (1989).

² Exact trend test (by OEHHA).

NS: not significant

Table 4. Tumor Incidence¹ in Female Wistar KFM-Han Rats Administered 1,3-DCP in Drinking Water in the Chronic Toxicity 78-Week Study.

Tumor Site/Type	Dose (mg/kg bw/day)				Trend test ² p-value
	0	3.4	9.6	30	
LIVER					
Hepatocellular carcinoma	0/10	0/10	0/10	7/10*	p<0.001
TONGUE					
Squamous cell papilloma	0/10	0/10	0/10	1/10	NS

* p<0.01, pairwise comparison with controls by Fisher exact test (performed by OEHHA)

¹As reported in Hercules (1989).

²Exact trend test (by OEHHA)

NS: not significant

Non-neoplastic findings

Progressive 1,3-DCP-related non-neoplastic changes observed in the livers of male and female rats in these chronic toxicity and carcinogenicity studies were as follows:

- After 26 weeks of treatment, eosinophilic foci were observed in the livers of one mid-dose male and one mid-dose female rat.
- After 52 weeks of treatment, peliosis hepatis (accumulation of fat in the sinuses) was observed in all treatment groups (but not in the controls) in both sexes of rats.
- After 78 weeks, peliosis hepatis was increased compared with the incidence at 52 weeks. Lipidosis was increased in the mid- and high-dose groups in both sexes.
- After 78 weeks, eosinophilic foci were observed in the high-dose male rats only.
- In the 104-week carcinogenicity studies, 1) eosinophilic foci were observed in high-dose males and females, 2) glycogen-free foci were observed in high-dose males and females, 3) dose-dependent increases in peliosis hepatis were observed in males and females in all treatment groups, and 4) an increased incidence of slight to moderate fatty change was observed in the livers of mid- and high-dose males.
- Absolute liver weights and liver to body weight ratios were increased in the mid- and high-dose males and females at 26, 52, 78 and 104 weeks.
- Slightly increased total cholesterol levels were observed in high-dose males after 26 or 52 weeks, and in high-dose females from 26 weeks up through 104 weeks.
- Slight to moderate increases in liver enzyme levels (aspartate- and alanine-aminotransferase, alkaline phosphatase, gamma-glutamyl transferase) were observed in high-dose female rats after 52, 78 and 104 weeks.

- Cytochrome P450 content decreased slightly in mid-dose and high-dose female rats at 104 weeks.
- Slightly increased glutathione levels were observed in high-dose male rats at 26 and 52 weeks, in high-dose female rats at 52 and 78 weeks, and in high-dose groups of both sexes at 104 weeks.
- There was an increased incidence of hepatic Kupffer cell siderosis (accumulation of iron) in mid- and high-dose males and in high-dose females.

Overall, the histopathological and biochemical findings suggest that hepatotoxicity occurred in the treated rats of both sexes, progressing both in terms of time of treatment and dose.

Non-neoplastic findings in the kidney and urinary system were as follows:

- Absolute kidney weights and kidney-to-body weight ratios were increased throughout the study in the treated groups, starting at 26 weeks with the mid- and high-dose males and the high-dose females.
- After 52, 78 and 104 weeks of exposure to 1,3-DCP, slight increases in urinary protein and amylase levels were observed in high-dose female rats.

Non-neoplastic findings in the thyroid were as follows:

- There was an increased incidence of thyroid follicular hyperplasia in high-dose males.

3.3 Other Relevant Data

3.3.1 Genotoxicity

The genotoxicity of 1,3-DCP has been investigated in various *in vitro* and *in vivo* studies, the majority of which have been reviewed and summarized in ILS (2005). The findings of these reviews are presented in Tables 5 and 6 and briefly described below.

1,3-DCP (0.1 to 130 mg/plate) induced mutations in the presence and absence of metabolic activation (rodent liver microsomes or "S9") in *Salmonella typhimurium* strains TA 100, TA 1535 and TM 677 (summarized in ILS, 2005) and in the presence of S9 in TA 97 and TA 98. Other studies have found that 1,3-DCP did not produce mutations in certain *S. typhimurium* strains (TA 1537, TA 1538) with or without S9 activation (ILS, 2005). TA 1535 and TA 100 detect mutagens that induce base-pair substitutions, while TA 97 and TA 98 detect frameshift mutations. These data indicate that 1,3-DCP is capable of inducing base-pair substitutions with or without S9 activation. The data also indicate that 1,3-DCP is capable of inducing frameshift mutations (based on TA 97 and TA 98 data) with S9 activation, but may not be able to induce frameshift mutations without S9 activation, as TA 97 and TA 98 were negative in all assays in which S9 was omitted.

1,3-DCP tested positive (with and without S9) in *S. typhimurium* strain TM 677 which tests for forward mutations.

1,3-DCP has tested positive for mutagenicity in *E. coli*. It caused reverse mutations in *E. coli* strain TM 930, and produced DNA damage in *E. coli* strains PM 21 and GC 4798.

1,3-DCP has also tested positive in mammalian cell assays. It caused gene mutation in mouse lymphoma cells, and in HeLa (human fibroblast) cells (measured by DNA synthesis inhibition). It induced sister chromatid exchanges (SCE) in Chinese hamster V79 cells, and in Chinese hamster ovary (CHO) cells with and without S9 activation. Also in CHO cells it induced chromosomal aberrations with and without S9 activation.

