CPSC Staff Statement on Toxicology Excellence for Risk Assessment (TERA) Report "Toxicity Review of Tris(2-Chloroethyl) Phosphate (TCEP)"[1]

December 2013

The report titled, "Toxicity Review of Tris(2-Chloroethyl) Phosphate (TCEP)," presents toxicological and exposure information on TCEP conducted by TERA under Contract CPSC-D-12-0001, Task Order 0006.

First, TERA provides information on TCEP's physico-chemical characteristics, manufacture, supply, and use. Next, TERA provides toxicokinetic (absorption, distribution, metabolism, and excretion) information. The hazard characterization assessment includes acute toxicity (oral, dermal, inhalation, intraperitoneal, primary eye and skin irritations, respiratory irritation, and sensitization); repeated dose toxicities (oral, inhalation, and dermal); endocrine activity; neurotoxicity; reproductive and developmental toxicity; genotoxicity; carcinogenicity; and lowest hazard endpoints. TCEP exposure was also assessed and includes levels in ambient and indoor air, water, food, house dust, consumer products, children's products, and non-United States products. Exposure studies and a report discussion are also provided.

Based on this report, the three main sources of exposure to consumers are indoor air, dust, and polyurethane foam. In terms of toxicity, animal data were sufficient to support the conclusion that TCEP fits the designation of "acutely toxic" under the Federal Hazardous Substances Act (FHSA) following single oral exposures. Additionally, sufficient animal data exist to support the conclusion that TCEP can be considered "toxic" under the FHSA due to its toxicity following short-term, subchronic, and chronic exposures. TCEP causes adverse effects in a variety of organs and is a rodent reproductive toxicant and carcinogen. There is sufficient evidence to support the conclusion that TCEP is not a direct acting genotoxicant.

Confidence in the database for systemic toxicity and carcinogenicity is medium to high, as it includes well-conducted subchronic and chronic studies in both sexes of two species of rodents by the oral route. While the database includes reproductive studies in mice and a developmental study in rats, study designs for many of the endpoints did not include multiple doses, and no no observed adverse effect level (NOAEL) is available for the reproductive endpoints for which multiple doses were tested. The database is missing a full neurotoxicity assessment, and mechanistic and toxicokinetic information is incomplete, making confidence in the database for other endpoints low to medium. Confidence in the database for inhalation and dermal routes is low, as there are large data gaps and inadequacies.

[1] This statement was prepared by the CPSC staff, and the attached report was produced by TERA for CPSC staff. The statement and report have not been reviewed or approved by, and do not necessarily represent the views of, the Commission.

Toxicity Review of Tris(2-Chloroethyl) Phosphate (TCEP)

Toxicology Excellence for Risk Assessment (TERA)

And

The Lifeline Group

Under Contract

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1 Introduction

This document is a review of current hazard information for TCEP (Tris(2-chloroethyl phosphate)). This assessment was prepared from a variety of review articles (ATSDR 2012; ECHA 2009; IPCS 1997; IUCLID 2000) as well as supplemental, independent studies retrieved from literature searching.

2 Physico-Chemical Characteristics and Identity

TCEP (CAS: 115-96-8) is a phosphate ester comprised of a phosphorus-oxygen pi bond with three chlorinated, straight-chain substituents (OC_2H_4Cl) and an overall chemical formula of $C_6H_{12}Cl_3O_4P$. TCEP is a transparent liquid that possesses a faint odor. It is soluble in many organic solvents, somewhat soluble in aliphatic hydrocarbons, and insoluble in benzene (ATSDR 2012).

Table 2.1. Structural Descriptors and Chemical Formulas of TCEP (ChemIDplus 2009)

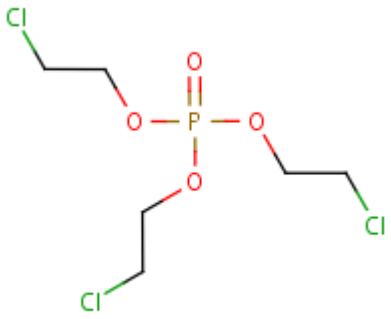
lnChl Notation	InChI=1S/C6H12Cl3O4P/c7-1-4-11-14(10,12-5-2-8)13-6-3-9/h1-6H2
Smiles Notation	P(=O)(OCCCl)(OCCCl)OCCCl
Molecular Structure	

Table 2.2. Chemical Names and Synonyms of TCEP (ATSDR 2012, ChemIDplus 2009)

Synonyms	TCEP; trichloroethyl phosphate; phosphoric acid; tris (2-chloroethyl) ester; tri(2-chloroethyl) phosphate; ethanol, 2-chloro-, phosphate (3:1); tris(2-chloroethyl) orthophosphate
Registered Trade Names	Antiblaze 100; Celluflex CEF; Disflamoll TCA; Fyrol CEF; Niax 3CF, Tolgard TCEP; Genomoll P; Hostaflam UP810; Levagard EP
Systematic Name	Ethanol, 2-chloro, phosphate (3:1); Tris(2-chloroethyl) phosphate; Tris(2-chloroethyl)phosphate
Superlist Name	Ethanol, 2-chloro, phosphate (3:1); Tris(2-chloroethyl) phosphate; Tris(2-chloroethyl)phosphate

Table 2.3. Registry Numbers for TCEP (ATSDR 2012, ChemIDplus 2009)

CAS registry	115-96-8
Other Registry Number	21343-84-0
RTECS	KK2450000
HSDB	2577
EINECS	204-118-5
NCI	C60128

Table 2.4. Physico-Chemical Properties of TCEP (ATSDR 2012)

Molecular Formula	$C_6H_{12}Cl_3O_4P$
Molecular Weight	285.50
Melting Point	-55° C
Boiling Point	330° C (1 atm)
Density	1.425 g/cm^3 (20° C)
Solubility (in water)	7.0 g/L (unspecified temp.)
Log K_{ow}	1.44
Vapor Pressure	6.125x10^{-2} mm Hg (25° C)
Autoignition Temp.	1,115° C
Flashpoint	216° C (420.8° F)

3 Manufacture, Supply and Use

Production of TCEP involves a synthesis reaction between phosphorus oxychloride and 2-chloroethanol via condensation. This must be conducted at relatively low pressure and temperature to avoid formation of undesired alkyl chloride byproducts.

Global production of flame retardants has risen to estimates of above 1 million metric tons (with phosphate ester flame retardant compounds comprising 186,000 tons) (Hartman et al. 2004, as

cited in ATSDR 2012). The manufacture of TCEP specifically has risen in the past several decades to 500,000-1,000,000 pounds, as estimated by the 2006 Inventory Update Reporting (IUR) (USEPA 2010, as cited in ATSDR 2012). This is a sizeable increase from the estimated >908 kg or roughly 1 metric ton of TCEP produced in 1975 (HSDB 2009, as cited in ATSDR 2012).

TCEP has been used as a flame retardant on a variety of commercial products, as well as in paints/glue and in industrial environments (Marklund et al. 2003, as cited in ATSDR 2012). TCEP has largely been used as a flame retardant for flexible and rigid polyurethane foams and for some textiles and clothing (Anderson et al. 2004, as cited in ATSDR 2012). When applied to plastics, such as polyvinylchloride (PVC), it can contribute to a lower corrosivity of combustion gases, an increased PVC susceptibility to hydrolysis, and alteration of heat distortion temperature (Wolf and Kaul 2005, as cited in ATSDR 2012). According to the USEPA (2010, as cited in ATSDR 2012), TCEP usage in the manufacture of commercial products has been greatly reduced in recent years.

4 Toxicokinetics

No *in vivo* human data for absorption, distribution, metabolism, or elimination of TCEP by any route of exposure were located, although there are some limited *in vitro* data on metabolism in liver slices or via microsomes.

Oral studies in rats and mice showed that TCEP is well-absorbed following gavage (Herr et al. 1991; Burka et al. 1991). While no animal studies were identified that directly assessed toxicokinetics following inhalation exposure for TCEP, indirect evidence of absorption is provided by several medial lethal concentrations (LC_{50}) and longer-term studies showing adverse systemic effects (Stauffer Chemical Company 1974, 1979 as cited in IUCLID 2000; Smyth et al. 1951, as cited in IUCLID 2000, ATSDR 2012; Shepel'skaia and Dyschinegvich 1981, as cited in ECHA 2009). Studies by the dermal route suggest that TCEP is systemically available (USEPA 1989). Distribution studies via oral (Minegishi et al. 1988; Chadwick et al. 1989, as cited in ECHA 2009; Herr et al. 1991) and intravenous routes (Dix et al. 1994) show wide and rapid distribution throughout the body. Radioactivity did not accumulate in any tissues; however, tissue/blood ratios were highest in the liver and kidneys. In rodents, enterohepatic circulation was identified.

In vivo (oral administration) and *in vitro* studies show that metabolism of TCEP involves phase I and phase II pathways (Herr et al. 1991; Burka et al. 1991). Phase I metabolism occurs via both an oxidative pathway, likely via a cytochrome P450, and via a hydrolytic pathway via a beta-esterase. Some products of the oxidative pathway undergo glucuronidation, a phase II process. Metabolic differences were identified between mice and rats and between male and

female rats. Female rats were found to have higher parent TCEP/metabolite ratios in brain cortical tissue compared to males. Male rat liver slices were able to metabolize TCEP while those of females did not (Chapman et al. 1991, as cited in ATSDR 2012). TCEP was metabolized by human liver slices and microsomes, but not by human plasma or whole blood. There are no data available regarding metabolism of TCEP following inhalation or dermal exposure, but it is expected that once TCEP is absorbed systemically, similar metabolic processes will apply regardless of the route of exposure, after accounting for first-pass metabolism.

Urine is the main route of excretion for TCEP in rodent studies following oral and intravenous (i.v.) administration, with minimal excretion in exhaled air and feces (Burka et al. 1991; Herr et al. 1991). Following oral gavage, species differences in elimination rate were identified between rats and mice, with mice eliminating TCEP faster than rats. Differences in excretion between sexes in rats were identified. Females eliminate certain metabolites of TCEP faster than males at lower doses, and, at high doses, female rats excreted less than male rats, indicating saturation of metabolism. No data are available for the inhalation or dermal routes.

4.1 Absorption

Oral Exposure

Rats and Mice

Oral gavage doses of 175-700 mg/kg TCEP administered in corn oil were absorbed and rapidly quantifiable in blood and plasma (Burka et al. 1991; Herr et al. 1991). Oral studies with ^{14}C-labeled TCEP showed >90% absorption based on quantification of radioactivity in urine, feces, and expired air (Burka et al. 1991; Herr et al. 1991). Following gavage with 175 mg/kg, female rats reached a maximum concentration of TCEP in plasma (106.1±13.8 nmol/mL plasma) within 15 minutes, while the maximum in male rats (75.8±29.1 nmol/mL plasma) was not reached until 1 hour post-dose (Herr et al. 1991). For the first two hours post-dosing, blood and plasma levels were higher in females than in males given equivalent doses, after which concentrations were similar in males and females (Herr et al. 1991).

Oral absorption in rats can also be inferred from the wide distribution of radioactivity of ^{14}C-TCEP (Chadwick et al. 1989, as cited in ECHA 2009).

The estimated absorption in male mice receiving 175 mg/kg radiolabeled TCEP via oral gavage was similar to that in rats (Burka et al. 1991).

Other Species

Studies in White Leghorn hens orally gavaged with 14.2 g/kg TCEP (neat) reported physiological effects, indicating that TCEP is bioavailable via the oral route (Sprague et al. 1981).

Inhalation

A number of studies reported systemic toxicity following inhalation exposure of rats to TCEP. They indicated that TCEP is absorbed via the inhalation route, but no information is available on the extent or rate of absorption. Inhalation studies are not available in mice.

One 4-hour inhalation study in Sprague Dawley rats showed systemic toxicity, including lethargy, depression and decreased body weight (Stauffer Chemical Company 1974, 1979 as cited in IUCLID 2000). In a 4-month inhalation study, male rats showed histopathological changes in the testes (NTP 1991b; Shepel'skaia and Dyschinegvich 1981, as cited in ECHA 2009). Taken together these studies provide indirect evidence as to the absorption of TCEP via the inhalation route.

Dermal

No quantitative data are available on the extent or rate of dermal absorption in any species. Some information about dermal absorption can be inferred, however, from dermal toxicity studies. There are limited data on other alkyl phosphate flame retardants to provide support for dermal absorption of TCEP (ATSDR 2012). An unpublished study submitted under the Toxic Substances Control Act (TSCA) rule reported a median lethal dose (LD_{50}) greater than 200 mg/kg but less than 5000 mg/kg in albino New Zealand rabbits (USEPA 1989). The sex, dose, number of animals, and lethality incidence were not reported. However, the observation of some death(s) at the tested dose(s) indicates that toxicity (and death) was observed, and, therefore, that TCEP was dermally absorbed in rabbits. More definitive data on absorption in rabbits comes from a primary skin irritation study, where the applied doses were not reported, but sufficient TCEP was absorbed to cause narcosis and paralysis in 4/6 of the treated rabbits (USEPA 1989).

As discussed in Section 5.7, there are two dermal carcinogenicity studies in mice. In one, 5 or 50% TCEP (volume not available) was applied to the skin of female ddY mice for 79 weeks (Takada et al. 1991). There were no increases in neoplasms or effects on systemic toxicity with the exception of decreased spleen weight in the high dose group. The observation of the effect on spleen weight suggests that TCEP was absorbed and caused systemic effects. However, the absence of this effect in oral studies, as well as the absence of effects in this study that were seen in oral studies (e.g., effects on the liver and kidney), raises questions about whether the change in

spleen weight was chemical-related[2]. Sala et al. (1982) conducted an initiation/promotion study with female Swiss mice. Although the authors reported an increased incidence of lung adenomas (not statistically significant) in the TCEP complete carcinogen group relative to the other groups, the absence of a concurrent control makes a conclusion that TCEP was absorbed and caused systemic effects less definitive.

Overall, the data in rabbits indicate that TCEP is systemically available following dermal application. The data on dermal exposure of mice is less clear; there is some evidence of systemic effects, but study limitations preclude definitive conclusions.

4.2 Distribution

Oral Exposure

Rats

Male Wistar rats were administered a single oral dose of 14 mg/kg ^{14}C-TCEP and radioactivity in organs was measured up to 168 hours post-dosing. Radioactivity was reported in all organs measured, indicating wide distribution. Low tissue/blood ratios of radioactivity for brain, heart, muscle, and testes were identified. Moderate ratios were identified for adipose tissue, spleen, and lung, and higher ratios were found for the liver and kidney. Peak radioactivity was reported in the liver and kidneys at 6 hours. At 168 hours post-dosing, the highest remaining ^{14}C level was found in the liver suggesting TCEP undergoes enterohepatic circulation (Minegishi et al. 1988).

Rats that received 88 mg ^{14}C-TCEP/kg via the oral route had an even distribution of radioactivity throughout the tissues, with higher concentrations in the liver, kidney, adipose tissue, and gastrointestinal tract contents (Chadwick et al. 1989, as cited in ECHA 2009).

Herr et al. (1991) compared blood and brain radioactivity in male and female F344 rats at 2 hours (peak seizure time point) following a single dose of vehicle, 175, 350, or 700 mg/kg TCEP; at 24 hours after female rats received doses of vehicle, 175 or 350 mg/kg; and 24 hours after the last dose in females receiving 14 consecutive doses of 175 mg/kg. At 2 hours post-dosing, the concentration of ^{14}C in blood significantly increased with dose and differed by sex. Males had higher blood levels of radioactivity than females at 2 hours post administration of the single dose. Radioactivity was detected in the blood and brain after 24 hours in both dosing regimens. The radiolabel was distributed to all regions of the brain, and there were no dose-related differences in brain levels after 24 hours. In the repeated dosing scenario, levels of ^{14}C were evenly distributed throughout all brain regions evaluated. The similarity of the blood/brain

[2] An alternative explanation might be that the difference in targets reflects the absence of first-pass metabolism via the dermal route, with the spleen effect being due to the parent chemical, but this seems less likely, based on the metabolism data.

ratios independent of dose in males and females suggests that TCEP does not accumulate in the brain.

Intravenous

Rats

Distribution of TCEP in rats was compared by Dix et al. (1994) using conventional methods of blood sampling or a new method of microdialysis and tandem mass spectroscopic (MS) analysis. Animals remained awake or were anesthetized (with methoxyfluorane). Male and female F344 rats were administered a single dose of 20 mg/kg TCEP. Values for free TCEP area under the curve (AUC) and other pharmacokinetic parameters were not statistically significantly different between males and females using the conventional methods, but differences were seen between awake and anesthetized male rats and were attributed to physiological differences in metabolism. The authors calculated an AUC of 393 min mg/L in awake females, 291 min mg/Lin awake males, and 677 min mg/L in anesthetized males. The authors also noted that at high TCEP plasma concentrations, binding sites are saturated, leading to a higher unbound TCEP fraction in blood. The fraction of free TCEP was about 0.4 to 0.5 (depending on the method) at 5-10 mg/L, and ~0.56-0.58 at 220 to 400 mg total TCEP/L plasma.

4.3 Metabolism

Oral Exposure

Rats and Mice

Two hours after oral gavage of up to 350 mg/kg radiolabeled TCEP, the radioactivity in the liver and brain of F344 rats was identified as primarily parent compound (Herr et al. 1991). In the brain cortical tissue, the parent compound TCEP:metabolite ratio was greater in female rats (16% metabolite) than in males (31% metabolite). Metabolic profiles in the plasma were comparable between male and female rats, and unmetabolized parent compound accounted for roughly 44% of the ^{14}C in plasma. Metabolites in the urine were identified as the predominant species.

Burka et al. (1991) evaluated the metabolites in urine and feces of male B6C3F1 mice and male and female F344 rats that received a single dose of 175 mg/kg ^{14}C-labeled TCEP via gavage in corn oil. They found that TCEP undergoes extensive metabolism and is excreted primarily in the form of metabolites. Metabolism was not induced or inhibited by nine consecutive daily doses. There was qualitative evidence that the metabolic pathways in rats and mice were similar, although quantitative differences in the amounts of different metabolites were observed. A proposed metabolic scheme was presented to account for identified metabolites based on female rat metabolism and confirmed in male rats and mice (Figure 4.1). As shown in Figure 4.1, TCEP

can be metabolized via a hydrolytic pathway or via an oxidative pathway, and some products of the oxidation pathway undergo glucuronidation (a phase II process) prior to elimination in urine. The enzymes responsible for the oxidative pathway and hydrolytic pathways were considered to be a cytochrome P450 and a beta-esterase, respectively. One metabolite [bis(2-chloroethyl) hydrogen phosphate - BCHP] can be produced either by direct hydrolysis of TCEP or by oxidation followed by hydrolysis.

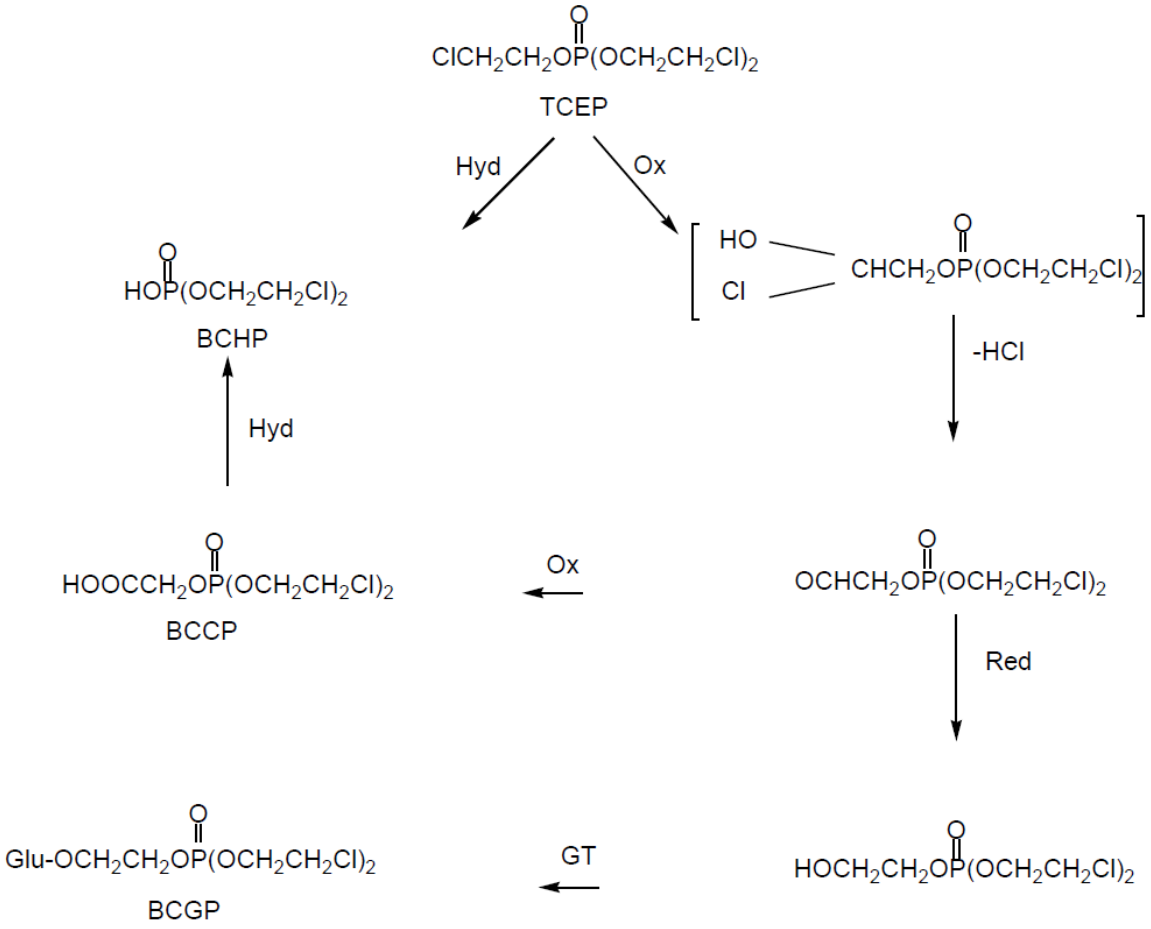
Enzyme inhibitors were used to determine the relationship between acute neurotoxicity (as manifested by clinical signs of "wet dog shakes") and metabolism, and to identify the toxic form(s) (Burka et al. 1991). Male and female F344 rats were pretreated via intraperitoneal (i.p.) injection with SKD 525-A (mixed function oxidase inhibitor), or the aldehyde dehydrogenase inhibitors butanal oxime or disulfiram (which interfere with the pathway leading to the major metabolite BCCP) prior to administration of 175 mg/kg ^{14}C-labled TCEP for 7 consecutive days. All three enzyme inhibitors altered the metabolic profile of TCEP by increasing the hydrolysis product (BCHP) compared to the oxidative product [bis(2-chloroethyl) carboxymethyl phosphate – BCCP]. Clinical signs of toxicity were substantially increased by the two aldehyde dehydrogenase inhibitors. The authors suggested that the increased toxicity was related to increased levels of a reactive metabolite, possibly the aldehyde. Inhibition of the mixed function oxidase pathway did not increase toxicity, but did slow the elimination of radioactivity in the urine and inhibited production of BCCP. The authors interpreted the results as suggesting that a metabolite, rather than the parent TCEP, is responsible for the acute neurotoxicity. However, they noted that SKD 525-A can interfere with neural transmission, and so a definitive result is not possible. In addition, they noted that inhibition of the oxidative pathway would lead to increased metabolism via the hydrolysis pathway. The lack of data on how much higher the internal dose of the parent compound TCEP was after dosing with SKD 525-A also precludes a definitive assignment of toxicity to the metabolite. Finally, as noted by ATSDR (2012), implication of a metabolite as the toxic form appears contrary to the results from the same laboratory published by Herr et al. (1991) that most of the radioactivity in the brain cortex at the time of seizures was in the form of the parent (therefore implicating the parent compound TCEP as the toxic agent).

