EVIDENCE ON THE CARCINOGENICITY OF

TRIS(1,3-DICHLORO-2-PROPYL) PHOSPHATE

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PREFACE

Proposition 65 requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity.[1] 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 Board.[2]

The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. OEHHA selected *tris(1,3-dichloro-2-propyl) phosphate (TDCPP)* for preparation of hazard identification materials. Upon selection, the public was given the opportunity to submit information relevant to the assessment of the evidence on the carcinogenicity of TDCPP. OEHHA reviewed and considered those submissions in preparing this document.

OEHHA developed this document to provide the CIC with comprehensive information on TDCPP's carcinogenicity for use in its deliberations on whether or not the chemical should be listed under Proposition 65.

[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

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1 EXECUTIVE SUMMARY

The halogenated phosphate triester tris(1,3-dichloro-2-propyl) phosphate (TDCPP) is a high-production volume chemical used primarily as an additive flame retardant in flexible polyurethane foams. It is also used as a flame retardant and plasticizer in rigid polyurethane foams, resins, plastics, textile coatings, and rubber.

TDCPP has been detected in both indoor and outdoor environments in the U.S. and abroad. It has been measured in household and office dust, indoor air, and in streams, sewage influents, effluents, and sludge. In humans, TDCPP has been measured in adipose tissue, seminal plasma and breast milk.

TDCPP has been tested for carcinogenicity in two-year studies in male and female Sprague-Dawley rats. Statistically significant increases in the incidence of benign and malignant tumors were observed in both male and female rats:

- In both sexes, the incidences of benign, malignant, and combined malignant and benign liver tumors were significantly increased among TDCPP treated animals.
- Benign kidney tumors were significantly increased in both sexes.
- In males, benign interstitial tumors of the testes were significantly increased.

TDCPP is genotoxic in multiple *in vitro* studies of bacterial and mammalian cells. It induced mutations in *Salmonella* and mouse lymphoma cells, induced chromosomal aberrations in mouse lymphoma and Chinese hamster fibroblast cells, and induced sister chromatid exchange (SCE) in mouse lymphoma cells. There is also evidence for DNA binding in mouse kidney, liver and muscle following *in vivo* exposure.

TDCPP induced malignant cell transformation of Syrian hamster embryo cells in culture.

TDCPP is metabolized to several chemicals identified as carcinogenic by IARC and listed under Proposition 65, namely 1,3-dichloro-2-propanol (1,3-DCP), 3-monochloropropane-1,2-diol (3-MCPD), epichlorohydrin and glycidol. TDCPP is structurally similar to two halogenated phosphate triester carcinogens identified under Proposition 65, tris(2,3-dibromopropyl) phosphate (TDBPP or Tris) and tris(2-chloroethyl) phosphate (TCEP).

Some of these metabolites and structurally similar compounds induce tumors at the same sites as TDCPP – liver, kidney, testes. 1,3-DCP induces liver tumors in rats; glycidol and TDBPP induce liver tumors in mice. 1,3-DCP, 3-MCPD, TDBPP and TCEP induce kidney tumors in rats; TDBPP and TCEP induce kidney tumors in mice. 3-MCPD induces interstitial cell tumors of the testes in rats.

2 INTRODUCTION

2.1 Identity of Tris(1,3-dichloro-2-propyl) phosphate (TDCPP)

Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) is a viscous, colorless liquid at temperatures greater than 27° C. It is soluble in water and most organic solvents (IPCS, 1998). Its structure is given in Figure 1 and physical and chemical characteristics are given below.

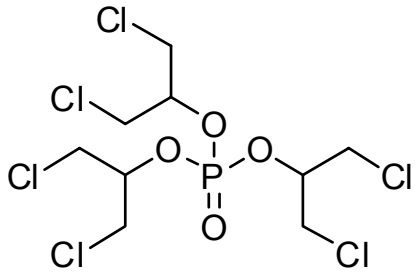


Figure 1. Chemical Structure of TDCPP.

Molecular Formula:	$C_9H_{15}Cl_6O_4P$
Molecular Weight:	430.91
CAS Registry Number:	13674-87-8
IUPAC Systematic Name:	2-Propanol, 1,3-dichloro-, phosphate (3:1)
Synonyms:	TDCPP; TDCP; chlorinated Tris; 2-Propanol, 1,3-dichloro-, phosphate (3:1); Fyrol FR-2; Antiblaze 195®, Tris[2-chloro-1-(chloromethyl)ethyl]phosphate; Tris(1,3-dichloroisopropyl)phosphate
Chemical Class:	Phosphate ester
Chemical Appearance:	Colorless
Melting Point:	27°C
Boiling point:	236-237°C (at 5 mmHg)
Water Solubility:	7 mg/L (at 24°C)
Vapor pressure:	0.01 mmHg at 30°C
Octanol-water coefficient:	$LogK_{OW}$ = 3.65

2.2 Occurrence and Use

TDCPP is produced by the epoxide opening of epichlorohydrin in the presence of phosphorus oxychlorine (HSDB, 2001). It is a high production volume chemical, primarily used as an organophosphate flame retardant in flexible polyurethane foams (U.S. EPA, 2006; European Commission, 2009; Levchik and Weil, 2004). Other reported uses are as flame retardants and plasticizers in rigid polyurethane foams,

resins, plastics, textile coatings, and rubber for use in the U.S. and Europe (IPCS, 1998; NRC, 2000). As a flame retardant, TDCPP is an additive, meaning it is not chemically reacted but physically combined with the material being treated.

Most of TDCPP's current use can be attributed to flexible polyurethane foams for upholstered furniture and automotive products such as seat cushions and headrests (European Commission, 2009). TDCPP was commonly used in children's sleepwear in the 1970s until manufacturers voluntarily withdrew it in 1977 due to concerns regarding its mutagenicity (CPSC, 1977; IPCS, 1998). More recently, in order to meet California's upholstered furniture flammability standard, Technical Bulletin 117 (California Bureau of Home Furnishings and Thermal Insulation, 2000), TDCPP has been used as a replacement for the flame retardant pentabromodiphenyl ether (pentaBDE), which was banned in 2006 (California Health and Safety Code, Section 108922). A 2011 study identified TDCPP in more than a third of the 101 baby products analyzed (*e.g.*, car seats, changing table pads) (Stapleton *et al.*, 2011).

The use of TDCPP as an additive flame retardant suggests it may be released from the treated product throughout the product life cycle into the indoor environment (*e.g.*, in dust), leading to human exposure (Marklund *et al.*, 2003; U.S. EPA, 2005; Stapleton *et al.*, 2009). Indeed, TDCPP has been detected in household dust in the U.S. and abroad (Stapleton *et al.*, 2009, Takigami *et al.*, 2009; Marklund *et al.*, 2003; Meeker and Stapleton, 2010).

In a study of 50 homes in Boston, Massachusetts, concentrations of TDCPP in dust were comparable to, and in some cases higher than, concentrations of polybrominated diphenyl ethers, with a geometric mean of 1.89 micrograms per gram (µg/g) of dust (maximum: 56.08 µg/g) (Stapleton *et al.*, 2009). TDCPP was detected in both dust and air samples in a variety of indoor environments such as homes, day care centers, hospital wards and offices in Sweden (Marklund *et al.*, 2003).

TDCPP's use as a flame retardant and plasticizer for many decades has resulted in widespread distribution in the environment. In a study of 139 streams across the U.S., including California, TDCPP was detected in over half (Kolpin *et al.*, 2002). An analysis of Swedish sewage treatment facilities found detectable concentrations of TDCPP in the influents, effluents and sludge from each of the plants studied (Marklund *et al.*, 2005).

Biomonitoring studies have detected TDCPP in human tissues. In the 1980s, levels were measured in human adipose tissue (maximum of 260 nanograms (ng)/g) (LeBel and Williams, 1983; LeBel *et al.*, 1989) and in human seminal plasma (Hudec *et al.*, 1981). More recently, TDCPP was detected in the lipids of human milk with a median level of 4.3 ng/g and a maximum level of 5.3 ng/g (Sundkvist *et al.*, 2010).