Table 5. *In Vitro* Genotoxicity Studies on 1,3-DCP¹

Endpoint	Assay System	Concentrations Tested	Results		References as cited in ILS (2005)
			+ S9	- S9	
Reverse mutation (bacteria/ <i>S. typhimurium</i>)	TA 97 and TA 98	100 - 6700 µg/plate	+	-	Zeiger <i>et al.</i> (1988)
	TA 98	≤1.2 mg/plate	NT	-	Ohkubo <i>et al.</i> (1995)
	TA 98, TA 1537, TA 1538	0.26-26 mg/plate	-	-	Silhanková <i>et al.</i> (1982)
	TA 100	10 – 100 µg/plate	+	-	Gold <i>et al.</i> (1978)
	TA 100	100 – 1000 µg/plate	NT	+	Lynn <i>et al.</i> (1981)
	TA 100	≤ 500 µg/plate	+	NT	Majeska and Matheson (1983)
	TA 100	0.13 – 8.1 mg/plate	+	+	Hahn <i>et al.</i> (1991)
	TA 100	1.3 – 130 mg/plate	+	+	Stolzenberg and Hine (1980)
	TA 1535	0.13 – 10 mg/plate	+	+	Hahn <i>et al.</i> (1991)
	TA 1535	0.26 – 26 mg/plate	+	+	Silhanková <i>et al.</i> (1982)
	TA 100, TA 1535	100 – 6700 µg/plate	+	+	Zeiger <i>et al.</i> (1988)
	TA 100, TA 1535	0.39 – 39 mg/plate	+	+	Nakamura <i>et al.</i> (1979)
	TA 100, TA 1535	≤ 1.2 mg/plate	+	+	Ohkubo <i>et al.</i> (1995)
Forward mutation (bacteria/ <i>S. typhimurium</i>)	TM 677	≤ 0.1 mg/plate	+	+	Ohkubo <i>et al.</i> (1995)
Reverse mutation (bacteria/ <i>E. coli</i>)	TM 930	0.26 – 26 mg/plate	+	-	Silhanková <i>et al.</i> (1982)
DNA repair	<i>E. coli</i> PM 21, GC 4798	0.3 – 3.9 mg/sample	+	-	Hahn <i>et al.</i> (1991)

Endpoint	Assay System	Concentration tested	Results		References as cited in ILS (2005)
			+ S9	- S9	
Mutations (mammalian cells)	Mouse lymphoma cells, <i>Tk</i> locus	2 – 9 mg/mL	+	+	Henderson <i>et al.</i> (1987)
	Mouse lymphoma cells, <i>Tk</i> +/- locus	0.1 – 0.6 µL/mL (+S9) 0.4 - 1.9 µL/mL (-S9)	+	+	Hercules (1989)
	HeLa cells (DNA synthesis inhibition)	320 µg/mL	+	NT	Painter and Howard (1982)
Sister chromatid exchanges (SCE)	Chinese hamster V79 cells	16 – 430 µg/mL	+	+	Von der Hude <i>et al.</i> (1987)
	Chinese hamster ovary (CHO) cells	0.005 – 5 µL/mL	+	+	Hercules (1990)
Chromosomal aberrations	CHO cells	0.063 – 1 µL/mL	+	+	Hercules (1990)

[†]Adapted from ILS (2005); NT: not tested

The three *in vivo* studies reviewed by ILS (2005) are summarized in Table 6. 1,3-DCP did not induce somatic mutations in *Drosophila*, micronuclei in rat bone marrow, or unscheduled DNA synthesis in rat liver.

Table 6. *In Vivo* Genotoxicity Studies on 1,3-DCP¹

Endpoint	Assay System	Concentrations/Doses Tested	Results	References as cited in ILS (2005)
Somatic mutation (wing spot test)	<i>D. melanogaster</i>	0.006 – 1.3 mg/mL	-	Frei and Würgler (1997)
Bone marrow micronucleus	Wistar Han rats	25 – 100 mg/kg once daily for two consecutive days	-	Howe (2002)
Liver unscheduled DNA synthesis	Wistar Han rats	40 – 100 mg/kg	-	Beevers (2003)

¹Adapted from ILS (2005)

In summary, 1,3-DCP yielded positive results for gene mutations and DNA damage in the majority of *in vitro* assays. It was negative in the small number of *in vivo* assays performed, which consisted of one study in fruit flies and studies for two endpoints in only one strain of one mammalian species (Wistar Han rats).

Taking the *in vitro* and *in vivo* results together, because 1,3-DCP is positive in the preponderance of *in vitro* assays and has received only limited study *in vivo*, it is reasonable to conclude that 1,3-DCP is genotoxic.

3.3.2 *In Vitro* Transformation Study

1,3-DCP was assayed for malignant transformation of mouse M2 fibroblasts by Piasecki *et al.* (1990). This assay is designed to detect a change in the growth pattern of fibroblasts that is indicative of loss of contact inhibition, a phenotype that is characteristic of cancer cells. The results of this assay are shown in Table 7. The number of morphologically transformed foci in the treated plates was significantly increased over the untreated control plates at 100 micrograms/milliliter ($\mu\text{g/mL}$) ($p < 0.001$), 250 $\mu\text{g/mL}$ ($p < 0.001$), and 500 $\mu\text{g/mL}$ ($p < 0.05$). In addition, a dose-dependent reduction in plating efficiency (from 22% to 3%), indicative of cytotoxicity, was observed. The decrease in plating efficiency may explain the decrease in number of transformed foci.