In vitro

In vitro data provide some information on the human metabolites of TCEP. Although the data are less complete than the rodent data, the available data are consistent with at least a portion of the human metabolic pathway and possibly the entire pathway being the same as in rats. Human liver slices (which contain cytosolic enzymes) and microsomes (which contain only enzymes from the endoplasmic reticulum) metabolize TCEP to BCHP, 2-chloroethanol (formed by the hydrolysis of TCEP to BCHP), and three unknown metabolites (Chapman et al. 1991, as cited in ATSDR 2012). The same spectrum of metabolites was seen with male rat liver slices and

microsomes. Female liver slices but not microsomes also metabolized TCEP, and sex-specific differences were identified, as male liver slices metabolized TCEP 1.7 times faster than females. TCEP hydrolysis was localized primarily in the liver cytosol, but the difference between the metabolic profile described by Chapman et al. (1991, as cited in ATSDR 2012) and that described by Burka et al. (1991) indicates that *in vivo* oxidation may occur extrahepatically. ATSDR (2012) further indicated that cytochrome P-450 was responsible for approximately 38% of the microsomal hydrolytic activity, while the majority of activity was associated with

Figure 4.1. Proposed metabolic pathway for TCEP in rats and mice. Hyd – hydrolysis; Ox – oxidation; Red – reduction; GT – glucuronyl transferase. BCCP - bis(2-chloroethyl) carboxymethyl phosphate; BCGP - bis(2-chloroethyl) 2-hydroxyethyl phosphate; BCHP - bis(2-chloroethyl) hydrogen phosphate; Taken from ATSDR (2012); originally sourced from Burka et al. (1991).



beta-esterase (Chapman et al. 1991, as cited in ATSDR 2012). The presence of beta-esterase in rat serum but not in human serum is consistent with the observation by Chapman et al. (1991, as cited in ATSDR 2012) that TCEP was metabolized by rat plasma but not human plasma or whole blood.

4.4 Excretion

Oral Exposure

Rats and Mice

Urine is the main route of excretion for TCEP in rodent studies following oral and i.v. administration, with minimal excretion in exhaled air and feces. In male and female F344 rats that received a single gavage dose of ^{14}C-TCEP, more than 75% of the radiolabel excreted within the first 24 hours was in the urine and less than 10% was excreted in the feces (Burka et al. 1991). In another study of ^{14}C-TCEP orally administered to rats, >90% was excreted in the urine, 7% in feces, and 1% as CO_2 within 72 hours (Chadwick et al. 1989, as cited in ECHA 2009). Similarly, administration of a single dose of ^{14}C-TCEP resulted in about 90% of radioactivity excreted in the urine within 7 days, with minimal excretion in feces or expired air (Minegishi et al. 1988). Herr et al. (1991) reported that in female rats gavaged with up to 350 mg/kg TCEP, the majority of TCEP was excreted in the urine in 24 hours, with 1% being expired in air as volatiles or as $^{14}CO_2$, and less than 10% excreted in the feces over 3 days.

Herr et al. (1991) found no difference between urinary excretion in male and female rats dosed with 175 mg/kg, but there were significant differences between sexes at 350 mg/kg. At this higher dose, females excreted less cumulative ^{14}C in the urine and also excreted less fecal ^{14}C than males over 24 hours (Herr et al. 1991). These results indicate a longer excretion half-life for females at higher doses, a result that Burka et al. (1991) suggested may reflect saturation of metabolism in females. Daily dosing for 9 consecutive days did not change the elimination or metabolic profile in male or female rats (Burka et al. 1991). In female rats, elimination in urine followed first-order kinetics with averaged half lives (across 1, 4 and 7 days of dosing) of roughly 6.3 hrs; the elimination half life in males was roughly 7.5 hrs. These differences were significant between sexes after 1 and 4 consecutive daily doses with females more rapidly excreting than males, but not after 7 consecutive daily doses (Burka et al. 1991).

Elimination from blood was biphasic (Chadwick et al. 1989, as cited in ECHA 2009; Minegishi et al. 1988). The maximum average concentration in tissues occurred by 6 hours post-exposure, with adipose tissue having the longest tissue elimination half-life of 87 hours; no accumulation was expected (Minegishi et al. 1988). No indication of long-term sex differences in clearance from the brain were identified (Herr et al. 1991).

Peak biliary excretion occurred 2 hours post-dosing with 25% TCEP excreted in the bile within 48 hours. The biliary/fecal excretion ratio reported for TCEP was 4.62 after 48 hours, suggesting reabsorption of biliary metabolites from the gastrointestinal tract and enterohepatic circulation (Minegishi et al. 1988). Biphasic plasma elimination half lives of 3 and 3.4 hours and red blood

cell elimination half lives of 1.8 and 10.8 days were reported (Chadwick et al. 1989, as cited in ECHA 2009).

Species differences in elimination were noted between rats and mice, with excretion of ^{14}C in the urine significantly higher in mice (three times faster than rats) (Burka et al. 1991). There were also quantitative differences in the urinary excretion between species, with mice eliminating about 70% of the radioactivity as the major metabolite (identified as BCCP - bis(2-chloroethyl) carboxymethyl phosphate), while this metabolite represented 46% (females) or 55% (males) of the radioactivity in rats.

Intravenous

Rats

TCEP was not consistently detected in blood sampled at 30-45 minutes after a single i.v. dose of 20 mg/kg TCEP, suggesting rapid elimination of TCEP (Dix et al. 1994). The authors calculated that the clearance was 53 mL/min per kg, 74 mL/min per kg, and 30 mL/min per kg in awake females, awake males, and anesthetized males, respectively. The corresponding volumes of distribution were (L/kg) 1.634, 1.4141, and 1.537, respectively (Dix et al. 1994).

5 Hazard Characterization

5.1 Acute toxicity

5.1.1 Acute Oral Toxicity (Table 5.1)

Manufacturers of TCEP have performed numerous acute toxicity studies (see Tables 5.1-5.4). Acute oral studies conducted at high doses reported depression, convulsions and lethality in Sprague-Dawley and F344/N rats given single doses of TCEP; reliable LD_{50} values ranged from 430 to 1410 mg/kg (Eldefrawi et al. 1977; Kynoch and Denton 1990; Smyth et al. 1951; Ulsamer et al. 1980; USEPA 1989). A lower LD_{50} was reported for a study that lacked experimental details, so its reliability could not be ascertained (USEPA 1989). Herr et al. (1991) performed a range-finding study in F344 rats (6/sex/group) at doses of 0, 175, 350 or 700 mg/kg. Female rats experienced seizures 1 hour after dosing with 350 or 700 mg/kg. Some seizures progressed to tonic-clonic convulsions over 12 hours post dosing; two females in the 350 mg/kg group died. No lethality was reported in males by Herr et al. (1991).

Two single-dose oral gavage studies were conducted to assess central nervous system (CNS) and behavioral effects (Tilson et al. 1990). For both studies, groups of female F344 rats were dosed with 0 or 275 mg/kg of TCEP. Epileptiform convulsions and loss of pyramidal cells in the CA1 region of the hippocampus were seen in the treated group in the CNS effect study (Tilson et al.

1990). Rats receiving 275 mg/kg of TCEP in the behavioral study showed mild impairment of their performance in the acquisition of a water maze task compared to controls. Lehner et al. (2010) reported accidental acute poisoning in four canines following ingestion of car seat cushions that contained TCEP, other flame retardants, phthalates and other chemicals. The dogs exhibited signs of CNS excitation and seizures before death; however, these effects cannot definitively be attributed to TCEP alone.

5.1.2 Acute Dermal Toxicity (Table 5.4)

An unpublished study submitted under the TSCA rule reported an LD_{50} greater than 200 but less than 5000 mg/kg in albino New Zealand rabbits (Aceto Chemical Co. Inc. 1989; USEPA 1989). The sex, dose, number of animals, and lethality incidence were not reported.

5.1.3 Acute Inhalation Toxicity (Table 5.2)

An inhalation study exposed Sprague-Dawley rats (10/sex) to 5 mg/L (5000 mg/m^3) of aerosolized TCEP for 4 hours (Stauffer Chemical Company 1974, 1979, as cited in IUCLID 2000). Rats exhibited decreased physical activity, bloodlike flecks around the face, and matted fur, but no mortality. Another study exposed rats (sex and strain not reported) to saturated TCEP vapor (concentration not reported) for 8 hours; no clinical signs of toxicity were seen (Smyth et al. 1951). No lethality occurred in any of the acute inhalation studies.

5.1.4 Acute Intraperitoneal Toxicity (Table 5.3)

In an unpublished single-dose toxicity study, rats were injected with TCEP intraperitoneally (Smith 1936, as cited in Aceto Chemical Co. Inc. 1989). The sex, strain, number of rats, and doses were not reported, but an LD_{50} of 280 mg/kg was reported; 250 mg/kg was reported as "lethal" in mice, with no additional information. Other effects included prolonged epileptiform convulsions, but the species in which these convulsions were observed was not reported.

5.1.5 Primary Skin Irritation (Table 5.4)

TCEP was applied to occluded, intact and abraded skin of six New Zealand rabbits for 4 hours (USEPA 1989). The rabbits were observed at 4, 24, and 48 hours following exposure; irritation was classified as none. The dose and sex were not reported, but a sufficiently high dose was tested, based on the observation of systemic toxicity. The authors reported that 4/6 of the treated rabbits experienced narcosis and paralysis that led to death within 120 hours.

5.1.6 Primary Eye Irritation (Table 5.4)

Several studies where 10 mg or 0.1 mL of TCEP was administered into one eye of New Zealand rabbits found no irritation at durations up to 72 hours post exposure (USEPA 1989).

5.1.7 Respiratory Irritation

No relevant studies were located.

5.1.8 Sensitization

No relevant studies were located.

Table 5.1. Acute Oral Toxicity Data Table*

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*	*Citation*	*Comments*
Sprague-Dawley Albino Rat (M, F) Oral gavage Single dose	Not reported	Lethality	LD_{50} reported as $46.4<LD_{50}<100$; males LD_{50} reported as $46.4<LD_{50}<1000$; females (values in mg/kg)	USEPA 1989	Dose, number of animals and lethality incidence were not reported. Due to these data gaps, the reliability of this study could not be ascertained.
Sprague-Dawley Albino Rat (M, F) Oral Single dose	Nominal dose: 215, 464, 1000 or 2150 mg/kg (5/sex/group)	Lethality, spasmodic contractions, acute depression	LD_{50} – 500 mg/kg males LD_{50} – 430, 500 or 790 mg/kg females	Ulsamer et al. 1980; USEPA 1989** (as cited in WHO 1998; NTP 1991b)	Lethality was seen in the 464 mg/kg dose group for both sexes. Different LD_{50} values in females are the result of 3 different lots of chemicals

Table 5.1. Acute Oral Toxicity Data Table*

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*	*Citation*	*Comments*
Sprague-Dawley Rat (M, F) Oral gavage Single dose	Nominal dose: 800, 1000 or 1260 mg/kg (5/sex/group)	Lethality, piloerection, increased salivation, hunched posture, abnormal gait, lethargy, labored breathing, ptosis, pale extremities; microscopic pathology	LD_{50} – 1150 mg/kg	Kynoch and Denton 1990 (as cited in WHO 1998)	14-day observation period. 1 female in 1000 mg/kg group died on day 2; 4/5 of both sexes died by day 4 in the 1260 mg/kg group. Clinical signs > 1000 mg/kg. No additional signs of toxicity after day 4.
Albino Rat Oral Single dose	Not reported	Lethality	LD_{50} – 1230 mg/kg	Eldefrawi et al. 1977 (as cited in ATSDR 2012)	Dose, strain, number of animals and sex not reported. Lethality incidence not reported.
Rat Oral Single dose	Not reported	Lethality	LD_{50} – 1410 mg/kg	Smyth et al. 1951 (as cited in WHO 1998; ATSDR 2012; NTP 1991b)	Dose, strain, sex and number per group not reported. Lethality incidence was not reported.

Table 5.1. Acute Oral Toxicity Data Table*

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*	*Citation*	*Comments*
F344 Rat (M, F) Oral gavage Single dose	Nominal dose: 0, 175, 350 or 700 mg/kg (6/sex/group)	Lethality, seizures, tonic-clonic convulsions	Lethality at 350 mg/kg in females	Herr et al. 1991	Seizures within 1 hour of dosing in the 350 and 700 mg/kg groups. Seizures in females progressed to tonic-clonic convulsions over 12 hours. 2 females in the 350 mg/kg group died.
F344 Rat (F) Oral gavage Single dose	Nominal dose: 0 or 275 mg/kg (6 control; 12 per group – hippocampal) (8 per group – behavioral)	1st experiment: Epileptiform convulsions, loss of pyramidal cells in CA1 region of hippocampus. 2nd experiment: behavioral effects in water maze	CNS and behavioral effects, LOAEL – 275 mg/kg	Tilson et al. 1990	Number of animals not reported. No lethality, single dose selected for CNS effects. Seizures occurred within 1-2 hours. Treated rats showed mild impairment of their performance in the acquisition of a water maze task compared to controls.

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*	*Citation*	*Comments*
2 Pitbulls, 1 Rottweiler, 1 German Shepherd Canine Oral Single exposure	Acute poisoning of 4 dogs (2 separate incidents)	Lethality, CNS excitation, seizures	CNS, analyzed stomach contents contained >2 ppm of TCEP	Lehner et al. 2010	Animals were exposed to a mixture of chemicals from ingesting car seat cushions, including other flame retardants and phthalates; cannot definitively attribute to TCEP alone.

[*]A control group is not always utilized in acute toxicity studies; studies without a control are not an error.

[**]The EPA/OTS report was sanitized to remove names and trademarks. It was determined that the data from EPA/OTS are the same as that from Ulsamer et al. (1980) study after comparison of doses and study details.

Table 5.2. Acute Inhalation Toxicity Data Table*

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*	*Citation*	*Comments*
Sprague-Dawley Albino Rat (M, F) Inhalation Single exposure 4 hours	5 mg/L (10/sex)	No lethality occurred, decreased activity and matted fur	LC_{50} >5 mg/L (5000 mg/m^3)	Stauffer Chemical Company 1974, 1979 (as cited in IUCLID 2000)	No lethality; >94% respirable particles.
Rat Inhalation Single exposure 8 hours	Saturated vapor (6 rats)	Lethality	Not determined	Smyth et al. 1951 (as cited in IUCLID 2000; ATSDR 2012; WHO 1998)	Sex and strain not reported. No deaths and no other clinical signs reported

*A control group is not always utilized in acute toxicity studies; studies without a control are not an error.

Table 5.3. Acute Intraperitoneal Toxicity Data Table*

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*	*Citation*	*Comments*
Rat Intraperitoneal Single dose	Not reported	Lethality, prolonged epileptiform convulsions	LD_{50} – 280 mg/kg	Smith 1936 as cited in Aceto Chemical Co. Inc. 1989	Sex, strain, number per group, and doses not reported. Species in which convulsions seen not clear.
Mouse Intraperitoneal Single dose	Not reported	Lethality, prolonged epileptiform convulsions	250 mg/kg was lethal	Smith 1936 as cited in Aceto Chemical Co. Inc. 1989	Sex, strain, number per group, and doses not reported. Species in which convulsions seen not clear.

*A control group is not always utilized in acute toxicity studies; studies without a control are not an error.

Table 5.4 Acute Dermal and Irritation Toxicity Data Table

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*	*Citation*	*Comments*
New Zealand Albino Rabbit Dermal	Not reported	Lethality	LD_{50} reported as $200<LD_{50}<5000$ (values in mg/kg)	Aceto Chemical Co. Inc. 1989; USEPA 1989	Sex, dose, number of animals, duration, and lethality incidence were not reported.
New Zealand Albino Rabbit Dermal (occluded intact and abraded) 4 hours	(6/group)	Primary skin irritation; clinical signs	Lethality (4/6)	USEPA 1989	Sex and dose were not reported. Animals observed at 4-, 24- and 48-hours; no skin irritation reported. 4/6 rabbits experienced narcosis and paralysis causing death within 120 hrs.
New Zealand Albino Rabbit Ocular	10 mg or 0.1 mL	Ocular irritation	None identified	USEPA 1989	No irritation at durations up to 72 hours post exposure

5.2 Repeated Dose Toxicity

5.2.1 Oral (Table 5.5)

Male and female F344/N rats (5/sex/group) were exposed to 0, 22, 44, 88, 175 or 350 mg/kg/day (equivalent to 0, 15.7, 31.4, 62.9, 125, and 250 mg/kg/day after duration-adjustment to daily exposure by multiplying by 5/7) by oral gavage in corn oil 5 days/week for 16 days[3] (NTP 1991b; Matthews et al. 1990). Male rats in the 125 and 250 mg/kg/day groups experienced an increase in mean absolute and relative kidney weights, and absolute and relative liver weights were increased in females in the 250 mg/kg/day group, but without any accompanying histopathology. These organ weight changes were all 10% or greater, except that the relative kidney weight in males at 125 mg/kg/day was increased by 8%. Serum cholinesterase activity was decreased by 18% ($p \leq 0.01$) and 20% ($p \leq 0.05$) in females dosed at 125 mg/kg/day and 250 mg/kg/day, respectively, without any gross or histopathologic lesions (Matthews et al. 1990; NTP 1991b). Serum cholinesterase was not reduced in males at any dose. Although the changes in organ weights may have been adaptive, the magnitude of the changes after acute exposure suggests that 125 mg/kg/day was a marginal LOAEL for kidney effects in males, and that 250 mg/kg/day was a minimal LOAEL for liver effects in females. This determination is supported by similar findings in the subchronic and chronic studies. ATSDR (2012) noted the organ weight changes, but did not comment on their adversity. However, it did not use this study as the basis for an acute minimal risk level (MRL), perhaps because the exposure duration was slightly longer than 2 weeks.

A second study exposed B6C3F1 mice (5/sex/group) to 0, 44, 88, 175, 350 or 700 mg/kg/day (0, 31.4, 62.9, 125, 250, or 500 mg/kg/day duration-adjusted) by oral gavage in corn oil 5 days/week for 14 days[2] (NTP 1991b; Matthews et al. 1990). Convulsive movements and ataxia were reported for male and female mice in the 250 and 500 mg/kg/day groups. All signs of toxicity were resolved by post exposure day 3. A single death occurred in each of three different exposure groups (125 mg/kg/day males, 250 mg/kg/day males, and 500 mg/kg/day females) and was attributed to gavage trauma. The LOAEL in this study was 250 mg/kg/day in both males and females, based on clinical signs of neurotoxicity.

In a range finding study (NTP 1991a), CD-1 mice (8/sex/group) were gavaged (frequency unclear, but appears to be daily, based on the broader study design) with 0, 87.5, 175, 350, 700 or 1000 mg/kg/day of TCEP in corn oil for 14 days. Water consumption was significantly greater in the 700 and 1000 mg/kg/day dose groups. One male death in the 714 mg/kg/day dose group was treatment related; the other 4 deaths were gavage trauma. The study authors reported a maximum tolerated dose (MTD) of approximately 700 mg/kg/day.

[3] The NTP report and Mathews et al. (1990) are not consistent in their report of the dosing duration. The former reported dosing for 12 doses over 16 days, while the latter reported dosing for 14 days.

Several studies (subchronic and chronic oral gavage, dietary, and continuous breeding studies) were conducted in rats and/or mice that evaluated the potential of TCEP to cause systemic toxicity (NTP 1991a; NTP 1991b; Matthews et al. 1990; Matthews et al. 1993). In a subchronic oral toxicity study in rats, TCEP treatment resulted in increases in the incidence of lesions in the brains (significant incidence in females, non-significant in males). Increases in liver and kidney weights (with renal histopathology developing after a 2-year exposure) were also noted in the rat. In mice, subchronic exposures resulted in significant decreases in kidney weights accompanied by renal lesions, and increased absolute (male and female) and relative (female) liver weights without accompanying histopathological changes. In 2-year oral gavage studies, TCEP treatment also caused microscopic lesions in the brain (degenerative) and the kidneys (focal hyperplasia) in rats, and kidney lesions (karyomegaly – nuclear enlargement – of tubule epithelial cells) in both sexes and liver lesions (foci of cytologic alteration) in female mice. In dietary studies, the predominant effects included testicular effects in male rats (short-term exposure), mean body weight changes and kidney effects (tubular hyperplasia) in rats (subchronic exposure), and kidney effects (hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei) in mice (chronic exposure). In the continuous breeding study, TCEP treatment at high doses resulted in increased incidences of cytomegaly of the renal tubular epithelium and hepatocytomegaly in both sexes of mice, fluid/sloughed degenerated cells in the lumen of the epididymis and reduced sperm content in males, and cystic ovaries in female mice.

These studies are discussed in detail below and summarized in Table 5.5. The text and corresponding table present the key studies first, followed by the supporting studies.

NTP conducted subchronic toxicity studies in rats and mice (NTP 1991b; Matthews et al. 1990). These studies have been reviewed by ATSDR (2012) and ECHA (2009). In the rat study, groups of F344/N rats (10/sex/group) were administered 0, 22, 44, 88, 175, or 350 mg/kg (0, 15.7, 31.4, 62.9, 125, or 250 mg/kg/day duration-adjusted) TCEP by gavage in corn oil 5 days per week for 16 weeks (females) or 18 weeks (males). Similarly, groups of B6C3F1 mice (10/sex/group) were administered 0, 44, 88, 175, 350, or 700 mg/kg (0, 31.4, 62.9, 125, 250, or 500 mg/kg/day duration-adjusted) TCEP by gavage in corn oil 5 days per week for 16 weeks. Feed and water were available *ad libitum*. Animals were observed twice daily for morbidity and mortality. Individual animal weights were recorded at the start, then weekly, and at termination of the studies. Clinical examination was performed weekly. Serum cholinesterase activity was determined at termination in surviving rats and mice. Necropsy was performed on all animals and organ weights (brain, heart, liver, lung, right kidney, and thymus) were obtained from all animals surviving until necropsy. Complete histopathological examination was performed on all control animals as well as on the two highest dose groups of rats (125 mg/kg/day, 250 mg/kg/day) and all high-dose mice (500 mg/kg/day). Tissues and organs examined were those

that are typically examined in NTP subchronic toxicity studies. In addition, histopathological examination was performed on the brain of female rats in the mid-dose group (62.9 mg/kg/day) and kidneys of mice receiving 31.4, 62.9, 125, or 250 mg/kg/day.

During week 4 of the rat study, an error in test substance preparation resulted in the rats in the two highest dose groups receiving a double dose on the first three days of this week dose (i.e., actual doses for those days were 350 and 700 mg/kg/day; these doses are not duration-adjusted, because they were delivered for only 3 days). The rats in these two groups were not dosed on the 4^{th} day of week 4 to allow them to recover; dosing was resumed according to protocol on the following day. The overdosing resulted in the death of two female rats each in the overdosed groups in the 4^{th} week, and gavage trauma also resulted in the death of one male rat in the 250 mg/kg/day group and one male and two female rats in the 15.7 mg/kg/day dose group. In rats, one out of 10 males in the 125 mg/kg/day group and 5/10 males and 3/10 females in the 250 mg/kg/day died; these deaths were not associated with overdosing or gavage errors.

Occasional periods of hyperactivity were reported in female rats that received doses of 125 and 250 mg/kg/day, while periodic convulsions were observed in 250 mg/kg/day dosed females but not in males during week 12.

There were no significant differences in the subchronic study in final body weight between dosed and control male rats, but final body weights in females at 250 mg/kg/day were 20% greater than those of controls. Treatment-related ($p \leq 0.01$) increases in the absolute and relative liver and kidney weights were noted in males at 250 mg/kg/day (absolute, but not relative liver weight in males was also significantly increased at 125 mg/kg/day) and in females at 31.4 to 250 mg/kg/day (relative liver weight was also significantly increased in females at 15.7 mg/kg/day). At the high dose, there were increases of 20% and 25% in relative liver and kidney weights, respectively, in males, while in females, relative liver weights increased from 13% to 51% and relative kidney weights increased from 9% to 22% at 31.4 to 250 mg/kg/day. Microscopic examination revealed no treatment-related lesions in either the liver or the kidney. However, there were increases in the incidence of lesions in the brains of male and female rats. Treatment-related increases in neuronal necrosis were noted in the hippocampus and thalamus of female rats in the 125 and 250 mg/kg/day groups. Although similar lesions were observed in male rats in the 250 mg/kg/day group, the authors described the lesions as mild and not significant. The incidences of loss of neurons in the hippocampus were 10/10 females and 2/10 males in the 250 mg/kg/day group and in 8/10 females in the 125 mg/kg/day group, indicating that the lesions occur at a higher incidence and are more severe in female rats than in males. In addition, two female rats in the 250 mg/kg/day group had necrosis and loss of thalamic nuclei. Serum cholinesterase activity determined at necropsy was 75% and 59% of the control value in female rats in the 125 and 250 mg/kg groups, respectively. However, this activity was not

reduced in male rats. The authors reported that technical difficulties prevented accurate analysis of sperm morphology in the rats.