3 DATA ON CARCINOGENICITY

3.1 Carcinogenicity Studies in Humans

An unpublished retrospective cohort cancer mortality study of workers employed at a TDCPP manufacturing plant for the years 1956 to 1980 was conducted by Stauffer

Chemical Company (Stauffer Chemical Company, 1983b, as described by the European Commission, 2009, and ATSDR, 2009). The cohort consisted of 289 workers. Ten deaths were reported in the cohort over the course of the study period. Three deaths due to lung cancer were observed among the ten deaths (deaths from other malignant cancers were observed by the study authors, but not described in the European Commission, 2009, and ATSDR, 2009 reports). When the observed deaths from the study were compared to a similar population of U.S. males, standard mortality ratios (SMR) were higher than expected for all cancers and lung cancer, although p-values were not calculated due to small sample size. The average time-weighted concentration of TDCPP in air within the work environment was assessed at the end of the study period and described as very low (0.4–0.5 μg/m^3). The authors concluded that although the SMR from lung cancer was higher than expected, overall there was no evidence linking the lung cancers to TDCPP exposure because all three cases with lung cancer were heavy to moderate cigarette smokers. Small sample size and the inability to account for confounding factors make it difficult to draw conclusions from this study.

3.2 Carcinogenicity Studies in Animals

A review of the scientific literature regarding the carcinogenicity of TDCPP in experimental animals identified one set of studies conducted in rats.

Male and female Sprague-Dawley CD rats (60/sex/group) were fed a diet containing TDCPP at concentrations intended to achieve dose rates of 0, 5, 20, or 80 mg TDCPP/kg-day (Bio/dynamics, 1981; Freudenthal and Henrich, 2000). Ten male and female rats from each group were sacrificed after 12 months on the diet for interim evaluation. At 24 months, all remaining surviving animals were sacrificed. At both 12 and 24 months, control and high-dose animals were examined microscopically for lesions in a broad suite of tissues. However, for animals in the low- and mid-dose groups only the liver, kidneys, testes, and adrenal glands were examined microscopically at the 12- and 24-month sacrifices.

Survival among male rats in the high-dose group (80 mg/kg-day) was significantly lower compared to control male rats. Among high-dose male rats, body weights were 20% lower than control animals at the end of the study. Body weights of high-dose male rats were significantly lower than control rats throughout the study. Survival was not significantly affected by TDCPP treatment in female rats at any dose. Body weights of high-dose female rats were also significantly lower that control rats throughout the study, with a similar 20% decrease in body weight observed by the end of the study. Food intake was not affected by treatment in either male or female rats.

Among male rats treated with TDCPP, benign and malignant tumors were seen (see Table 1 below for all tumor incidence data). Statistically significant increases were observed in the high-dose group for hepatocellular adenoma ($p < 0.01$), hepatocellular carcinoma ($p < 0.05$), and combined hepatocellular adenoma and carcinoma ($p < 0.01$) by pairwise comparison with the control group. The incidences across dose groups showed statistically significant positive trends with dose for adenomas ($p < 0.001$), carcinomas ($p < 0.01$), and combined adenomas and carcinomas ($p < 0.001$). Three hepatocellular adenomas were also observed in high-dose male rats at the 12-month interim sacrifice.

Also among male rats treated with TDCPP, statistically significant increases in renal cortical adenomas were increased in both the mid- ($p < 0.05$) and high-dose groups ($p < 0.01$) by pairwise comparison with the control group. The incidences across all groups showed a statistically significant positive trend with dose ($p < 0.001$).

In addition, statistically significant increases in benign interstitial (Leydig) cell tumors of the testes were observed in both the mid- and high-dose male rats by pairwise comparison with the control ($p < 0.01$). The incidence across all groups showed a statistically significant positive trend with dose ($p < 0.001$). Three interstitial cell tumors were observed in each of the mid- and high-dose groups at the 12-month interim sacrifice.

Among female rats in the high-dose group treated with TDCPP, statistically significant increases in hepatocellular adenomas ($p < 0.05$) and combined hepatocellular adenomas and carcinomas ($p < 0.01$) were observed. The incidences across dose groups showed a statistically significant positive trend with dose for hepatocellular adenomas ($p < 0.005$), carcinomas ($p < 0.05$), and combined adenomas and carcinomas ($p < 0.001$). One hepatocellular adenoma was also observed in high-dose female rats at the 12-month interim sacrifice.

Also among female rats treated with TDCPP, statistically significant increases in renal cortical adenomas were observed in both the mid- and high-dose groups by pairwise comparison with the control group ($p < 0.01$). The incidences across all groups showed a statistically significant positive trend with dose ($p < 0.001$).

In addition, statistically significant increases in cortical adenomas of the adrenal gland were observed in high-dose female rats by pairwise comparison with the control group ($p < 0.05$). The incidences across all groups showed a statistically significant positive trend with dose ($p < 0.001$). Two malignant adrenal cortical carcinomas were found in the control group and one in the mid-dose group. No treatment related increase in adrenal cortical carcinomas was observed. An increased incidence of combined cortical adenomas and carcinomas was observed in the high dose by pairwise comparison ($p < 0.05$) and by trend ($p < 0.01$).

At the 12-month interim sacrifice, five animals with adrenal cortical adenomas were observed in the control group and one in the high dose group of female rats. The presence of animals with tumors in the control group at the interim sacrifice warranted further analysis. If all animals are considered together (*i.e.*, interim, unscheduled, and terminal deaths), the incidence of adrenal cortical adenomas in the high-dose group does not show a statistically significant increase above controls by pairwise comparison, but still shows a statistically significant positive trend with dose ($p < 0.01$; data not shown). Similarly, combined incidence of adrenal cortical adenomas and carcinomas among all animals (interim, unscheduled, and terminal deaths) are not significantly increased by pairwise comparison, but there is a significant positive trend with dose.

In summary, exposure to TDCPP in male and female rats caused statistically significant increases in tumors at multiple sites. Treatment-related increases in combined benign and malignant liver tumors were observed in both male and female rats. Increased incidences of benign tumors of the kidneys were also observed in both male and female rats. Interstitial cell tumors of the testes were increased in male rats. An increased

Table 1. Tumor Incidences in Male and Female Sprague-Dawley Rats Treated with TCDPP.

Organ	Tumor [a]	Dose group (mg/kg/day)				Trend test (p-value)[b]
		0	5	20	80	
Male rats						
Liver	Hepatocellular adenoma	2/45	7/48	1/48	13/46[c]	0.00055
	(Interim)[e]	0	0	0	3	
	Hepatocellular carcinoma	1/45	2/48	3/48	7/46[d]	0.0069
	(Interim)[e]	0	0	0	0	
	Combined hepatocellular adenoma and carcinoma	3/45	9/48	4/48	20/46[c]	0.0000065
Kidney	Renal cortical adenoma	1/45	3/49	9/48[d]	32/46[c]	7.0 E-17
	(Interim)[e]	0	0	0	0	
Testes	Interstitial cell tumor	7/43	8/48	23/48[c]	36/46[c]	5.0 E-12
	(Interim)[e]	0	0	3	3	
Female rats						
Liver	Hepatocellular adenomas	1/49	1/47	4/47	8/50[d]	0.0025
	(Interim)[e]	0	0	0	1	
	Hepatocellular carcinoma	0/49	2/47	2/47	4/50	0.045
	(Interim)[e]	0	0	0	0	
	Combined hepatocellular adenoma and carcinoma	1/49	2/47	5/47	12/50[c]	0.00013
Kidney	Renal cortical adenoma	0/49	1/48	8/48[c]	29/50[c]	1.3 E-15
	(Interim)[e]	0	0	0	0	
Adrenal gland	Cortical adenoma	8/48	5/48	2/36	19/49[d,f]	0.00012
	(Interim)[e]	5	0	0	1	
	Cortical carcinoma	2/48	0/48	1/36	0/49	Not significant
	(Interim)[e]	0	0	0	0	
	Combined cortical adenoma and carcinoma	9/48	5/48	3/36	19/49[d,f]	0.003

[a] Incidences presented next to tumor types represent the combined incidences from all unscheduled deaths plus the terminal sacrifice at two years (Bio/dynamics, 1981). The 12-month interim sacrifice tumor incidences are not included in the statistical analyses.
[b] Exact test for linear trend.
[c] Statistically significant increase in incidence compared to control ($p < 0.01$, by Fisher's exact test).
[d] Statistically significant increase in incidence compared to control ($p < 0.05$, by Fisher's exact test).
[e] Numbers of animals with tumors in the 12-month interim sacrifice groups were calculated by OEHHA by subtracting the combined terminal and unscheduled incidences from the combined terminal, unscheduled, and interim incidences that were presented in Bio/dynamics (1981).
[f] No statistically significant pairwise comparisons between dosed and control animals were found for adrenal tumors when the incidences from interim sacrifice were combined with the unscheduled and terminal sacrifice incidences; however, the trends for adenomas and combined adenomas and carcinomas were still significant.

incidence of adrenal gland tumors in female rats among terminal and unscheduled deaths was tempered by a finding of these tumors in control animals at the one-year interim sacrifice.