Table 7. *In Vitro* Mouse Cell Transformation Assay (Piasecki *et al.*, 1990)

No. of transformed foci / No. of treated dishes	1,3-DCP Concentration ($\mu\text{g/mL}$)			
	Control	100	250	500
	0/24	7/14**	15/15**	3/14*

* $p < 0.05$; ** $p < 0.001$ (Fisher exact test by OEHHA)

3.3.3. *Pharmacokinetics and metabolism*

The metabolism of 1,3-DCP has been studied in rats (Jones and Fakhouri, 1979; Koga *et al.*, 1992) and rat liver microsomes (Garle *et al.*, 1999). The metabolic scheme presented in Figure 2 is based on the findings of these studies. There are two main pathways by which 1,3-DCP can be metabolized. One pathway leads directly to 1,3-dichloroacetone, a mutagen and skin tumor initiator. The other pathway leads to epichlorohydrin which is a genotoxic carcinogen and which can either be conjugated with glutathione and further converted to a mercapturic acid, or it can be metabolized to 3-MCPD. 3-MCPD may be metabolized to glycidol or to β -chlorolactaldehyde, which leads to the formation of 1,2-propanediol or oxalic acid. Glycidol can either be conjugated with glutathione and further converted to a mercapturic acid, or it can be metabolized to glycerol. A chlorine atom is lost (as a chloride ion) in the conversion of 3-MCPD to glycidol and in the conversion of β -chlorolactic acid to either oxalic acid or 1,2-propanediol. The pathway from β -chlorolactic acid to oxalic acid involves the loss of a carbon atom in the form of CO_2 , as does the further metabolism of glycerol. A discussion of the studies supporting the metabolic scheme laid out in Figure 2 follows.

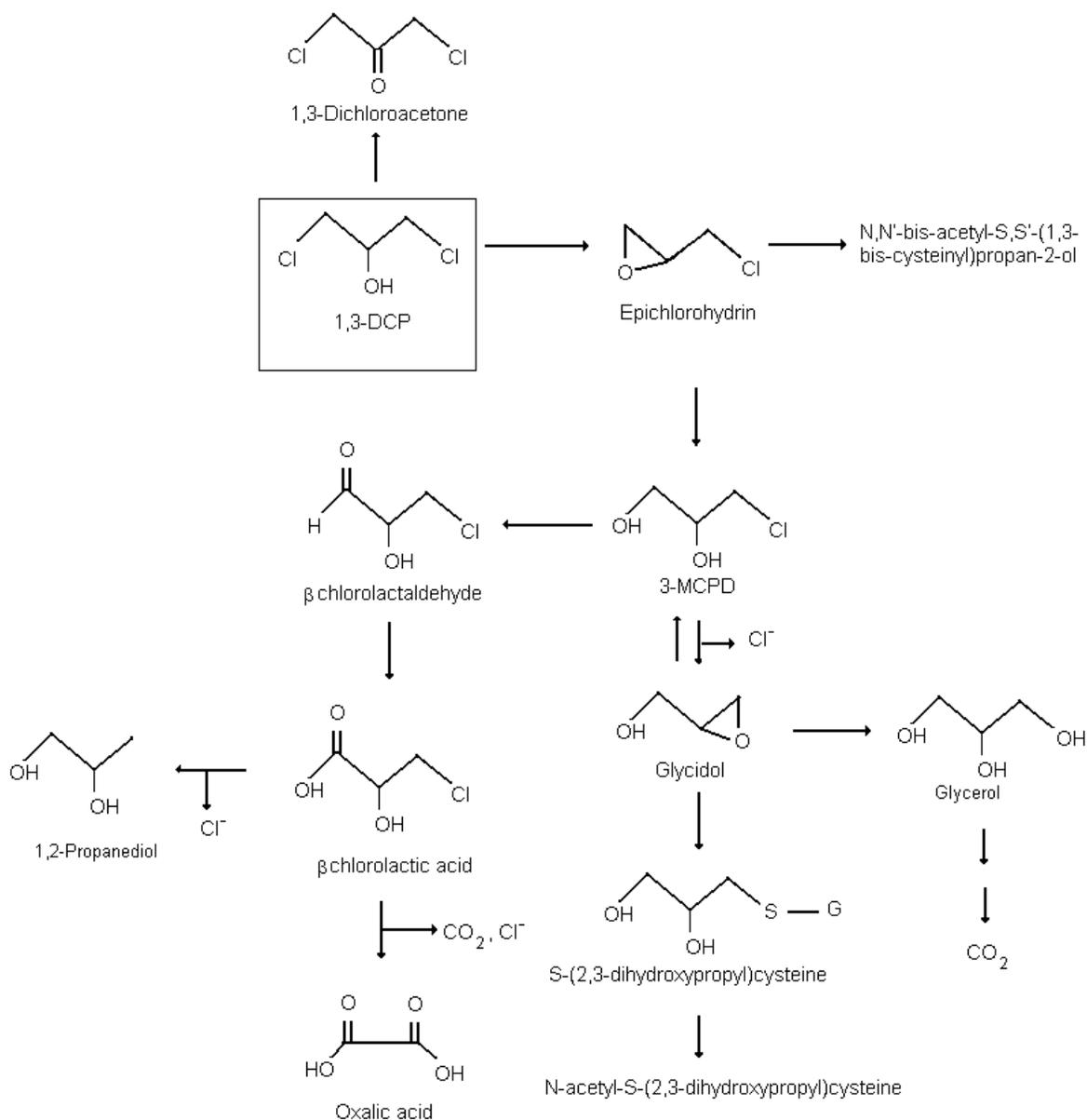
In studies conducted in male Sprague-Dawley rats, animals were administered 1,3-DCP by gavage daily for five days, and urine was collected over 10 days, beginning on the first day of dosing. Three urinary metabolites of 1,3-DCP were identified. These were β -chlorolactate (accounting for approximately 5% of the administered dose) and two mercapturic acids, *N,N*-bis-acetyl-*S,S'*-(1,3-bis-cysteinyl)propan-2-ol and *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine (accounting for approximately 1% of the administered dose) (Jones and Fakhouri, 1979). These same three urinary metabolites were observed in rats administered epichlorohydrin. The authors proposed that 1,3-DCP forms epichlorohydrin, which may either conjugate with glutathione to yield the mercapturic acid (i.e., *N,N*-bis-acetyl-*S,S'*-(1,3-bis-cysteinyl)propan-2-ol) or hydrolyze to 3-MCPD. The latter can undergo oxidation to β -chlorolactate, which may be further oxidized to oxalic acid (Figure 2).

