Albemarle Corporation
&
ICL North America

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

Comments on July 2011 "Evidence of Carcinogenicity" Document Prepared by OEHHA and Arguments Opposing Listing TDCPP as a Carcinogen Under Proposition 65

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1. Executive Summary

The CIC's Guidance Criteria for Identifying Chemicals for Listing compounds as "Known to the State to Cause Cancer" summarizes that "if the weight of scientific evidence clearly shows that a certain chemical causes invasive cancer in humans, or that it causes invasive cancer in animals (unless the mechanism of action has been shown not to be relevant to humans), the committee will normally identify that chemical for listing." (CIC, 2001) As we detail below, TDCPP does not satisfy this criteria and should not be listed as a carcinogen under Proposition 65. We support our conclusion with the following arguments

- **TDCPP is differentiated from structurally similar chemicals that are carcinogens by metabolism to structurally distinct, lower toxicity, metabolites.** The body of research on TDCPP metabolism, including a new comparative metabolism study on TDCPP and structurally similar compounds (Fabian & Landsiedel, 2009), demonstrates that TDCPP, in contrast to the other compounds, is very rapidly and nearly completely conjugated prior to further metabolism. These results are consistent with previous assessments of TDCPP metabolism and elimination (Lynn et al., 1981 and Nomeir et al., 1981) which detect the production of the "bi-ester" from the "tri-ester" phosphate, but not the corresponding propanyl moieties which are presumed to be pre-conjugated and rapidly eliminated as conjugated "polar metabolites" or exhaled as CO2 following metabolism of the conjugated compounds. This apparent process results in a metabolic profile that is distinct in structure and biological activity from the metabolites generated by structurally similar compounds. The data from these metabolism studies in conjunction with the lack of TDCPP genotoxicity in mammals indicate that the

biological actions of these structurally similar compounds vary, and caution should be exercised in extrapolating among structurally similar molecules. The biologically active metabolites noted by OEHHA (2011) would not be expected to be present at any significant level upon exposure to TDCPP and, therefore, presumptive extrapolations among these compounds regarding potential carcinogenicity are inappropriate.

- **The weight of the evidence demonstrates that TDCPP is not genotoxic.** The two most recent and comprehensive assessments of the *in vitro* and *in vivo* database on TDCPP genetic toxicity (EBRC, 2005; ECHA, 2010) conclude, respectively, that "it is reasonable to assume that TDCPP is devoid of genotoxic potential" and that "Regarding notably the five negative *in vivo* assays, it is considered that TDCP[P] is not genotoxic *in vivo* and thus no classification for mutagenicity is proposed [for the EU]." The 2005 assessment of the database used "Klimisch et al" ranking for study reliability and the 2010 assessment used the standards established by the European Chemicals Agency. Guideline studies produced recently in the key areas of unscheduled DNA synthesis in hepatocytes (Cifone, 2005) and chromosomal aberrations in CHO cells (Murli, 2004) were both negative, providing further support for this conclusion.

- **No evidence in Humans.** Epidemiological data on TDCPP is limited and these data do not provide any evidence that TDCPP causes cancer of any type, including invasive cancer, in humans (Stauffer, 1983).

- **The single carcinogenicity study in animals does not report any relevant invasive tumors**. Only a single animal bioassay report is available on TDCPP (Bio/dynamics, 1981, later published as Freudenthal & Henrich, 2000). The study was conducted prior to the implementation of Good Laboratory Practices and US EPA Guidelines for carcinogenicity testing. In this study, tumors were reported at several sites. However, the tumors were either non-invasive, were misclassified with respect to modern histological protocol, and/or were only observed at the high dose level where the Maximum Tolerated Dose (MTD) was severely exceeded.

- **Kidney tumors were non-invasive.** The renal cortical adenomas reported in Bio/dynamics (1981) were non-invasive, and no progression was observed in these lesions over time.

- **Adrenal gland tumors were non-invasive**. The cortical adenomas of the adrenal gland that were reported in the high dose group of female rats, where the MTD was significantly exceeded, were non-invasive and did not progress to malignancy.

- **Liver tumors generally within historical range for Sprague-Dawley CD rats.** The incidence of non-invasive hepatocellular adenomas in Bio/dynamics (1981) for the mid and low dose groups are in the expected range based on the concurrent and historical control data (McMartin *et al*., 1992). The high dose male and female incidence rates were elevated to 27.1% and 15.5% respectively, with the males somewhat above the upper historical control range for Sprague-Dawley rats from this era (1984-1991) of 16.7% in males and 21.7% in females (McMartin *et al.*, 1992). This increase is not unexpected given the exposure was clearly above the MTD. The small incidence of lesions identified in the original report as hepatocellular carcinomas were only noteworthy at the high dose level in males where the dose level was clearly excessive.

- **Hepatocellular neoplasia classification has changed since the histopathology was recorded in 1981**. The lesions identified in Bio/dynamics (1981) as neoplastic nodules would have been classified differently had the histological examination and classification been performed after 1986, when the standards for liver neoplasia classification were modified (Maronpot *et al*., 1986). At this time, the term hepatocellular neoplastic nodule was replaced by two terms: hepatocellular hyperplasia, and hepatocellular adenoma. Though a reassessment of the tissues is not warranted, it is likely that such a reevaluation would substantially alter the adenoma classifications and, accordingly, the resulting incidences. Although Freudenthal & Henrich (2000) referred to the lesions as hepatocellular adenomas it is not known how many of the “neoplastic nodules” were, in fact, neoplastic.

- **MTD was significantly exceeded in rat bioassay, resulting in spurious findings**. The MTD was clearly and significantly exceeded in Bio/dynamics (1981). Body weights were down more than 20% in the high dose for both males and females. Mortality in high dose male animals was also significantly higher, at 38% than was observed in the control animals. In Sprague-Dawley CD rats, this level of toxicity often exacerbates already high level of hepatocellular and other neoplasia that are observed in this strain.

- **The weight of evidence conclusion is that TDCPP has not been clearly shown to be carcinogenic**. The weight of the evidence is that TDCPP is non-genotoxic, differs in important ways from other structurally similar compounds, has not been shown to have carcinogenic potential in any study in humans, and in the single animal bioassay on TDCPP, is not "clearly shown to be carcinogenic" using the criteria establish by the CIC.

2. Occurrence and Use

a. OEHHA's "Evidence of Carcinogenicity" Document.

The summary document prepared by OEHHA (2011) summarizes the uses of TDCPP and makes several suggestions regarding the potential scope of exposures to TDCPP in several statements. They include the following:

> *"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)."*

b. Comments on TDCPP's Occurrence and Use.