Non-neoplastic findings

In addition to the body weight (males and females) and survival (males only) effects noted above, there were several other signs of toxicity from treatment of the rats observed in the TDCPP studies.

Potentially pre-neoplastic lesions were observed in some organs in which tumors occurred. The incidence of altered hepatocellular foci was significantly increased in high-dose female rats following long-term treatment (Bio/dynamics, 1981; $p < 0.05$, by Fisher's exact test). In high dose male rats they were increased and of borderline statistical significance ($p = 0.07$). Significant increases in the incidences of hyperplasia of the convoluted tubules of the kidney were observed in both male and female rats ($p < 0.05$).

TDCPP treatment resulted in significant decreases in the hemoglobin, hematocrit, and total erythrocyte counts among high dose male and female rats (Freudenthal and Henrich, 2000). High dose male and female animals also showed lower serum alkaline phosphatase levels than the control groups. Absolute liver and kidney weights were significantly higher among both mid-and high-dose male rats. Relative weights of kidney, brain, and thyroid were increased among mid- and high-dose male rats and relative liver weight was increased among high-dose male rats. Absolute kidney weights were significantly higher among female rats in the mid- and high-dose groups, while the high-dose group of female rats also showed significant increases in relative weights of liver, brain, and thyroid. Freudenthal and Henrich (2000) also reported the animals showed "a variety of abnormalities in the livers, kidneys, and testes of the treated animals, including discoloration, masses, nodules, and cysts" and that "mid- and high-dose male animals exhibited a higher incidence of small seminal vesicles and testicular enlargement, as compared to control males."

3.3 Other Relevant Data

3.3.1 Genotoxicity

Multiple *in vitro* and *in vivo* studies have investigated the genotoxicity of TDCPP. The findings are presented in Tables 2, 3, and 4 below.

TDCPP's ability to induce reverse mutations was examined across various strains of *Salmonella typhimurium* and in the yeast *Saccharomyces cerevesiae* in the presence and absence of metabolic activation systems (S9 fraction of rodent liver microsomes). Results are summarized in Table 2. Studies in *Salmonella* strains sensitive in detecting frameshift mutations (TA 97, TA 98, TA 1537, and TA 1538) indicate that TDCPP induces frameshift mutations with or without metabolic activation. TDCPP treatment of *Salmonella* strains TA 100 and TA 1535, sensitive to base-pair substitution mutations, produced mutations with and without S9 metabolic activation. Discrepancies in results between studies of the same strain may be due to the method of metabolic activation

(Babich, 2006; Gold *et al.*, 1978). Overall, TDCPP induced mutations with and without S9 activation across multiple *Salmonella* strains. TDCPP did not induce mutations in *Saccharomyces cerevesiae*.

In *in vitro* mammalian cell assays for gene mutation, TDCPP gave both positive and negative results (Table 3). TDCPP induced gene mutations in one study in L5178Y mouse lymphoma cells (Inveresk Research International, 1985) and not in another (Brusick *et al.*, 1980). TDCPP was negative for gene mutations in V79 Chinese hamster cells (Soderlund *et al.*, 1985).

TDCPP caused an increase in chromosomal aberrations *in vitro* in mouse lymphoma and Chinese hamster fibroblast cells (Brusick *et al.*, 1980; Ishidate, 1983), but not in Chinese hamster ovary cells (Covance Laboratories Inc., 2004). TDCPP weakly induced sister chromatid exchanges (SCE) in mouse lymphoma cells using two methods of metabolic activation in one set of experiments (Brusick *et al.*, 1980), but not in another (Stauffer Chemical Company, 1977). TDCPP induced a weakly positive response in the *in vitro* rat hepatocyte DNA synthesis (UDS) assay, in the absence, but not in the presence, of phenobarbital induction (Soderlund *et al.*, 1985).

For the most part, *in vivo* genotoxicity assays of TDCPP have been negative (Table 4). Studies in *Drosophila melanogaster* did not result in an increase of sex-linked recessive lethal (SLRL) mutations (Stauffer Chemical Company, 1978). TDCPP did not induce chromosome aberrations in mouse bone marrow or chick embryos, or micronuclei in mouse bone marrow erythrocytes (Brusick et al., 1980; Bloom, 1984, Thomas and Collier, 1985). *In vivo* exposure of rats to TDCPP did not induce UDS in hepatocytes (Cifone, 2005).

In an *in vivo* study designed to evaluate covalent binding, TDCPP readily bound to DNA and proteins in liver, kidney and muscle in mice intravenously treated with TDCPP and sacrificed 6 hours later (Morales and Matthews, 1980).

Table 2. *In Vitro* Genotoxicity Studies in Non-Mammalian Species.

Endpoint	Strain	Concentrations Tested	Results		Activation System	References
			+S9	−S9		
Reverse mutations (*Salmonella typhimurium)*	TA 97	Variable: Upper limit of 10 mg/plate	**+**	**+**	Not described	Mortelmans *et al.*, 1986
	TA 98	50– 1000 µg/plate	**+**	**NT**	Not described	Ishidate, 1983*
	TA 98	Variable: Upper limit of 10 mg/plate	**+**	**+**	Not described	Mortelmans *et al.*, 1986
	TA 98	20 – 15200 µg/plate	**−**	**−**	Not described	Safepharm Laboratories Ltd., 1984*; Safepharm Laboratories Ltd., 1985*
	TA 1537	50– 1000 µg/plate	**+**	**NT**	Not described	Ishidate, 1983*
	TA 1537	Variable: Upper limit of 10 mg/plate	**+**	**+**	PCB-induced hamster S9	Mortelmans *et al.*, 1986
	TA 1537	20 – 15200 µg/plate	**−**	**−**	Not described	Safepharm Laboratories Ltd., 1984*; Safepharm Laboratories Ltd., 1985*
	TA 1538	0, 1, 10 µl/plate	**−**	**−**	PCB-induced rat S9	Prival *et al.*, 1977
	TA 1538	20 – 15200 µg/plate	**−**	**−**	Not described	Safepharm Laboratories Ltd., 1984*; Safepharm Laboratories Ltd., 1985*
	TA 100	50-250 µg/plate	**+**	**NT**	PB-induced rat S9	Brusick *et al.*, 1980
			−	**NT**	PB-induced mouse S9	
			−	**NT**	PCB-induced mouse & rat S9	
	TA 100	0-1000 µg/plate	**−**	**NT**	Human S9	Brusick *et al.*, 1980
	TA 100	50-250 µg/plate	**+**	**NT**	PB-induced mouse S9	Gold *et al.*, 1978
		20-50 µg/plate	**±**	**NT**	PCB-induced mouse & rat S9	
	TA 100	50– 1000 µg/plate	**+**	**NT**	Not described	Ishidate, 1983*

Endpoint	Strain	Concentrations Tested	Results		Activation System	References
			+S9	−S9		
	TA 100	0, 50, 125, 500, 750, 1000 µg/plate	±	−	PB-induced mouse S9	Lynn *et al.*, 1981
	TA 100	High dose: 500 µg/plate	±	NT	PCB-induced rat S9 or PB-induced mouse S9	Majeska and Matheson, 1983
	TA 100	Variable: Upper limit of 10 mg/plate	+	+	Not described	Mortelmans *et al.*, 1986
	TA 100	0-300 µmole/plate	+	−	PCB-induced rat S9	Nakamura *et al.*, 1979
	TA 100	20 – 15200 µg/plate	−	−	Not described	Safepharm Laboratories Ltd., 1984*; Safepharm Laboratories Ltd., 1985*
	TA 100	50, 250, 500, 1000 µg/plate	+	NT	PB-induced rat S9	Soderlund *et al.*, 1985
			−	NT	PB-induced rat hepatocyte monolayer activation	
	TA100	0.98 – 500 µg/plate	±	−	PCB- induced rat S9	Stauffer Chemical Company, 1983a*
			±	−	PB- induced mouse S9	
	TA 1535	0 – 50 µg/plate	−	NT	PCB-induced rat S9	Brusick *et al.*, 1980
	TA 1535	50– 1000 µg/plate	+	NT	Not described	Ishidate, 1983*
	TA 1535	0-300 µmole/plate	+	±	PCB-induced rat S9	Nakamura *et al.*, 1979
	TA 1535	Variable: Upper limit of 10 mg/plate	+	+	PCB-induced rat S9	Mortelmans *et al.*, 1986
	TA 1535	20 – 15200 µg/plate	−	−	Not described	Safepharm Laboratories Ltd., 1984*; Safepharm Laboratories Ltd., 1985*
Reverse mutations (Saccharomyces cerevesiae)	Strain S4	1.5 – 7565 µg/plate	−	−	Not described	Stauffer Chemical Company, 1976*; Stauffer Chemical Company, 1977*