In other studies, male Wistar rats were administered a single subcutaneous injection of 1,3-DCP, and urine collected over 24 hours was analyzed following ethyl acetate extraction. 1,3-DCP and two metabolites, 3-MCPD and 1,2-propanediol, were identified in the urine. The parent compound accounted for 2.4% of the administered dose, 3-MCPD for 0.35%, and 1,2-propanediol for 0.43% (Koga *et al.*, 1992). These findings are consistent with the proposed metabolism of 1,3-DCP to epichlorohydrin, giving rise to 3-MCPD, and with subsequent metabolism of 3-MCPD via β -chlorolactate to form either 1,2-propanediol or oxalic acid.

These urinary metabolism studies employed different analytical techniques and identified two unique sets of urinary excretion products in the rat. The urinary excretion products measured by Jones and Fakhouri (1979) accounted for approximately 6% of the administered dose, while those measured by Koga *et al.* (1992) accounted for less than 4% of the administered dose. These findings suggest that other metabolic pathways and excretion routes are likely for 1,3-DCP.

In vitro incubation studies with rat liver microsomes have identified an additional metabolic pathway for 1,3-DCP, involving cytochrome P450 mediated oxidation of 1,3-DCP to form 1,3-dichloroacetone (1,3-DCA) (Garle *et al.*, 1999). 1,3-DCA is a potent depletor of reduced glutathione, a skin tumor initiator in SENCAR mice, and a reactive mutagenic metabolite of the carcinogen 1,2,3-trichloropropane (IARC, 1995). Earlier work from Garle and colleagues, using *in vivo* and *in vitro* rat liver models, implicated 1,3-DCA as a metabolite responsible for 1,3-DCP-induced hepatotoxicity in acute human occupational poisoning cases (Hammond and Fry, 1997). 1,3-DCP-induced hepatotoxicity in the rat *in vivo* and in hepatocyte cultures is dependent upon cytochrome P450, and is associated with depletion of reduced glutathione (Hammond *et al.*, 1996; Stott *et al.*, 1997; Hammond and Fry, 1997; Fry *et al.*, 1999). These investigators showed that both 1,3-DCP and 1,3-DCA deplete glutathione in rat liver microsomes and hepatocyte cultures and that the primary cytochrome P450 isoform involved in the conversion of 1,3-DCP to 1,3-DCA is CYP2E1 (Hammond and Fry, 1997; Stott *et al.*, 1997). CYP1A2 has also been shown to convert 1,3-DCP to a metabolite that depletes glutathione (Stott *et al.*, 1997).

Figure 2. Metabolic pathways for 1,3-DCP³



3.3.4 Animal Tumor Pathology

1,3-DCP significantly increased the incidence of malignant tumors of the liver and tongue in male and female Wistar KFM-Han rats and the combined incidence of benign and malignant tumors of the liver, tongue, and thyroid in both sexes, as well as the kidney in males (Section 3.2).

³ This diagram is based on the following references: Jones and Fakhouri (1979), Koga *et al.* (1992), Lynch *et al.* (1998), and Garle *et al.* (1999).

The liver tumors observed in treated male and female rats were hepatocellular adenomas, hepatocellular carcinomas, and hemangiosarcomas. Hepatocellular adenomas and carcinomas arise from the same cell type, and adenomas can progress to carcinomas. For this reason, these two tumor phenotypes are aggregated when evaluating the results of a study (IARC, 2006; McConnell *et al.*, 1986). In these studies, one hepatocellular adenoma occurred in the male controls, and another in the female controls. Twenty-five percent of the hepatocellular carcinomas occurring in treated females had metastasized to the lungs, indicating that these tumors were highly malignant.

The kidney tumors observed in treated male rats were renal tubular cell adenomas and carcinomas. These tumor phenotypes arise from the same cell type, and adenomas can progress to carcinomas. They also are aggregated for study evaluation (IARC, 2006; McConnell *et al.*, 1986). No kidney tumors were observed in male controls, and none were observed in the studies in female rats.

The researchers who carried out these studies stated that squamous cell papillomas and carcinomas of the tongue constituted a rare tumor type in these rats (Hercules, 1989). Indeed, no squamous cell tumors of the tongue were observed in the controls in these studies. Arising from the same cell type, squamous cell papillomas of the tongue can progress to carcinomas, and were aggregated for study evaluation.

A few hepatocellular tumors were observed in the chronic toxicity studies in male and female rats, after only 26 and 52 weeks of exposure to 1,3-DCP in the drinking water (Hercules, 1989). After 78 weeks, liver, kidney, thyroid and tongue tumors were observed in treated males, but not in controls. Similarly, in the 78-week study in female rats, liver and tongue tumors were observed only in treated animals. Thus, the tumor findings in the chronic toxicity studies are consistent with the findings of the 104-week carcinogenicity studies.

3.3.5 Structure-Activity Comparisons

1,3-DCP is a chlorinated three-carbon alcohol. Information on cancer bioassay findings in mice and rats, *in vitro* and *in vivo* genotoxicity findings, and cancer classification (Proposition 65 and IARC) for 1,3-DCP and ten structurally related compounds is presented in Table 8. 1,3-DCA is a chlorinated three-carbon compound that is a direct metabolite of 1,3-DCP. 1,3-DCA has not been tested in long-term carcinogenesis studies, but it has been shown to be a skin tumor initiator in SENCAR mice and is positive in a wide range of *in vitro* and *in vivo* genotoxicity assays. These include observations of induction of mutations in *S. typhimurium*, with and without S9 metabolic activation, and production of micronuclei in peripheral erythrocytes⁴ of the newt, *Pleurodeles waltl* (IARC, 1995). IARC considers 1,3-DCA to be “the reactive and mutagenic metabolite” of 1,2,3-trichloropropane.