Essentially, the only use of TDCPP in the United States is as a flame retardant in flexible polyurethane foams. Accordingly, these foams, which are fully enveloped when present within products, result in insignificant exposure to consumers, as summarized in the most recent and most comprehensive assessment of TCDPP, produced by the European Union (EU, 2008). For example, in the EU (2008) summary of exposure studies, they conclude from a study that measured TDCPP and TCPP in foams for more than 8 years, that "From this ageing study, it can be seen that flame retardants are contained within the foam and so consumer exposure to the flame retardants in these foams are expected to be low." (EU, 2008, pg 149)

The OEHHA (2011) summary suggests that TDCPP was a major replacement for pentaBDE for furnishings and insulation in California. However, TDCPP is rarely if ever used as a replacement for the uses previously covered by pentaBDE.

The study cited by OEHHA (2011) as illustrating that a third of the baby products analyzed had TDCPP (Stapleton *et al*., 2011), was based on the chemical analyses of the foam's chemical composition, not an assessment of the potential exposures to the consumers using these products. The foams are not in direct contact with consumers since they are enveloped in plastics and other materials.

3. Carcinogenicity Studies in Humans

a. OEHHA's "Evidence of Carcinogenicity" Document.

The OEHHA summary of the studies in humans (OEHHA, 2011) ends with the following statements regarding the single study that is available, a retrospective cohort cancer mortality study (Stauffer, 1983). OEHHA states that:

> *"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."*

b. Comments on OEHHA Document and Assessment of Studies in Humans.

We agree with OEHHA that there are no studies that would suggest that TDCPP causes invasive cancer in humans. This is the conclusion drawn by all authorities that have ever assessed the data on TDCPP.

4. Carcinogenicity Studies in Animals

a. OEHHA's "Evidence of Carcinogenicity" Document.

The OEHHA summary (OEHHA, 2011) makes the following conclusion regarding the single carcinogenicity study in animals (Bio/dynamics, 1981; Freudenthal and Henrich, 2000):

> *"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."*

Freudenthal and Henrich (2000) is the published summary version of the single 1981 unpublished full length study (Bio/dynamics, 1981) sponsored by the Stauffer Chemical Company. Only this one study of carcinogenicity is available on TDCPP and it suffers from the limitations and deficiencies regarding dose level selection, study conduct, and study reporting.

b. **Comments on the Study in Animals.**

The highest dose level in the single available carcinogenicity study far exceeded the Maximum Tolerated Dose recommended for this type of testing. Dose levels in carcinogenicity studies should be selected to result in no increased mortality and no more 15-20% decreased body weight gain (Rhomberg *et al*., 2007). In this case, absolute body weights were decreased more than 30% for males and 20% for females and weight gains were decreased approximately 48% and 34% in males and females, respectively). Mortality was also increased in males at the high dose level (38/60 and 26/60 rats died during the course of the study in the high dose and control groups, respectively).

Males. Table 1 summarizes the tumor incidence for those sites in males with significant elevations compared with the control group (Bio/dynamics, 1981) and provides our comments regarding the observations:

Table 1: Tumor Incidences in Male Rats Treated with TDCPP

(Data from Bio/dynamics, 1981)

Organ	Tumor	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day	Comment
Liver	Hepatocellular adenoma	2/45	7/48	1/48	13/46	Noninvasive lesion originally described as a "nodule" would now be separated into hyperplasia and adenoma
	12 months	0/15	0/12	0/13	3/14	
	Hepatocellular carcinoma	1/45	2/48	3/48	7/46#	Increased only at excessive dose level
	12 months	0/15	0/12	0/13	0/14	
Kidney	Renal cortical adenoma	1/45	3/49	9/48*	32/46*	Noninvasive tumor that may be associated with tubular epithelial cell hyperplasia
	12 months	0/15	012	0/13	0/13	
Testes	Interstitial (leydig) cell tumor	7/43	8/48	23/47*	36/45*	Noninvasive tumor that has limited relevance for humans
	12 months	0/14	0/12	3/13	3/11	

* Identified by the study authors as significantly different from control ($p<0.05$).

Identified by the study authors as different from control ($p=0.06$).

Females. No significant increase in invasive tumors (carcinomas) was observed in female rats at any dose level. Table 2 summarizes the tumor incidence for those sites in females with significant elevations compared with the control group (Bio/dynamics, 1981,):

Table 2. Tumor Incidences in Female Rats Treated with TDCPP
(Data from Bio/dynamics 1981)

Organ	Tumor	0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	80 mg/kg/day	Comment
Liver	Hepatocellular adenoma 12 months	1/49 0/11	1/47 0/13	4/46 0/9	8/50 1/10	Noninvasive lesion originally described as a "nodule" would now be separated into hyperplasia and adenoma.
	Hepatocellular carcinoma 12 months	0/49 0/11	2/47 0/13	2/46 0/9	4/50 0/10	Not significant increase only at excessive dose level
Kidney	Renal cortical adenoma 12 months	0/49 0/11	1/48 0/13	8/48* 0/9	29/50* 0/10	Noninvasive tumor that may be associated with tubular epithelial cell hyperplasia
Adrenal	Cortical adenoma 12 months	8/48 5/11	5/27 0/0	2/33 0/0	19/49* 1/10	Noninvasive tumor with high spontaneous incidence that is only increased at excessive dose level

* Identified by the study authors as significantly different from control (P<0.05)

Liver. Tables 1 and 2 show that hepatocellular adenomas are elevated in both sexes and that hepatocellular carcinoma was slightly elevated in males (data from Bio/dynamics, 1981). The hepatic adenoma incidence rates for the low and mid-dose groups were well within historical control ranges for Sprague Dawley rats from the study era of 0 to 16.7% for males and 1.4 to 21.7% for females (McMartin *et al*., 1992). These lesions were only significantly elevated compared to controls at the dose level which exceeded the MTD. The incidence of lesions identified as hepatocellular carcinomas was only increased at the high dose level in males where the dose was clearly excessive.

Testes. Benign interstitial cell tumors of the testes were increased at a dose level that was not associated with excessive toxicity, but these tumors are relatively common in aging rats, as indicated by the comparatively high incidence

rate of 16% in the concurrent controls (Bio/dynamics, 1981). In addition, these lesions are difficult to distinguish from hyperplasia. This tumor type has little potential to progress to malignancy and carcinomas derived from interstitial cells are rare (McConnell *et al*., 1992).

Kidney. Renal cortical adenoma incidence also increased compared to control at a dose level that was not associated with excessive toxicity (Bio/dynamics, 1981). Renal cortical adenomas, although less common than testicular interstitial cell tumors, also have little potential to progress to malignancy (Kurata *et al*., 1993). Renal cysts were observed in mid- and high-dose level female and in low-, mid- and high-dose level male rats. The observed renal cortical adenomas may be secondary to chronic irritation in the renal convoluted tubules. Hyperplasia in the tubular epithelium was common in this TDCPP exposure study and may have exacerbated the formation of the renal tumors.

Adrenal Gland. An increase in non-invasive adrenal cortical adenomas occurred in female rats exposed at the high TDCPP dose, a dose considered to be above the MTD based on the decrease in body weight compared to controls (Bio/dynamics, 1981). A relatively high incidence of this lesion type occurred in the concurrent female control group, suggesting that the excessive dose of TDCPP exacerbated the development of spontaneously occurring noninvasive tumors.