+ = positive result; − = negative result; ± = weakly positive result

NT= not tested; S9= supernatant fraction from liver homogenate; PB = phenobarbital; PCB = polychlorinated biphenyls

** As reported in European Commission (2009).*

Table 3. In Vitro Genotoxicity Studies in Mammalian Species.

Endpoint	Assay System	Conc. Tested	Results		Activation System	References
			+S9	−S9		
Gene mutations	L5178Y mouse lymphoma cells (forward mutation at *Tk* locus)	0 – 0.07 µl/ml	**−**	**−**	PB-induced mouse S9	Brusick *et al.*, 1980
	L5178Y mouse lymphoma cells (forward mutation at *Tk* locus)	1.25 – 60 µg/ml; 10 – 120 µg/ml	**+**	**−**	PCB-induced mouse S9	Inveresk Research International, 1985*
	V79 Chinese hamster cells (point mutation)	0.02 mM high dose	**−**	**NT**	PB-induced rat S9	Soderlund *et al.*, 1985
Chromosomal aberrations	L5178Y mouse lymphoma cells	0.05 – 0.1 µl/ml	**+**	**±**	PCB-induced mouse S9	Brusick *et al.*, 1980
			+	**±**	PB-induced mouse S9	
	Chinese hamster fibroblast cells	Not reported	**+**	**NT**	Not described	Ishidate, 1983*
	Chinese hamster ovary cells	6.78 – 1000 µg/ml	**−**	**−**	PCB-induced rat S9	Covance Laboratories Inc., 2004*
Sister chromatid exchanges (SCE)	L5178Y mouse lymphoma cells	0.004 – 0.072 µg/ml	**±**	**±**	PCB-induced mouse S9	Brusick *et al.*, 1980
			±	**±**	PB-induced mouse S9	
	L5178Y mouse lymphoma cells	0.0047 – 0.072 µl/ml	**−**	**NT**	PCB & PB-induced mice	Stauffer Chemical Company, 1977*
Unscheduled DNA synthesis	Rat hepatocytes	0.025, 0.05, 0.10 mM	**−**	**±**	PB-induced rat hepatocytes	Soderlund *et al.*, 1985

+ = positive result; − = negative result; ± = weakly positive result
NT= not tested; S9= supernatant fraction from liver homogenate; PB = phenobarbital; PCB = polychlorinated biphenyls
** As reported in European Commission (2009).*

Table 4. *In Vivo* Genotoxicity Studies.

Endpoint	Assay System	Conc. Tested	Results	References
Sex-linked recessive lethal (SLRL) mutations	*Drosophila melanogaster*	2.5%, 25% TDCPP	−	Stauffer Chemical Company, 1978*
Chromosomal aberrations	CD-1 mouse bone marrow	0.05, 0.17, or 0.5 mL/kg for 1 or 5 days	−	Brusick *et al.*, 1980
	Chick embryo/ neonate	50 – 100 µl/embryo	−	Bloom, 1984
Micronuclei	CFLP mouse bone marrow erythocytes	200, 630, 2000 mg/kg	−	Thomas and Collier, 1985**
Unscheduled DNA synthesis	Rat hepatocytes	500, 1000, 2000 mg/kg	−	Cifone, 2005**
DNA binding assay	CD-1 mouse liver, kidney and muscle	94.4 µmol/kg	+	Morales and Matthews, 1980

+ = positive result; − = negative result; ± = weakly positive result
** As reported in European Commission (2009).*
*** As reported in Babich (2006).*

3.3.2 In Vitro Transformation Studies

TDCPP was tested in *in vitro* cell transformation assays using BALB/c 3T3 cells and Syrian hamster embryo (SHE) cells (see Table 5 below). These assays are designed to detect a change in growth pattern of fibroblasts that is indicative of loss of contact inhibition, a phenotype that is characteristic of cancer cells.

TDCPP did not induce transformed foci in the BALB/c 3T3 cells (Brusick *et al*., 1980), but was positive in SHE cells in two separate experiments (Soderlund *et al.*, 1985). In the first SHE cell experiment, 20 µM TDCPP resulted in a transformation frequency of 1.85%. In the second experiment, 30µM TDCPP induced a transformation frequency of 1.35%. A transformation frequency greater than one percent was considered by the authors to be a positive response.

Table 5. *In Vitro* Cell Transformation Assays.

Endpoint	Assay System	Conc. Tested	Results	References
Morphological transformation	BALB/c 3T3 cells	0.02 – 0.312 µl/ml	**–**	Brusick *et al.*, 1980
	Syrian hamster embryo cells	Control, 20 µM	**+**	Soderlund *et al.*, 1985
		Control, 10 µM, 30 µM	**+**	

+ = positive result; − = negative result; ± = weakly positive result

3.3.3 Pharmacokinetics and Metabolism

TDCPP is readily absorbed following dermal application and oral administration of ^{14}C-labeled compound to Sprague-Dawley rats (Nomeir *et al.*, 1981). More than 90% of an oral dose of TDCPP was absorbed within 24 hours of administration to rats. Oral dosing or dermal application of TDCPP resulted in rapid distribution through the blood and to the liver, lung, kidney, adipose tissue and muscle. The half-life for clearance from various tissues ranged from 1.5 to 5.4 hours. TDCPP is also reported to be well absorbed from dermal application to rabbit skin (Ulsamer *et al.*, 1980). Rabbit liver and kidney were also reported to accumulate the highest levels of radiolabeled TDCPP.

The elimination of TDCPP from Sprague-Dawley rats administered *propyl*-1,3-^{14}C-labeled TDCPP has also been reported in several studies. Within 10 days, 47% of radioactivity from an applied dose of TDCPP was found in the urine, with less than half that amount found in feces (~21%) (Nomeir *et al.*, 1981). The finding of substantial levels of radiolabeled compound in bile relative to feces (~27% within four hours), suggests that a portion of that present in bile was subsequently reabsorbed from the gastrointestinal tract. Approximately 20% of the applied intravenous dose was eliminated as carbon dioxide in exhaled air (Nomeir *et al.*, 1981). In other studies, approximately 98% of an oral dose of ^{14}C-labeled TDCPP administered to Wistar rats was eliminated after seven days, with radioactivity appearing in urine (~43%), feces (~39%), and expired air (~16%) (Minegishi *et al.*, 1988). Similarly, Lynn *et al.* (1981) report that 92% of an intravenous dose of *propyl*-1,3-^{14}C-labeled TDCPP administered to Sprague-Dawley rats was excreted within five days in urine (~54%), feces (~16%), and expired air (22%).

In the Nomeir *et al.* studies, less than one percent of the applied intravenous dose was eliminated intact as TDCPP after 24 hours. Isolation and identification of urinary metabolites was carried out by acidification, drying, then reaction with diazomethane, followed by thin-layer chromatography (isolation), then extraction and high-performance liquid chromatography (HPLC) analysis (identification). Analysis of urine collected from rats up to 120 hours following treatment showed the diester, bis(1,3-dichloro-2-propyl) phosphate (BDCPP), to be the primary metabolite (67%), with much of the balance an unidentified polar metabolite (see Figure 2) (Nomeir *et al.*, 1981). Trace amounts of the monoester, 1,3-dichloro-2-propyl phosphate, and unmetabolized TDCPP were also found in the urine (0.29 and 0.45%, respectively).