Thus, TCEP caused increased absolute and relative organ weight changes (liver and kidney weight changes) in both males and females. However, the interpretation of the adverse effect levels differs based on agency guidelines. Because the changes were not accompanied by histological effects, ECHA (2009) did not consider the effects to be toxicologically relevant. Instead, ECHA (2009) focused on the brain lesions observed in the hippocampal region of female rats, and identified a NOAEL of 62.9 mg/kg/day in female rats and 125 mg/kg/day in male rats, with corresponding LOAELs of 125 mg/kg/day and 250 mg/kg/day, respectively. Although ATSDR (2012) agreed that liver and kidney weight changes without accompanying histopathological changes could in some cases be considered not to be toxicologically adverse, the authors reached a different determination about the toxicological relevance of the liver and kidney weight changes, based on consideration of the magnitude of the changes and the potential for progression. Based on the progression to more severe kidney effects in the related NTP (1991b) 2-year oral toxicity study (see below), ATSDR (2012) concluded that the kidney effects in the subchronic study can represent a minimal LOAEL (125 mg/kg/day). ATSDR did not specify the criteria for the effect level, but it appears to be based on a dose-related 10% increase in both absolute and relative organ weight, an approach consistent with common practice (Andrews, 2005). ATSDR (2012) further concluded that although no such progression was seen for the liver, 125 mg/kg/day represented a minimal LOAEL for liver changes in males and females, with a NOAEL of 62.9 mg/kg/day, based on the magnitude of the liver weight changes. We note, however, that although a 10% increase in liver weight was seen in females at 125 mg/kg/day, a 10% or greater change in male liver weight was seen only at 250 mg/kg/day. Thus, the minimal LOAEL for both liver and kidney effects in females is 125 mg/kg/day and the corresponding NOAEL is 62.9 mg/kg/day. In males, the minimal LOAEL for both liver and kidney effects is 250 mg/kg/day, and the NOAEL is 125 mg/kg/day. ATSDR (2012) analyzed the kidney data using the benchmark dose (BMD) approach to determine the point of departure for MRL derivation, but the data were not amenable to BMD modeling. ATSDR (2012) developed a $BMDL_{10}$ of 85.07 mg/kg/day for the brain lesions. To adjust for continuous exposure, the $BMDL_{10}$ was multiplied by 5 days/7 days, resulting in a duration-adjusted $BMDL_{10}$ of 60.76 mg/kg/day.

In the subchronic study with B6C3F1 mice treated with TCEP at 31.4 to 500 mg/kg/day, there were no treatment-related deaths, but gavage trauma resulted in the death of one male each in the 125, 250, and 500 mg/kg/day groups and one female each in the 125 and 250 mg/kg/day groups (NTP 1991b; Matthews 1990). TCEP treatment did not affect body weight gain, final body weight or serum cholinesterase activity at any dose level. There were statistically significant increases of >10% in mean relative liver weight in males at 500 mg/kg/day and in absolute and

relative liver weight in females at 125 mg/kg/day and above, although the increase in females was not fully dose-related. There were dose-related and significant decreases in mean absolute, but not relative, kidney weights in males only at 125 mg/kg/day (5%), 250 mg/kg/day (10%) and 500 mg/kg/day (20%). There were decreases in absolute and relative testis weights (NTP 1991b) and a slight decrease in sperm count only in the 500 mg/kg/day males compared to that of controls (ECHA 2009). Microscopic examination did not reveal any morphological changes in the liver, but TCEP treatment resulted in epithelial cells with enlarged nuclei (mild cytomegaly and karyomegaly) in the kidney of all animals of both sexes at 500 mg/kg/day. Unlike the findings in the rat, there were no brain or thalamic lesions in mice.

Identification of an effect level for the liver is problematic in light of the absence of accompanying histopathological lesions, the sporadic dose-response, and the absence of progression in the accompanying chronic study. Based on the magnitude of the change, it appears that the liver weight changes in mice were of minimal toxicological adversity, and could be the basis for a minimal LOAEL in females of 125 mg/kg/day. In addition, the dosing errors during week 4 (double dosage at the top two doses) confound the identification of a LOAEL or NOAEL. The only clearly adverse effect in this study (NTP 1991b; Mathews et al. 1990) was in the kidney, for which a NOAEL of 250 mg/kg/day and a LOAEL of 500 mg/kg/day can be determined for both sexes of mice, based on significant decreases in the mean absolute kidney weights and by renal lesions (mild cytomegaly and karyomegaly).

Chronic Studies

Two-year studies were conducted in F344/N rats and B6C3F1 mice to characterize the chronic toxicity of TCEP (purity 98%) (NTP 1991b; Matthews et al. 1993b). These studies have also been reviewed by ATSDR (2012) and ECHA (2009). In the rat study, 60 animals/sex/group were administered 0, 44, or 88 mg/kg/day (0, 31.4, or 62.9 mg/kg/day duration-adjusted) TCEP in corn oil by gavage, 5 days per week, for up to 103 weeks[4]. Similarly, 60 mice/sex/group received 0, 175, or 350 mg/kg/day (0, 125, or 250 mg/kg/day duration-adjusted) TCEP by oral gavage on the same dosing schedule for 103 weeks. For both rats and mice, 10 animals/sex/group were predesignated for interim evaluation (necropsy, histopathology, hematology, and clinical chemistry) after 66 weeks. All animals were observed twice a day for morbidity and mortality. Body weights were recorded weekly for the first 13 weeks and monthly thereafter. Clinical observations, including palpable tissue masses or other lesions, were recorded monthly. Necropsy and complete histopathological examinations were performed as is routinely conducted in 2-year NTP studies (NTP 1991b; Matthews et al. 1993).

[4] For both the rat and mouse 2-year studies, dosing was for 103 weeks, and sacrifice was at the beginning of week 104. Therefore, NTP lists exposure duration as 103 weeks, and sacrifice at week 104, with effects as those seen at the 104-week sacrifice. This document describes exposures as 103 weeks, with sacrifice at week 104.

In the 66-week interim evaluation rat group, one female rat in the high-dose group died on day 261 and one control died on day 408 (NTP 1991b; Matthews et al. 1993). TCEP treatment did not affect body weight gain or hematology. However, serum alkaline phosphatase and alanine aminotransferase were significantly decreased in the high-dose female rats only; while increases in these enzymes can indicate liver damage, *decreases* are generally not toxicologically significant. Significant increases in relative liver (14%) and kidney (20%) weights were seen in males at the high dose; there was no effect on organ weights in females. Microscopic evaluation at interim sacrifice noted focal brain lesions in the cerebrum and thalamus. There were local necrosis and accumulation of inflammatory cells, reactive gliosis, and endothelial hypertrophy and hyperplasia in the cerebrum and thalamus of the 3/10 female rats in the high-dose group (ECHA 2009). In mice, the 66-week interim evaluation found no effects on mortality, body weight gain, hematology, or clinical chemistry. Microscopic evaluation of the kidneys revealed hyperplasia of tubule epithelial cells in two males in the high-dose group.

Survival was reduced in male and female rats treated with TCEP for 2 years, with the decrease significant at the high dose (NTP 1991b; Matthews et al. 1993). The percent survival in males was 78%, 68%, and 51%, at 0, 31.4 mg/kg/day, and 62.9 mg/kg/day, respectively. The corresponding percent survival in females was 66%, 71%, and 37%, respectively. The authors attributed the reduced survival of the high-dose females, in part, to the neurotoxicity of TCEP and to a marginally increased incidence of mononuclear cell leukemia. Microscopic examination revealed lesions in the brain of females, but not males. Over 40% of the females in the high-dose group had degenerative lesions in the brain stem and cerebrum (thalamus, hypothalamus, and basal ganglia), while at the low dose there were no lesions. These lesions were reported to be focal, located in the cerebrum and brain stem, and involved both grey and white matter. In males, the incidence of the brain lesions approximated that in controls. Treatment-related proliferative lesions were reported primarily in the kidney of both sexes. Significant and dose-related increases in the incidence of focal hyperplasia in the renal tubule epithelium of both sexes were noted. The hyperplasia occurred in the convoluted tubules of the cortex and was characterized by stratification of the epithelial cells, with partial to complete obliteration of the tubule lumens. The incidences of the focal hyperplasia at 0, 31.4, and 62.9 mg/kg/day, respectively, were 0/50 (0%), 2/50 (4%), and 24/50 (48%) in males and 0/50 (0%), 3/50 (6%), and 16/50 (32%) in females.

Based on the results of the 2-year bioassay, the NOAEL for brain lesions in rats is 31.4 mg/kg/day, with a LOAEL of 62.9 mg/kg/day (NTP 1991b; Matthews et al. 1993). The LOAEL for the dose-related increase in kidney lesions in rats of both sexes was 62.9 mg/kg/day, with a NOAEL of 31.4 mg/kg/day. For deriving a chronic-duration MRL for TCEP, ATSDR (2012) analyzed the incidences of cerebrum gliosis in female rats and of renal hyperplasia in both male

and female rats using the BMD approach. The estimated $BMDL_{10}$ values were 31.1 mg/kg/day[5] and 23.44 mg/kg/day for renal tubule hyperplasia in male rats and female rats, respectively. ATSDR (2012) also estimated a $BMDL_{10}$ of 42.8[4] mg/kg/day for cerebral gliosis in female rats. Thus, based on the BMD analysis, the most sensitive endpoint was renal lesions in female rats, with a $BMDL_{10}$ of 23.44 mg/kg/day; this was the overall most sensitive chronic effect level.

In mice treated with TCEP for 2 years, there were no effects on mortality or body weight gain and no alterations in hematology or clinical chemistry in either sex at any dose level (NTP 1991b; Matthews et al. 1993). The kidney was the main target organ in mice following TCEP treatment. There were dose-related increases in the incidence of karyomegaly (nuclear enlargement) of tubule epithelial cells, with significance at 125 mg/kg/day and above. The incidence of karyomegaly at 0, 125, and 250 mg/kg/day was 2/50 (4%), 16/50 (32%) and 39/50 (78%), respectively, in males and 0/50 (0%), 5/49 (10%) and 44/50 (88%), respectively, in females. The incidence of hyperplasia was not significant at any dose in either sex. Microscopic examination of the liver revealed a dose-dependent increase in the incidence of foci of cytologic alteration, particularly eosinophilic, but not basophilic or clear cell, foci in males [0/50 (0%), 3/50 (6%), and 8/50 (16%) at 0, 125, and 250 mg/kg/day respectively]. There were no increases in the incidences of hepatocellular foci in treated females compared to controls.

Based on the results from the 2-year bioassay in mice, the LOAEL for kidney lesions in both male and female mice and liver foci in males was 125 mg/kg/day, the lowest dose tested. No NOAEL could be determined.

Other Studies

Task 2 of a continuous breeding protocol provided additional information on systemic toxicity. Swiss CD·1 mice (20-40 breeding pairs/group) were dosed by oral gavage with 0, 175, 350, or 700 mg TCEP/kg/day (apparently daily, based on the statement that the animals were "continuously exposed") for 18 weeks (1 week prior to cohabitation, 14 weeks of cohabitation, and 3 weeks thereafter – see Section 5.5 for the reproductive/developmental portion) (NTP 1991a). During the cohabitation phase, 4 females and 2 males (two females in the control group, two males and one female in the 175 mg/kg/day group, and one female in the 700 mg/kg/day group) died or were killed for humane reasons. The deaths were attributed to gavage trauma or due to septicemia or lymphoma (not chemical-related). Adult male body weights varied slightly but always were within 10% of the control values. For females, a body weight difference between controls and those receiving 700 mg/kg/day was observed after 6 weeks of treatment,

[5] ATSDR provided only the BMDL for male rat renal tubule hyperplasia based on nominal dose, of 43.58 mg/kg/day. The duration-adjusted value of 31.1 mg/kg/day was calculated for this assessment. Similarly, ATSDR provided a BMDL of 59.86 mg/kg/day for cerebral gliosis in females, and the duration-adjusted value was calculated for this assessment.

but the authors indicated that the difference could be related to gestation status. Absolute live pup weights (male, female, and combined) were significantly increased in the mid and high doses, but the differences were not significant when adjusted for litter size. The histopathological examination conducted on animals that died did not reveal any treatment-related effects. Histopathological examination of selected organs from 10-13 representative rats/sex/group in the control and 700 mg/kg/day groups (from Tasks 2 and 3 of the continuous breeding protocol) revealed minimal to mild cytomegaly of the renal tubular epithelium in 10/12 treated males and 5/13 of the treated females. Hepatocytomegaly was present in 10/12 treated males and 2/13 of the treated female mice, with the lesions being more frequent and more severe in males than in females. Lesions in reproductive organs are discussed in Section 5.5. Based on the result of this study, an effect level of 700 mg/kg/day can be determined based on cytomegaly of the renal tubular epithelium and hepatocytomegaly in both sexes of mice, fluid/sloughed degenerated cells in the lumen of the epididymis and reduced sperm content in males, and cystic ovaries in female mice. It cannot be determined whether 350 mg/kg/day is a NOAEL, since histopathological examination was not performed on this group.

Three dietary studies [28-day and 3-month studies in rats (Stauffer Chemical Company 1980a,b, as cited in ECHA 2009) and an 18-month study in mice (Takada et al. 1989)] were identified. Limited details were available for another dietary study (Stauffer Chemical Company 1972, as cited in ECHA 2009).

The 28-day dietary study was a range-finding study in which groups of Sprague-Dawley CD rats (10/sex/group) received TCEP (purity not reported) at dietary concentrations of 0, 500, 850, 1500, or 2000 ppm (Stauffer Chemical Company 1980b, as cited in ECHA 2009). The dose levels were reported by ECHA (2009) to be equivalent to 0, 42, 72, 125, and 163 mg/kg/day, respectively, in males and 0, 50, 88, 144, and 191 mg/kg/day, respectively, in females; calculation of intake was based on nominal concentrations. ECHA (2009) reported that, "Additionally two weeks after initiation of dosing, the 200 ppm (sic) dose level (equivalent to 19 mg/kg bw/d in males, and 20 mg/kg bw/d in females) was increased to 4000 ppm (equivalent to 293 mg/kg bw/d in males and 334 mg/kg bw/d in females), and in another dose group receiving 350 ppm (sic) (equivalent to 30 mg/kg bw/d in males and 38 mg/kg bw/d in females) for three weeks the dose level was increased to 8000 ppm (equivalent to 495 mg/kg bw/d in males and 508 mg/kg bw/d in females) for one week." It is not entirely clear when the 200 ppm and 350 ppm dose levels were begun, when these dose levels were increased to the 4000 ppm and 8000 ppm levels, and whether or not these animals were dosed for the entire 28 days. However, ECHA (2009) noted that the study was not conducted in accordance with the requirements of the guideline testing protocols of B.7/ OECD TG 407 and that it differed in some respects from the published guidelines. Significant deficiencies identified by ECHA in this study included evaluation of only a small selection of parameters in clinical biochemistry and lack of organ

weight assessment and histopathology. ECHA (2009) acknowledged that basic data were provided in this study that can be used as supporting information. Results from this range-finding study indicated no treatment-related deaths or changes in mean body weights. Food consumption was significantly decreased in males and females receiving 495 mg/kg/day and 508 mg/kg/day, respectively, after one week of treatment. Treatment with TCEP did not result in biologically significant changes in hematology or clinical chemistry. However, 1/10 males in the 125 mg/kg/day group and 3/10 males in the 495 mg/kg/day group had smaller than normal seminal vesicles and/or prostate. ECHA (2009) determined that ingestion of TCEP may have caused the testes of one male to be dissimilar in size and weight. Based on the results from this study, the NOAEL was 72 mg/kg/day, with a LOAEL of 125 mg/kg/day for testicular effects in male rats. The NOAEL for systemic toxicity appears to be 495 mg/kg/day in male rats and 508 mg/kg/day in female rats, without a LOAEL identified, although it is unclear what duration of dosing these doses apply to. If the doses were increased at 2 and 3 weeks in males and females, respectively, this would suggest that the rats received the higher doses for only 2 and 1 week, respectively; ECHA (2009) did not report time-weighted average doses.

In an early dietary study (Stauffer Chemical Company 1972, as cited in ECHA 2009), groups of rats (strain not specified) (10/sex/group) received 0.5% (250 mg/kg/day) TCEP for 30 days in the diet. Results indicate no adverse effects on growth, appearance and behavior, liver and kidney weights, or changes in pathological examinations of survivors. This indicates a NOAEL of 250 mg/kg/day for both sexes of rats in this study. This same study was also summarized without detailed information in Ulsamer et al. (1980).

A 3-month dietary study was conducted based on the results of the 28-day range-finding dietary study (Stauffer Chemical Company 1980a, as cited in ECHA 2009). In this OECD test and guideline-compliant study (but without microscopy of the brain), groups of Sprague-Dawley CD rats (20/sex/group) were fed TCEP (purity commercial grade) in a commercial diet at 0, 400, 1000, 3000, or 8000 ppm (equivalent to 0, 26, 65, 192, and 506 mg/kg/day in males; and 0, 30, 75, 215, and 586 mg/kg/day in females, calculation of intake based on nominal concentrations). There were no deaths or clinical signs attributable to TCEP treatment. There were significant decreases in body weights and the mean weekly food consumption in males at 506 mg/kg/day and in females at 586 mg/kg/day. In addition, there was a 5% decrease in food consumption in females at 215 mg/kg/day. Compared with control values, final body weights in males were reduced by 7% and 18% at 192 and 506 mg/kg/day, respectively, and in females by 8% and 17% at 215 and 586 mg/kg/day, respectively. The decreased body weight was similar to the decrease in food consumption and may have been related to poor palatability, although a general malaise leading to decreased food consumption cannot be ruled out. Treatment with TCEP did not alter clinical chemistry, hematology, urinalysis, or cholinesterase activity at any dose level. Compared to control values, the mean relative liver weight in males was significantly higher at 192 mg/kg/day (19%) and 506 mg/kg/day (22%) and in females at 215 mg/kg/day (9%) and at

586 mg/kg/day (30%). There was also a dose-related increase in the mean relative kidney weights in males. Increases of 9%, 15%, and 22%, compared to controls, were noted in the males at 65, 192, and 506 mg/kg/day, respectively; the latter two changes are considered biologically significant. In females, increases of 12% and 13% were reported at 215 and 586 mg/kg/day, respectively. There was a slight increase over controls in tubular hyperplasia with total incidences of 6/20, 4/20, 7/20, 8/20 and 11/20, respectively, although there was not a dose-related increase in severity. Apparently these increases were in males, although ECHA (2009) is not clear, and it appears that increases occurred in both sexes. ECHA (2009) suggested that the increased incidence at the two middle doses may not have been treatment-related but did consider the increase at the high dose to be meaningful. Therefore, ECHA (2009) listed 192 mg/kg/day in males and 215 mg/kg/day in females as the NOAELs; the corresponding LOAELs are 506 mg/kg/day for males and 586 mg/kg/day for females.

Although the relative mean weights of the gonads and the brain were reported to be reduced at the two highest doses in males and females, ECHA (2009) did not report the extent of the reduction. High-dose males and females were also reported to have lower absolute heart weights, but the extent of the reduction was not reported. Based on lack of changes in absolute mean weights of other organs in treated animals compared to controls and the absence of other treatment-related gross or histological legions, ECHA (2009) indicated that the relative increases in organ weights noted in this study might be due to the lower body weight gain at the two highest doses. Therefore, ECHA (2009) identified 192 mg/kg/day in males and 215 mg/kg/day in females as the NOAELs, with LOAELs of 506 mg/kg/day in males and 586 mg/kg/day in females, based on mean body weight changes and kidney effects (tubular hyperplasia). The NOAELs for liver effects were 506 mg/kg/day in males and 586 mg/kg/day in females, with no LOAEL identified. As noted above, ECHA (2009) used a more stringent definition of adversity based on organ weight changes than that used by ATSDR or elsewhere in this assessment. Based on a 10% change in organ weight, it appears that there may have been adverse effects at lower doses than those identified by ECHA (2009), but a full independent assessment is not possible based on the data in the ECHA report. In light of the decreased body weight at the high dose, interpretation of the results would require information on the degree of change for both the absolute and relative organ weights at each dose.

In an 18-month dietary study, Slc:ddY mice (50/sex/group) were administered TCEP in the diet at 0, 0.012, 0.06, 0.3, or 1.5% (Takada et al. 1989). This study has been reviewed by ECHA (2009) and ATSDR (2012). The study is in Japanese, with an English abstract and tables. ATSDR (2012) reported the dose levels to be approximately 0, 11, 53, 267, and 1333 mg/kg/day, based on assuming a mean body weight of 0.0045 kg and daily food consumption of 0.004 kg/day from graphs in the Takada et al. (1989) paper. Survival was reduced in males from week 70 and in females from week 60 in the 1333 mg/kg/day group, with the final survival in both

sexes being approximately 49% as compared to the 65% survival in controls. Body weight gain was 60% lower than the control value in both males and females in the 1333 mg/kg/day group; ATSDR (2012) stated that there was a 32% reduction in the final body weight at 1333 mg/kg/day. Food consumption was not affected at any dose level in any of the sexes. Hematology revealed only a significant increase in platelets (no further detail) in males in the 267 mg/kg/day group; the effect was not dose-related. Heart and testes weights in males and kidney weights in females at 1333 mg/kg/day were significantly reduced, but percent changes were not provided. Microscopic examination revealed the kidney (predominant) and the liver as target organs. Focal necrosis, vacuolation of the liver cells and extramedullary hematopoiesis were observed in the liver of animals in all groups including the control group. Males and females of all treatment groups, but not the control group, showed hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei, the latter being described as polymorphic. Abnormal division, degeneration and necrosis were also observed several times. However, the incidences of these findings were not reported. Animals in the 1333 mg/kg/day group also had cysts, necrosis of the urinary tubule epithelium, and interstitial fibrosis. In the liver of all treated and control groups, there were focal necrosis, vacuolation of the liver cells, and extramedullary hematopoiesis. No information on noncancer lesions was provided in the abstract or tables.

Overall, the highest dose, 1333 mg/kg/day, resulted in reduced survival, reduced body weight gain, and severe toxicity in this mouse strain, while microscopic examination showed kidney effects (hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei) in treated animals in all dose groups. These effects were absent in control animals, but liver effects were identified in all dose groups including controls, and so could not be attributed to TCEP exposure. Although incidence data for the microscopic changes were not available, ECHA (2009) determined that no NOAEL for the kidney effects could be established in this study. Therefore, based on the data reported, the LOAEL for the kidney effects in both sexes of Scl:ddY mice in this study can be identified as 11 mg/kg/day, the lowest dose tested. However, it is noted that this dietary study appears to identify an effect level well below that identified in the chronic gavage studies. The reason for the difference between studies is unclear, but could reflect differences in strain sensitivity or saturable mechanisms (e.g., saturation of absorption or metabolic activation in the gavage study). ATSDR (2012) reported only the body weight changes and tumors and did not report any effects on other organs, perhaps because it did not have a translation of the paper.