Epichlorohydrin is a chlorinated three-carbon epoxide compound, and a direct metabolite of 1,3-DCP. Epichlorohydrin is carcinogenic in male and female rats (forestomach, nasal cavity) and male mice (lung) and is genotoxic *in vitro* without

⁴ Newts have nucleated erythrocytes.

metabolic activation and in several *in vivo* assays (ILS, 2005; IARC, 1999). Thus there are two direct metabolites of 1,3-DCP, epichlorohydrin and 1,3-DCA, that are direct acting genotoxic compounds.

3-MCPD is a chlorinated three-carbon alcohol, and a metabolite of 1,3-DCP via the intermediate epichlorohydrin (OEHHA, 2010). 3-MCPD induced tumors in male and female rats (kidney, Leydig cell, mammary gland), and is genotoxic in *in vitro*, but not *in vivo* assays.

Glycidol is a three-carbon epoxide compound that is a metabolite of 1,3-DCP, epichlorohydrin, and 3-MCPD. Glycidol is carcinogenic in both sexes of rats and mice, and is genotoxic *in vitro* and *in vivo* (IARC, 2000).

2,3-Dibromo-1-propanol is a brominated three-carbon alcohol that is metabolized similarly to 1,3-DCP, forming the same two mercapturic acid metabolites, and β -bromolactic acid, instead of β -chlorolactic acid. This compound is carcinogenic in both sexes of rats and mice and is genotoxic *in vitro*, but not in *in vivo* assays (IARC, 2000).

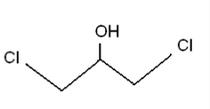
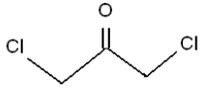
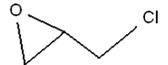
1,2,3-Trichloropropane is a chlorinated three-carbon compound that shares two metabolites with 1,3-DCP, namely 1,3-DCA and the mercapturic acid precursor S-(2,3-dihydroxypropyl)cysteine. 1,2,3-Trichloropropane is carcinogenic in both sexes of rats and mice and is genotoxic *in vitro* and *in vivo* (IARC, 1995).

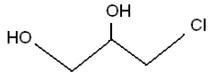
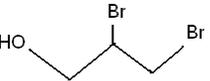
1,3-Dichloropropene is a chlorinated three-carbon compound that is carcinogenic in both sexes of rats and mice and is genotoxic *in vitro*, but not in *in vivo* assays (IARC, 1999).

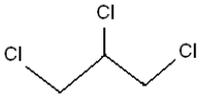
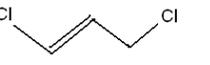
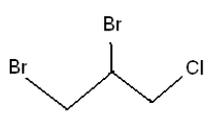
1,2-Dibromo-3-chloropropane (DBCP) is a chlorinated and brominated three-carbon compound that shares two metabolites with 1,3-DCP, namely epichlorohydrin and 3-MCPD. It is carcinogenic in both sexes of rats and mice and is genotoxic *in vitro* and *in vivo* (IARC, 1999).

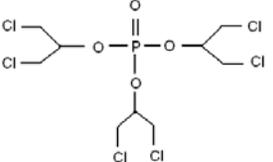
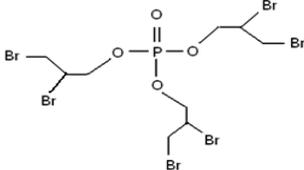
Two phosphate triesters -- TDCPP and tris(2,3-dibromopropyl)phosphate (TDPP) -- are much larger molecules than 1,3-DCP but still share some structure-activity similarities with 1,3-DCP. 1,3-DCP is a metabolite of TDCPP. TDCPP induced tumors in male and female rats (liver, kidney, Leydig cell, adrenal) and is genotoxic in *in vitro*, and in some *in vivo* assays (Babich, 2006). 2,3-Dibromo-1-propanol is a metabolite of the other triester, TDPP. TDPP is carcinogenic in both sexes of rats and mice and is genotoxic *in vitro* and *in vivo* (IARC, 1999).

Table 8. Structure-Activity Comparisons for 1,3-DCP

Chemical	Target Tumor Sites		Genotoxicity	Cancer Classification	
	Mice	Rats		Proposition 65	IARC
<p>1,3-DCP</p>  <p>Metabolized to: 1,3-dichloroacetone; epichlorohydrin; 3-MCPD; glycidol; 1,2-propanediol; <i>N,N</i>-bis-acetyl-<i>S,S'</i>-(1,3-bis- cysteinyl)propan-2-ol; <i>N</i>-acetyl-<i>S</i>-(2,3- dihydroxypropyl)cysteine; β- chlorolactic acid</p>	Not tested	<p><i>Males:</i> liver, tongue, thyroid, kidney</p> <p><i>Females:</i> liver, tongue, thyroid</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> negative (<i>Drosophila</i> wing spot mutation, rat bone marrow MN, rat UDS)</p>	Currently under evaluation	Not evaluated
<p>1,3-Dichloroacetone¹</p>  <p>Metabolite of: 1,3-DCP & 1,2,3-trichloropropane</p>	Not tested Skin tumor initiator	Not tested	<p><i>In vitro:</i> positive <i>In vivo:</i> positive (newt MN)</p>	Not evaluated	Not evaluated
<p>Epichlorohydrin</p>  <p>Metabolite of: 1,3-DCP Metabolized to: 3-MCPD; <i>N,N</i>-bis- acetyl-<i>S,S'</i>-(1,3-bis-cysteinyl)propan-2- ol; <i>N</i>-acetyl-<i>S</i>-(2,3- dihydroxypropyl)cysteine; β- chlorolactic acid</p>	<i>Male:</i> lung	<p><i>Males:</i> forestomach, nasal cavity</p> <p><i>Females:</i> forestomach</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> positive (binds in rats and mice to DNA, <i>Drosophila</i> SLRL mutation; mouse bone marrow SCE and CA) and negative (mouse MN, mouse dominant lethal)</p>	Listed	Group 2A ² (1999)