In the Lynn *et al.* (1981) studies, urine from rats administered TDCPP intravenously was analyzed for metabolites by gas chromatographic-electron impact-mass spectroscopy. Briefly, urine samples were extracted with ether, acidified, and re-extracted. The aqueous residue was dissolved in pyridine and N,O-bis-(trimethylsilyl) trifluoroacetamide with trimethylchlorosilane to produce derivatives of the possible metabolites. The monotrimethylsilyl derivative of BDCPP was identified, accounting for 63% of the radioactivity present in the urine (Lynn *et al.*, 1981).

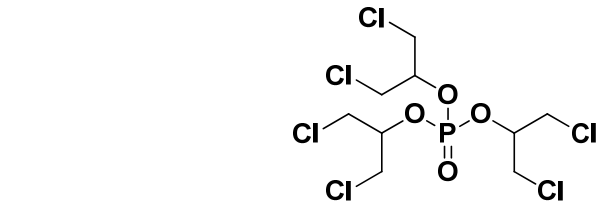
Lynn *et al.* also evaluated metabolites of rats treated either intraperitoneally or intravenously with TDCPP, creating both trimethylsilyl and methylation derivatives for analysis by HPLC and gas chromatography mass spectrometry (GC/MS) following extraction (Lynn *et al.*, 1981). BDCPP was identified in the urine from the i.p. studies, as well as in urine, feces, and bile from the i.v. administration studies. A methylated derivative of the monoester, 1,3-dichloro-2-propyl phosphate (MDCPP), was also identified in urine and bile. 1,3-Dichloro-2-propanol (1,3-DCP) was also identified as a urinary metabolite by chloroform extraction of urine followed by GC/MS analysis (no derivatization step).

Ulsamer *et al.* reported 1,3-DCP as the only metabolite detected in the urine of TDCPP-treated animals (rats and rabbits; Ulsamer *et al.*, 1980). Experimental details were not provided.

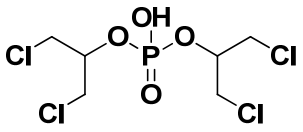
In vitro studies by Nomeir *et al.* (1981) examined the ability of various rat liver fractions and rat blood plasma to metabolize TDCPP. Soluble liver fractions (both 10,000*g* and 100,000*g* supernatants) were able to metabolize TDCPP, and this was true to a lesser extent for microsomal and mitochondrial fractions. Rat blood plasma had relatively weak ability to metabolize TDCPP. Metabolism by liver fractions was enhanced by the addition of either glutathione or NADPH. In microsomal fractions, NADPH substantially increased the metabolism of TDCPP. Microsomal metabolism was decreased by the addition of SKF 525A, an inhibitor of cytochrome P450 mixed function oxidase activity.

Several products of metabolism were identified by *in vitro* reaction of TDCPP with microsomal fractions of male rat liver (Nomeir *et al.*, 1981). Metabolites were isolated using silica gel plates that were then identified using HPLC. The primary metabolites identified from this *in vitro* system were 1,3-DCP, 3-MCPD [3-chloro-1,2-propanediol], and BDCPP, as well as unknown metabolites. 3-MCPD were identified by chromatography, while 1,3-DCP was identified following methylation or acetylation, then thin-layer chromatography and HPLC. BDCPP, 3-MCPD, and the unknown metabolites increased over time in this system, though levels of 1,3-DCP remained somewhat steady. The authors hypothesized that this was due to the conversion of 1,3-DCP to 3-MCPD. Neither 3-MCPD nor 1,3-DCP was detected in the urine of TDCPP-exposed rats in the *in vivo* Nomeir *et al.* studies. The authors hypothesized that these metabolites identified in the *in vitro* studies were further metabolized and released (or reincorporated by other metabolic processes) as carbon dioxide before they could be excreted *in vivo*.

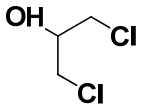
Figure 2. TDCPP and its Metabolites.



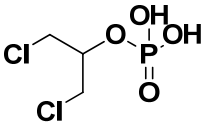
tris(1,3-dichloro-2-propyl) phosphate
(TDCPP)



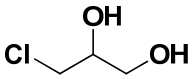
bis(1,3-dichloro-2-propyl) phosphate
(BDCPP)



1,3-dichloro-2-propanol
(1,3-DCP)



1,3-dichloro-2-propyl phosphate
(MDCPP)



3-monochloro-1,2-propanediol
(3-MCPD)

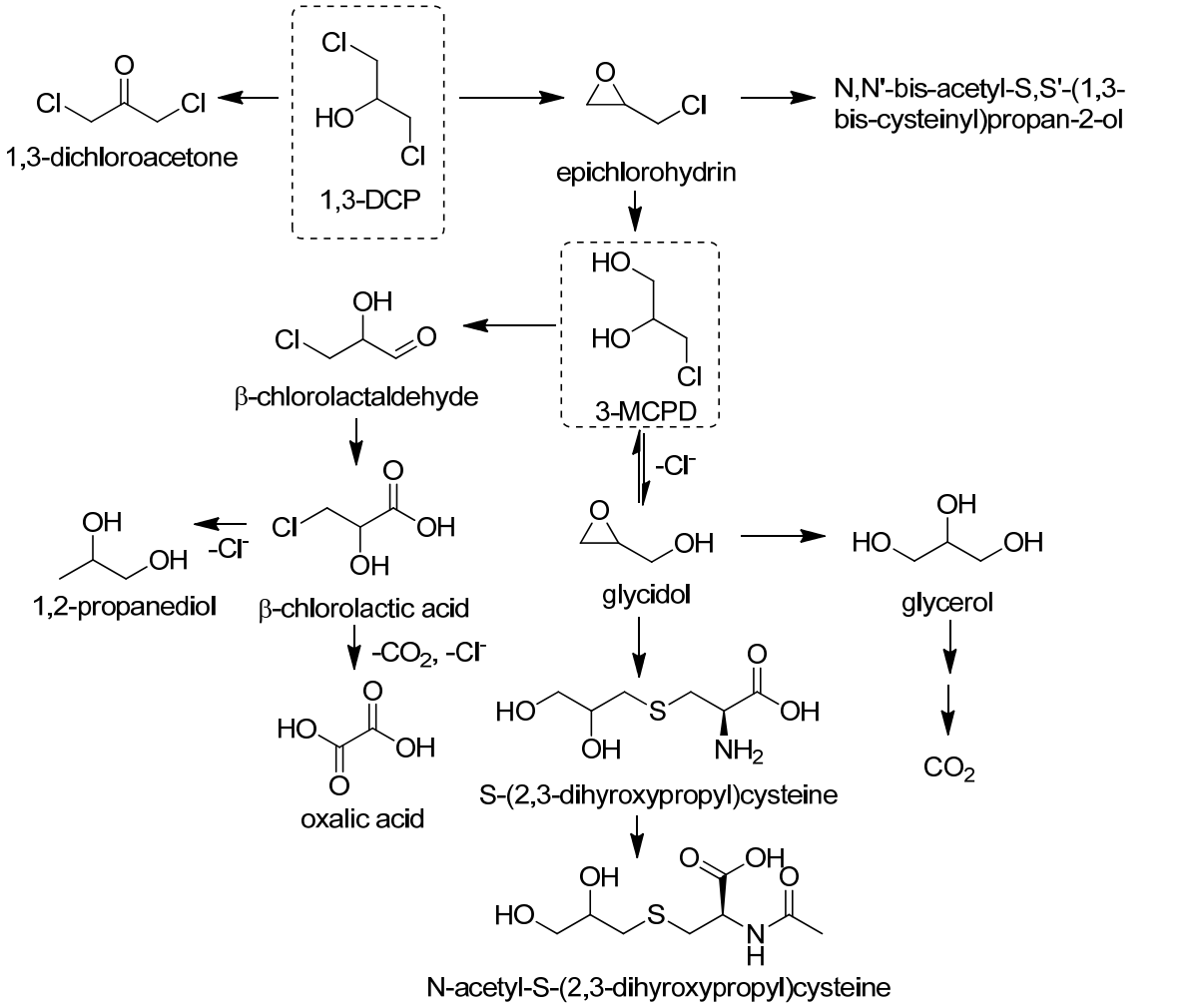
Soluble fraction metabolism *in vitro* was increased by the addition of glutathione. The one major metabolite from this reaction was tentatively identified as glutathione-conjugated TDCPP. This was based on evidence that this metabolite was ninhydrin-positive, indicating the presence of an unprotected amine-containing group, and heating the metabolite produced two breakdown products, BDCPP, and another ninhydrin-positive product.