Summary. The weight of evidence to support carcinogenicity of TDCPP based on laboratory animal bioassays is weak according to the CIC criteria (CIC, 2001). TDCPP exposure in the only laboratory animal bioassay available is not associated with invasive tumors at dose levels that do not exceed the maximum tolerated dose. Chronic TDCPP exposure of rats to dose levels below the MTD is not associated with an increase in invasive tumors. In addition, there was nothing unusual about the types of tumors that were identified in the rats on study in terms of tumor location or age of onset, as demonstrated by the occurrence of the same tumor types in treated rats and concurrent and historical controls (e.g., McMartin *et al.,* 1992). Only a single bioassay in one species is available to evaluate the potential for carcinogenicity of TDCPP, though according to CIC guidance, the CIC normally relies on studies in two distinct species, or on two distinct studies carried out in different laboratories under

different protocols, to make a determination. The exception to this rule is if the bioassay is heavily supported by indirect evidence (CIC, 2001). The strengths and weaknesses of the indirect evidence are addressed elsewhere in this document.

5. Genotoxicity

a. OEHHA's "Evidence of Carcinogenicity" Document.

The summary document by OEHHA (2011) makes the following conclusion regarding the genetic toxicity data on TDCPP:

> *"Positive findings in multiple in vitro genotoxicity test systems indicate that TDCPP may be carcinogenic through a genotoxic mechanism."*

OEHHA's assessment is based on selected results from *in vitro* studies.

b. Comments on the Weight of Evidence for TDCPP Genotoxicity.

Over thirty genotoxicity studies are available for TDCPP and it is important to consider both the quality and reporting of the individual studies and the overall weight of the evidence. Comprehensive summaries of the available genotoxicity studies of this chemical were compiled by EBRC (2005) and the European Chemicals Agency (ECHA, 2010). Both the EBRC and ECHA summary tables are enclosed for your convenience (Appendix A and B, respectively).

A number of TDCPP genotoxicity studies have been conducted in whole mammals that have resulted in negative conclusions regarding genotoxicity, while none have been positive (Cifone, 2005; Thomas & Collier, 1985; Brusick *et al.*, 1980). According to the CIC Guidance Criteria (2001), these results in whole mammals "must be considered more pertinent" than "studies of genetic toxicology or DNA repair using *in vitro* methods." The available genotoxicity data can be summarized as follows, though the first three bullets that summarize the *in vivo* study data largely supersede the data from the *in vitro* studies that follow:

- An Unscheduled DNA Synthesis (UDS) study in rats identified no increased UDS in rat hepatocytes harvested 2 to 4 or 14 to 16 hours after rats received a gavage dose of 500, 1,000 or 2,000 mg/kg TDCPP (Cifone, 2005).

- An assay in mice detected no increase in micronuclei of bone marrow red blood cells following treatment of the mice with 200, 630 or 2,000 mg/kg TDCPP (Thomas & Collier, 1985).

- No increase in chromosomal aberrations in bone marrow was reported in mice treated for 5 days with 0.05, 0.17, or 0.5 mL/kg TDCPP (Brusick *et al*., 1980).

- Ames studies were generally negative or resulted in weak positive responses in Strain TA 100 with metabolic activation. Ames studies with the highest reliability rating (Klimish reliability rating "1") were negative for genotoxicity.

- Gene mutation studies using the mouse lymphoma assay with similar concentrations gave conflicting results (McGregor & Brown, 1985; Matheson, 1977).

- Ishidate (1983) reported positive results from an *in vitro* chromosomal aberration study but the concentrations used and the extent of cytotoxicity were not reported in that publication (Klimish reliability rating "4"). Other chromosomal aberration studies were negative or equivocal. A well-reported recent chromosomal aberration study (Murli, 2004) was negative.

- *In vitro* DNA damage and repair assays and cell transformation assays were negative or equivocal. Soderlund (1985) reported a positive result in an Unscheduled DNA assay but the dose range was not specified in that publication (Klimish reliability rating "4"). Cifone (2005), in a recent, well-reported and reliable study, found no effect on this same endpoint in rat hepatocytes.

Based upon their evaluation of the weight of the evidence and giving particular weight to the negative *in vivo* studies, the European Chemicals Agency (ECHA) concluded that no classification for mutagenicity was necessary (ECHA, 2010).

In its evidence document, OEHHA (2011) indicated that discrepancies in the results among Ames assays may be due to the method of metabolic activation as suggested

by Gold and coworkers (1978) and reiterated by Babich (2006). Gold and coworkers (A. Blum and B. Ames) recommended that the Ames Assay be run using a variety of metabolic activation homogenates. They suggested using several liver S9 preparations from different species, and stimulated with various inducers, to obtain the most thorough test of the potential mutagenicity of a chemical. To support this modification of the Ames Assay, Gold *et al*. (1978) noted that TDCPP tested negative when an S9 preparation of PCB-induced rat liver was used and weakly positive when an S9 preparation of phenobarbital-induced mouse liver homogenate was used.

In an evaluation of the potential mutagenic metabolites of TDCPP, Gold and coworkers (1978) postulated the existence of a possible TDCPP metabolite (1,3-dichloropropanone) they identified as strongly mutagenic. Researchers have actively searched for this purported metabolite in exposed animals but not detected it (e.g., Lynn *et al*., 1981). Both TDCPP and one confirmed metabolite tested weakly positive in the Gold *et al*. (1978) study, when TDCPP was used with optimized S9 preparation only.

In its evidence document, OEHHA (2011) noted a study by Morales and Matthews (1980) intended to evaluate potential covalent binding of TDCPP to macromolecules of the mouse liver, kidney and muscle. In this study, [^{14}C]-TDCPP was administered intravenously to 3 CD-1 male mice that were sacrificed 6 hours later (Morales & Matthews, 1980). At sacrifice, protein, DNA, ribosomal RNA and low molecular weight RNA were extracted from the tissues of interest, and radioactivity was greatest in the liver, followed by the kidney and muscle. More than 95% of the radioactivity associated with the macromolecules was covalently bound to low molecular weight RNA, protein and DNA. According to the EU (2008) interpretation of the Morales and Matthews (1980) study, TDCPP was metabolized to [14]CO2 that was incorporated into endogenous molecules via the carbon pool or exhaled. Similarly, the incorporation of radiolabeled alkyl groups of TDCPP into endogenous tissue components was suggested by Nomier *et al.* in 1981 based on their data. Thus, the covalent binding in these tissues likely reflects the background rates of cell synthesis, and not adduction of TDCPP to macromolecules.

6. Structure-Activity Comparisons

a. OEHHA's "Evidence of Carcinogenicity" Document.

The summary document by OEHHA (2011) makes the following conclusion statement regarding TDCPP's structural, and activity relationship to other compounds:

> *"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)."*

b. Comments on Structure-Activity Comparisons.