Chemical	Target Tumor Sites		Genotoxicity	Cancer Classification	
	Mice	Rats		Proposition 65	IARC
<p>3-MCPD</p>  <p>Metabolite of: 1,3-DCP & epichlorohydrin Metabolized to: glycidol; S-(2,3-dihydroxypropyl) cysteine; β-chlorolactic acid</p>	No treatment related tumors	<p><i>Males:</i> kidney, Leydig cell, mammary</p> <p><i>Females:</i> kidney</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> negative (<i>Drosophila</i> wing spot mutation assay, dominant lethal assay in mice and rats, bone marrow MN in mice and rats, UDS in rats, DNA damage (comet assay) in rats)</p>	Currently under evaluation	Not evaluated
<p>Glycidol</p>  <p>Metabolite of: 1,3-DCP, 3-MCPD, and epichlorohydrin Metabolized to: S-(2,3-dihydroxypropyl)cysteine; β-chlorolactic acid</p>	<p><i>Males:</i> liver, lung, mammary, skin, thyroid, subcutis, Harderian gland, forestomach</p> <p><i>Females:</i> mammary, subcutis, Harderian gland, uterus</p>	<p><i>Males:</i> thyroid, mammary, tunica vaginalis, brain, forestomach, intestine, skin, Zymbal's gland</p> <p><i>Females:</i> mouth/tongue, mammary, brain, forestomach, leukemia, clitoral gland</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> positive (mouse MN) and negative (mouse bone marrow CA)</p>	Listed	Group 2A (2000)
<p>2,3-Dibromo-1-propanol</p>  <p>Metabolized to: N,N-bis-acetyl-S,S'-(1,3-bis-cysteinyl)propan-2-ol; N-acetyl-S-(2,3-dihydroxypropyl)cysteine; β-bromolactic acid</p>	<p><i>Males:</i> skin, forestomach, liver, lung</p> <p><i>Females:</i> skin, forestomach</p>	<p><i>Males:</i> liver, mouth, skin, esophagus, forestomach, small intestine, large intestine, nose, Zymbal's gland</p> <p><i>Females:</i> liver, mouth, skin, esophagus, forestomach, large intestine, nose, mammary, Zymbal's gland, clitoral gland</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> negative (mouse bone marrow MN)</p>	Listed	Group 2B ³ (2000)

Chemical	Target Tumor Sites		Genotoxicity	Cancer Classification	
	Mice	Rats		Proposition 65	IARC
<p>1,2,3-Trichloropropane</p>  <p>Metabolized to: 1,3-dichloroacetone; S-(2,3-dihydroxypropyl) cysteine</p>	<p><i>Males:</i> forestomach, liver, Harderian gland</p> <p><i>Females:</i> forestomach, liver, Harderian gland, oral mucosa, uterus</p>	<p><i>Males:</i> oral cavity, kidney, forestomach, preputial gland, pancreas</p> <p><i>Females:</i> oral cavity, forestomach, mammary, clitoral gland</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> positive (binds in rats to DNA, RNA and protein, rat DNA strand breaks) and negative (rat UDS, rat dominant lethal)</p>	Listed	Group 2A (1995)
<p>1,3-Dichloropropene (Telone II)</p> 	<p><i>Males:</i> bladder, lung forestomach,</p> <p><i>Females:</i> bladder, lung, forestomach</p>	<p><i>Males:</i> liver, forestomach</p> <p><i>Females:</i> forestomach</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> negative (mouse bone marrow MN, <i>Drosophila</i> SLRL)</p>	Listed	Group 2B (1999)
<p>1,2-Dibromo-3-chloropropane (DBCP)</p>  <p>Metabolized to: epichlorohydrin, 3-MCPD</p>	<p><i>Males:</i> lung, nasal cavity, stomach and forestomach</p> <p><i>Females:</i> lung, nasal cavity, stomach and forestomach</p>	<p><i>Males:</i> tongue, kidney, nasal cavity, stomach or forestomach</p> <p><i>Females:</i> tongue, kidney, nasal cavity, stomach or forestomach, pharynx, adrenal cortex, mammary</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> positive (<i>Drosophila</i> SLRL mutation, mitotic recombination, heritable translocation; rat and mouse bone marrow MN, rat dominant lethal, rat and guinea pig DNA strand breaks)</p>	Listed	Group 2B (1999)

Chemical	Target Tumor Sites		Genotoxicity	Cancer Classification	
	Mice	Rats		Proposition 65	IARC
<p>Tris(1,3-dichloro-2-propyl)phosphate (TDCPP)</p>  <p>Metabolized to: 1,3-DCP, 3-MCPD</p>	Not tested	<p><i>Males:</i> liver, kidney, Leydig cell, adrenal</p> <p><i>Females:</i> liver, kidney</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> positive (binds in mice to DNA) and negative (mouse bone marrow MN and CA; rat UDS)</p>	Not evaluated	Not evaluated
<p>Tris(2,3-dibromopropyl) phosphate (TDPP)</p>  <p>Metabolized to: 2,3-Dibromo-1-propanol</p>	<p><i>Males:</i> forestomach, lung, skin</p> <p><i>Females:</i> forestomach, lung, liver, kidney, skin, oral cavity</p>	<p><i>Males:</i> oral mucosa, kidney, skin, esophagus, stomach, small intestine, large intestine, nasal mucosa, Zymbal's gland</p> <p><i>Females:</i> Liver, oral mucosa, kidney, skin, esophagus, stomach, large intestine, nasal mucosa, Zymbal gland</p>	<p><i>In vitro:</i> positive <i>In vivo:</i> positive (DNA single strand breaks, <i>Drosophila</i> mutations, mouse and hamster bone marrow MN, rat liver MN, mouse kidney mutations)</p>	Listed	Group 2A (1999)

CA – chromosomal aberrations, MN—micronuclei , SCE – sister chromatid exchange, SLRL – sex-linked recessive lethal, UDS — unscheduled DNA synthesis

¹ As reported in the IARC review of 1,2,3-trichloropropane (IARC, 1995).