5.2.2 Inhalation (addressed in Table 5.7)

No data on systemic effects of inhalation exposure were located. A non-standard inhalation study was conducted in which male rats (strain and number of rats exposed not specified) were exposed to 0, 0.5 or 1.5 mg TCEP/m^3 around-the-clock for 4 months

Table 5.5. Repeat-dose toxicity studies – Noncancer Endpoints

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
Short-term Studies				
F344/N Rats (M, F) Oral gavage 5 days/week 16 days (12 doses) (See footnote 2 in the text regarding dosing regimen) NTP 1991b; Matthews et al. 1990	Nominal dose: 0, 22, 44, 88, 175, or 350 mg/kg/day Duration adjusted dose: 0, 15.7, 31.4, 62.9, 125, or 250 mg/kg/day (5/sex/group)	Increase in mean absolute and relative kidney weights (M); absolute and relative liver weights (F), but without any accompanying histopathology Decrease in serum cholinesterase activity in females, but without any gross or histopathologic lesions	Kidney marginal LOAEL = 125 mg/kg/day (M); ND (F); NOAEL = 62.9 mg/kg/day (M), 250 mg/kg/day (F) Liver minimal LOAEL = 250 mg/kg-day (F), ND (M); NOAEL = 125 mg/kg/day (F), 250 mg/kg/day (M)	Serum ChE activity was decreased by 18% and 20% in females dosed at 125 ($p \leq 0.01$) and 250 mg/kg/day. Since inhibition is not greater than 20% (to be regarded as toxicologically adverse), ECHA did not call out neurotoxicity as an adverse endpoint. There were no histopathological findings in the brain. ATSDR (2012) noted the organ weight changes, but did not comment on their adversity, but did not use them as the basis for an acute MRL.

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
B6C3F1 mice (M, F) Oral gavage 5 days/week 16 days (12 doses) (See footnote 2 in the text regarding dosing regimen) NTP 1991b; Matthews et al. 1990	Nominal dose: 0, 44, 88, 175, 350, or 700 mg/kg/day Duration adjusted dose: 0, 31.4, 62.9, 125, 250, 500 mg/kg/day (5/sex/group)	Convulsive movements and ataxia in both sexes	CNS; LOAEL = 250 mg/kg-day (M,F); NOAEL = 125 mg/kg-day (M,F)	Signs of toxicity were not seen on post exposure day 3
CD-1 mice (M, F) Oral gavage 14 days (frequency not clear, but appears to be daily) NTP 1991a (Task 1 repro study)	Nominal dose: 0, 87.5, 175, 350, 700, or 1000 mg/kg/day (8/sex/group)	Lethality, increased water consumption	MTD – approximately 700 mg/kg/day	Water consumption was significantly greater in the two highest dose groups. One animal died in the 1000 mg/kg/day group.
Longer-term studies				

344/N Rats (M, F) Oral gavage 5 days/week, 16 weeks (F), 18 weeks (M) NTP 1991b; Matthews 1990	Nominal dose: 0, 22, 44, 88, 175, or 350 mg/kg/day Duration adjusted dose: 0, 15.7, 31.4, 62.9, 125, or 250 (10/sex/group)	Increased mortality: 1/10 males at 125 mg/kg/day and 5/10 males, 3/10 females at 250 mg/kg/day. Hyperactivity/ Convulsions: periods of hyperactivity in females at 125 and 250 mg/kg/day; periodic convulsions were observed at 250 mg/kg/day in females during week 12. Loss of neurons in the hippocampus in females at 125 and 250 mg/kg/day and non-significantly in males at 250 mg/kg/day. Decreased serum cholinesterase at 125 and 250 mg/kg/day in females Body weight changes (20% increase) in females at 350 mg/kg/day; final mean body weights of the remaining groups of dosed female rats and dosed male rats were similar Increase in relative liver weight >10%, unaccompanied by histopathology Increase in relative kidney weight >10%, unaccompanied by histopathology at this duration	Mortality – LOAEL = 250 mg/kg/day (M, F); NOAEL = 125 mg/kg/day (M, F) Brain – LOAEL = 250 mg/kg/day (M), 125 mg/kg/day (F); NOAEL = 125 mg/kg/day (M), 62.9 mg/kg/day (F) $BMDL_{10}$ for brain lesions = 60.76 mg/kg/day (F) (ATSDR, 2012) Liver – LOAEL = ND; NOAEL = 250 mg/kg/day (M, F) (ECHA 2009) Liver – LOAEL (minimal) = 125(F) or 250 (M) mg/kg/day; NOAEL = 62.9 (F) or 125 (M) mg/kg/day (ATSDR 2012). Kidney – LOAEL = ND; NOAEL = 250 mg/kg/day (M, F) (ECHA 2009) Kidney – LOAEL (minimal) = 125 (F) or 250 (M) mg/kg/day; NOAEL = 62.9 (F) or 125 (M) mg/kg/day (ATSDR 2012). 2012).	Dosing and gavage errors during week 4 resulted in rats in the two highest dose groups receiving a double dose on the first three days of this week, presenting uncertainty in the effect level identification; Interpretation of the adverse effect levels differs based on agency guidelines. ECHA (2009) focused on the brain lesions. ATSDR (2012) reached a different determination about the toxicological relevance of the liver and kidney weight changes, based on consideration of the magnitude of the changes and the potential for progression

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
B6C3F1 Mice (M, F) Oral gavage 5 days/week, 16 weeks NTP 1991b; Matthews 1990	Nominal dose: 0, 44, 88, 175, 350, or 700 mg/kg/day Duration adjusted dose: 0, 31.4, 62.9, 125, 250, or 500 (10/sex/group)	Increased mean absolute (M, F) and relative (F only) liver weights in females but without accompanying histopathology changes Decreases in absolute and relative testis weights and a slight decrease in sperm count at 500 mg/kg/day Decreased absolute kidney weights in males and renal lesions (mild cytomegaly and karyomegaly) in all animals of both sexes	Liver – minimal/ questionable LOAEL = 125 (F) or 500 (M) mg/kg/day; NOAEL = 62.9 (F) or 250 (M) mg/kg/day Male repro – LOAEL = 500 mg/kg/day; NOAEL = 250 mg/kg/day Kidney – LOAEL = 500 mg/kg/day; NOAEL = 250 mg/kg/day	Received double dosage during week 4 for 350 and 700 mg/kg/day groups, presenting uncertainty in the effect level identification
F344/N rats (M, F) Oral gavage 5 days per week, 66 weeks (interim sacrifice) NTP 1991b; Matthews et al. 1993	Nominal dose: 0, 44, or 88 mg/kg/day Duration adjusted dose: 0, 31.4, or 62.9 mg/kg/day (10/sex/group)	Decrease in alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) in females Increase >10% in relative kidney and liver weights in males Brain lesions in females One female at 62.9 mg/kg/day died prior to sacrifice	Liver – LOAEL = 62.9 mg/kg/day (M), ND (F); NOAEL = 31.4 mg/kg/day (M), 62.9 (F) Kidney – LOAEL = 62.9 mg/kg/day (M), ND (F); NOAEL = 31.4 mg/kg/day (M), 62.9 (F) Brain – LOAEL = ND (M), 62.9 mg/kg/day (F); NOAEL = 62.9 mg/kg/day (M), 31.4 mg/kg/day (F)	Decreases in ALP and ALT not considered adverse

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
B6C3F1 mice (M, F) Oral gavage 5 days per week, 66 weeks (interim sacrifice) NTP 1991b; Matthews et al. 1993	Nominal Dose: 0, 175, or 350 mg/kg/day Duration adjusted dose: 0, 125, or 250 (10/sex/group)	Hyperplasia of tubule epithelial cells in males	Kidney – LOAEL = 250 mg/kg/day (M); NOAEL = 125 mg/kg/day (M)	
F344/N rats (M, F) Oral gavage 5 days per week, 103 weeks NTP 1991b	Nominal dose: 0, 44, or 88 mg/kg/day Duration adjusted dose: 0, 31.4, or 62.9 mg/kg/day (60/sex/group)	Decreased survival in both sexes Focal hyperplasia of tubule epithelium of the kidney in both sexes Degenerative lesions in the brain of females	Mortality – LOAEL = 62.9 mg/kg/day (M, F); NOAEL = 31.4 mg/kg/day (M, F) Kidney – LOAEL = 62.9 mg/kg/day (M, F); NOAEL = 31.4 mg/kg/day (M, F) $BMDL_{10}$= 31.1 mg/kg/day (M); 23.4 mg/kg/day (F) (ATSDR 2012) Brain/Neuro – LOAEL = 62.9 mg/kg/day (F); NOAEL = 31.4 mg/kg/day (F) $BMDL_{10}$ = 42.8 mg/kg/day (ATSDR 2012)	Renal lesions in female rats were the most sensitive chronic endpoint.

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
B6C3F1 mice (M, F) Oral gavage 5 days per week, 103 weeks NTP 1991b	Nominal dose: 0, 175, or 350 mg/kg/day Duration adjusted dose: 0, 125, or 250 mg/kg/day (60/sex/group)	Kidney morphology [karyomegaly (nuclear enlargement) of tubule epithelial cells] in both sexes Foci of cytologic alteration, particularly eosinophilic foci in the liver of males	Kidney – LOAEL = 125 mg/kg/day (lowest dose tested) (M, F) Liver – LOAEL = 125 mg/kg/day (lowest dose tested) (M)	
Swiss CD·1 Mice (M, F) Oral gavage Continuous Breeding Protocol: 18 weeks (1 week prior to cohabitation, 14 weeks of cohabitation, and 3 weeks thereafter) (dosing frequency not clear, but appears to be daily) NTP 1991a	Task 2: Nominal doses: 0, 175, 350, or 700 mg/kg (20-40 breeding pairs/group)	Minimal to mild cytomegaly of the renal tubular epithelium Hepatocytomegaly Fluid/sloughed degenerated cells in the lumen of the epididymis and reduced sperm content Cystic ovaries	Kidney – AEL – 700 mg/kg/day (M and F) Liver – AEL – 700 mg/kg/day (M and F) Epididymis – AEL – 700 mg/kg/day Ovary – LOAEL – 700 mg/kg/day	Histopathology conducted only at 700 mg/kg/day, so NOAEL cannot be identified AEL = adverse effect level

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
Sprague-Dawley CD rats (M, F) Diet 28 days Stauffer Chemical Company 1980b (as cited in ECHA, 2009)	Nominal dose: 0, 500, 850, 1500, or 2000 ppm (M: 0, 42, 72, 125, or 163 mg/kg/day; F: 0, 50, 88, 144, or 191 mg/kg/day; **after 2 weeks**, concentration increased 200 → 4000 ppm (M: 19 →293 mg/kg/day; F: 20 → 334 mg/kg/day) **after 3 weeks**, concentration increased 350 → 8000 ppm (M: 30 → 495 mg/kg/day; F: 38→ 508 mg/kg/day) (10/sex/group) (equivalent doses in mg/kg/day as reported by ECHA 2009)	No treatment-related deaths or changes in mean body weights Smaller seminal vesicles and/or prostate (males) Food consumption decreased in males (495 mg/kg/day) and females (508 mg/kg/day) after one week of treatment	Seminal vesicles/prostate – LOAEL = 125 mg/kg/day (M); NOAEL = 72 mg/kg/day (M) NOAEL for systemic effects = 508 mg/kg/day (F); 495 mg/kg/day (M)	A range-finding study, but not conducted in line with guideline testing protocols of B.7/ OECD TG 407 (ECHA, 2009); No mortality; significant study deficiencies include: changes in dose within a group during the study; only a small selection of parameters in clinical biochemistry; no organ weight assessment, no histopathology

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
Rats (M, F) Diet, daily 30 days Stauffer Chemical Company 1972 (as cited in ECHA, 2009); Ulsamer 1980	Nominal dose: 0.5% (250 mg/kg/day) (10/sex/group)	No treatment-related effects	NOAEL = 250 mg/kg/day (M, F),	Strain not reported. Limitations in testing and reporting.

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
Sprague-Dawley CD rats (M, F) Diet, daily 3 months Stauffer Chemical Company 1980a (as cited in ECHA, 2009) Anonymous (1977) (as cited in ATSDR 2012)	Nominal dose: 0, 400, 1000, 3000, or 8000 ppm (equivalent to: M – 0, 26, 65, 192, or 506 mg/kg/day; F – 0, 30, 75, 215, or 586 mg/kg/day) (20/sex/group)	Decrease in food consumption and body weight (M, F) Increase in relative kidney weights (M, F); Increased incidence in tubule hyperplasia in the renal cortex (affected sex unclear) Increase in relative liver weights (M, F)	Body weight and food consumption – LOAEL = 506 mg/kg/day (M), 586 mg/kg/day (F); NOAEL = 192 mg/kg/day (M), 215 mg/kg/day (F) Kidney – LOAEL = 506 mg/kg/day (M), 586 mg/kg/day (F); NOAEL = 192 mg/kg/day (M), 215 mg/kg/day (F) Liver – LOAEL – ND; NOAEL = 506 mg/kg/day (M), 586 mg/kg/day (F) Effect levels listed were identified by ECHA. A full independent assessment is not possible based on the data in the ECHA report. In light of the decreased body weight at the high dose, interpretation of the results would require both information on the degree of change for both the absolute and relative organ weights at each dose.	Conducted mostly according to OECD guidelines The decreased body weight was similar to the decrease in food consumption, and so may have been related to poor palatability, although a general malaise leading to decreased food consumption cannot be ruled out.

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
Scl:ddY mice (M, F) Diet, daily 18 months Takada et al. 1989 (as cited in ATSDR 2012 and ECHA 2009)	Nominal dose: 0, 0.012, 0.06, 0.3, 1.5% (equivalent to 0, 11, 53, 267, or 1333 mg/kg/day (ATSDR 2012) (50/sex/group)	Reduced survival Marked reduction in body weight gain Hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei Focal necrosis, vacuolation of the liver cells and extramedullary hematopoiesis were observed in the liver of animals in all groups including the control group	Mortality – LOAEL = 1333 mg/kg/day (M, F); NOAEL = 267 mg/kg/day (M, F) Body weight – LOAEL = 1333 mg/kg/day (M, F); NOAEL = 267 mg/kg/day (M, F) Kidney – LOAEL = 11 mg/kg/day (M, F); NOAEL = ND	Doses estimated by ATSDR 2012 ECHA 2009 estimated slightly different doses, based on a food factor of 10%
Slc:ddY mice (F) Dermal 2 times per week Up to 18 months Takada et al. 1991	Nominal dose: 0, 5, 50% (20-27/group)	Decrease in spleen weight	Spleen – LOAEL = 50% TCEP; NOAEL = 5% TCEP	Publication in Japanese with abstract in English. Applied volume not in English abstract, so doses not available. Interim sacrifices at 6 and 12 months but time to effect on spleen weight unclear

[1] All NOAELs, LOAELs, and AELs expressed in terms of duration-adjusted doses.

(Shepel'skaia and Dyschinegvich 1981, as cited in NTP 1991b). However, the study was designed to investigate reproductive outcome and a detailed description of systemic effects was not available, precluding determination of effect levels in this study.

5.2.1 Dermal (Table 5.5)

Takada et al. (1991) conducted a 79 week dermal carcinogenicity study in which TCEP was dissolved in ethanol at concentrations of 0, 5, or 50% and was topically applied to the skin of female Slc:ddY mice (20-27/group) twice a day (Takada et al. 1991). The article is in Japanese with an English abstract. The volume applied to the skin was not available in the English abstract, and so the total applied doses are not available. Five animals from each group were killed at 6 and 12 months to evaluate the systemic effects of TCEP. The authors noted that TCEP treatment caused a decrease in spleen weight in the 50% group. It is not clear whether the decrease occurred at all sacrifice times, but tabular data showed a substantial decrease in absolute and relative spleen weight at 18 months.

5.3 Endocrine Activity

No *in vivo* studies evaluating endocrine activity of TCEP were located. *In vitro* studies evaluating possible endocrine effects are addressed in Section 5.8, Other Mechanistic Studies.

5.4 Neurotoxicity (Table 5.6)

Neurotoxicity of TCEP has been observed in several studies in rats, mice, and hens [Mathews et al. 1990; Matthews et al. 1993b; NTP 1991b, NTP 1991a; Stauffer Chemical Company 1980b (as cited in ECHA, 2009); Anonymous 1977 (as cited in ATSDR 2012); Sprague et al. 1981]. These studies found that TCEP administered orally causes clinical signs of neurotoxicity, and brain lesions in rats, but not in mice. TCEP did not appear to cause delayed neurotoxicity in hens. A summary of the available studies is shown in Table 5.6. Two single-dose oral studies in one publication reported clinical signs of neurotoxicity, brain histopathology, and mild performance impairment (Tilson et al. 1990). With the exception of the hen study, other specialized neurotoxicity studies (e.g., including a functional observational battery) have not been conducted (with the exception of a study in Japanese but with an English abstract and tables that evaluated pups of exposed mothers – Kawashima et al. 1983). However, there is clear evidence of neurotoxic effects based on histopathology and clinical signs at high doses, and decreases in serum and red blood cell (RBC) cholinesterase levels. There are no available studies of neurotoxicity of TCEP via the inhalation or dermal routes.

Although decreased serum or RBC cholinesterase is not a neurologic effect, it is considered a biomarker for effects on nervous system cholinesterase, for which measurements are much less commonly available. RBC cholinesterase is considered more reflective of neural enzyme levels

than serum cholinesterase. With regard to plasma or red blood cell (RBC) cholinesterase, the USEPA's Office of Pesticide Programs (OPP) states that, while no fixed percentage of change is predetermined to separate adverse from non-adverse effects, "differences between pre- and postexposure of 20% or more in enzyme levels is nearly always statistically significant and would generally be viewed as biologically significant" (USEPA 2000). Assuming that a similar approach would apply to differences between exposed and control groups, and in light of other neurotoxicity of TCEP and its organophosphate structure, the current report considers decreases in serum or RBC cholinesterase as potentially adverse.

Two single dose oral gavage studies were conducted to assess CNS and behavioral effects (Tilson et al. 1990). For both studies, groups of female F344 rats were dosed with 0 or 275 mg/kg of TCEP. Epileptiform convulsions and loss of pyramidal cells in the CA1 region of the hippocampus were seen in the treated group in the CNS effect study (Tilson et al. 1990). Rats receiving 275 mg/kg of TCEP in the behavioral study showed mild impairment of their performance in the acquisition of a water maze task compared to controls. In a developmental toxicity study for which only the abstract and tables are in English, Kawashima et al. (1983) reported no effects in a variety of functional neurodevelopmental toxicity tests of the offspring of dams treated with doses up to 200 mg/kg/day on gestation days (GD) 7–15. Limited additional details of this study are available and provided in Section 5.5.

In a 16-18-week study (duration varied depending on species and sex) (Matthew et al. 1990; NTP 1991b), TCEP was administered to rats and mice by oral gavage in corn oil for 5 days/week to groups of 10 animals of each sex and species. F344/N rats were dosed at 0, 22, 44, 88, 175, or 350 mg/kg/day (0, 15.7, 31.4, 62.9, 125, or 250 mg/kg/day duration-adjusted) and the B6C3F1 mice received 0, 44, 88, 175, 350, or 700 mg/kg/day (0, 31.4, 62.9, 125, 250, or 500 mg/kg/day duration-adjusted). In female rats, serum cholinesterase levels decreased significantly to 25 and 41 % of controls in the 125 and 250 mg/kg/day groups, respectively. There were no significant changes in serum cholinesterase levels in male rats or either sex of mice. Examination of the hippocampus at termination revealed that TCEP caused necrosis (loss of pyramidal neurons of the CA1 region of the hippocampus) in 8/10 and 10/10 female rats at 125 mg/kg/day and 250 mg/kg/day, respectively, but the incidence was 2/10 in male rats at 250 mg/kg/day. The lesion was more common and more severe in females than males, indicating that the females were more sensitive than the males. Ataxia and convulsions were identified in the 125 and 250 mg/kg/day groups during the first 3 days of dosing in week 4, when a dosing error resulted in these groups receiving a double dose (i.e., actual doses for those days were 350 and 700 mg/kg/day; these doses are not duration-adjusted, because they were delivered for only 3 days). In mice, TCEP administration did not produce hippocampal lesions in either sex at any dose level. In rats, the LOAEL for brain lesions was (females) 125 mg/kg/day and (males) 250 mg/kg/day, with NOAELs of (females) 62.9 mg/kg/day and (males) 125 mg/kg/day. The subchronic $BMDL_{10}$ for

brain lesions in female rats was $BMDL_{10}$ was 60.76 mg/kg/day. In mice, the highest dose tested, 500 mg/kg/day, did not cause any neurotoxic effects in males or females.

In contrast to the gavage study, TCEP administered to male and female Sprague-Dawley CD rats in the diet at estimated doses of 0, 26, 65, 192, and 506 mg/kg/day (males) or 0, 30, 75, 215, and 586 mg/kg/day (females) for 3 months did not affect RBC cholinesterase activity or induce brain lesions (Stauffer Chemical Company 1980a, as cited in ECHA 2009; Anonymous 1977, as cited in ATSDR 2012). NOAELs for neurotoxicity were reported as the highest dose tested, or 506 mg/kg/day in males and 586 mg/kg/day in females.

The potential of TCEP to induce brain lesions was evaluated in 2-year bioassays in rats and mice (NTP 1991b; Matthews et al. 1993). F344/N rats (60/sex/group, including interim sacrifice group) received TCEP at doses of 0, 44, or 88 mg/kg/day by oral gavage 5 days/week (0, 31.4, or 62.9 mg/kg/day duration-adjusted). B6C3F1 mice (60/sex/group, including interim sacrifice group) received TCEP at doses of 0, 175, or 350 mg TCEP/kg/day (0, 125, or 250 mg/kg/day duration-adjusted) using the same dosing schedule. An interim evaluation was conducted on 10 animals/sex/group after a treatment period of 66 weeks that revealed that female rats in the high dose group had focal brain lesions that were located in the cerebrum and thalamus. Local necrosis and accumulation of inflammatory cells, reactive gliosis, and endothelial hypertrophy and hyperplasia were noted in 3/10 females at the high dose of 250 mg/kg/day. However, no brain lesions were observed in mice at the 66 weeks interim or at the 104 weeks terminal evaluation. At the 104-week evaluation, microscopic examination revealed a marked increase in the incidence of brain lesions [degenerative lesions of the brain stem and cerebrum (thalamus, hypothalamus, and basal ganglia)] in over 40% of female rats in the 62.9 mg/kg/day group. The lesions were also seen in dosed males but to a lesser extent. These lesions were reported to be focal and were characterized by neuronal necrosis with accumulation of inflammatory cells, reactive gliosis, and endothelial hypertrophy and hyperplasia. The lesions varied in severity from minimal to marked and often involved extensive areas. Based on the results from the 2-year bioassay, the NOAEL for brain lesions is 31.4 mg/kg/day and the LOAEL is 62.9 mg/kg/day for male and female F344/N rats (NTP 1991b; Matthews et al. 1993). ATSDR (2012) analyzed the incidences of cerebrum gliosis (the lesion with the highest incidence) in female rats and estimated a $BMDL_{10}$ (duration-adjusted) of 42.8 mg/kg/day.

The hen is the model organism for evaluation of organophosphorus-induced delayed neurotoxicity (OPIDN). This is a syndrome characterized by limb weakness and upper motor neuron spasticity, correlative signs of distal axonopathy of peripheral nerve and spinal cord, and inhibition and aging of neurotoxic esterase (neuropathy target esterase, NTE) in neural tissues (USEPA 1998). The potential for TCEP to cause delayed neurotoxicity was tested on 12- to 14-month-old white Leghorn hens (Sprague et al. 1981; Stauffer Chemical Company 1979, as

cited in ECHA 2009). In the acute study, 18 hens were gavaged with a single dose of TCEP (neat) at 10 mL/kg (~14.2 g/kg or 14,200 mg/kg, as determined by the authors), and were killed 24 hours after dosing; brain NTE activity was determined. In the delayed neurotoxicity study, the same investigators dosed 18 hens once at 14,200 mg/kg and observed the animals for walking behavior over a two-week period. The treatment was repeated three weeks later and the hens killed three weeks after the second treatment. Positive and negative control groups (10/group) indicated that the assay was performing as intended. Nerve tissues were obtained for analysis. A single oral dose of TCEP produced significant inhibition of plasma cholinesterase (87.1%) and brain NTE (30.0%). Marked inhibition (>75%) of NTE activity after single administration of a compound is interpreted as indicating that TCEP would produce delayed neurotoxicity in hens (Johnson 1975, as cited in Sprague et al. 1981). Thus, the magnitude of the decrease in NTE activity in the short-term study (<75%) is consistent with the absence of evidence of behavioral evidence of delayed neurotoxicity in the repeated dose study and the absence of histopathological evidence of delayed neurotoxicity. The repeated dose study tested a dose higher than the limit dose of 1,000 mg/kg, but the dosing should have been daily for 28 days, followed by an additional 21 days of observation. The tested dose did cause systemic and reproductive toxicity, as evidenced by a loss of body weight, transient reductions in food consumed immediately after treatment, cessation of egg production shortly after the first administration, and severe feather loss. Although these effects cannot be extrapolated to human toxicity, they do indicate that a sufficiently high dose was tested. This study suggests that TCEP does not cause delayed neurotoxicity, although the intermittent nature of the dosing decreased the confidence in that conclusion.