The European Union, at the conclusion of the most recent and most comprehensive assessment of TDCPP to date, stated that when comparing TDCPP with TCEP (tris(2-chloroethyl) phosphate) and TCPP (Tris(2-chloro-1-methylethyl)-phosphate), that "Although the structures and physiochemical properties of the three substances may be seen as sufficiently comparable to suggest a read-across approach, some differences in the target organs and critical effects for the three substances do not support a full direct read-across from data on either TCEP or TCPP[P]." (ECHA, 2010, pg 7)

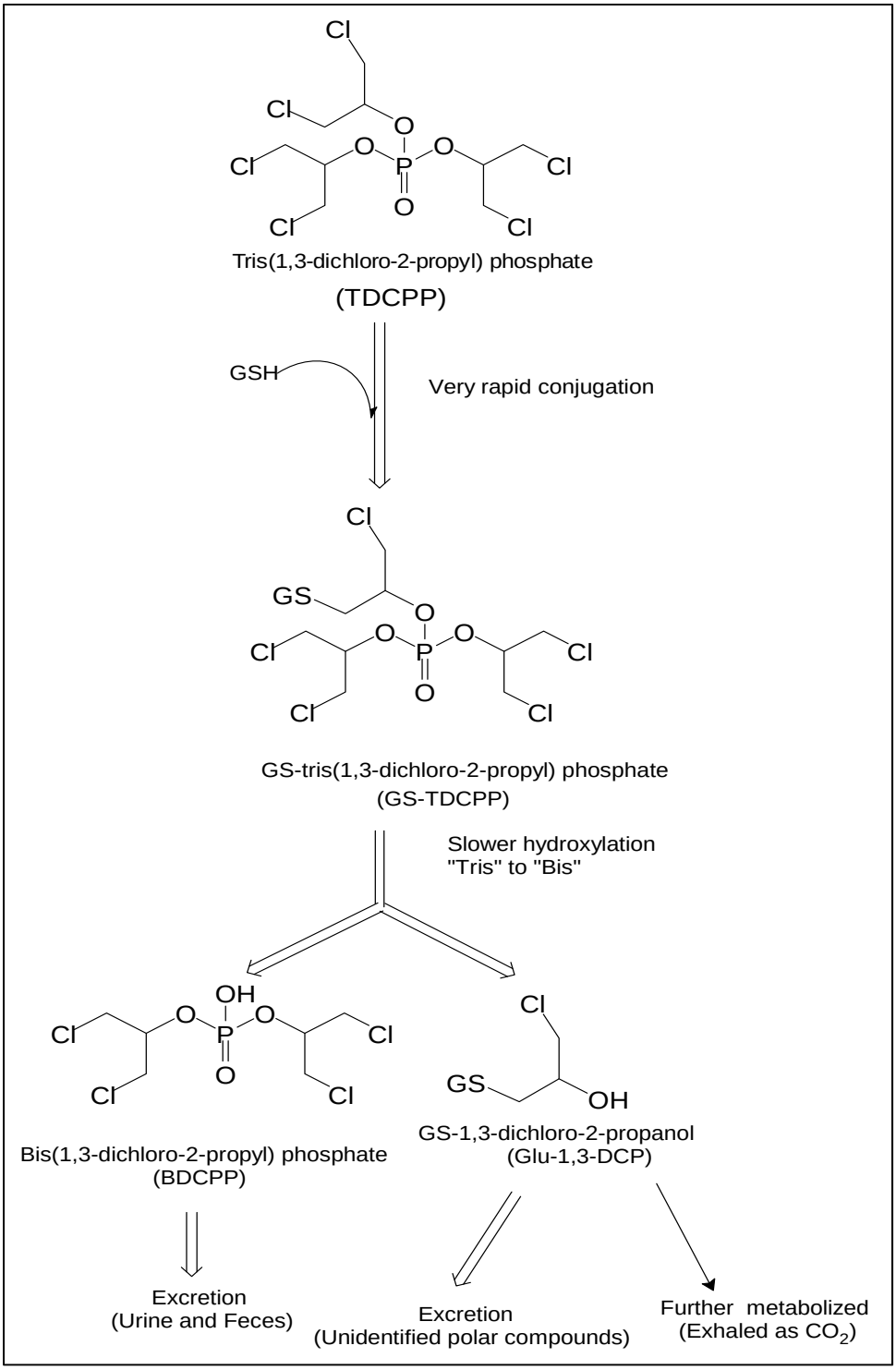
Metabolism significantly different. As illustrated in Figure 1, the collective evidence indicates that TDCPP, in contrast to structurally similar compounds noted in OEHHA (2011), is rapidly conjugated with glutathione (GSH) followed by loss of the conjugated propyl group to form the primary bi-ester ("bis") metabolite, bis(1,3-dichloro-2-propyl) phosphate, or BDCPP. This pathway is supported by the *in vitro* metabolism study of TDCPP with liver preparations that resulted in the rapid GSH conjugation of TDCPP (Fabian & Landsiedel, 2009), and the *in vivo* metabolism studies that demonstrated rapid metabolism of TDCPP, with a lag in the formation of BDCPP, and elimination of polar metabolites (Lynn *et al.*, 1981; Nomeir *et al.*, 1981). The first *in vivo* study showed that 46% of TDCPP was metabolized in the first 5 minutes after iv administration, and 16% of this was converted to BDCPP; over 80% of administered TDCPP was metabolized

within 30 minutes, and BDCPP accounted for 27%; ultimately, 63% of TDCPP was recovered as BDCPP, which suggests that the initial metabolite was the intact TDCPP molecule conjugated with GSH, as demonstrated *in vitro* (Fabian & Landsiedel, 2009; Lynn et al., 1981). The conjugated TDCPP is metabolized from the tri-ester to the bi-ester form, with the leaving group being the conjugated arm of GSH-TDCPP, making the biester (BDCPP) and the conjugated 1,3-dichloro-2-propanol (which appears in the urine as an "unidentified polar metabolite". This is why studies on TDCPP metabolism (Nomeir et al., 1981; Lynn et al., 1981) find the bi-ester breakdown product, but only trace amounts, if any of the corresponding free 1,-3-dichloro-2-propanol. The propyl moiety is already conjugated, categorized analytically as a "polar metabolite", and rapidly eliminated in the urine and feces. The free propyl moiety (1,3-dichloro-2-propanol) and its potential metabolites (e.g., 3-chloro-1,2-propanediol), some of which are listed in the OEHHA (2011) summary as potential carcinogens, are not detected.

Figure 1: TDCPP Proposed Metabolism Summary

(From Fabian & Landsiedel, 2009; Lynn *et al*., 1991; Nomeir *et al*., 1981)

("GSH" is glutathione. "GS-" is glutathione conjugate attachment.)