² IARC Group 2A: Probably carcinogenic to humans

³ IARC Group 2B: Possibly carcinogenic to humans

In summary, the compounds discussed here are structurally similar to 1,3-DCP, are metabolized to 1,3-DCP or a structurally similar compound, or share common metabolites with 1,3-DCP. All have some positive carcinogenicity data in rodent studies, and seven are currently listed under Proposition 65 as causing cancer and classified by IARC as either Group 2A or Group 2B carcinogens (i.e., epichlorohydrin, glycidol, 2,3-dibromo-1-propanol, 1,2,3-trichloropropane, 1,3-dichloropropene, DBCP, and TDPP). Most of the compounds included in Table 8 induce tumors at multiple sites, and in most cases in more than one sex/species. Certain tumor sites such as liver, kidney, tongue, mouth or oral cavity, and mammary gland are common target sites shared by many of these compounds. All of the compounds in Table 8 have positive results in genotoxicity assays performed *in vitro*. All but 1,3-DCP, 3-MCPD, 2,3-dibromo-1-propanol, and 1,3-dichloropropene also have some positive genotoxicity results *in vivo*. Interestingly, several of the chemicals tested negative in the same *in vivo* assay systems in which 1,3-DCP tested negative:

- 1,3-DCP and 3-MCPD were negative in the *Drosophila* wing spot mutation assay,
- 1,3-DCP, 3-MCPD, and 1,3-dichloropropene were negative in the rat bone marrow MN assay,
- 1,3-DCP, 3-MCPD, 1,2,3-trichloropropane, and TDCPP were negative in the rat liver UDS assay.

If structurally similar compounds share similar genotoxic or carcinogenic mechanisms, it may be for either of two reasons: 1) similar structures may result in similar reactions with macromolecules, or 2) similar structures may be converted by metabolism to common active metabolites.

4. MECHANISMS

1,3-DCP was found to induce tumor formation in the kidneys, liver, thyroid and tongue of male Wistar rats, and in the liver, thyroid and tongue of female Wistar rats. The evidence suggests that 1,3-DCP may be carcinogenic in rats by a genotoxic mechanism or mechanisms. The chemical has tested positive in a variety of genotoxicity assays described above (Section 3.3.1). 1,3-DCP is also metabolized to a number of mutagenic and carcinogenic metabolites (most notably epichlorohydrin) as described in the metabolism section above. The fact that 1,3-DCP induces tumors in a variety of tissues in male and female rats suggests that the mechanism may be a general one such as mutagenesis, rather than organ-specific toxicity.

It should also be considered that 1,3-DCP may cause tumors by more than one mechanism and that different mechanisms may operate in different tissues. 1,3-DCP is hepatotoxic in the Wistar rats, as shown by the chronic toxicity studies in these rats. The hepatotoxicity of 1,3-DCP is likely to be mediated principally by CYP2E1 (Stott *et al.*, 1997).

1,3-DCP was found to cause peliosis hepatitis in the livers of both sexes of exposed Wistar rats after 52 or 78 weeks, and dose-dependent increases were observed in males and females in the 104 week studies (Hercules, 1989). Peliosis hepatitis has been related to the formation of malignant hemangioendotheliomas in *Mastomys* mice

(Wayss *et al.*, 1979). One hemangiosarcoma each was observed in treated rats of both sexes in the 104-week studies (Hercules, 1989), and these tumors may have been related to the observed increase in peliosis hepatis.

An increased incidence of slight to moderate “hepatocellular fatty change” was reported in the two highest dose groups among the treated male rats (Hercules, 1989). Most cases of hepatocellular carcinoma in humans develop after a pre-existing chronic liver disease due to hepatitis B or C virus or alcohol toxicity (Petta and Craxi, 2010). There is increasing evidence that in the absence of these well-known causative factors, another pre-existing condition that may dispose toward hepatocellular carcinoma is non-alcoholic fatty liver disease (NAFLD) (Petta and Craxi, 2010; Siegel and Zhu, 2009). NAFLD is associated with insulin resistance, oxidative stress and other changes that may lead to liver carcinogenesis by promoting cellular growth and DNA damage (Petta and Craxi, 2010).

As the Wistar rats were exposed via drinking water, the tongue tumors (squamous cell papillomas and carcinomas, rare in rats) may have resulted from direct contact of the test substance with the lingual epidermis.

In summary, while the mechanisms of carcinogenic action of 1,3-DCP remain unclear, the evidence suggests that genotoxicity is involved. The evidence for 1,3-DCP acting by a genotoxic mechanism includes: evidence from a number of *in vitro* test systems, metabolism to genotoxic carcinogens, and similarity to other genotoxic carcinogens with respect to metabolism to reactive species. Other mechanisms, yet to be elucidated, may also be operative.

5. REVIEWS BY OTHER AGENCIES

The data relating to the carcinogenicity of 1,3-DCP has been reviewed by the World Health Organization (WHO, 2002; 2007). Based on the data reviewed, the WHO concluded there is evidence that 1,3-DCP is genotoxic and carcinogenic.