Overall, rats appear to be more sensitive than mice to the neurotoxic effects of TCEP, with female rats being more sensitive than males. In a subchronic study (NTP 1991b), based on a significant reduction in serum cholinesterase levels and necrosis of the neurons in the hippocampal region of the brain, the LOAEL for neurotoxic effects in female rats was 125mg/kg/day, with a NOAEL of 62.9 mg/kg/day. The subchronic $BMDL_{10}$ for brain lesions in female rats was 60.76 mg/kg/day. The LOAEL and NOAEL for male rats were 250 mg/kg/day and 125 mg/kg/day, respectively. No neurotoxicity was seen in mice up to the highest dose tested, 500 mg/kg/day. In the 2-year bioassay, the NOAEL for brain lesions was 31.4 mg/kg/day for male and female F344/N rats (NTP 1991b, Matthews et al. 1993); the corresponding BMDL10 was 42.8 mg/kg/day for cerebral gliosis in female rats.

5.5 Reproductive and Developmental Toxicity (Table 5.7)

One reproductive toxicity study using a continuous breeding protocol, and two developmental toxicity studies in rodents were available for evaluation of TCEP (NTP 1991a; Hardin et al. 1987; Kawashima et al. 1983). In addition, one non-standard inhalation study that evaluated

Table 5.6. Summary of Neurotoxicity Studies

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
F344 Rat (F) Oral gavage Single dose	0 or 275 mg/kg (6 control; 12 exposed – hippocampal) (8 control and 6-8 exposed – behavioral)	1st experiment: Epileptiform convulsions, loss of pyramidal cells in CA1 region of hippocampus. 2nd experiment: behavioral effects in water maze	CNS and behavioral effects, LOAEL – 275 mg/kg	Tilson et al. 1990	Total number of animals not clear, but the number/group was reported for individual tests. No lethality, single dose selected for CNS effects. Seizures occurred within 1-2 hours. Treated rats showed mild impairment of their performance in the acquisition of a water maze task compared to controls.

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
F344 Rats (M, F) Oral gavage 5 days/week, 16 weeks (F), 18 weeks (M)	Nominal dose: 0, 22, 44, 88, 175, or 350 mg/kg/day Duration adjusted dose: 0, 15.7, 31.4, 62.9, 125, or 250 mg/kg/day (10/sex/group)	Decreased serum cholinesterase levels (F only) Necrosis of the neurons in the hippocampal region of the brain at 125 mg/kg/day and 250 mg/kg/day (F) and at lower incidence in male rats at 250 mg/kg/day	Neurotoxicity– LOAEL = 125 mg/kg/day (F), 250 mg/kg/day (M); NOAEL = 62.9 mg/kg/day (F), 125 mg/kg/day (M) $BMDL_{10}$ for brain lesions = 60.76 mg/kg/day (F) (ATSDR, 2012)	Matthews et al. 1990; NTP 1991b	Brain lesions were the most sensitive subchronic effect Ataxia, convulsions in two highest dose groups during first 3 days of dosing in week 4, when a dosing error resulted in these groups receiving a double dose
B6C3F1 Mice (M, F) Oral gavage 5 days/week, 16 weeks	Nominal dose: 0, 44, 88, 175, 350, or 700 mg/kg/day Duration adjusted dose: 31.4, 62.9, 125, 250, or 500 mg/kg/day (10/sex/group)	No histopathological alterations in the brain and no effect on serum cholinesterase	Neurotoxicity – LOAEL = ND (M, F); NOAEL = 500 mg/kg/day (M, F)	Mathews et al. 1990; NTP 1991b	

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
Sprague-Dawley CD rats (M, F) Diet, daily 3 months	Nominal dose: 0, 400, 1000, 3000, or 8000 ppm (equivalent to: M: 0, 26, 65, 192, or 506 mg/kg/day; F: 0, 30, 75, 215, or 586 mg/kg/day) (20/sex/group)	No effect on brain lesions	Neurotoxicity – LOAEL = ND; NOAEL – 506 mg/kg/day (M), 586 mg/kg/day (F)	Stauffer Chemical Company 1980a (as cited in ECHA, 2009) Anonymous 1977 (as cited in ATSDR 2012)	There was also no effect on red blood cell cholinesterase
F344/N Rats (M, F) Oral gavage 5 days/week, 66 weeks (interim evaluation)	Nominal dose: 0, 44, or 88 mg/kg/day Duration adjusted dose: 0, 31.4, or 62.9 mg/kg/day (10/sex/group)	Brain lesions (f)	Neurotoxic – LOAEL = 62.9 mg/kg/day (F); NOAEL = 31.4 mg/kg/day (F)	Matthews et al. 1993; NTP 1991b	

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
F344/N Rats (M and F) Oral gavage 103 weeks	Nominal dose: 0, 44, or 88 mg/kg/day Duration adjusted dose: 0, 31.4, or 62.9 mg/kg/day (50/sex/group)	Brain lesions (f)	Neurotoxicity – LOAEL = 62.9 mg/kg/day (M, F); NOAEL = 31.4 mg/kg/day (M, F) Cerebral gliosis (F) $BMDL_{10}$ = 42.8 mg/kg/day (ATSDR 2012, duration adjustment done for this document)	Matthews et al. 1993; NTP 1991b	
B6C3F1 Mice (M and F) Oral gavage 5 days/week, 103 weeks	Nominal dose: 0, 175, or 350 mg/kg/day Duration adjusted dose: 0, 125, or 250 mg/kg/day (50/sex/group)	No neurotoxicity	Neurotoxicity – LOAEL = ND (M and F); NOAEL = 250 mg/kg/day (M and F)	Matthews et al. 1993; NTP 1991b	Interim evaluation at 66 weeks showed no neurotoxic effects
White Leghorn Hens Oral gavage; single dose; hens killed 24 hours after dosing	Nominal dose: 0, or 14,200 mg/kg/day (10 mL/kg neat) (18/group)	Inhibition (87.1% of control) of plasma cholinesterase activity Inhibition (30.0% of control) of brain NTE	Neurotoxicity – LOAEL = 14,200 mg/kg (only dose tested)	Sprague et al. 1981	

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
White Leghorn Hens Oral gavage 2 treatments (on day 1 and again 3 weeks later); two weeks observation period; hens killed 3 weeks after second treatment	Nominal dose: 0, or 14,200 mg/kg/day (10 ml/kg neat) (18 test animals, 10/negative and 10/positive control group)	No neurotoxicity noted	Neurotoxicity – LOAEL = ND; NOAEL = 14,200 mg/kg/day	Stauffer Chemical Company 1979 (as cited in ECHA 2009) ; Sprague et al. 1981	

[1] All NOAELs and LOAELs expressed in terms of duration-adjusted doses.

ND = not determined

NTE =neuropathy target esterase or neurotoxic esterase

male reproductive and developmental endpoints was available (Shepel'skaia and Dyschinegvich 1981, as cited in NTP 1991a). However, there were no studies evaluating the development and reproductive effects of TCEP by the dermal exposure route. Oral dosing of F0 and F1 generation rodents with TCEP resulted in adverse reproductive effects in the form of reduced fertility, with males reported to be more sensitive than females to TCEP treatment. In the inhalation study, TCEP reportedly affected fertility in both sexes (based on decreased litter size) and development. TCEP was not teratogenic or fetotoxic in mice or rats treated during organogenesis.

A continuous breeding protocol study (using the method of Chapin and Sloane 1997) was conducted where CD-1 mice were dosed by oral gavage with 0, 175, 350, or 700 mg TCEP/kg/day (apparently daily, based on the statement that the animals were "continuously exposed") (NTP 1991a). The continuous phase of the protocol (Task 2) consisted of treating a control group (40 breeding pairs) and 3 dose groups (20 pairs/group) with TCEP for 18 weeks (1 week prior to cohabitation, 14 weeks of cohabitation resulting in multiple litters per breeding pair, and 3 weeks thereafter). Figure 5.1 shows the reproductive assessment by continuous breeding design. Endpoints measured included clinical signs of toxicity, parental body weight and average consumption of water during representative weeks, fertility (number producing a litter/number of breeding pairs), litters per pair, live pups per litter, proportion of pups born alive, sex of live pups, and the pup body weights immediately after birth. A 1-week crossover mating (Task 3) was performed to determine the affected sex after the last Task 2 litter was weaned by mating: control males x control females, control males x high dose (700 mg/kg/day) females, and high dose males (700 mg/kg/day) x control females. Task 4 of this protocol assessed the fertility of F1 animals. In this task, F1 animals at 74 ± 10 days of age, were cohabited for 7 days and then housed singly until delivery. Animals (20 non-siblings per treatment group) were treated with 0, 175 or 350 mg/kg/day. In addition to the Task 2 endpoints, necropsy and microscopic evaluations of selected organs were performed on Task 3 and 4 animals, in addition to evaluation of sperm morphology and vaginal cytology.

TCEP reduced fertility in the parental (F0) generation (Task 2), as evidenced by a dose-related decrease in the number of litters per pair and the number of live pups per litter at doses ≥350 mg/kg/day. The high dose group was almost completely infertile by the end of the exposure period.

In the cross-mating study (Task 3), adverse effects were noted in both treated groups at 700 mg/kg/day, the only dose tested. TCEP significantly reduced pregnancy and fertility indices and adversely affected the number of live pups per litter when treated males were mated with control females. In addition, the epididymal and testicular weights, epididymal sperm motility, epididymal sperm count, average spermatid head count, total spermatid heads per testis were significantly decreased, and the incidence of abnormal sperm significantly increased in the males. When treated females were mated with control males, the number of live pups per litter,

the number of live male pups per litter, and average dam weights were decreased, while there was no effect on estrous cycle length in treated females.

In this same study, histopathologic examination of selected organs from 10-13 representative mice/sex/group in the control and 700 mg/kg/day groups from Tasks 3 and all animals that died or were sacrificed early revealed histopathology in both male and female reproductive organs. Six out of 10 male mice in the control group and 4/10 male mice in the 700 mg/kg/day group had degeneration of seminiferous tubules, with 3/10 treated male mice having interstitial cell hyperplasia. Fluid/sloughed degenerated cells in the lumen of the epididymis (2/10 and 7/12 in control and treated groups, respectively) and reduced sperm content (1/10 and 5/12 in the control and treated groups, respectively) were noted in both groups, but were relatively more severe in treated mice. Cystic ovaries were reported in 3/10 and 6/10 females in the control and 700 mg/kg/day groups, respectively. Effects in the liver and kidney were described in Section 5.2.

In Task 4, treatment of the F1 generation with TCEP did not affect sperm endpoints, estrous cyclicity, or the average estrous cycle length at any dose level. However, the number of live pups per litter and the number of live male pups per litter were significantly reduced at ≥175 mg/kg/day, the lowest dose tested.

In summary, TCEP affected fertility in both males and females, but males were more severely affected. The study authors noted that mating of the treated F1 generation (Task 4) revealed an effect not seen in the F0 mating (altered sex ratio), and that effects on fertility were seen in Task 4 in the absence of effects on sperm parameters. In light of minimal histopathology observed in the liver of the F1 animals, the authors also concluded that TCEP has the potential for selective reproductive toxicity. ATSDR (2012) derived a $BMDL_{10}$ of 167.83 mg/kg/day for the decrease in live male F2 pup in Task 4 of this study. However, it appears that ATSDR did not use the models for nested data that are designed for developmental toxicity studies for this modeling. TCEP was not teratogenic or fetotoxic in a combined developmental toxicity/perinatal and postnatal (old Japanese-style combined Segment II and III) study in Wistar rats, when tested at doses of 0, 50, 100, or 200 mg/kg/day with an olive oil vehicle and where 15 dams/group were C-sectioned and 8 dams/group delivered (Kawashima et al. 1983); nor using a Chernoff/Kavlock approach with CD-1 mice at 940 mg/kg/day (Hardin et al. 1987). In the rat study, Kawashima et al. (1983) reported maternal toxicity [piloerection, general weakness, and reduced food consumption and maternal death (7 out of 30 died)] in rats at 200 mg/kg/day on GD 7–15. There were no increases in fetal death or malformations on GD 20 at any TCEP dose level. The authors also reported no effects in a variety of functional neurodevelopmental toxicity tests of the offspring, although details of the testing protocol were not available. The developmental NOAEL from this study was 200 mg/kg/day, with no LOAEL identified. This article is in Japanese with an English abstract and tables.

In the Hardin et al. (1987) study, pregnant time-mated mice (50/group dosed, 35/group evaluated) were gavaged with 940 mg TCEP/kg/day (the calculated LD_{10} and only dose tested) on GD 6–13 (and killed on postnatal day [PND] 3) exhibited a 12% reduction in body weight gain between GD 6 and PND 3 relative to controls. In this study, the number of viable litters, number of live pups born per litter, percent survival of pups, pup birth weight, and pup weight gain were not significantly affected. The single dose tested was a maternal LOAEL and a developmental NOAEL.

The three reproductive/developmental studies via the oral route indicate that TCEP is a reproductive toxicant, adversely effecting reproductive organ weights and sperm parameters and decreasing litter size. The reproductive LOAEL is considered 125 mg/kg/day based on reproductive effects in NTP (1991a). No reproductive NOAEL was determined. No developmental toxicity was observed at doses up to 940 mg/kg/day, even though maternal toxicity occurred, as evidenced by decreased body weight gain (Hardin et al. 1987).

In a non-standard inhalation toxicity study (Shepel'skaia and Dyschinegvich 1981, as cited in NTP 1991a), male rats (strain unspecified) were exposed to 0, 0.5, or 1.5 mg TCEP/m^3 around-the-clock for 4 months. It appears that after the exposure, the males were mated with naïve females to determine the effects of TCEP on fertility and development. Results indicated no differences in male fertility index. Litter size was decreased and pre- and post-implantation loss was increased in the 1.5 mg/m^3 group. Pups sired by males in the 0.5 mg/m^3 group were reported to have significantly reduced average fetal size (weight and crown-rump measurements). Histopathological examination of the testes showed effects mainly in "meiosis, postmeiotic growth stage, and the maturity of the spermatozoids" (NTP 1991a). The LOAEL from this study appears to be 0.5 mg/m^3, the lowest concentration tested. However, the reliability of the study is unconfirmed, with limited available data, and the results are inconsistent with well-conducted oral developmental toxicity studies.

5.6 Genotoxicity (Tables 5.8 and 5.9)

TCEP has been tested for genotoxicity in numerous test systems (Table 5.8). In bacterial systems, TCEP produced mostly negative results with and without metabolic activation. One of twelve Ames assays indicated equivocal evidence of mutagenicity (Nakamura et al. 1979), although there are conflicting results reported in secondary sources. Nakamura et al. (1979) stated that "most of the test samples showed mutagenic activity in the strains TA100 and TA1535…", but WHO (1998) and IARC (1999) both suggest that TCEP was mutagenic only with metabolic activation. ATSDR (2012) interpreted the results of Nakamura et al. (1979) as providing weak or equivocal evidence of mutagenicity. *In vitro* assays using mammalian

Table 5.7. Reproductive and Developmental Toxicity

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
Swiss CD·1 Mice (M and F) Oral gavage Continuous Breeding Protocol: 18 weeks (1 week prior to cohabitation, 14 weeks of cohabitation, and 3 weeks thereafter) (Dosing frequency not clear, but appears to be daily) NTP 1991a	Task 2: (20-40 breeding pairs/group) Nominal dose: 0, 175, 350, or 700 mg/kg/day Task 3 (crossover study): (20 pairs/group) Nominal dose: 0 or 700 mg/kg/day Task 4: (20 non-siblings/group) Nominal dose: 0, 175 or 350 mg/kg/day	Task 2: Reduced number of F1 litters produced by the parental generation Task 3: Reduced fertility, adverse sperm effects (decreased concentration, motility, total spermatid heads per testis, and percent abnormal), fluid/ sloughed degenerated cells in the lumen of the epididymis, cystic ovaries, reduced epididymal and testicular weights; degeneration of seminiferous tubules Task 3: When treated females were mated with control males, the number of live pups per litter, the number of live male pups per litter, and average dam weights were decreased. Task 3: When treated males were mated with control females, reduced pregnancy and fertility indices and decreased number of live pups per litter Task 4: Reduced number of live pups per litter in F1, altered sex ratio	Task 2: F0: Reproductive LOAEL = 350 mg/kg/day; NOAEL = 175 mg/kg/day Task 3: Reproductive AEL (M and F) = 700 mg/kg/day (only dose tested) Task 4: Reproductive LOAEL = 175 mg/kg/day (lowest dose tested) NOAEL –ND $BMDL_{10}$ = 167.83 mg/kg/day based on decreased live male pups/litter in offspring of treated F1 mice (ATSDR, 2012)	Fertility severely reduced at 700 mg/kg/day; 4 females and 2 males died (2 females in the control group; 1 female each in the low and high dose groups and 2 males in the middle dose group). Cause of death for 1 female (700 mg/kg) and for 1 male was (same dose) attributed to gavage trauma.

Strain/Species (Sex)/Route/ Duration *Citation*	*Dose (No. of Animals/dose group)*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Comments*
Wistar rats (F) Oral gavage GD 7-15 Kawashima et al. 1983	0, 50, 100, or 200 mg/kg/day; (23-30/group, with additional rats at high dose, but disposition unclear)	Decreased food consumption, piloerection and general weakness, mortality in dams; no fetal deaths, malformation, or functional deficits	Maternal (general) – LOAEL = 200 mg/kg/day; NOAEL = 100 mg/kg/day Developmental NOAEL = 200 mg/kg/day; LOAEL = ND	Vehicle was olive oil; 15 dams/group C-section and 8 dams/group delivered and evaluated in neurobehavioral tests
CD-1 Mice (F) Oral gavage GD 6-13 Hardin et al. 1987	0, 940 mg/kg-day 50/group (35 selected)	12% reduced body weight gain in dams	Maternal (general) – LOAEL = 940 mg/kg-day (lowest dose tested); NOAEL NA Developmental NOAEL = 940 mg/kg/day	Dosed mid-gestation (GD 6-13) and killed PND 3
Rats (strain unspecified) (M) Inhalation 24 hours/day, 4 months, then mated to naïve females Shepel'skaia and Dyschinegvich 1981 (cited in NTP 1991a)	0, 0.5 or 1.5 mg TCEP/m^3 (number unspecified)	Decrease in average fetal size (weight and crown-rump measurements) for pups sired by treated males Decreases in litter size and increases in pre- and post-implantation loss in the 1.5 mg/m^3 group Histopathological examination of the testes showed effects mainly in "meiosis, postmeiotic growth stage, and the maturity of the spermatozoids" (NTP 1991a)	Developmental – LOAEL = 0.5 mg/m^3 (lowest concentration tested); NOAEL = ND	The reliability of the study is unconfirmed, with limited available data, and the results are inconsistent with well-conducted oral developmental toxicity studies

[1] All NOAELs and LOAELs are expressed in terms of duration-adjusted doses. ND = not determined

Figure 5.1. The reproductive assessment by continuous breeding design

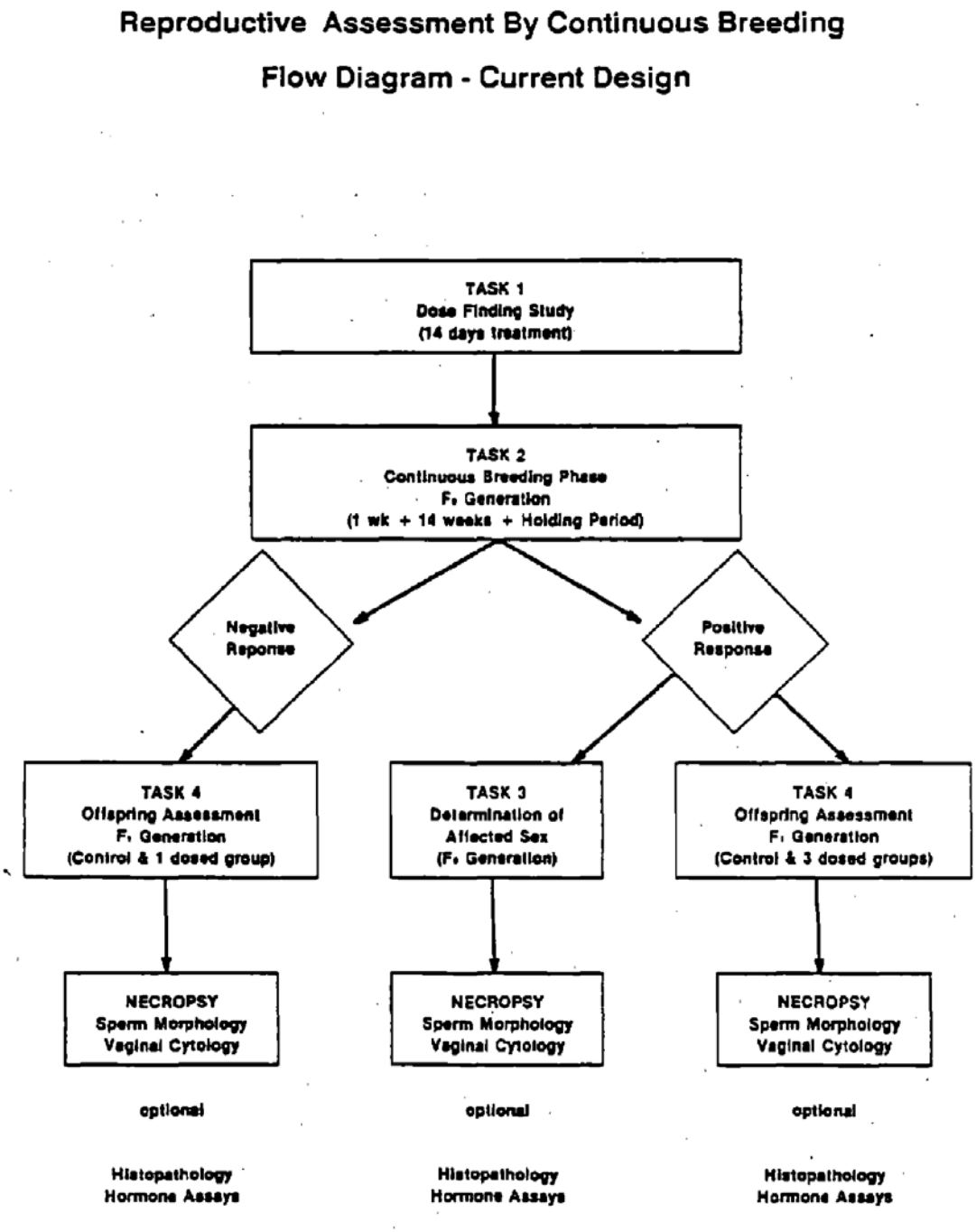


Figure 1 from NTP (1991a)

systems, including mouse lymphoma (L5178Y/TK +/-) and Chinese hamster V79 cells were negative for mutagenicity (Albright and Wilson Inc 1984a-d, as cited in USEPA 1989; Sala et al. 1982, as cited in ATSDR 2012). There was no evidence of clastogenicity in an *in vitro* chromosomal aberration analysis with CHO cells (Galloway et al. 1987; Föllmann and Wober 2006). TCEP was positive for cell transformation in hamster embryo cells (Sala et al. 1982), but not in two mouse cell lines (Sala et al. 1981; Matthews et al. 1993a). Sala et al. (1982) hypothesized that the positive result observed in the Syrian hamster embryo cells was caused by their higher metabolic activity, as compared to murine and human cells. Assays detecting primary DNA damage via sister chromatid exchange found positive evidence both with and without metabolic activation in Chinese hamster V79 cells (Sala et al. 1982), but negative in CHO cells without activation and marginal in CHO cells with activation (Galloway et al. 1987). TCEP was negative for unscheduled DNA synthesis (UDS) in a human cell line (Stauffer Chemical Company 1979, as cited in ECHA 2009).