This proposed metabolic pathway of TDCPP can be contrasted to the activity of the structurally similar compounds noted in OEHHA (2011). TDCPP's apparent very rapid and near complete conjugation via glutathione transferase can be contrasted to the other structurally similar compounds that are not subject to rapid or complete conjugation, such that these others compounds have a higher potential for biological and toxicological activity than TDCPP. For example, the Proposition 65-listed compound TCEP apparently eliminates its ethyl moiety in going from trimester to the biester primarily prior to conjugation, resulting in the presumed production of the free ethanol moiety, 2-chloroethanol, as well as the singularly dechlorinated TCEP, bis(2-chloroethyl)2-hydroxyethyl phosphate.

Although OEHHA noted that TDCPP is structurally similar to TCEP, Fabian & Landsiedel (2009) showed that TDCPP has a different metabolic pathway than TCEP. TDCPP is metabolized by substitution of the chlorine atom of the 1,3—dichloropropyl group by glutathione followed by hydrolysis of glutathione to yield cysteine conjugates. TCEP, which is genotoxic *in vitro* and *in vivo* and is carcinogenic in rats and mice, is poorly metabolized but forms low levels of ester hydrolysis products. The structurally related TCPP (Tris(2-chloro-1-methylethyl)-phosphate) illustrates a third metabolic pathway. TCPP was shown to be hydroxylated and then conjugated with glucuronic acid (Fabian & Landsiedel (2009)).

TDCPP metabolites are rapidly conjugated and eliminated. TDCPP is rapidly absorbed and metabolized (Nomeir *et al*., 1981). The metabolism and metabolites of TDCPP are well-characterized, both *in vitro* and in live animals, and the primary metabolites are confirmed to be non-genotoxic (Nomeir *et al*., 1981; Lynn *et al*., 1981; Fabian & Landsiedel, 2009). The primary metabolite identified in exposed rats is bis(1,3-dichloro-2-propyl)phosphate (BDCP), which makes up 63% to 67.2% of TDCPP recovered after radiolabeled TDCPP administration (Lynn *et al*., 1981; Nomeir *et al*., 1981). Trace amounts of 1,3-dichloro-2-propyl phosphate (0.29%) and unmetabolized TDCPP (0.45%) were detected in the urine (Nomeir et al., 1981). Another minor metabolite (1,3-dichloro-2-propanol) detected in trace amounts not quantified, was the major chlorinated component identified in extracted urine of the TDCPP-exposed rats (Lynn *et al*., 1981). The detection of 1,3-dichloro-2-propanol in rat urine below

quantification levels using GC/MS indicates that this is a particularly minor metabolite. In addition, efforts to find the hypothetical genotoxic metabolite: 1,3-dichloro-2-propanone proposed by Gold *et al*. (1978) were not successful (Lynn *et al*., 1980).

OEHHA suggests that the detection of 1,3-dichloro-2-propanol as a TDCPP metabolite supports a determination of carcinogenicity for TDCPP, based on the actions of this compound and also on the metabolites this compound can be metabolized to form. None of the proposed metabolites identified by OEHHA, such as epichlorohydrin or glycidol have been detected following TDCPP exposure.

Phosphorous-based compounds share flame retardant capacities. Finally, it is worth noting that, even though these structurally similar compounds do not behave similarly biologically or toxicologically, they do all have similar abilities to retard flammability. These tri-substituted phosphate compounds act in the solid phase. When heated, they release a polymeric form of phosphoric acid, which causes the materials to char, forming a glassy layer of carbon which inhibits the pyrolysis process. So, even though these structurally similar and economically important compounds are not biologically or toxicologically similar, they do behave similarly within the environment for which they were designed.

7. Summary and Conclusion

TDCPP does not satisfy the CIC's criteria for "Known to the State to cause Cancer" and should not be listed as a carcinogen under Proposition 65.

- TDCPP is differentiated from structurally similar chemicals that are carcinogens by rapid conjugation and metabolism to structurally distinct, lower toxicity, metabolites.

- TDCPP is not genotoxic using weight of evidence and supported by all-negative *in vivo* data.

- There is no supporting evidence of carcinogenicity in humans.

- The single carcinogenicity study in animals does not report any relevant invasive tumors:

    - Kidney tumors were noninvasive.

    - Adrenal gland tumors were noninvasive.

    - Liver tumors generally within historical range for Sprague-Dawley CD rats.

    - Hepatocellular neoplasia classification has changed since the histopathology was recorded in 1981, so not all of the "neoplastic nodules" would likely be neoplastic.

    - MTD was significantly exceeded in rat bioassay, resulting in spurious findings.

The weight of evidence conclusion is that TDCPP has not been clearly shown to be carcinogenic. The weight of the evidence is that TDCPP is non-genotoxic, differs in important ways from other structurally similar compounds, has not been shown to have carcinogenic potential in any study in humans, and in the single animal bioassay on TDCPP, is not "clearly shown to be carcinogenic" using the criteria establish and used by the CIC.

8. References

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Appendix A

EBRC (2005)

Summary Table

Tabulated summary of studies investigating the mutagenicity of TDCP

Reference	GLP	Method	Guideline	Test system	Dose range	Result	Remarks	Study rating[a]
Bacterial test systems								
Johnson, (1984); Safepharm Laboratories Ltd.	No [c]	Reverse mutation assay	Ames test. The conduct of the study is consistent in all important aspects to EU method B.13/14 (2000/32/EC), with exception that no TA 102 or *E. coli* strain was tested.	*S. typh*. (TA98, 100, 1535, 1537, 1538)	20 to 12,500 µg/plate with and without metabolic activation (vehicle DMSO)	**negative**	Single assay	**1**
Johnson, (1985); Safepharm Laboratories Ltd.	No [c]	Reverse mutation assay	Ames test. The conduct of the study is consistent in all important aspects to EU method B.13/14 (2000/32/EC), with exception that no TA 102 or *E. coli* strain was tested.	*S. typh*. (TA98, 100, 1535, 1537, 1538)	20 to 12,500 µg/plate with and without metabolic activation (vehicle DMSO)	**negative**	Single confirmatory assay	**1**
Majeska, J.B. (1983)	No [c]	Reverse mutation assay	Ames test. The conduct of the study is similar to EU method B.13/14 (2000/32/EC), with exception that only one strain was tested, cytotoxicity and solubility were not taken into account for determining the highest amount of test substance to be used, and that no confirmatory assay was performed. Standard plate assay and plate assay modified to permit quantitative measurement of revertants and cell survival.	*S. typh*. (TA100)	0.98 to 500 µg/plate with metabolic activation (standard plate assay) 50 to 10000 µg/mL with metabolic activation (quantitative suspension assay), vehicle DMSO	ambiguous	- only one strain tested -the extreme toxicity accompanying the mutagenic response (> 1000 µg/plate) indicates that this effect may not be biologically significant and thus the result cannot be regarded as truly positive.	2