The metabolism of two TDCPP metabolites, 1,3-DCP and 3-MCPD, was recently characterized (see Figure 3 below) (OEHHA, 2010a; OEHHA, 2010b). 1,3-DCP can be metabolized by two main pathways, one leading directly to the formation of 1,3-dichloroacetone, a mutagen and skin tumor initiator. The other pathway leads to the formation of epichlorohydrin, a genotoxic carcinogen. Epichlorohydrin can either be conjugated with glutathione then further converted to a mercapturic acid or it can be metabolized to 3-MCPD. 3-MCPD can be metabolized to glycidol, another genotoxic carcinogen, or to β-chlorolactaldehyde. β-Chlorolactaldehyde can form either 1,2-propanediol or oxalic acid. Glycidol can either be conjugated with glutathione then further converted to a mercapturic acid or it can be metabolized to glycerol.

Diester phosphate formation in urine has been found to occur in rats from metabolism of another halogenated phosphotriester compound, tris(2,3-dibromopropyl) phosphate (Lynn *et al.*, 1980). Early studies of the urine of rats and mice administered tri-alkyl phosphate compounds with simple alkyl groups, such as trimethyl-, triethyl-, tri-*n*-propyl-, tri-isopropyl-, and tri-*n*-butylphosphate, identified the presence of diesters (Jones, 1970). Little mono-ester was observed in the urine in the Jones study. Diester

phosphate compounds including bis(2-chloroethyl)-, diphenyl-, di-*m*-cresyl-, and di-*p*-cresyl-, bis(2-chloropropyl)-, and di-*n*-butyl phosphate have been detected in human urine (Schindler *et al.*, 2009a; Schindler *et al.*, 2009b). These are likely metabolites resulting from exposure to the corresponding phosphotriester flame retardants.

Figure 3. Metabolism of 1,3-DCP and 3-MCPD.



(Adapted from OEHHA, 2010a & 2010b)

In an early publication that addressed the subject of TDCPP's metabolism, Gold *et al.* (1978), hypothesized several possible metabolites and mechanisms based on the similarities to other phosphotriester compounds. Oxidative dealkylation was proposed to produce 1,3-dichloropropanone [1,3-dichloroacetone] with subsequent 1,3-DCP formation. Phosphotriester hydrolase acting on TDCPP was proposed to produce 1,3-DCP directly. Glutathione S-transferases were proposed to result in the formation of glutathione chloro-thio-ethers. Lynn *et al.* (1980) hypothesized that three enzyme systems are capable of cleaving ester bonds of organophosphorus alkyl triesters: mixed function oxidase, hydrolase, and glutathione-S-alkyl transferase.

The overall evidence from *in vitro* and *in vivo* studies, along with similarities to evidence from related compounds, suggests that multiple metabolic systems may play a role in the metabolism of TDCPP. The phosphodiester metabolite is the most prevalent urinary metabolite, though 1,3-DCP and the monoester have also been detected in rats and rabbits (1,3-DCP only). *In vitro* studies report production of 1,3-DCP and 3-MCPD from liver homogenate fractions. Differences in experimental methods of detection or rapid metabolism to further breakdown products may explain the lack of detection of products of the moiety cleaved from the phosphotriester in *in vivo* systems.

3.3.4 Animal Tumor Pathology

TDCPP significantly increased the incidence of combined benign and malignant liver tumors in male and female rats, benign renal tumors in male and female rats, and testicular interstitial cell tumors in male rats. An increase in benign adrenal tumors was observed in female rats, but this increase was not statistically significant by pairwise comparison when tumors observed at the 12-month sacrifice were included (Bio/dynamics, 1981; Freudenthal and Henrich, 2000).

The liver tumors observed in treated male and female rats were identified as neoplastic nodules and hepatocellular carcinomas in the original report (Bio/dynamics, 1981). The more recent publication of these studies' results in the open literature refers to the nodules as hepatocellular adenomas (Freudenthal and Henrich, 2000), consistent with current pathology nomenclature. 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 study results (IARC, 2006; McConnell *et al.*, 1986).

The benign kidney tumors observed in treated male and female rats were identified as "renal cortical tumors" in the original report (Bio/dynamics, 1981), and as "renal cortical adenomas" in the more recent publication of these studies' results (Freudenthal and Henrich, 2000). These proliferative lesions of the renal cortex tend to be characterized currently as renal cell adenomas. While no malignant renal cell *carcinomas* were observed in these studies, renal cell adenomas are known to progress to carcinomas.

Testicular interstitial cell tumors, also referred to as Leydig cell tumors, were observed in male Sprague-Dawley rats. Leydig cells are located in the interstitium of the testis, between the seminiferous tubules. There is a continuum of Leydig cell proliferative response, ranging from hyperplasia to adenomas and carcinomas (Boorman *et al.*, 1990). Differential diagnosis is based on size of the lesion. Leydig cell adenomas and carcinomas are aggregated for carcinogen identification (IARC, 2006; McConnell *et al.*, 1986). In Sprague-Dawley rats, the spontaneous incidence of Leydig cell tumors is generally low (~1% incidence).

The benign adrenal gland tumors observed in treated female rats were identified as "adrenal cortical adenomas" by Freudenthal and Henrich (2000). While no increases in malignant adrenal cortical tumors were observed in the female rats, the adenomas are considered to have the potential to progress from benign to malignant phenotypes (Duprat *et al.*, 1990).

3.3.5 Structure-Activity Comparisons

TDCPP is a halogenated phosphate triester that shares structural similarity with several other compounds. TDCPP's metabolites also present concerns for potential carcinogenicity.

TDCPP Metabolites

Several compounds that are potential products of the metabolism of TDCPP are known to cause cancer (see Figure 3 and Table 6).

1,3-DCP, a metabolite of TDCPP detected in rat and rabbit urine, is a chlorinated three-carbon alcohol that is further metabolized to 3-MCPD via the formation of epichlorohydrin. 1,3-DCP induced tumors in male and female rats (kidney, liver, tongue, thyroid) and is genotoxic in *in vitro*, but not *in vivo* assays (OEHHA, 2010a).

3-MCPD, a metabolite of 1,3-DCP, and therefore also a metabolite of TDCPP[3], is a chlorinated three-carbon alcohol (OEHHA, 2010b). 3-MCPD induced tumors in male and female rats (kidney, Leydig cell tumors of testes, mammary gland), and is genotoxic in *in vitro*, but not *in vivo* assays.

Epichlorohydrin is a chlorinated three-carbon epoxide compound that is a direct metabolite of 1,3-DCP and an intermediate in the formation of 3-MCPD. Epichlorohydrin is carcinogenic in male and female rats (forestomach, nasal cavity) and male mice (lung) and is genotoxic *in vitro* without metabolic activation and in several *in vivo* assays (ILS, 2005; IARC, 1999).

Another direct metabolite of 1,3-DCP is 1,3-dichloroacetone (1,3-DCA). 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 erythrocytes of the newt, *Pleurodeles waltl* (IARC, 1995).

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, inducing tumors at multiple sites, and is genotoxic *in vitro* without metabolic activation and *in vivo* (IARC, 2000).

The primary metabolite of TDCPP found in the urine of exposed animals is the diester BDCPP. This compound has not been tested for carcinogenicity in experimental animals. Limited testing in *S. typhimurium in vitro* has provided no evidence for mutagenicity.

Chemicals Structurally-Related to TDCPP

Tris(2,3-dibromopropyl) phosphate (TDBPP; Tris), a brominated analogue of TDCPP, is a phosphate triester that is halogenated with bromine instead of chlorine (see Table 6 below). TDBPP is carcinogenic in both sexes of rats and mice, inducing tumors at multiple sites in mice, and is genotoxic *in vitro* and *in vivo* (IARC, 1999).

[3] Detected following *in vitro* incubation of rat liver homogenate fractions with TDCPP (Nomeir *et al.*, 1981).