Four *in vivo* micronucleus assays in rats and mice were negative (Table 5.9) (BG Chemie 1994, Fraunhofer Institute 1984, Otto 1984, as cited in IUCLID 2000, ECHA 2009), as was one *in vivo* chromosome aberration assay in rats (Stauffer Chemical Company 1978, as cited in IUCLID 2000). One micronucleus assay in Chinese hamsters indicated a weakly positive response for chromosomal aberrations (Sala et al. 1982, as cited in ATSDR 2012; ECHA 2009; WHO 1998). In Drosphilia, no evidence of somatic cell damage was identified (Vogel and Nivard 1993, as cited in ATSDR 2012).

Overall, the weight of the evidence indicates that TCEP is not mutagenic in bacterial or mammalian systems, and not clastogenic *in vitro* or *in vivo*. Based on the results of the sister chromatid exchange assays, the evidence is mixed regarding the potential for TCEP to cause primary DNA damage, but based on the results of mutagenicity tests, it appears that any such damage is repaired before it results in mutations.

5.7 Carcinogenicity (Table 5.10)

The potential of TCEP to be carcinogenic has been investigated in rats and mice following oral and dermal exposures (Matthews et al. 1993b; NTP 1991b; Takada et al. 1989, as cited in ATSDR 2012; Takada et al. 1991; Sala et al. 1982) (Table 5.10). Results from these studies indicated that rats developed renal tubular and thyroid follicular tumors following oral doses of TCEP for 2 years (Matthews et al. 1993b; NTP 1991b). Mice administered TCEP by oral gavage for 2 years had increased renal tumors (males) and Harderian gland tumors (females) (Matthews et al. 1993b; NTP 1991b). Mice fed a diet containing TCEP for 18 months developed tumors in the kidney and liver (males) and forestomach (females), as well as leukemia (females) (Takada et al. 1989, as cited in ATSDR 2012). When dermally treated with TCEP, mice showed no

Table 5.8 TCEP – In Vitro Summary

Species / Test System (Strain)	*End Point*	*Conclusion WITHOUT Activation*[1]	*Conclusion WITH Activation*[1]	*Citation*	*Comments*
S. typhimurium, TA98	Gene mutation	-	-	Abe and Urano 1994 [ATSDR 2012]	
S. typhimurium, TA97a, TA98, TA100, TA104, TA1535, TA1537, and TA1538	Gene mutation	-	-	Föllmann and Wober 2006 [ATSDR 2012]	
S. typhimurium, TA1535, TA1537, TA98, and TA100	Gene mutation	-	-	Haworth et al. 1983 [ATSDR 2012; WHO 1998; ECHA 2009]	Up to 3333 µg/plate, using the preincubation method
S. typhimurium, TA98, TA100, TA1535, TA1537	Gene mutation	-	-	Miltenburger 1984 [IUCLID 2000]	
S. typhimurium, TA98, TA100, TA1535, TA1537	Gene mutation[2]	+	+	Nakamura et al. 1979; [ECHA 2009; IARC 1999; WHO 1998]	"weak evidence of mutagenicity", up to 8550 µg/plate, 7.6-fold and 1.8-fold increase in revertants over control at 2850 µg/plate for TA1535 and TA100, respectively
		±	±	Nakamura et al. 1979; [ATSDR 2012]	
		+	+	Nakamura et al. 1979; [ECHA 2009]	
		-	+	Nakamura et al. 1979; [IARC 1999]	
		-	+	Nakamura et al. 1979; [WHO 1998]	
S. typhimurium, TA98, TA100, TA1537, TA1538	Gene mutation	-	-	NTP 1991b [ECHA 2009; IARC 1999]	

Species / Test System (Strain)	*End Point*	*Conclusion WITHOUT Activation*[1]	*Conclusion WITH Activation*[1]	*Citation*	*Comments*
S. typhimurium, TA100, TA1535, TA1538	Gene mutation	-	-	Prival 1977 [ECHA 2009]	
S. typhimurium, TA1535, TA1537, TA1538, TA98, and TA100	Gene mutation	-	-	Simmon et al. 1977 [WHO 1998]	Up to 5 mg/plate
S. typhimurium, TA98, TA100, TA1535, TA 1537	Gene mutation	-	-	Spiegelberg 1982 [IUCLID 2000]	
S. typhimurium, TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	-	-	Stauffer Chemical Company 1976 [ECHA 2009]	May be unpublished version of Ulsamer et al. (1980)
S. typhimurium, TA98, TA100, TA1535, TA1537, and/or TA97	Gene mutation	-	-	Tennant and Ashby 1991 [ATSDR 2012, WHO 1998]	
S. typhimurium, TA98, TA100, TA1535, TA1537	Gene mutation	-	-	Ulsamer et al. 1980 [IUCLID 2000]	
S. cerevisiae	Gene mutation	-	-	Stauffer Chemical Co. 1977, 1978 [IUCLID 2000]	
Mouse lymphoma (L5178Y/TK +/-)	Mutagenesis	-	-	Albright & Wilson Inc. 1984a (Study No: 40531) [USEPA 1989]	
Mouse lymphoma (L5178Y/TK +/-)	Mutagenesis	-	-	Albright & Wilson Inc. 1984c (Study No: 40711) [USEPA 1989]	.

Species / Test System (Strain)	*End Point*	*Conclusion WITHOUT Activation*[1]	*Conclusion WITH Activation*[1]	*Citation*	*Comments*
Mouse lymphoma (L5178Y/TK +/-)	Mutagenesis	-	-	Albright & Wilson Inc. 1984b (Study No: 40712) [USEPA 1989]	
Mouse lymphoma (L5178Y/TK +/-)	Mutagenesis	-	-	Albright & Wilson Inc. 1984d(Study No: 41031) [USEPA 1989]	
Mouse lymphoma (L5178Y/TK +/-)	Mutagenesis	-	-	Stauffer Chemical Company 1978 [ECHA 2009]	
Chinese hamster V79 cells	Forward gene mutation	-	-	Sala et al. 1982 [ATSDR 2012; ECHA 2009; WHO 1998]	Forward mutation HRPT locus
CHO cells	Chromosomal aberrations (clastogenicity)	-	-	Galloway et al. 1987; NTP 1991a [ATSDR 2012, WHO 1998; ECHA, 2009]	
CHO cells	Sister chromatid exchange	-	±	Galloway et al. 1987; NTP 1991a [ATSDR 2012, WHO 1998; ECHA 2009]	Incorrectly listed as chromosomal aberrations/ SCE in ATSDR. Chromosomal aberrations were not induced in CHO cells (see previous row).
Chinese hamster V79 cells	Sister chromatid exchange	+	+	Sala et al. 1982 [ATSDR 2012, WHO 1998; ECHA 2009]	
A-31-1-13 BALB/c-3T3 cells	Transformation assay	-	NA	Matthews et al. 1993a	Did not use an exogenous activation system

Species / Test System (Strain)	*End Point*	*Conclusion WITHOUT Activation*[1]	*Conclusion WITH Activation*[1]	*Citation*	*Comments*
Mouse C3H10T1/2 cells	Transformation assay	-	-	Sala et al. 1982 [ATSDR 2012, WHO 1998]	Induction of transformation
Syrian hamster embryo cells	Transformation assay	+	+	Sala et al. 1982 [ATSDR 2012, WHO 1998]	Hamster cells have higher metabolic activity, could explain why this result is positive and mouse is negative
Human WI-38 cells	Unscheduled DNA synthesis	-	-	Stauffer Chemical Company 1979 [ECHA 2009]	0, 0.005. 0.01, 0.05, 0.1 ul/ml
Chinese hamster V79 cells	DNA damage (Comet analysis)	-	-	Föllmann and Wober 2006 [ATSDR 2012]	Strand Breakage

[1] positive +; negative -; equivocal ±

[2]Conflicting interpretations were provided by different secondary sources, and so the conclusions of each secondary source are provided separately.

Table 5.9 TCEP – In Vivo Summary

Species / Test System (Strain)	*End Point*	*Conclusion (Neg/Weak-Equiv/Pos/ND)*	*Citation*	*Comments*
Mouse (unspecified strain)	Chromosomal aberrations (micronucleus assay)	-	BG Chemie 1994 [IUCLID 2000]	Gavage; up to 700 mg/kg
NMRI mice	Chromosomal aberrations (micronucleus assay)	-	Fraunhofer Institute 1984 [as cited by BUA report 20; IUCLID 2000]	Gavage; 1000 mg/kg
CD-1 mice; bone marrow erythrocytes	Chromosomal aberrations (micronucleus assay)	-	BG Chemie 1994 [IUCLID 2000]	i.p.; 175, 350, 700 mg/kg
NMRI mice; bone marrow erythrocytes	Chromosomal aberrations (micronucleus assay)	-	Otto 1984 [ECHA 2009]	1000 mg/kg; single oral dose
Chinese hamster (male/female, 2/ sex/dose); bone marrow erythrocytes	Chromosomal aberrations (micronucleus assay)	±	Sala et al. 1982 [ATSDR 2012; ECHA 2009; WHO 1998]	Significant increase in micronuclei at 62.5-250 mg/kg with dose-response in males
Sprague-Dawley rats; bone marrow	Chromosomal aberrations	-	Stauffer Chemical Company 1978 [IUCLID 2000]	Gavage; 0.062, 0.021, 0.0062 mg/kg bw
Drosophila melanogaster; mitotic recombination	Somatic cell damage	-	Vogel and Nivard 1993 [WHO 1998; ATSDR 2012; ECHA 2009]	w/w+ bioassay; feeding 2.5-40 mmol/L

[1] positive +; negative -; equivocal ±

significant treatment-related increases in neoplasms or lung adenomas (Takada et al. 1991). TCEP did not show initiator, promoter, or complete carcinogenic properties when dermally applied to mice (Sala et al. 1982), but this study is limited by the absence of negative controls.

A 2-year study was conducted by NTP where male and female F344/N rats or B6C3F1 mice were orally gavaged with TCEP (Mathews et al. 1993b; NTP 1991b). Groups of 60 rats of each sex received TCEP at a dose of 0, 44, or 88 mg/kg/day (0, 31.4, or 62.9 mg/kg/day duration-adjusted), 5 days per week for up to 103 weeks and were sacrificed at week 104. Similar numbers of mice of each sex received a dose of 0, 175 or 350 mg/kg/day (0, 125, or 250 mg/kg/day duration-adjusted) for 103 weeks and were sacrificed at week 104. Necropsy was performed on all animals killed or found dead and all organs and tissues were examined. Complete histopathological examinations were performed on all groups of rats, all controls, in high-dose mice, and in selected tissues of low-dose mice.

Survival (Kaplan-Meier) of rats was reported to be 78% of control, 68% of low-dose, and 51% of high-dose males and 66% of control, 71% of low-dose, and 37% of high-dose females (statistically significantly decreased in high-dose females relative to controls) (Mathews et al. 1993b; NTP 1991b). The authors attributed the reduced survival of the high-dose female rats in part to the neurotoxicity of TCEP and also to a marginally increased incidence of mononuclear cell leukemia. TCEP-related proliferative lesions (both sexes), renal tubular adenomas (both sexes) and carcinomas (males) were observed in the kidneys. TCEP caused a dose-related increase in the incidence of focal hyperplasia and adenoma or carcinoma of the renal tubule epithelium in male and female rats, with both effects being significant at 62.9 mg/kg/day, compared to respective controls: renal tubule hyperplasia, male rats: 0/50, 2/50, 24/50; female rats: 0/50, 3/50, 16/50; renal tubule adenoma, male rats: 1/50, 5/50, 24/50; female rats: 0/50, 2/50, 5/50); renal tubular adenomas or carcinomas, male rats 2/50, 5/50, 25/50; there were no renal tubule carcinomas in female rats. In the thyroid gland of rats, there were marginally increased incidences of follicular cell neoplasms (adenoma or carcinoma) in both sexes (male rats: 1/50, 2/48, 5/50; female rats: 0/50, 3/50, 4/50), but the incidence was significant only in high-dose females compared to controls. There was a significant positive trend in the incidence of mononuclear cell leukemia in both male and female rats (male rats: 5/50, 14/50, 13/50; female rats: 14/50, 16/50, 20/50). However, the study authors noted that the increase in males was not clearly dose related and partially due to an unusually low response in the control group, while the response in females was marginal. Overall the authors considered the increases in leukemia to be marginal and not clearly chemical-related. In the rat, the LOAEL for renal tubular adenoma, renal tubular adenoma/carcinoma, and thyroid follicular cell adenoma or carcinoma was 62.9 mg/kg/day (both sexes), with a NOAEL of 31.4 mg/kg/day (both sexes). NTP (1991b) concluded that there was “clear evidence of carcinogenic activity for male and female rats in this study, based on increased incidences of renal tubule adenomas.” NTP noted that thyroid

follicular cell neoplasms and mononuclear cell leukemia in male and female rats may have been related to chemical administration.

In mice, survival of the treated animals was not significantly different in either sex compared with their respective control groups (Mathews et al. 1993b; NTP 1991b). An increased incidence of renal tubule adenomas or carcinomas was reported in high-dose males (1/50, 1/50, and 4/50). The study authors stated that the incidence was not statistically significant even at the high dose, but that "the marginal increase in both tubule cell hyperplasia and tubule cell neoplasms is suggestive of a chemical-related effect." Adenomas or carcinomas of the Harderian gland, primarily adenomas, were marginally increased in the high-dose female mice (3/50; 8/50; 7/50), reaching significance when interim and terminal sacrifice incidence was combined (3/59, 8/60, 10/60). However, there was no corresponding TCEP-related increase in the incidence of hyperplasia of the Harderian gland. There was a positive trend in hepatocellular adenomas, but none of the results were significant in pairwise comparisons (20/50; 18/50; 28/50). Sensitivity to detect an effect was decreased by the high background, a common finding in this strain of mice. The following effect levels were determined: LOAEL for kidney adenoma was 250 mg/kg/day and the NOAEL was 125 mg/kg-day in males; the LOAEL for Harderian gland adenoma or carcinoma was 250 mg/kg/day, with a NOAEL of 125 mg/kg/day in females. NTP concluded that there was "equivocal evidence of carcinogenic activity for male B6C3Fl mice as shown by a marginally increased incidence of renal tubule cell neoplasms and equivocal evidence of carcinogenic activity for female B6C3F1mice as shown by a marginally increased incidence of Harderian gland adenomas." NTP did not mention the liver tumors in its conclusions regarding mice.

In an 18-month dietary study (Takada et al. 1989, as cited in ATSDR 2012) in which male and female ddY mice were exposed to TCEP at dietary concentrations of 0, 0.012, 0.06, 0.3, or 1.5% (estimated by ATSDR (2012) to correspond to 0, 11, 53, 267, or 1333 mg/kg/day), TCEP significantly increased the incidences of renal cell adenomas or carcinomas in high-dose males (2/50, 0/49, 2/49, 5/47, and 41/50), hepatocellular adenoma or carcinomas in the two highest male groups (4/50, 5/49, 7/49, 12/47, and 19/50), forestomach papillomas or squamous cell carcinomas in high-dose females (0/49, 0/49, 0/50, 1/49, and 7/50), and leukemia in the two highest female groups (1/49, 3/49, 6/50, 9/49, and 9/50). Based on these results, the following effect levels were determined: LOAEL for kidney tumors of 1333 mg/kg/day (males) and NOAEL of 267 mg/kg/day (males); LOAEL for liver tumors of 267 mg/kg/day (males), with a NOAEL of 53 mg/kg/day (males); LOAEL for forestomach tumors of 1333 mg/kg/day (females) and NOAEL of 267 mg/kg/day (females); and LOAEL for leukemia of 267 mg/kg/day (females) and NOAEL of 53 mg/kg/day (females).

In a 79-week dermal study in Slc:ddY mice (20-27/group) exposed to 0, 5, or 50% TCEP twice a week (volume not available, and so applied dose cannot be determined), there were no significant treatment-related neoplasms at any dose (Takada et al. 1991), indicating the 50% TCEP was the NOAEL in this study. This is a study in Japanese with an abstract and tables in English, and so full study details were not available.

In a 78-week dermal initiation/promotion study, female Swiss mice (32-33 per group) were either (a) initiated with TCEP (71 mg) in acetone then promoted with tetradecanoyl phorbol acetate (TPA) (1 µg) in acetone 2 times per week; (b) initiated with 7,12-dimethyl-benz(a)anthracene (DMBA) (50 µg) in acetone, then promoted with TCEP (21 mg) in acetone 2 times per week; or (c) treated with TCEP (21 mg) in acetone as the complete carcinogen by applying it to the skin 2 times per week (Sala et al. 1982). No solvent or initiator-only control groups were used, but a control group with TPA alone was used. The authors compared data from treated animals to historical control data. The total dose of TCEP (as a promoter) applied to the skin of mice was 3.2 g/mouse. The incidence of skin tumors were as follows: 0/32 (TCEP alone), 17/33 (TCEP/TPA), 0/32 (DMBA/TCEP), 12/28 (TPA alone). The incidence of lung adenomas were as follows: 12/32 (TCEP alone), 7/33 (TCEP/TPA), 6/32 (DMBA/TCEP), 5/26 (TPA alone). There were no increases in skin lesions; however, there was an increased incidence of lung adenomas (not statistically significant) in the TCEP complete carcinogen group relative to the other groups. The authors concluded that TCEP did not show an initiating, promoting, or complete carcinogenic activity on mouse skin, but the overall conclusions are limited by the lack of concurrent solvent-only and initiator-only control groups.

Overall, chronic administration of 31.4 or 62.9 mg/kg/day TCEP in corn oil via gavage in rats resulted in an increase in the incidence of renal tubular adenoma in both sexes; renal tubular adenomas or carcinomas in males; increases in thyroid adenoma or carcinoma (females) and in mononuclear cell leukemia (both sexes) may have been related to TCEP (Mathews et al. 1993b; NTP 1991b). Administration of 250 mg/kg/day in corn oil by gavage in mice resulted in a marginally increased incidence of renal tubular cell neoplasms in males and Harderian gland neoplasms in female mice (Mathews et al. 1993b; NTP 1991b). Dietary administration of 267 mg/kg/day TCEP to mice caused liver tumors in males and leukemia in females; increases in kidney tumors in males and forestomach tumors in females was seen at 1333 mg/kg/day (Takada et al. 1989, as cited in ATSDR 2012). No carcinogenic response, and no initiating or promoting activity was reported in the available dermal studies, but the studies are limited by the information available and by inadequate controls (Takada et al. 1991; Sala et al. 1982).

5.8 Other Mechanistic Studies

There is limited information about the mechanism of action for the primary effects of TCEP on the reproductive system, the nervous system and the development of renal, liver and other

Table 5.10 Repeat-dose toxicity studies – Cancer Endpoints

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
F344/N rats (M, F) Oral gavage 5 days per week 103 weeks	Nominal dose: 0, 44, or 88 mg/kg/day Duration adjusted dose: 0, 31.4, or 62.9 mg/kg/day (60/sex/group)	Renal tubule adenomas (M, F), renal tubule adenomas or carcinomas (M) Decreased survival (F) Adenomas or carcinomas in the thyroid (F)	Kidney tumors– LOAEL = 62.9 mg/kg/day (M, F); NOAEL = 31.4 mg/kg/day (M, F) Thyroid tumors – LOAEL = 62.9 mg/kg/day (F); NOAEL = 31.4 mg/kg/day (F)	NTP 1991b; Matthews et al. 1993b	Number/sex/group includes interim sacrifice group. Mononuclear cell leukemia in males and females may have been related to chemical administration.

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
B6C3F1 mice (M, F) Oral gavage 5 days per week 103 weeks	Nominal dose: 0, 175, or 350 mg/kg/day Duration adjusted dose: 0, 125, or 250 mg/kg/day (60/sex/group)	Renal tubule adenomas or carcinomas (M) Harderian gland adenomas or carcinomas (F)	Kidney adenoma – marginal LOAEL = 250 mg/kg/day (M); NOAEL = 125 mg/kg/day (M) Harderian gland adenoma or carcinoma (interim and terminal incidence combined) – LOAEL = 250 mg/kg/day (F); 125 mg/kg/day (F)	NTP 1991b; Matthews et al. 1993b	Number/sex/group includes interim sacrifice group. While tumor incidence not statistically significant relative to controls, NTP concluded these tumors constitute "equivocal" evidence in mice Liver tumors were significant in trend test but not pairwise comparisons, and were not noted in the NTP summary.

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
ddY mice (M and F) Diet 18 months	Nominal dose: 0, 0.012, 0.06, 0.3, or 1.5% (equivalent to 0, 11, 53, 267, or 1333 mg /kg/day (50/sex/group)	Liver tumors (M) Renal tumors (M) Forestomach tumors (F) Leukemia (F)	Liver tumors – LOAEL = 267 mg/kg/day (M); NOAEL = 53 mg/kg/day (M) Kidney tumors – LOAEL = 1333 mg/kg/day (M); NOAEL = 267 mg/kg/day (M) Forestomach tumors – LOAEL = 1333 mg/kg/day (F); NOAEL = 267 mg/kg/day (F) Leukemia – LOAEL = 267 mg/kg/day (F); NOAEL = 53 mg/kg/day (F)	Takada et al. 1989, as cited in ATSDR 2012	Doses estimated by ATSDR 2012
Slc:ddY mice (F) Dermal 2x/week for 79 weeks	Nominal dose: 0, 5, or 50% (20-27/group)	No significant treatment-related neoplasms	Tumors – LOAEL = ND; NOAEL = 50% TCEP	Takada et al. 1991	Publication in Japanese with abstract and tables in English. Volume (and therefore dose) not available.

Strain/Species (Sex)/Route/ Duration	*Dose (No. of Animals/dose group*	*Endpoints*	*Organ System or Target/Effect Levels*[1]	*Citation*	*Comments*
Swiss mice (F) Dermal 2x/week for 78 weeks	Initiation /promotion TCEP (71 mg)/TPA (1 µg); total dose of TCEP (as a promoter) applied to the skin of mice was 3.2 g/mouse Initiation/ promotion – DMBA (50 µg)/TCEP (21 mg) Complete carcinogen – TCEP (21 mg) (32-33/group)	No initiating activity No promoting activity Increase in the incidence of lung adenomas (not statistically significant)	Tumors – LOAEL = ND; NOAEL = 71 mg/mouse Tumors – LOAEL = ND; NOAEL = 3200 mg/mouse Tumors – LOAEL = ND; NOAEL = 3200 mg/mouse	Sala et al. 1982	Study is limited by the absence of concurrent solvent-only and initiator-only control groups.

[1] All NOAELs and LOAELs expressed in terms of duration-adjusted doses.

ND= not determined

tumors. Although several studies have attempted to address the mechanism of neurotoxicity from both the toxicokinetic and toxicodynamic perspectives, a number of questions remain. Studies on the mechanism for other toxic effects are even more limited. Ongoing research focused on elucidating the mode of action (MOA) for TCEP is limited.