Reference	GLP	Method	Guideline	Test system	Dose range	Result	Remarks	Study rating[a]
Brusick, D. J. (1976); Litton Bionetics Inc. no. T5960	No	Reverse mutation assay	Ames test. The conduct of the study is similar to EU method B.13/14 (2000/32/EC), with exception that no TA 102 or *E. coli* strain was tested, less than five analysable concentrations were investigated, cells were plated singly and only one assay was performed.	*S. typh*. (TA98, 100, 1535, 1537, 1538)	0.001 to 1 µL/plate (= 1.5 – 1513 µg/plate*) with and without metabolic activation, vehicle DMSO * rel. density: 1.513	negative	The study does not meet current regulatory standards.	3
		Test for point mutagenic effects in *S. cerevisiae*	Ames test. The conduct of the study is similar to EU method B.16 (88/303/EEC), with exception that less than five analysable concentrations were investigated, cells were plated singly and only one assay was performed.	*Saccharomyces cerevisiae* D4	0.001 to 1 µL/plate (= 1.5 – 1513 µg/plate*) with and without metabolic activation, vehicle DMSO * rel. density: 1.513	negative		3
Brusick, D.J. (1977); Litton Bionetics Inc. no. T6254	No	Reverse mutation assay	Ames test. The conduct of the study is similar to EU method B.13/14 (2000/32/EC), with exception that no TA 102 or *E. coli* strain was tested, cells were plated singly and only one assay was performed.	*S. typh*. (TA98, 100, 1535)	0.001 to 5 µL/plate (= 1.5 – 7565 µg/plate*) with and without metabolic activation, vehicle DMSO * rel. density: 1.513	negative	The study does not meet current regulatory standards.	3
Brusick, D.J. (1977); Litton Bionetics Inc. no. T6255	No	Reverse mutation assay	Ames test. The conduct of the study is similar to EU method B.13/14 (2000/32/EC), with exception that no TA 102 or *E. coli* strain was tested, cells were plated singly and only one assay was performed.	*S. typh*. (TA98, 100, 1535, 1537, 1538)	0.001 to 5 µL/plate (= 1.5 – 7565 µg/plate*) with and without metabolic activation, vehicle DMSO * rel. density: 1.513	negative	The study does not meet current regulatory standards.	3
		Test for point mutagenic effects in *S. cerevisiae*	Ames test. The conduct of the study is similar to EU method B.16 (88/303/EEC), with exception that cells were plated singly and only one assay was performed.	*Saccharomyces cerevisiae* D4	0.001 to 5 µL/plate (= 1.5 – 7565 µg/plate*) with and without metabolic activation, vehicle DMSO * rel. density: 1.513	negative		3

Reference	GLP	Method	Guideline	Test system	Dose range	Result	Remarks	Study rating[a]
Jagannath, D.R. (1977); Litton Bionetics Inc. no. T6300	No	Reverse mutation assay	Ames test. The conduct of the study is similar to EU method B.13/14 (2000/32/EC), with exception that no TA 102 or *E. coli* strain was tested, cells were plated singly and only one assay was performed.	*S. typh*. (TA98, 100, 1535, 1537, 1538)	0.001 to 5 µL/plate (= 1.5 – 7565 µg/plate*) with and without metabolic activation, vehicle DMSO * rel. density: 1.513	negative	The study does not meet current regulatory standards.	3
		Test for point mutagenic effects in *S. cerevisiae*	Ames test. The conduct of the study is similar to EU method B.16 (88/303/EEC), with exception that cells were plated singly and only one assay was performed.	*Saccharomyces cerevisiae* D4	0.001 to 5 µL/plate (= 1.5 – 7565 µg/plate*) with and without metabolic activation, vehicle DMSO * rel. density: 1.513	negative		3
Mortelmans, K. et al. (1986), Environmental Mutagenesis 8, (7), p1-119. [b]	No	Reverse mutation assay	Pre-incubation assay (Haworth et al., 1983).	*S. typh*. (TA97, 98, 100, 1535)	10 – 10000 µg/plate with and without metabolic activation (vehicle DMSO)	positive (+ S9 only)	secondary literature; Inter-laboratory comparison	4
Gold, M.D. et al. (1978); Science 200, p785-787. [b]	No	Reverse mutation assay	not specified	*S. typh*. (TA100)	not stated with and without metabolic activation	positive (+ S9 only)	review article; no information is given for cytotoxicity or precipitation	4
Ishidate, M. (1983), Tests courts de cancerogenese/ Short-term tests for carcinogenesis: Montpellier 4-5 Feb. 1981 [b]	No	Reverse mutation assay	Ames test	*S. typh*. (TA98, 100 or other strains)	not stated with and without metabolic activation	positive (+ S9 only)	review article	4

Reference	GLP	Method	Guideline	Test system	Dose range	Result	Remarks	Study rating[a]
Lynn, R.K. et al. (1981), Am. Society for Pharm. and Exp. Therapeutics [b]	No	Reverse mutation assay	Ames test	*S. typh*. (TA100)	not specified with and without metabolic activation (vehicle DMSO)	positive (+ S9 only)	BDCP and 1,3-dichloro-2-propyl phosphate were not mutagenic. 1,3-dichloro-2-propanol was mutagenic without S9-mix. At concentrations > 1000 µg TDCP/plate, extensive toxicity was observed. very short description	4
Soderlund, E.J. et al. (1985), Acta pharmacol. et toxicol. 56, p20-29. [b]	No	Reverse mutation assay	Ames test	*S. typh*. (TA100)	not stated with and without metabolic activation (vehicle DMSO)	positive (+ S9 only)	very short description	4
Studies with *Drosophila*								
Jagannath, D.R. (1977), Litton Bionetics Inc.	No	Sex-linked recessive lethal assay	not stated. The conduct of the study was consistent in all important aspects to OECD 477 (1984), with exception that the results were not confirmed in a separate experiment.	*Drosophila melanogaster*	2.5%, 25% vehicle: gum tragacanth	negative	Positive control flies were lost in this experiment.	