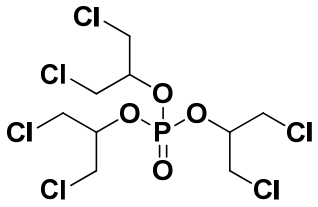
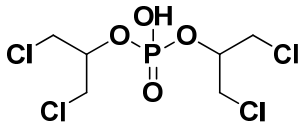
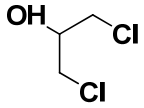
Tris(2-chloroethyl) phosphate (TCEP) is a chlorinated phosphate triester. TCEP induces tumors in both sexes of rats and mice, inducing tumors at multiple sites in rats, and is genotoxic *in vitro* and *in vivo* (IARC, 1999).

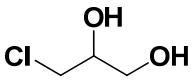
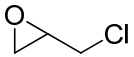
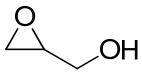
Tris(1-chloro-2-propyl) phosphate (TCPP) is another chlorinated phosphate triester. TCPP has not been tested in long-term studies for carcinogenicity in experimental animals. TCPP is genotoxic in *in vitro*, but not *in vivo* assays (European Commission, 2008).

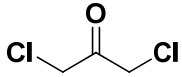
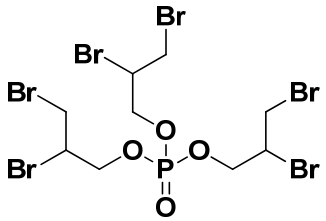
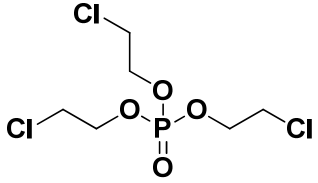
Structure-Activity Summary

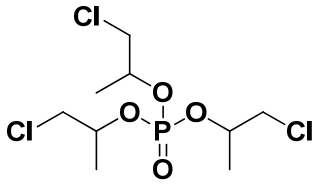
The compounds discussed here are metabolites of TDCPP or are structurally similar to TDCPP. Several of the compounds included in Table 6 have positive carcinogenicity data in rodent studies and are listed under Proposition 65 as causing cancer and/or are classified by IARC as Group 2A or Group 2B carcinogens (*i.e.,* 1,3-DCP, 3-MCPD, epichlorohydrin, glycidol, TDBPP, TCEP). Most of the compounds included in Table 6 induce tumors at multiple sites, and in most cases in more than one sex/species. Liver and/or kidney tumors were induced by TDCPP and several TDCPP metabolites (*i.e*., 1,3-DCP, 3-MCPD, glycidol) and structurally similar halogenated phosphate triesters (*i.e*., TDBPP, TCEP). Interstitial cell tumors of the testes were induced by TDCPP and 3-MCPD. All of the compounds in Table 6, except BDCPP, have positive results in genotoxicity assays performed *in vitro*. Of the compounds in Table 6 tested for *in vivo* genotoxicity, all but three, 1,3-DCP, 3-MCPD, and TCPP, have some positive results.

Table 6. Structure-Activity Comparisons for TDCPP and its Metabolites.

Chemical	Target Tumor Sites		Genotoxicity	Cancer Classification	
	Mice	Rats		Prop. 65	IARC
TDCPP  Metabolized to: BDCPP, 1,3-DCP, and 3-MCPD, among others	Not tested	*Males:* Liver, kidney, testes *Females:* Liver, kidney, adrenal gland	*In vitro*: positive *In vivo* : positive (mouse kidney, liver and muscle DNA binding) and negative (*Drosophila* SLRL, mouse bone marrow and chick embryo CA, mouse MN, rat UDS)	Under evaluation	Not evaluated
Metabolites of TDCPP					
Bis(1,3-dichloro-2-propyl)phosphate (BDCPP)[1]  Metabolite of: TDCPP	Not tested	Not tested	*In vitro:* negative	Not evaluated	Not evaluated
1,3-DCP[2]  Metabolized to: 1,3-dichloroacetone; epichlorohydrin; 3-MCPD; glycidol; among others	Not tested	*Males*: liver, kidney, tongue, thyroid *Females*: liver, tongue, thyroid	*In vitro*: positive *In vivo*: negative (*Drosophila* wing spot mutation, rat bone marrow MN, rat UDS)	Listed	2B[3] (Grosse *et al.*, 2011)

Chemical	Target Tumor Sites		Genotoxicity	Cancer Classification	
	Mice	Rats		Prop. 65	IARC
3-MCPD[2]  Metabolite of: 1,3-DCP & epichloro-hydrin Metabolized to: glycidol; among others	No treatment related tumors	*Males*: kidney, testes, mammary *Females*: kidney	*In vitro*: positive *In vivo*: negative (*Drosophila* 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)	Listed	2B (Grosse *et al.*, 2011)
Epichlorohydrin[2]  Metabolite of: 1,3-DCP Metabolized to: 3-MCPD; among others	*Male*: lung	*Males:* forestomach, nasal cavity *Females*: forestomach	*In vitro:* positive *In vivo*: positive (binds in rats and mice to DNA, *Drosophila* SLRL mutation; mouse bone marrow SCE and CA) and negative (mouse MN, mouse dominant lethal)	Listed	Group 2A[4] (1999)
Glycidol[2]  Metabolite of: 1,3-DCP, 3-MCPD, and epichlorohydrin	*Males*: liver, lung, mammary, skin, thyroid, subcutis, Harderian gland, forestomach *Females:* mammary, subcutis, Harderian gland, uterus	*Males:* thyroid, mammary, tunica vaginalis, brain, forestomach, intestine, skin, Zymbal's gland *Females*: mouth/tongue, mammary, brain, forestomach, leukemia, clitoral gland	*In vitro:* positive *In vivo*: positive (mouse MN) and negative (mouse bone marrow CA)	Listed	Group 2A (2000)

Chemical	Target Tumor Sites		Genotoxicity	Cancer Classification	
	Mice	Rats		Prop. 65	IARC
1,3-Dichloroacetone (1,3-DCA; 1,3-Dichloropropanone)[5]  Metabolite of: 1,3-DCP	Not tested Skin tumor initiator	Not tested	*In vitro:* positive *In vivo:* positive (newt MN)	Not evaluated	Not evaluated
Chemicals Structurally-Related to TDCPP					
Tris(2,3-dibromopropyl) phosphate (TDBPP, Tris) 	*Males*: kidney, lung, forestomach *Females*: liver, lung, forestomach, skin, oral cavity	*Males*: kidney *Females*: kidney	*In vitro*: positive (*Salmonella* mutations, V79 Chinese hamster lung cell mutations, Chinese hamster lung cells SCE, rat liver and testicular cell DNA strand breaks, rat kidney and liver DNA binding, SHE and C3H mouse cell transformation) *In vivo*: positive (*Drosophila* SLRL, somatic and germ cell mutations, mouse kidney mutations, rat kidney DNA single strand breaks, B6C3F$_1$ mouse and Chinese hamster bone marrow MN, rat liver MN, rat kidney and liver DNA binding)	Listed	Group 2A (1987; 1999)
Tris(2-chloroethyl) phosphate (TCEP)[6] 	*Males:* kidney (marginal increase) *Females:* Harderian gland (marginal increase)	*Males:* kidney, thyroid, leukemia, brain *Females:* kidney, thyroid, brain	*In vitro:* positive (*Salmonella* mutations, Chinese hamster lung V79 cells SCE, SHE and C3H mouse cell transformation) *In vivo:* positive (rat dominant lethal)	Listed	Group 3[7] (1999)

Chemical	Target Tumor Sites		Genotoxicity	Cancer Classification	
	Mice	Rats		Prop. 65	IARC
Tris(1-chloro-2-propyl) phosphate (TCPP; Tris(2-chloro-1-methylethyl) phosphate)[8] 	Not tested	Not tested	*In vitro:* positive (*Salmonella* and mouse lymphoma cell mutations, mouse BALB/c 3T3 cell transformation) *In vivo:* negative (mouse MN, rat bone marrow CA, UDS, DNA damage [comet assay] in rat liver)	Not evaluated	Not evaluated

CA = chromosomal aberrations; MN = micronuclei; SCE = sister chromatid exchange; SLRL = sex-linked recessive lethal; UDS = unscheduled DNA synthesis.