Mechanisms for Neurotoxicity

NTP (1991b) reported that female F344 rats were substantially more sensitive to the neurotoxic effects of TCEP than male rats of the same strain, and neurotoxic effects were not seen in B6C3F1 mice, even at higher doses. A major purpose of the toxicokinetic studies conducted by Herr et al. (1991) and Burka et al. (1991) (two separate publications from the same laboratory) with radiolabeled TCEP was to investigate the possibility of kinetic explanations for these observations. They wanted to determine whether the species- and sex-related differences in response (particularly the hippocampal lesions) are due to differences in the internal dose of the form of TCEP responsible for the neurotoxic effects. As a rapid measure of neurotoxicity, Herr et al. (1991) used the clinical sign of seizures typified by rearing and "wet dog shakes." Comparing hippocampal levels of radioactivity and the time and dose profile for the seizures, Herr et al. (1991) noted that TCEP did not accumulate in the hippocampus, and most of the radioactivity in the brain was in the form of the parent chemical, suggesting that the parent chemical (TCEP, rather than a metabolite) is responsible for the observed seizures. They also found that female rats are less efficient than males at metabolizing TCEP at higher doses (i.e., 350 mg/kg and above), consistent with toxicity being due to the parent compound rather than a metabolite. In the related study, Burka et al. (1991) suggested that the decreased metabolic efficiency results from saturation of metabolic pathways. Based on comparing TCEP metabolism in rats and mice, Burka et al. (1991) suggested that the toxic agent responsible for the neurotoxicity in rats is also present in mice, but that more rapid metabolism of the agent may prevent the toxicity. Burka et al. (1991) noted that mice dosed i.v. with 175 mg/kg TCEP in a preliminary experiment did exhibit seizures.

Despite coming from the same laboratory, somewhat different results and interpretations were provided by Burka et al. (1991). Based on a quantitation of how treatment with inhibitors of various metabolic enzymes affected seizure activity (see Section 4.3 for details), Burka et al. (1991) interpreted their results as suggesting that a metabolite, rather than the parent TCEP, is responsible for the acute neurotoxicity. However, their conclusion included several caveats, including the potential for one of the inhibitors to interfere with neural transmission, the presence of multiple pathways for metabolism of the parent compound, and lack of data on the internal dose of TCEP (as the parent) following dosing with the inhibitors.

Although these studies do not provide definitive conclusions regarding whether the parent compound TCEP or its metabolite is responsible for the acute neurotoxic effects of TCEP (and

possibly the hippocampal lesions), they do illustrate how evaluation of toxicokinetics and differences in internal dose can be used to aid in understanding differences in toxicity between species and between sexes. A refined understanding of these aspects, including information on human metabolic capacity, could be used to aid in the quantitative extrapolation of effect levels from rodents to humans.

Less information is available regarding toxicodynamic explanations (i.e., how TCEP interacts with cellular targets to cause the effects) for the development of brain lesions, convulsions, and delayed and other neurotoxic effects. Based on results with pharmacological probes, Tilson et al. (1990, as cited in ATSDR 2012) suggested that TCEP seizures are related to morphological damage in the brain. This suggests that the kinetic results described in the previous paragraphs, using seizures as a marker of neurotoxicity, are also relevant to the brain lesions. Results of a study with pharmacological probes in mice (Umezu et al. 1998, as cited in ATSDR 2012) suggest that TCEP may act as a GABA antagonist, and that observed clinical effects were not due to inhibition of acetylcholinesterase. As noted by ATSDR (2012), it would be useful to replicate these results in rats, particularly since marked inhibition of serum acetylcholinesterase has been observed in female rats treated for 16 weeks (Matthews et al. 1990; NTP 1991b). As noted by ATSDR (2012), TCEP inhibits acetylcholinesterase by directly phosphorylating a serine hydroxyl group at the active site.

Sprague et al. (1981) evaluated the potential for TCEP to cause delayed neurotoxicity based on the biochemical, histological, and clinical evidence for the absence or presence of delayed neurotoxicity in adult hens following a single or repeated oral exposure. Single-dose treatment significantly inhibited plasma cholinesterase activity but did not cause marked inhibition of brain NTE, indicating that TCEP would not produce delayed neurotoxicity via brain NTE alteration. Consistent with this finding, there were no clinical signs of delayed neurotoxicity and no histological changes in nerve sections. Sprague et al. (1981) built on earlier work by Johnson (1975), who concluded that delayed neurotoxicity increased in a homologous series of chlorinated alkyl phosphates with the size and hydrophobic nature of the substituents. Sprague et al. (1981) considered TCEP to behave differently chemically from the compounds evaluated by Johnson, consistent with the absence of observed delayed neurotoxicity.

Mechanisms for Reproductive Toxicity

Less information on the toxicokinetic or toxicodynamic mechanisms are available for reproductive effects of TCEP. TCEP decreased fertility in mice regardless of whether one or both sexes were exposed (NTP, 1991a). Although both sexes were affected, males were more sensitive than females, with altered sperm quality and morphology and decreased fertility. Fertility was also decreased when only females were treated, but there was no effect on estrous cyclicity, and the only reported histological effect on female reproductive organs was the

presence of cystic ovaries. Morrissey et al. (1988) reported on rodent sperm and vaginal cytology data from 13-week NTP studies, and reported decreased sperm motility in rats exposed to TCEP. However, the basis for the statement is unclear, since this endpoint is not mentioned in the full NTP report (NTP 1991b; Matthews 1990).

One potential mechanism for altered reproductive endpoints in rodents exposed to TCEP is interference with sex hormone balance, including alterations of steroidogenesis or estrogen metabolism. No *in vivo* data addressing this potential mechanism were located. However, Liu et al. (2012) evaluated the mechanisms underlying the endocrine disruption potential of TCEP in human cell lines and in zebrafish by examining (1) sex hormone synthesis and steroidogenic gene transcriptions in the H295R cell line (a human adrenocortical carcinoma cell line that is used to evaluate effects of chemicals on steroidogenesis), (2) estrogen receptor binding activities in the MVLN cell line, and (3) sex hormones and related gene transcriptions in male and female zebrafish. TCEP increased 17β-estradiol (E2) and testosterone (T) concentrations in H295R cells, upregulated transcription of four major steroidogenic genes (CYP11A1, CYP11B2, CYP19A1, and HSD3β2), but down-regulated two sulfotransferase genes (SULT1E1 and SULT2A1). However, TCEP did not act as estrogen receptor agonist in MVLN cells. In zebrafish, TCEP did not affect plasma T or E2, or 11-ketotestosterone (11-KT) concentrations in any of the sexes and had no effect on CYP17, CYP19A, and vitellogenin 1 (VTG1) gene transcription. These results suggest that TCEP may have the potential for altering steroidogenesis and resulting levels of sex hormones. However, interpretation of the results are difficult without information on how the concentrations tested compare with those associated with *in vivo* effects. Studies evaluating hormone levels in rodents exposed to TCEP *in vivo* and comparing the dose-response for any such changes with observed reproductive effects would be of interest.

Other mechanisms for adverse reproductive effects have not been investigated.

Mechanisms for Tumorigenesis

F344 rats developed renal tubular (males and females) and thyroid follicular tumors (females), and B6C3F1 mice had marginal increases in renal tubular tumors (males) and Harderian gland tumors (females) following exposure to TCEP by oral gavage for 2 years (NTP 1991b). Among mice exposed to TCEP in the diet, increased tumor incidences were observed in the kidney and liver (males), while females had increases in forestomach tumors and leukemia (Takada et al. 1989, as cited by ATSDR 2012). When female Slc:ddY mice were dermally treated with TCEP for 79 weeks, there were no significant treatment-related increases in tumors (Takada et al. 1991). Dermal treatment of female Swiss mice with TCEP did not exhibit initiator, promoter, or complete carcinogen properties (Sala et al. 1982), although this study is limited by incomplete controls. There are no carcinogenicity studies by the inhalation route.

While the mode of tumor induction is not known, several lines of evidence provide information as to what modes are not likely. TCEP does not appear to be genotoxic, as most mutation tests in bacterial systems as well as studies of genetic damage in mammalian cells and *in vivo* mammalian tests were negative. Induction of renal tubular adenomas/carcinomas does not appear to be related to alpha-2u-globulin, since this mechanism affects only male rats, and renal tumors were increased in both male and female as well as in male mice (NTP 1991b). In addition, pathology reports did not describe hyaline droplets associated with alpha-2u-globulin formation. Renal hyperplasia and karyomegaly, considered preneoplastic lesions in the kidney, were associated with the renal tumors, suggesting a nongenotoxic MOA, such as a proliferative process may be a possible underlying mechanism. Toxicokinetic factors, such as the high blood flow to the kidneys and metabolic capability of the kidney, may also have a role in the renal tubular tumorigenesis of TCEP.

Results of mechanistic studies indicate that cell proliferation may be a possible MOA for the induced renal tumors, but it appears that insufficient data are available to identify likely key events or evaluate the dose-response for those key events relative to the tumor dose-response. Two oral gavage studies and two *in vitro* tests evaluated a possible underlying mechanism/MOA for the renal carcinogenic potential of TCEP (Taniai et al. 2012a,b,; Ren et al. 2008; Ren et al. 2012). Taniai et al. (2012a, b) found that exposure to male F344 rats to 350 mg/kg TCEP by oral gavage for 28 days caused proximal tubule cell proliferation. Observation of cell proliferation at this early time point is consistent with a direct effect of TCEP on cell proliferation, but it would be useful to have data on tubular proliferation at the lower doses that were carcinogenic in the NTP (1991b) assay. As described by ATSDR (2012), Ren et al. (2008) exposed primary cultured rabbit renal proximal tubule cells to TCEP and found decreased cell viability, decreased the expression of some regulatory proteins (CDK4, cyclin D1, CDK2, cyclin E), increased expression of others ($p21^{WAF/Cip1}$, $p27^{Kip1}$), and decreased DNA synthesis and cell numbers. In follow-up work with the same system, Ren et al. (2012) reported that TCEP may have a proapoptotic effect, based on decreases in anti-apoptotic regulatory protein (Bcl-2 and cIAP-2) and increases in proapoptotic proteins (JNK, caspase-3, and caspase-9). Since inhibition of apoptosis, rather than increased apoptosis, is a possible MOA for tumorigenesis, these results suggest that an effect on apoptosis is not related to kidney tumorigenesis. However, since the test system was rabbit tubule cells, it would be useful to confirm the results with rat or mouse cells.

Minimal data are available regarding the mechanism of action for the liver tumors that were seen in male mice in the dietary study (Takada et al. 1989, as cited in ATSDR 2012), but not at a statistically elevated incidence in the gavage study (NTP 1991b). Liver tumors are prevalent in male B6C3F1 mice with a high background incidence. This can make it harder to identify an effect, but conversely means that there is a higher incidence of initiated cells for tumor promoters to act on. The liver tumors in mice in Takada et al. (1989) were associated with necrosis and

vacuolation of liver cells, suggesting that cellular damage and reparative processes could be involved, although there are no supporting data.

No data are available to discern the mechanism for thyroid carcinogenesis in female rats (NTP, 1991b) or forestomach tumors or leukemia in female mice (Takada et al. 1989).

Overall, the greatest degree of mechanistic data is available for the neurotoxic effects of TCEP, and even that process is not fully understood. Few studies have evaluated the underlying mechanism or MOA for TCEP carcinogenesis or reproductive toxicity.

5.9 Lowest Hazard Endpoints (LOAELs, NOAELs) by Organ System and Exposure Duration

Acute Oral toxicity- single dose

Numerous oral acute toxicity studies have reported depression, convulsions, and lethality in rats given single doses of TCEP. For most studies, dose, strain, sex, and numbers of animals were not reported. The lowest reliable LD_{50}s of 500 mg/kg (males) and 430, 500, or 790 (females, three different lots of chemicals) were reported in a study available under two different citations (Ulsamer et al. 1980; USEPA 1989) (as cited in WHO 1998 and NTP 1991b) for Sprague Dawley rats. A lower LD_{50} was reported for a study that lacked experimental details, so its reliability could not be ascertained.

Two single dose oral gavage studies were conducted to assess CNS and behavioral effects (Tilson et al. 1990). Epileptiform conversions and loss of pyramidal cells in the CA1 region of the hippocampus were seen in the treated group. In the behavioral study, rats showed mild impairment of their performance in the acquisition of a water maze task compared to controls. The LOAEL for this study was 275 mg/kg, the only dose tested.

Acute Dermal Toxicity

LOAEL/NOAELs for dermal toxicity could not be established, as the only data available were from an unpublished study submitted to EPA under TSCA that reported a LD_{50} greater than 200 but less than 5000 mg/kg in albino New Zealand rabbits (Aceto Chemical Co. Inc. 1989; USEPA 1989). The sex, dose, number of animals and lethality incidence were not reported.

Acute Inhalation Toxicity

An inhalation study exposed Sprague-Dawley rats (10/sex) to 5.0 mg/L (5000 mg/m^3) of aerosolized TCEP for 4 hours (Stauffer Chemical Company 1974, 1979, as cited in IUCLID 2000). There was no mortality, but decreased activity was noted. Another study exposed rats (sex and strain not reported) to saturated TCEP vapor (concentration not reported) for 8 hours;

no clinical signs were seen (Smyth et al. 1951). A LOAEL could not be determined based on this limited data.

Primary Skin Irritation

No dermal irritation to New Zealand rabbits were reported for up to 48 hours following an unspecified dermally-applied dose of TCEP for 4 hours (USEPA 1989). Although the applied dose is not available from the existing reporting, and the lack of study details limits the study reliability, the evidence of substantial systemic toxicity (narcosis and paralysis leading to death) indicates that an adequately high dose was tested; supporting the conclusion that TCEP is not a skin irritant. No NOAEL or LOAEL could be established.

Primary Eye Irritation

Several studies where 10 mg or 0.1 mL of TCEP was administered into one eye of New Zealand rabbits showed no irritation up to 72 hours post exposure (USEPA 1989). No LOAELs or NOAELs were identified.

There were no studies located assessing respiratory irritation or sensitization to TCEP.

Repeated dose toxicity

All doses throughout this section are duration-adjusted as needed. Where ATSDR (2012) provided unadjusted BMDLs, the BMDL is noted as duration-adjusted, since the numbers shown did not appear in the ATSDR report.

Two-week

Male and female F344/N rats (5/sex/group) were exposed to duration-adjusted doses of 0, 15.7, 31.4, 62.9, 125 or 250 mg/kg/day by oral gavage 5 days/week for 14-16 days (primary sources are inconsistent) (Matthews et al. 1990; NTP 1991b). Male rats in the 125 and 250 mg/kg/day groups experienced an increase in mean absolute and relative kidney weights, and absolute and relative liver weights were increased in females in the 250 mg/kg/day group, but without any accompanying histopathology. Serum cholinesterase activity was decreased by 18% ($p \leq 0.01$) in females dosed at 125 mg/kg/day and 20% ($p \leq 0.05$) in females dosed at 250 mg/kg/day, but without any gross or histopathologic lesions (Matthews et al. 1990; NTP 1991b). These changes in cholinesterase were statistically significant, but just below the degree of change considered toxicologically significant. Although the changes in organ weight may have been adaptive, the magnitude of the change after acute exposure suggests that 125 mg/kg/day was a marginal LOAEL for kidney effects in males, and that 250 mg/kg/day was a minimal LOAEL for liver effects in females. This determination is supported by similar findings in the subchronic and chronic studies.

B6C3F1 mice (5/sex/group) were exposed to duration-adjusted doses of 0, 31.4, 62.9, 125, 250, or 500 mg/kg/day by oral gavage 5 days/week for 14-16 days (Matthews et al. 1990; NTP 1991b). Convulsive movements and ataxia were reported for male and female mice in the 250 and 500 mg/kg/day groups. There were no other indications of toxicity based on organ weight or necropsy, and no effect on serum cholinesterase. All signs of toxicity were resolved by post exposure day 3. Clinical signs of toxicity were not reported in the accompanying subchronic toxicity study in mice, although it is not clear whether they were monitored within the critical timeframe. This study suggests that 250 mg/kg/day is a severe LOAEL, based on clinical signs of neurotoxicity, and 125 mg/kg/day is a NOAEL.

Subchronic and chronic oral gavage and dietary studies were conducted in rats and/or mice to evaluate the potential of TCEP to cause systemic toxicity. Systemic toxicity studies have not been conducted by the inhalation route and one limited study was conducted by the dermal route.

Subchronic

In subchronic oral toxicity studies, TCEP treatment in both sexes of rats resulted in decreased brain weight accompanied by significant increases in the incidence of lesions in the brains (NTP 1991b; Matthews et al. 1990). Increases in liver and kidney weights (with kidney effects progressing to renal histopathology after exposure for 2 years) were noted in the rat, along with increased mortality and hyperactivity/convulsions. In mice, similar exposures resulted in significant alterations in kidney weights accompanied by renal lesions, as well as increased relative and absolute liver weights (no histopathological changes), and decreased testis weight and sperm count. In dietary studies with exposure for about a month, the predominant effects included testicular effects, mean body weight changes and kidney effects (tubular hyperplasia) in rats; no subchronic studies or other studies of duration between 2 weeks and subchronic were conducted in mice.

Significantly increased relative organ weight changes liver and kidney weight changes in male and female rats occurred in NTP (1991b; Matthews et al. 1990), but these changes were not accompanied by histological effects. Based on the progression to more severe kidney effects in the related NTP (1991b) 2-year oral toxicity study, ATSDR (2012) concluded that the kidney effects in the subchronic study can represent a minimal LOAEL (125 mg/kg/day). ATSDR (2012) further concluded that although no such progression was seen for the liver, 125 mg/kg/day represented a minimal LOAEL for liver changes in males and females, with a NOAEL of 62.9 mg/kg/day, based on the magnitude of the liver weight changes. ATSDR (2012) developed a $BMDL_{10}$ of 60.76 mg/kg/day (duration-adjusted) for the brain lesions in females; males were less sensitive. Testicular effects were not observed in rats, but a LOAEL of 500 mg/kg/day and a NOAEL of 250 mg/kg/day was noted in mice (NTP 1991b), based on decreased testis weight and decreased sperm count.

In mice, based on the absence of accompanying histopathological lesions, the sporadic dose-response, the absence of progression in the accompanying chronic study, and the magnitude of the change, it appears that the liver weight changes were not toxicologically adverse, although 125 mg/kg/day could be considered a minimal LOAEL based on the criteria that ATSDR applied for the rat study. The only clearly adverse effect in this study (NTP 1991b; Mathews et al. 1990) was in the kidney, for which a NOAEL of 250 mg/kg/day and a LOAEL of 500 mg/kg/day can be determined for both sexes of mice, based on statistically significant decrease in the mean absolute kidney weights accompanied by renal lesions (mild cytomegaly and karyomegaly).

Chronic

In 2-year oral gavage studies, TCEP treatment caused decreased survival in both sexes, degenerative lesions in the brain of female rats, and focal hyperplasia of the tubule epithelium in both sexes of rats (NTP 1991b). In mice, kidney effects (hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei) and male liver effects (foci of cytologic alteration) were also reported (NTP 1991b).

In the 2-year oral toxicity study, a LOAEL of 62.9 mg/kg/day and a NOAEL of 31.4 mg/kg/day were identified for the kidney, based on kidney hyperplasia in rats. Mortality was also increased at 62.9 mg/kg/day in both sexes of rats. Degenerative lesions in the brain occurred at a LOAEL of 62.9 mg/kg/day and a NOAEL of 31.4 mg/kg/day. Mice were less sensitive than rats (NTP 1991b). The LOAEL for kidney lesions (mild cytomegaly and karyomegaly) in both sexes and liver lesions (altered foci) in male mice was 125 mg/kg/day, the lowest dose tested; no NOAEL was identified.

For deriving a chronic-duration MRL for TCEP, ATSDR (2012) analyzed the incidences of cerebral gliosis in female rats and of renal hyperplasia in both male and female rats using the BMD approach. A $BMDL_{10}$ of 42.8 mg/kg/day for brain lesions was estimated by ATSDR (2012). For the incidence of renal lesions in female rats, ATSDR (2012) estimated a $BMDL_{10}$ of 23.4 mg/kg/day, which was the most sensitive chronic BMDL.

Neurotoxicity

The potential of TCEP to be neurotoxic has been evaluated in rats, mice, and hens in several studies (Tilson et al. 1990; Matthews et al. 1990, 1993b; NTP 1991b; Stauffer Chemical Company 1980a, as cited in ECHA 2009; Sprague et al. 1981). Subchronic and chronic gavage studies in rats and mice reported brain lesions in rats but not mice, with female rats more sensitive than males (Matthews et al. 1990; NTP 1991b). Decreased serum cholinesterase was also observed in female rats. A subchronic dietary study in rats did not show alterations in red blood cell acetyl cholinesterase (AChE) or brain lesions at the doses tested, which were higher than the gavage studies (Stauffer Chemical Company 1980a, as cited in ECHA 2009). In a test

for delayed neurotoxicity (Sprague et al. 1981), TCEP via oral gavage in hens did not appear to cause delayed neurotoxicity; mild decreases in brain NTE, below those considered indicative of delayed neurotoxicity, were reported. Based on decreases in serum AChE and necrosis of the neurons in the hippocampal region of the brain of female rats, the subchronic LOAEL for neurotoxic effects was identified to be 125 mg/kg/day and a NOAEL 62.9 mg/kg/day, and the $BMDL_{10}$ was 60.76 mg/kg/day (NTP 1991b). For chronic oral exposure, based on brain lesion in both sexes of rats, the LOAEL was 62.9 mg/kg/day, the NOAEL is 31.4 mg/kg/day, and the $BMDL_{10}$ is 42.8 mg/kg/day (NTP 1991b).

There are no available studies of neurotoxicity of TCEP via the inhalation or dermal routes.

Reproductive and Developmental toxicity

The three reproductive/developmental studies via the oral route indicate that TCEP is a reproductive toxicant, adversely effecting male reproductive organ weights and sperm parameters and decreasing litter size (NTP 1991a; Kawashima et al. 1983; Hardin et al. 1987). Fertility of both males and females was impaired but that of males was more severely affected. The reproductive LOAEL is considered 175 mg/kg/day based on reproductive effects in NTP (1991a). ATSDR (2012) derived a $BMDL_{10}$ of 167.83 mg/kg/day for the decrease in live male F2 pups (altered sex ratio) of this study. However, it appears that ATSDR did not use the models for nested data that are designed for developmental toxicity studies for this modeling. No reproductive NOAEL was determined. No developmental toxicity was observed at doses up to 940 mg/kg/day, even though maternal toxicity occurred, as evidenced by decreased body weight gain (Hardin et al. 1987).

A 4 month non-standard inhalation toxicity study, exposing male rats, evaluated the effects of TCEP on fertility and development (Shepel'skaia and Dyschinegvich 1981, as cited in NTP 1991a). While no differences in male fertility index were reported, there were significant decreases in litter size, increases in pre- and post-implantation loss, significantly reduced average fetal size, and histopathological effects on the testes and maturity of the spermatozoids. The LOAEL from this study based on significant decreases in average fetal size appears to be 0.5 mg/m^3, the lowest concentration tested. However, the reliability of the study is limited by limited available data, and inconsistency with well-conducted oral developmental toxicity studies.

There were no data available for reproductive or developmental effects via the dermal route.

Genotoxicity

TCEP has been tested for genotoxicity in numerous test systems. Overall, the weight of the evidence indicates that TCEP is not mutagenic in bacterial or mammalian systems, and not

clastogenic *in vitro* or *in vivo* (ATSDR 2012; WHO 1998; IARC 1999). Based on the results of the sister chromatid exchange assays (Sala et al. 1982; Galloway et al. 1987), the evidence is mixed regarding the potential for TCEP to cause primary DNA damage, but based on the results of mutagenicity tests, it appears that any such damage is repaired before it results in mutations.

Carcinogenicity

The potential of TCEP to be carcinogenic has been investigated in rats and mice (NTP 1991b; Matthews et al. 1993b; Takada et al. 1989, as cited in ATSDR 2012; Takada et al. 1991). Overall, chronic administration of 62.9 mg/kg/day TCEP in corn oil via gavage in rats resulted in an increase in the incidence of renal tubular adenoma in both sexes and renal tubule adenoma or carcinoma in males; increases in thyroid adenoma or carcinoma in females and in mononuclear cell leukemia in females may have been related to TCEP (NTP 1991b; Matthews et al. 1993b). Administration of 250 mg/kg/day in corn oil by gavage in mice resulted in a marginal LOAEL for increased incidence of renal tubular cell neoplasms in males and Harderian gland neoplasms in female mice, and a NOAEL of 125 mg/kg/day (NTP 1991b; Matthews et al. 1993b). Dietary administration of 267 mg/kg/day to mice caused liver tumors in males and leukemia in females; at 1333 mg/kg/day in the same study, males exhibited kidney tumors and females had increased forestomach tumors (Takada et al. 1989, as cited in ATSDR 2012). The lowest tumor NOAEL in this study was 53 mg/kg/day for males and females. No carcinogenic response, and no initiating or promoting activity was reported in the available dermal studies, but the studies are limited by the information available and by inadequate controls (Takada et al. 1991; Sala et al. 1982).