Reference	GLP	Method	Guideline	Test system	Dose range	Result	Remarks	Study rating[a]
***In vitro* studies**								
McGregor; Brown (1985); Inveresk	No [c]	Gene mutation (TK locus)	not stated. The conduct of the study is consistent in all important aspects to EU method B.17 (2000/32/EC) with exception that the test colonies were not scored using the criteria of small and large colonies (recommended in case of a positive response).	mouse lymphoma cells (L5178Y)	1.25 – 60 µg/mL (1st) 10- 120 µg/mL (2nd) with and without metabolic activation vehicle: acetone	**positive (+ S9 only)**	two independent assays	**1**
Murli, H. (2004); Covance	Yes	Chromosomal aberration	OECD 473 (1997), corresponding to EU method B.10 (2000/32/EC). Deviations: none	CHO cells	6.78 – 1000 µg/mL (1st) 1.25 – 150 µg/mL (2nd); with and without metabolic activation vehicle: DMSO	**negative**	two independent assays	**1**
Tong, C. (1982), Naylor Dana Institute	No	Unscheduled DNA Synthesis test	not stated. The conduct of the study is consistent in all important aspects to EU method B.18 (88/303/EEC).	primary rat hepatocytes	10^{-7} – 10^{-2} % (v/v) vehicle: DMSO	**negative**	Test substances: TDCP and potential metabolites (3-chloro-1,2-propanediol; 1,3-dichloro-2-propanol; 1,3-dichloro-2-propanone)	**2**
Matheson, D.W. (1977); Litton Bionetics Inc.	No	Gene mutation (TK locus)	not stated. The conduct of the study is similar to EU method B.17 (2000/32/EC), with exception that only single cultures were used for each experimental point and no independent experiment was performed.	mouse lymphoma cells (L5178Y)	0.002 – 0.098 µL/mL (= 3 – 150 µg/mL*) with and without metabolic activation, vehicle: DMSO * rel. density: 1.513	negative	-	2
		Sister chromatid exchange	not stated. The conduct of the study is similar to EU method B. 19 (88/303/EEC), with exception that only single cultures were used for each experimental point, that less than 25 well-spread metaphases per culture were scored and that no independent experiment was performed.		0.004 – 0.072 µL/mL (= 6 - 110 µg/mL*) with and without metabolic activation, vehicle: DMSO * rel. density: 1.513	negative	inadequately documented	3

Reference	GLP	Method	Guideline	Test system	Dose range	Result	Remarks	Study rating[a]
Matheson, D.W. (1977); Litton Bionetics Inc.	No	Chromosomal aberration	not stated. The conduct of the study is similar to EU method B. 10 (2000/32/EC) with exception that only single cultures were used for each experimental point, only one experiment was performed and that less than 200 well spread metaphases were scored.	mouse lymphoma cells (L5178Y)	0.004 – 0.125 µL/mL (= 6 – 189 µg/mL*) with and without metabolic activation, vehicle: DMSO * rel. density: 1.513	positive (+S9 only)	inadequately documented	3
Ishidate, M. (1983), Tests courts de cancerogenese/ Short-term tests for carcinogenesis: Montpellier 4-5 Feb. 1981 [b]	No	Chromosomal aberration	not stated. (exposure durations: 24 and 48 hours; 100 well-spread metaphases were scored)	CHL cells	not stated with and without metabolic activation	positive (+ S9 only)	inadequately documented secondary literature (review article)	4
Soderlund, E.J. et al. (1985), Acta pharmacol. et toxicol. 56, p20-29. [b]	No	Gene mutation (HPRT locus)	not specified	V79 Chinese hamster cells	0.02 mM with metabolic activation	negative	inadequately documented	4
		Unscheduled DNA Synthesis test	not specified	adult rat hepatocytes	0.025, 0.05, 0.1 mM vehicle: DMSO	ambiguous	very short description. Minimal response, but it was not possible to quantify the response from the information given in the report.	4
		Morphological transformation	not specified	Syrian hamster embryo cells	10, 20, 30 µM vehicle: DMSO	positive	inadequately documented	4
Brusick, D. et al. (1980), J. Envir. Path. Tox. 3, p207-226. [b]	No	Malignant transformation	not specified	BALB/3T3 cells	max. employed conc.: 0.312 µL/mL	negative	only short summary available; two independent tests	4

Reference	GLP	Method	Guideline	Test system	Dose range	Result	Remarks	Study rating[a]
Bloom, S.E. (1984); Basic Life Sciences 29B, p509-533. [b]	No	Sister chromatid exchange	not specified	chick embryos	not specified	negative	inadequately documented	4
***In vivo* studies**								
Thomas; Collier, (1985); Safepharm Laboratories Ltd.	No [c]	Micronucleus test	OECD 474. Deviation to OECD 474 (1997): 1000 immature erythrocytes per animal were scored	CFLP male and female mice	200 - 2000 mg/kg bw (one administration by gavage, vehicle: arachis oil)	**negative**	two independent assays; There was no alteration in PCE:NCE ratio. The highest dose was set at the maximum tolerated dose level in accordance with the guideline.	**1**
Matheson (1978); Litton Bionetics Inc.	No	Bone marrow cytogenetic test	not stated. The conduct of the study is consistent in all important aspects to EU method B.11 (2000/32/EC) with exception that less than 100 metaphases were scored per animal, the mitotic index was determined in less than 1000 cells per animal, sampling was performed at 6, 24 and 48 hours and colchicine was injected two hours prior to sacrifice.	CD1 male mice	0.5, 017, 0.05 mL/kg (= 0.76, 0.26, 0.076 mg/kg*) (by gavage, single dose and administration for 5 consecutive days; vehicle: DMSO) * rel. density: 1.513	**negative**	The administered doses were very low. However, the dose levels were selected based on LD_{50}, LD_5 and LD_1 values obtained in a preliminary test.	**2**

Reference	GLP	Method	Guideline	Test system	Dose range	Result	Remarks	Study rating[a]
Studies with metabolites of TDCP								
Majeska, J.B. (1982)	No [c]	Reverse mutation assay	Ames test. The conduct of the study is similar to EU method B.13/14 (2000/32/EC), with exception that only one strain was tested, cytotoxicity and solubility were not taken into account for determining the highest amount of test substance to be used, and that no confirmatory assay was performed. Standard plate assay and plate assay modified to permit quantitative measurement of revertants and cell survival.	*S. typh*. (TA100)	0.098 to 50 µg/plate with and without metabolic activation (standard plate assay) 10 to 100 µg/mL with and without metabolic activation (quantitative suspension assay) (vehicle DMSO)	weak positive	The study was conducted with the potential metabolite 1,3-Dichloro-2-propanone of TDCP. Only one strain tested	2
Jagannath (1978); Litton Bionetics Inc.	No	*In vivo*/ *in vitro* urine assay	non-standard test. Three non-toxic dose levels of TDCP were administered to mice. Urine was collected from these animals over a period of 16 h. Non-conjugated and deconjugated aliquots were tested in four *S. typh.* strains (plate incorporation test).	CD1 male mice *S. typh*. (TA98, 100, 1535, 1537)	0.5, 017, 0.05 mL/kg (by gavage)	negative	Study conducted with urinary (rat) excretion products considered to contain metabolites of TDCP. Inadequately documented non-standard test. A single plate was used for each data point and no positive control data were included.	3

a based on evaluation criteria developed by Klimisch *et al* (1997)

b published data

c The study was conducted in compliance with Good Laboratory Practice Regulations but prior to implementation of GLP in the EU.

Appendix B

ECHA (2010)

Summary Table

Genotoxicity

significantly higher than control males. Details of the histological observations on the male reproductive organs are discussed in section 6.9.

A **LOAEL of 5 mg/kg/day** (based on the hyperplasia, considered a pre-neoplastic lesion, observed in the kidneys in all treated groups and the testicular effects observed at this dose) can be derived from this study.