[1] Lynn *et al.*, 1981
[2] As reviewed in OEHHA (2010a)
[3] IARC Group 2B: Possibly carcinogenic to humans
[4] IARC Group 2A: Probably carcinogenic to humans
[5] As reported in the IARC review of 1,2,3-trichloropropane (IARC, 1995)
[6] NTP, 1991.
[7] IARC Group 3: Not classifiable as to its carcinogenicity to humans
[8] As reviewed by European Commission (2008)

4 MECHANISMS

TDCPP induced benign and malignant tumors in the liver and benign tumors of the kidney and testes in Sprague-Dawley rats, with evidence of a positive trend in benign tumors of the adrenal gland in females. The mechanism by which TDCPP induces tumors at these various tissues is unknown. However, a body of evidence suggests that TDCPP is likely to be carcinogenic by a genotoxic mechanism or mechanisms.

TDCPP tested positive in a variety of genotoxicity assays (described in Section 3.3.1 Genotoxicity above). Evidence for genotoxicity includes positive tests for mutagenicity in multiple strains of *Salmonella* and in mouse lymphoma cells, chromosomal aberrations in mouse lymphoma and hamster fibroblast cells, SCE in mouse lymphoma cells, and UDS in rat hepatocytes exposed *in vitro*. There is also evidence for DNA binding in mouse liver, kidney and muscle following *in vivo* exposure. Further, TDCPP is metabolized to several genotoxic and carcinogenic metabolites, including 1,3-DCP, epichlorohydrin, 3-MCPD, and glycidol (see Section 3.3.3 Pharmacokinetics and Metabolism above).

Potentially pre-neoplastic lesions were observed in some organs in which tumors occurred. The incidence of altered hepatocellular foci was significantly increased in high-dose female rats ($p < 0.05$, by Fisher's exact test) (Bio/dynamics, 1981), while altered foci were only slightly increased in male rats in the high-dose group ($p = 0.07$). Significant increases in the incidences of hyperplasia of the convoluted tubules of the kidney were observed in both male and female rats ($p < 0.05$). The triggers for these proliferative responses and their relationship to the development of tumors in the liver or kidney are unknown. It is possible that TDCPP causes tumors by more than one mechanism, and different mechanisms may be responsible for the tumors observed in the different tissues.

In summary, while the mechanism(s) of carcinogenic action of TDCPP remain unknown, the available evidence suggests that genotoxicity is involved. Evidence for TDCPP's genotoxic action includes evidence from a number of *in vitro* test systems, *in vivo* DNA binding studies, metabolism to genotoxic carcinogens, and similarity to two other genotoxic and carcinogenic halogenated phosphate triesters. Other mechanisms, yet to be elucidated, may also be operative.

5 REVIEWS BY OTHER AGENCIES

TDCPP has not been classified as to its potential carcinogenicity by the U.S. EPA, IARC, the U.S. Food and Drug Administration, the National Toxicology Program, or the National Institute for Occupational Safety and Health.

The data relating to the carcinogenicity of TDCPP has, however, been reviewed by several other agencies or organizations:

- The National Research Council, in a report prepared for the Consumer Product Safety Commission, concluded that "[t]he available animal data on TDCPP provide sufficient evidence of carcinogenicity in rats following chronic oral exposure" (NRC, 2000).

- A preliminary staff report prepared by the U.S. Consumer Product Safety Commission concluded: "[TDCPP] exposure also induced tumors at multiple doses in the kidneys and liver of both male and female rats. Therefore, TDCP [TDCPP] may be considered a probable human carcinogen based on sufficient evidence in animals … This conclusion is further supported by structural similarity to another animal carcinogen, TRIS. TDCP [TDCPP] and its metabolites were genotoxic in some assays, although the majority of tests were negative." (Babich, 2006)
- A report from the European Union prepared by Rapporteur Member States Ireland and the United Kingdom classified TDCPP as Carcinogen Category 3 (R40), "limited evidence of a carcinogenic effect" (European Commission, 2009).

6 SUMMARY AND CONCLUSIONS

6.1 Summary of Evidence

Chronic exposure to TDCPP significantly increased the incidence of combined benign and malignant liver tumors, and benign tumors of the kidney and testes in two-year dietary studies conducted in male and female Sprague-Dawley CD rats. A positive trend with dose in combined benign and malignant adrenal gland tumors was also observed in female rats.

The following increases in tumors were observed:

Liver tumors

- In male rats, TDCPP significantly increased the incidence of hepatocellular adenomas, hepatocellular carcinomas, and combined adenomas and carcinomas in the high-dose group as compared with the control group.
- In female rats, TDCPP significantly increased the incidence of hepatocellular adenomas and combined adenomas and carcinomas in the high-dose group as compared with the control group.

Kidney tumors

- In male rats, TDCPP significantly increased the incidence of renal cortical adenomas in the mid- and high-dose groups as compared with the control group.
- In female rats, TDCPP significantly increased the incidence of renal cortical adenomas in the mid- and high-dose groups as compared with the control group.

Testicular tumors

- Interstitial cell tumors of the testes were significantly increased among male rats treated with TDCPP (mid- and high-dose groups compared to the control group).

Adrenal gland tumors

- Cortical tumors of the adrenal gland were significantly increased by trend test among female rats treated with TDCPP, including when tumors observed at the 12-month interim sacrifice were included in the analysis. No positive trends in malignant cortical tumors of the adrenal gland were observed, however.

Evidence of TDCPP's genotoxicity comes from the following non-mammalian and mammalian test systems:

In vitro:

- TDCPP induced both base-pair substitution and frameshift mutations in *Salmonella typhimurium* in the presence or absence of exogenous metabolic activation.
- TDCPP induced forward mutations in mouse lymphoma cells.
- TDCPP induced chromosomal aberrations in mouse lymphoma and Chinese hamster fibroblast cells.
- TDCPP induced SCEs in mouse lymphoma cells.
- TDCPP induced unscheduled DNA synthesis in rat hepatocytes.

In vivo:

- TDCPP bound to DNA and proteins in mouse liver, kidney and muscle cells.

TDCPP induced malignant transformation of SHE cells in culture.

Metabolites of TDCPP and structurally similar halogenated phosphate triesters present concern regarding the carcinogenicity of TDCPP:

- Multiple metabolites of TDCPP are carcinogens identified by IARC and listed under Proposition 65: 1,3-DCP, 3-MCPD, epichlorohydrin and glycidol. Each are genotoxic *in vitro*. Epichlorohydrin and glycidol are genotoxic *in vivo.*
- TDCPP is structurally similar to the halogenated phosphate triesters TDBPP, TCEP and TCPP. TDBPP and TCEP are listed under Proposition 65 as carcinogens and are genotoxic *in vitro* and *in vivo*. TDBPP has been identified by IARC as a carcinogen.
- Several TDCPP metabolites and structurally similar halogenated phosphate triesters induce tumors at the same sites as TDCPP (liver, kidney, testes):
    - 1,3-DCP induces liver tumors in rats; glycidol and TDBPP induce liver tumors in mice
    - 1,3-DCP, 3-MCPD, TDBPP and TCEP induce kidney tumors in rats; TDBPP and TCEP induce kidney tumors in mice
    - 3-MCPD induces testes (interstitial cell) tumors in rats.

6.2 Conclusions

Evidence for carcinogenicity of TDCPP comes primarily from two-year diet studies conducted in both sexes of Sprague-Dawley rats. Exposure to TDCPP in male and female rats resulted in statistically significant increases in tumors at multiple sites. In male rats, an increased incidence of benign, malignant and combined benign and malignant liver tumors was observed. Increases in benign tumors of the kidneys and testes were also found in male rats. The incidence of benign and combined malignant

and benign liver tumors was significantly increased in female rats. Benign kidney tumors were also significantly increased in female rats.

Positive findings in multiple *in vitro* genotoxicity test systems indicate that TDCPP may be carcinogenic through a genotoxic mechanism. TDCPP induced mutations in multiple strains of *Salmonella typhimurium* and in mouse lymphoma cells. It induced chromosomal aberrations in mouse lymphoma and hamster fibroblast cells, increased the formation of SCE in mouse lymphoma cells, and induced unscheduled DNA synthesis in rat hepatocytes. In an *in vivo* study, TDCPP bound to DNA and proteins in mouse liver, kidney and muscle. TDCPP also induced malignant transformation of SHE cells in culture.

TDCPP is structurally similar to two halogenated phosphate triester carcinogens identified under Proposition 65 (TDBPP, TCEP) and is metabolized to several chemicals identified as carcinogenic by IARC and listed under Proposition 65 (1,3-DCP, 3-MCPD, epichlorohydrin, glycidol).

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