1,3-DCP has not been classified as to its potential carcinogenicity by the U.S. Environmental Protection Agency, the U.S. Food and Drug Administration, the National Toxicology Program, the National Institute for Occupational Safety and Health, or the International Agency for Research on Cancer.

6. SUMMARY AND CONCLUSIONS

6.1 Summary of Evidence

In 104-week drinking water studies conducted in male and female Wistar KFM-Han rats, 1,3-DCP significantly increased the incidences of benign and malignant tumors at multiple sites.

These 104-week carcinogenicity studies produced the following observations:

Liver tumors

- 1,3-DCP significantly increased the incidence of malignant, and benign and malignant liver tumors in male rats.

- The incidence of liver tumors in untreated males was low.
- 1,3-DCP significantly increased the incidence of malignant, and benign and malignant liver tumors in female rats.
 - Twenty-five percent of the malignant liver tumors occurring in treated females metastasized to the lungs.
 - The incidence of liver tumors in untreated females was low.

Tongue tumors

- 1,3-DCP significantly increased the incidence of malignant, and benign and malignant tongue tumors in male rats.
 - Tongue tumors are rare in untreated male Wistar KFM-Han rats.
- 1,3-DCP significantly increased the incidence of benign and malignant tongue tumors in female rats.
 - Tongue tumors are rare in untreated female Wistar KFM-Han rats.

Thyroid tumors

- 1,3-DCP increased the incidence of benign and malignant thyroid tumors in high-dose male rats (p=0.052).
 - A significant dose-related trend was observed in males.
 - No thyroid tumors were observed in untreated males.
- A significant dose-related trend in benign and malignant thyroid tumors was observed in 1,3-DCP treated females.
 - One benign thyroid tumor was observed in untreated females.

Kidney tumors

- 1,3-DCP significantly increased the incidence of benign and malignant kidney tumors in male rats.
 - No kidney tumors were observed in untreated males.

In the chronic toxicity studies using small group sizes (10 per group) conducted in male and female rats, increases in hepatocellular carcinomas were observed in treated males and in treated females after 78 weeks of exposure to 1,3-DCP in the drinking water. These findings are consistent with the findings of the 104-week carcinogenicity studies.

Evidence indicating that 1,3-DCP is genotoxic comes from a number of *in vitro* test systems:

- 1,3-DCP induced mutations in *Salmonella typhimurium*, in the presence or absence of exogenous metabolic activation, inducing both base-pair substitution and frameshift mutations.
- 1,3-DCP induced mutations in *E. coli*.
- 1,3-DCP induced DNA damage in *E. coli*.
- 1,3-DCP induced mutations in mouse lymphoma cells.
- 1,3-DCP induced mutations in HeLa (human) cells.
- 1,3-DCP induced SCEs in Chinese hamster V79 cells and CHO cells.
- 1,3-DCP induced CA in CHO cells.

1,3-DCP induced malignant transformation of mammalian cells in culture.

Structure-activity considerations include:

- 1,3-DCP is structurally related to seven carcinogens identified by IARC and listed under Proposition 65:
 - Glycidol
 - Epichlorohydrin
 - 2,3-Dibromo-1-propanol
 - 1,2,3-Trichloropropane
 - 1,3-Dichloropropene
 - DBCP
 - TDPP
- 1,3-DCP is metabolized to multiple genotoxic compounds, including two genotoxic carcinogens:
 - Epichlorohydrin (carcinogen identified by IARC, listed under Proposition 65)
 - Glycidol (carcinogen identified by IARC, listed under Proposition 65)
 - 1,3-Dichloroacetone (skin tumor initiator in mice; putative active metabolite of carcinogen 1,2,3-trichloropropane; not tested in long-term carcinogenesis studies)
 - 3-MCPD (induces kidney, Leydig cell and mammary tumors in rats)
- Six structurally-related chemicals that are metabolized in a similar manner as 1,3-DCP, share the same cysteine conjugate/mercapturic acid metabolite(s) as 1,3-DCP, and cause cancer in rodents are:
 - Epichlorohydrin
 - 3-MCPD
 - Glycidol
 - 1,2,3-Trichloropropane
 - 2,3-Dibromo-1-propanol
 - TDPP
- Many of these structurally-related chemicals induce tumors at the same sites in rats as 1,3-DCP:
 - Liver tumors
 - 2,3-Dibromo-1-propanol
 - 1,3-Dichloropropene
 - TDCPP
 - TDPP
 - Tongue and oral cavity tumors
 - Glycidol
 - 2,3-Dibromo-1-propanol
 - 1,2,3-Trichloropropane
 - DBCP
 - TDPP
 - Thyroid tumors
 - Glycidol
 - Kidney tumors
 - 3-MCPD
 - 1,2,3-Trichloropropene

- DBCP
- TDCPP
- TDPP

6.2 Conclusion

The evidence for carcinogenicity of 1,3-DCP comes from:

- Drinking-water studies in male and female Wistar Han rats
 - Tumors at multiple sites in males
 - Liver
 - Tongue
 - Thyroid
 - Kidney
 - Tumors at multiple sites in females
 - Liver
 - Tongue
 - Thyroid
- Positive findings in a variety of *in vitro* genotoxicity test systems
- Malignant transformation of mammalian cells in culture
- Metabolism of 1,3-DCP to multiple genotoxic compounds, including two genotoxic carcinogens
 - Epichlorohydrin (carcinogen identified by IARC, listed under Proposition 65)
 - Glycidol (carcinogen identified by IARC, listed under Proposition 65)
 - 1,3-Dichloroacetone
 - 3-MCPD
- Structure-activity considerations with seven carcinogens identified by IARC and listed under Proposition 65.

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