Elsewhere:

In a 90-day study to investigate the possible neurotoxicity of TDCP in hens, doses of 0, 4, 20 and 100 mg/kg/day TDCP were administered to hens. There were no mortalities in TDCP-treated birds. Under the conditions of the test, there was no evidence of TDCP induced delayed neurotoxicity (Stauffer Chemical Company, 1979b).

In an epidemiology study carried out on 289 male workers in a TDCP manufacturing plant as an adjunct to a mortality study, no adverse health effects linked to TDCP exposure were determined, but it should be underlined that all air samples were under the limit of detection of TDCP (Stauffer Chemical Co., 1983b.

No data are available on inhalation and dermal repeated dose toxicity (HSA/EA, 2008a).

Repeated dose toxicity has not been evaluated as part of this dossier and the above information is included as supporting information only. Further information on this endpoint can be found in the IUCLID file for TDCP.

5.7 Mutagenicity

5.7.1 In vitro data

The available in vitro mutagenicity data for TDCP is summarised in Table 6.1, below.

Table 6.1 Summary of in vitro mutagenicity data for TDCP

Test	Endpoint	Result	Comments	Ref.
In vitro plate incorporation assay, bacteria (Ames)	Gene mutation	Non-mutagenic	Test substance: TDCP: LV. Purity not stated	SafePharm Labs (1984 & 1985b)
In vitro plate incorporation assay, bacteria (Ames)	Gene mutation	Non-mutagenic	Studies did not meet current regulatory stds Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1976 & 1977a)
In vitro plate incorporation assay, bacteria (Ames)	Gene mutation	Significant positive response at 500 µg/plate +S9 (TA 100)	Test substance: Fyrol FR-2. Purity 95.7%	Stauffer Chem. Co. (1983a)
Ames modified quantitative suspension assay	Gene mutation	Mutagenic only at toxic doses (>1000µg/plate (+&-S9)	Not a true positive response Test substance: Fyrol FR-2. Purity 95.7%	Stauffer Chem. Co. (1983a)

Test	Endpoint	Result	Comments	Ref.
Ames assays	Gene mutation	Positive response +S9 in strains TA 100 & 1535 from 333 µg/plate.	Dose-related response (Interlaboratory comparison) Test substance: Tris(1,3-dichloro-2-propyl)phosphate. Purity 94.4%	Mortelmans et al. (1986)
Ames assays	Gene mutation	Weakly mutagenic +S9 with TA 100. Positive in 6 independent expts + PB-induced S9. Positive in 2 expts + PCB-induced S9 and in 3 expts +PB-induced S9. Confirmatory results with PCB-induced mouse & guinea pig liver S9.	Dose dependency observed in multiple assays Test substance: Fyrol FR-2. Purity not stated	Gold et al. (1978)
Ames (Pour plate assay)	Gene mutation	Weakly mutagenic + S9 with TA 100.	Test substance: TDCP. Purity not stated	Lynn et al. (1981)
Ames assay	Gene mutation	Positive at 0.5mg/ml +S9.	Test substance: Tris-dichloropropylphosphate. Purity not stated	Ishidate (1983)
In vitro plate incorporation assay, bacteria (Ames)	Gene mutation	Positive mutagenic response +S9 with TA 100 at 500 µg/plate	Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)
In vitro plate mutagenicity assay, fungi (	Gene mutation	Non-mutagenic in Sacc. cereviseriae	Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1976 & 1977a)
In vitro mouse lymphoma assay with L5178Y cells	Gene mutation	Positive +S9 at >80µg/ml. Non-mutagenic -S9.	Clear dose-related increase Test substance: TDCP LV. Purity not stated	Inveresk (1985)
In vitro chromosome aberration assay	Chromosome aberration	Negative with or without S9	Test substance: Fyrol FR-2. Purity not stated	Covance (2004)
In vitro mouse lymphoma assay	Gene mutation	Negative with or without S9	Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1977b)
Sister chromatid exchange assay (L5178Y $TK^{+/-}$ cells)	SCE	Negative	Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1977b)
Chromosome aberration assay (L5178Y $TK^{+/-}$ cells)	Chromosome aberration	Increase at highest dose analysed (118 µg/ml) +S9.	Considered equivocal. Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem. Co. (1977b)
Chromosomal aberration assay	Chromosome aberration	Positive +S9 at 0.5 mg/ml	Test substance: Tris-dichloropropylphosphate. Purity not stated	Ishidate (1983)
Sister chromatid exchange (CECT assay)	SCE	Negative	Test substance: Fyrol FR-2. Purity not stated	Bloom (1982 & 1984)
In vitro transformed foci in BALB/3T3 cells	Cell transformation	Negative	Test substance: Fyrol FR-2. Purity not stated	Stauffer Chem Co. (1978b)
In vitro point mutation assay in V79 cells	Gene mutation	Negative	Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)

Test	Endpoint	Result	Comments	Ref.
In vitro UDS assay	DNA damage & repair	Minimal response at 0.1mM	Not possible to quantify response Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)
In vitro transformation assay in Syrian hamster embryo cells	Cell transformation	Positive at 20 & 30µM	Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)
In vitro Salm. typhimurium mutagenicity assay with hepatocyte activation	Gene mutation	Small increase in revertants at 0.05 mM (non-induced rat livers). No increase using PB-induced hepatocytes	Test substance: Tris-CP. Purity not stated	Soderland et al. (1985)

5.7.2 In vivo data

The available in vivo mutagenicity data for TDCP is summarised in Table 6.2 below.

Table 6.2 Summary of in vivo mutagenicity data for TDCP

Test	Endpoint	Result	Comments	Ref.
In vivo Mouse micronucleus assay	Clastogenicity	Non-clastogenic	Test substance: Tolgard TDCP LV. Purity not stated.	SafePharm Labs Ltd. (1985c)
In vivo Mouse bone marrow cytogenetic assay	Chromosome aberration	Non-clastogenic	Test substance: Fyrol FR-2. Purity not stated,	Stauffer Chem Co. (1978c)
In vivo/in vitro urine mutagenicity assay	Mutation	Negative	Test substance: Fyrol FR-2. Purity not stated,	Stauffer Chem Co. (1978d)
In vivo/in vitro unscheduled DNA synthesis assay	DNA damage & repair	Negative	Test substance: TDCP. Purity >99% w/w	Covance Laboratories Inc. (2005)
Recessive lethal mutation assay in Drosophila	Chromosomal mutation	Negative	Test substance: Fyrol FR-2. Purity not stated,	Stauffer Chem Co. (1978e)

5.7.3 Human data

No data available for this dossier.

5.7.4 Other relevant information

No data available for this dosser.

5.7.5 Summary and discussion of mutagenicity

No data from humans are available on the mutagenicity of TDCP.

There is evidence to suggest that TDCP is mutagenic *in vitro*. Among the 22 reported in vitro assays, the Ames assays and mammalian cells (mouse lymphoma L5178Y), both in presence of metabolic activation (S9), are positive. The in vitro transformation assay in Syrian hamster embryo (SHE) cells is also positive; it should be noted that this assay points out earliest identifiable stage in