6 Human Exposure

The primary properties of TCEP that are relevant to exposure are its molecular weight (285.5g/mole), vapor pressure (6.1×10^{-2} torr), water solubility (7.0 g/L) and Log K_{ow} (1.44) (Household Substances Data Base (HSDB), 2009, 2011 as cited in ATSDR 2012). This vapor pressure translates to a room temperature (25^{o}C) saturation vapor concentration for pure TCEP of 940 mg/m^3.

Exposure to TCEP has been reviewed by ATSDR (2012). Other recent reviews are primarily non-U.S. based and focus on indoor air and dust (Garcia-Jares et al. 2009; Harrad et al. 2010; Destaillats 2007; Reemtsma et al. 2008). Two additional reviews examined multi-media TCEP exposures (van der Veen and de Boer 2012; Takahashi et al. 2013). According to ATSDR, food and/or water consumption is likely the main source of exposure to phosphate ester flame retardants (Fiserova-Bergerova et al. 1990; Hartman et al. 2004; Hughes et al. 2001; IPCS 1997 as cited by ATSDR 2012). However, for children, incidental oral exposure (non dietary exposures such as dust ingestion and mouthing of objects) may be the primary exposure pathway

(ATSDR 2012). TCEP has been detected in ambient and indoor air, surface and groundwater, food, house dust, and consumer products. Information regarding TCEP levels in soil and sediment are sparse; only one study is mentioned in the ATSDR report (ATSDR 2012).

This section summarizes the available concentration data reported for TCEP contamination within the potentially relevant media of inhalable air and ingestible water and dust. Age-specific average daily intake rates for each of these media (most of which are available in the EPA Exposure Factors Handbook (USEPA 2011) would allow for the calculation of average daily intake of TCEP for each but was considered beyond the scope of this review.

6.1 TCEP Levels in Ambient and Indoor Air

Air results are presented in Table 6-1. Likely sources of phosphate ester flame retardants in indoor air include: polyvinylchloride (PVC) plasticizers, floor polishes, electronics (plastic cabinets), polyurethane foams, upholstery, furniture, and textiles (ATSDR 2012, Reemtsma et al. 2008, Canada Gazette 2011; Marklund et al. 2005). Both particulates and vapors contribute to exposure (Garcia et al. 2007 as cited by ATSDR 2012).

Bradman et al. (2012) measured TCEP in outdoor air near 40 childcare facilities in California. Fourteen samples were collected, seven of which had measurable TCEP levels. In addition to the outdoor air samples, 40 indoor air samples were collected. TCEP levels in indoor air were significantly higher than those found in the outdoor air. Of the indoor air samples collected, 65% were at levels above the detection limit (0.3 ng/m^3) (Bradman 2012).

The ATSDR report contains indoor air studies from countries other than the U.S. In these studies, TCEP was been detected in indoor air in homes and offices (Garcia et al. 2007; Ingerowski et al. 2001; Otake et al. 2001, Otake et al. 2004; Saito et al. 2007; Sjodin et al. 2001, as cited by ATSDR 2012).

Several studies analyzed indoor air in a variety of locations in Sweden. Marklund et al. (2005) analyzed indoor air samples in homes, a day care center, a hospital ward, a hotel, a prison, a library, shops, and factories. TCEP was one of more frequently detected compounds. Marklund et al. (2005) noted that TCEP air levels were correlated with dust levels found in the same environments. TCEP levels in public buildings were three to four times the concentrations found in residential buildings (Marklund et al. 2005). Bergh (2011) and Bergh et al. (2011) measured TCEP in Swedish homes, day care centers, work places, and apartments. Staff and Ostman (2005) measured TCEP in indoor air at 24 locations in Sweden where humans may be exposed on a daily basis. These locations were grouped into five categories: private homes, workplaces, stores, health care facilities, and transportation. TCEP was detected in at least one location in each of the five categories.

Table 6.1. TCEP Levels in Outdoor and Indoor Air

Country	*Location*	*Media*	*TCEP Levels (ng/m^3)*	*Reference*
U.S. (California)	Child Care Centers	ambient air	mean: 0.72 median: 0.19 max: 1.60	Bradman et al. 2012
		indoor air	mean: 2.69 median: 0.91 max: 15.34	
Japan (Tokyo)	homes and offices	indoor air	homes: 0-136 offices: 0-42.1	Saito et al. 2007 as cited by ATSDR 2012
Sweden	homes, offices, day center, hospital, shops, hotel, prison, library, dance hall, factories, bowling alley, and laboratory	indoor air	0.4-730	Marklund et al. 2005
	homes, day care centers, work places, apartments	indoor air	median values homes: 4.8 daycare centers: 25 work places: 10 apartments: 3.7, (range ND-230)	Bergh 2011 Bergh et al. 2011
	schools, office buildings	indoor air	schools:18-250 offices: 7.4-11	Carlsson et al. 2000, as cited by Destaillats et al. 2008
	private homes, workplaces, stores, health care facilities, transportation	indoor air	private homes: 1-115 car: 20 garage:320 office: 6-870 workshop: 3-29 stores: 11-56 health care facility:9-350	Staaf and Ostman 2005
Switzerland	public buildings cars	indoor air	23-56	Hartmann et al. 2004 as cited by Garcia-Jares et al. 2009; Destaillats et al. 2008

6.2 TCEP Levels in Water

Water results are presented in Table 6.2.

Drinking Water

Conventional water treatment may not be effective in removing TCEP from drinking water (Meyer and Bester 2004; Reemtsma et al. 2006; Watts and Linden 2008; Watts and Linden 2009 as cited by ATSDR 2012). Another study by Westerhoff et al. 2005(2005) on treatment effectiveness supports the conclusion that water treatment methods may not be effective in TCEP removal. Bennotti et al. (2009) analyzed source water, finished water, and distribution water from 19 US water facilities for 51 compounds in 2006 and 2007. TCEP was one of the most frequently detected compounds and was detected in all three types of water (source, finished, and distribution) (Bennotti et al. 2009). Similar to the findings of the studies noted at the beginning of this paragraph, these data also suggest that conventional water treatment methods are not effective at removing or reducing TCEP levels in drinking water (Bennotti et al. 2009). Stackelberg et al. (2007) evaluated the effectiveness of water treatment methods in removing compounds from drinking water. Only one method tested, granulated activated charcoal filtration, was effective in reducing TCEP concentrations in finished water. TCEP was detected in 100% of source water and 8% of finished water (Stackelberg et al. 2007).

TCEP was detected in three out of 22 samples collected from 20 Cape Cod public drinking water wells. The maximum concentration was 20 ng/L (Schaider 2010). In an earlier study also conducted on Cape Cod, samples were collected from monitoring wells located near a waste water treatment facility, three public water supply wells, a semiprivate well, and four private drinking water wells. Samples were also collected in a standard septic-tank leachfield, and a sand recirculating system. TCEP was detected in three of the monitoring wells; however the concentrations, although reported, were less than the minimum reporting limit of 0.5 µg/L. TCEP was detected in one private water supply well but was below the minimum reporting limit of 0.5 µg/L (Zimmerman 2004).

The United States Geological Survey (USGS) sampled nine water supplies using surface water as their source. Both raw and treated samples were analyzed for TCEP. TCEP was detected in 33% of source water samples and 31% of finished water samples (Kingsbury 2008).

Surface and Groundwater

TCEP has been detected in streams across the U.S. (Lee and Rasmussen 2006; Kolpin et al. 2002 as cited by ATSDR 2012). TCEP was detected in 57.6% of the samples (Kolpin et al. 2002). Studies in Wisconsin and Kansas detected TCEP in streams and surface water (Kolpin et al. 2002; Peterman et al. 1980; Lee and Rasmussen 2006 as cited by ATSDR 2012).

The USGS also analyzed groundwater samples from 47 locations in 18 states. TCEP was detected in 29.5% of the samples collected (Barnes et al. 2008, as cited by ATDSR 2012). The USGS also collected both groundwater and surface water samples used as drinking water from 25 states and Puerto Rico. Although TCEP was detected in 20.3% of the samples, all levels were below the reporting limit of 0.5 µg/L (Focazio et al. 2008 as cited by ATSDR 2012).

TCEP has also been detected in rivers in Japan, rainwater collected in Germany, and groundwater in Canada (Andresen et al. 2004; Fukushima et al. 1992; Williams et al. 1981; Fries and Puttmann 2001 as cited by ATSDR 2012).

Waste Water

Waste water can be a source of TCEP. Samples were collected from 21 waste water samples from different sites in Oakland, California including: residential, commercial, industrial, and influent and effluent streams from waste water treatment facilities. TCEP was detected in waste water from an industrial laundry, an adhesives manufacturer, and two treated waste water samples (Jackson and Sutton 2008).

6.3 TCEP Levels in Food

Results from the Food and Drug Administration's (FDA) Total Dietary Study (TDS) from 1991-2003 and 2004-2005 are presented in Table 6-3. The source of TCEP in food has not been determined; however, Daft 1982 (as cited by ATSDR 2012) suggested that the wrapping material used in food packaging may be the source.

Another potential source of TCEP in food may be from plant uptake. Two recent studies evaluated TCEP uptake in plants. TCEP was added to the soil in known concentrations and both roots and leaves of plants were analyzed for TCEP. TCEP was found to accumulate in the leaves of plants. This may be relevant where biosolids are applied to agricultural land that is used for food crops (Trapp and Eggen 2013; Eggen et al. 2013).

6.4 TCEP Levels in House Dust

Multiple studies have detected TCEP in house dust (Garcia et al. 2007; Ingerowski 2001; Marklund 2003 as cited by ATSDR 2012). Garcia et al. (2007) detected an average concentration of TCEP in house dust of 1700 ng/g. Table 6-4 summarizes the house dust data from other studies, some of which are discussed below.

In California, two rounds of sampling were conducted in 16 homes; the first round in 2006 and the second in 2011. House dust samples were collected and analyzed for a variety of chemicals, including TCEP. Maximum concentrations of TCEP in house dust exceeded 0.01% (100 ng/mg). In 2006, TCEP concentrations ranged from 610-160,000 ng/g and in 2011 TCEP

Table 6.2. TCEP Levels in Water

Country	*Location*	*Media*	*TCEP Levels (ng/L)*		*Reference*
U.S.	19 drinking water treatment plants	source, finished, and distribution water	median: source: 120 finished: 120 distribution:150	max: source: 530 finished: 470 distribution:200	Benotti et al. 2009
	Cape Cod public wells	Water	max: 20		Schaider et al. 2010
	Cape Cod	monitoring wells; drinking water	monitoring wells: 81-240[a] private well: 110[a]		Zimmerman 2004
	drinking water supplies	surface water: raw and finished	max: source: 260 (Estimated) finished 220 (Estimated)		Kingsbury et al. 2008
	Kansas	streams	500 (average)		Lee and Rasmussen 2006 as cited by ATSDR 2012
	multiple locations	streams	540 (maximum)		Kolpin et al. 2002 as cited by ATSDR 2012
	multiple locations	groundwater	737 (maximum)		Barnes et al. 2008 as cited by ATSDR 2012
	drinking water supplies	groundwater and surface water	<500[b]		Focazio et al. 2008 as cited by ATSDR 2012
	drinking water treatment plants	drinking water	source water (max):120 finished water (max):50		Stackelberg et al. 2007
Germany	N/A	municipal waste water influent and effluent, river water, groundwater	mean: effluent:352 influent: 986 range: river water: ND-1,036 groundwater: ND-312		Fries and Puttmann 2003 as cited by ATSDR 2012
	N/A	river water untreated and finished	untreated:10-130 finished:0.3-30		Andresen and Bester 2006 as cited by ATSDR 2012
Italy	N/A	volcanic lakes	mean monthly range: ND-64		Bacaloni et al. 2008 as cited by ATSDR 2012

[a]Reported levels were below the minimum reporting limit (0.5 µg/L or 500 ng/L).[b] All samples were detected below the reporting limit. ND = non-detect. N/A = not applicable

Table 6.3. TCEP Levels in Food (ng/g)

Food	*mean*
Total Diet Study Market Baskets 1991-1993, 2003-2004	
Peas, green, frozen, boiled	1.82[a]
Oatmeal, plain, cooked	0.02[b]
Cream of wheat (farina), enriched, cooked	2.59[a]
Rolls, white, soft, enriched	0.08[b]
Broccoli, fresh/frozen, boiled	0.14[a]
Green beans, fresh/frozen, boiled	1.59[a]
Baby food, turkey and rice	0.48[a]
Baby food, peas	0.02[b]
Bread, cracked wheat	0.02[b]
Eggplant, fresh, peeled, boiled	1.75[a]
Candy, hard, any flavor	0.02[b]
Sweet cucumber pickles	0.05[b]
Baby food, teething biscuits	0.06[b]
Soup, Oriental noodles (ramen noodles), prepared with water	7.25[a]
baby food, pears, and pineapple	0.02[b]
Total Diet Study Market Baskets 2004-2005	
BF custard/pudding	28[a]
BF, juice, apple-banana	1.05[a]
BF, juice, apple-cherry	4.63[a]
BF, oatmeal w/fruit	2.37[a]
BF, veg w/turkey	0.88[a]

[a] Only one sample >=LQ [b] Trace amounts; sources: US Food and Drug Administration - Total Diet Study Market Baskets 1991-3 through 2003-4 and U.S Food and Drug Administration – Total Diet Study Market Baskets 2004-1 through 2005-4

concentrations ranged from 330-110,000 ng/g. After a new roof was installed on one home, TCEP levels in house dust increased 20-fold (Dodson et al. 2012). Fang et al. (2013) collected dust samples from 20 homes and cars. The study authors purchased a V6 commercial standard to develop the analytical methodology. While V6, another flame retardant, was the focus of this study, TCEP was found in the commercial standard as an impurity at a concentration of 14% by weight. Both V6 and TCEP were found in the dust samples and were highly correlated. TCEP was detected in 48% of house dust samples and 95% of the car dust samples. The authors suggest that use of V6 may be an important source of TCEP. The authors also suggested that since TCEP has a higher vapor pressure than V6, TCEP may result in higher dust concentrations and greater migration from consumer products with respect to V6 (Fang et al. 2013).

In 2013, house dust and hand wipe samples from toddlers were collected from 30 homes in Durham, NC. While the primary focus of this study was Tris (1,3-dichloro-2-propyl) phosphate (TDCPP), another flame retardant, samples were also analyzed for TCEP. TCEP was detected in 48.8% of the hand wipes and 97.6% of the house dust samples (Misenheimer 2013).

Overseas, TCEP has been detected in house dust in the Philippines, Sweden, Japan, Germany, and New Zealand (Kim et al. 2013; Bergh 2011; Ali et al. 2012; Araki et al. 2013; Haumann and Thumulla 2002). Contact with house dust by adults and children may lead to incidental oral ingestion of TCEP by hand to mouth activities. Young children typically engage in more hand to mouth activities than adults which increases their exposure to TCEP in dust.

6.5 TCEP Levels in Consumer Products

Unlike concentrations in environmental media (e.g., air, water, dust), TCEP levels in consumer products cannot be used as a proxy for concentration levels to which consumers are exposed. To develop realistic consumer exposure concentrations, TCEP levels in consumer products would need to be paired with experimental results on the availability of TCEP compound to leave these products and enter the body. To date these data are not available, so this review is limited to a discussion of levels in consumer products. Table 6-5 presents data on TCEP in various consumer products. Any consumer product that contains TCEP has the potential to contribute household dust levels.

Automobiles and Furniture

The ATSDR reported briefly summarized data on TCEP in products. TCEP was detected in polyurethane foam samples in concentrations ranging from 0.8-3.1 µg/g (Nagase et al. 2003 as cited by ATSDR 2012). TCEP was also detected condensed onto the inside of automobile windows, which was attributed to its use as a plasticizer in interior automobile plastics (Boethling and Cooper 1985 as cited by ATSDR 2012).

Table 6.4. TCEP Levels in House Dust

Country	*Location*	*Media*	*TCEP Levels ng/g*		*Reference*
U.S.	California homes	house dust	2006 sampling min:610 median:5100 max:160,000	2011 sampling min:330 median:2700 max:110,000	Dodson et al. 2012
	Boston MA 20 homes and cars	house dust car dust	<u>median:</u> house dust 50.2 car dust 1080.0	<u>range:</u> house dust <20-1350 car dust <20-50120	Fang et al. 2013
Philippines	Malate and Payatas	house dust	<u>Malate</u> median:34 min:<0.44 max:1200	<u>Payatas</u> median: 16 min:<0.44 max:140	Kim et al. 2012
Japan	182 single family homes	floor dust multi surface dust	<u>floor</u> min: <MDL median:5830 max:338,450	<u>multi surface dust</u> min: <MDL median:8260 max:2.32 x10^6	Araki et al. 2013
Sweden	home, day care, work	dust	<u>median</u> home:2100 day care: 30000 work:6700		Bergh 2011
Germany	1569 samples (1999-2001)	house dust	median: 600,000 95th: 8.4 x 10^6 max:3.3 x 10^8		Haumann and Thumulla 2002
New Zealand		floor dust mattress dust	<u>median</u> floor:110 mattress:10		Ali et al. 2012

Stapleton et al. (2012) collected 102 foam samples from US couches purchased between 1985 and 2012. TCEP was detected in one sample along with V6. According to the authors, the Material Safety Data Sheet (MSDS) for Antiblaze, a product containing V6, indicates that TCEP is present at 10% by weight. As such, V6 may be the source of TCEP in the sample (Stapleton et al. 2012).

6.5.1 Children's Products

Stapleton et al. (2011) analyzed 101 polyurethane foam samples collected from baby products. TCEP was detected at concentrations greater than 1 mg/g of foam in a car seat, one changing table pad, one sleep positioner, one portable mattress, 10 nursing pillows, one baby carrier, and two infant bath mats/slings. Of note is that V6 was detected along with TCEP in 15 of the 16 samples. The authors suggested that the results indicate that the products may have been treated with V6 and, since TCEP is an impurity of V6, its presence may be from the use of V6. TCEP was detected in lower levels than other flame retardants measured (Stapleton et al. 2011).

The Washington Toxics Coalition and Safer States purchased 20 baby products in 2011. The purchased products were tested for the presence of flame retardants. TCEP was detected in one product, a co-sleeper. TCEP was below the detection limit (0.04 mg/g) in all other products (Schreder 2012).

Fang et al. (2013) reanalyzed 12 foam samples from baby products collected in a previous study where V6 was identified but not quantified. Both V6 and TCEP were detected in the 12 foam samples. The reported concentrations in foam are consistent with reported application rates of 5.3 weight % for V6 in automobile foam (Fang et al. 2013).

6.5.2 Non-US Products

In 2009, Health Canada tested the following Canadian products for TCEP: 14 sofas, 4 mattresses, 10 children's products, 4 acoustical panels, and a seat from a car. TCEP was detected in four sofas, a car seat, and two children's products. The only reported TCEP levels were for the children's products. In 2010, additional testing on 30 children's products was conducted for Health Canada's Priority Substances List (PSL) evaluation. Again, TCEP was detected in a polyurethane foam book and a sleep positioner. Three other products contained TCEP levels below the quantitation limit (Canada Gazette 2011).

In Belgium, 64 toys were sampled and analyzed for flame retardants. TCEP was detected in 13% of the samples (Ionas et al. 2012). Two Danish studies did not find TCEP above the detection limits in toys or baby products (Borling et al. 2006 and Tonnig et al. 2008).

Table 6.5. TCEP Levels in Products

Country	*Location or item*	*Media*	*TCEP Levels*	*Reference*
U.S.	couches purchased between 1985 and 2010	foam	5.47 mg/g	Stapleton et al. 2012
	baby products	Foam	mean: 5.91 mg/g range: 1.08-5.94 mg/g	Stapleton et al. 2011
	Connecticut, Maryland, Massachusetts, Michigan, New York, Washington baby and children's products	foam	2.99 mg/g	Schreder 2012
	Boston, MA baby products	foam	1.1 $x10^6$-5.9 $x10^6$ ng/g	Fang et al. 2013
Canada	products	foam	<u>2009</u> PUF book 13,000 ng/g sleep positioner 21,000 ng/g <u>2010</u> PUF book 3800 ng/g sleep positioner 34 ng/g	Canada Gazette 2011
Belgium	64 toys	foam	75^{th}percentile: 70 ng/g median: < LOQ	Ionas et al. 2012
Germany	various products	foams, paints, mattresses, sealants	soft foams: 6400 mg/kg paints/finishes: 840 mg/kg mattresses: 890 mg/kg foam sealants: 89,000 mg.kg	Haumann and Thumulla 2002

6.6 Exposure Studies

Emissions

McKone et al. (2009) tested TCEP emissions from computers in test chambers. With a computer running for seven days, the air concentration of TCEP in the test chamber was over 20 ng/m^3. McKone also measured the emission rate of TCEP from five computers. The emission rates ranged from 50 ng/hr/unit to over 200 ng/hr/unit (McKone et al. 2009).

Several studies have measured TCEP in televisions. TCEP migration rate from the surface area of plastic housings of television sets was measured at 13 µg/m^2-hr at ambient air temperatures (Saito et al 2007 as cited by ATSDR 2012).

Wensing et al. (2005) also measured emission rates from televisions sets in a 1 m^3 test chamber with an air exchange rate of 0.5/hr over the course of 550 hours. The concentration of TCEP in the chamber rose steeply over the first 100 hours of testing, then rose at a more gradual rate (Figure 6 in Wensing et al. 2005). TCEP concentrations from computer monitors were measured in a 1 m^3 test chamber with an air exchange rate of 0.5/hr on different days (Table 11a in Wensing et al. 2005). TCEP levels ranged from 10 to 121 ng/m^3 over the course of 3 to 14 days (Wensing et al. 2005).

Malmgren-Hansen et al. (2003) conducted a study on chemicals found in electrical and electronic products. While new television sets did not emit TCEP above 0.01 ug/set-hr , aged TV sets emitted TCEP at levels ranging from <0.01 to 0.30 µg/set-hr. TCEP reached 75% of equilibrium after 100 hours (Malmgren-Hansen et al. 2003, SCHER 2012). The data strongly indicate that TCEP can migrate to the surface where it is subsequently released to the air or available for dermal transfer. TCEP migrates to the outer surface of plastic material via diffusion, a process known as "blooming". However, the rate of migration is not known (SCHER 2012). TCEP is a non-volatile compound and therefore is unlikely to be present in a gaseous state. Release of TCEP from consumer products likely occurs via abrasion, which contributes to TCEP levels in dust (EU 2009).

Consumer Products

There is a dearth of data from the US on emission rates from consumer products.

The European Union Risk Assessment Report for TCEP (2009) states that migration from various consumer products is generally unknown (ECHA 2009). According to an unpublished study presented in the EU RAR, 217 ng/cm^2/hr of TCEP may be released from upholstered furniture originally containing 8 mg/cm^2 of this flame retardant (Bruckert and Schoene 1990 as cited by ECHA 2009).

Health Canada published its Screening Assessment for the Challenge on TCEP in 2009. As part of this assessment, Health Canada derived exposure estimates for infants and toddlers who mouthed foam. The Canadian calculation is based on the US Environmental Protection Agency's Voluntary Children's Chemical Evaluation Program for two other flame retardants, pentabromodiphenyl ether and octabromodiphenyl ether (Health Canada 2009).

The default values used by Health Canada were as follows: water solubility of TCEP is 7820 mg/L, salivary flow rate in child's mouth is 0.22 mL/min, saliva extraction rate is 0.038, absorption factor is 0.5, mouthing behavior frequency is 9 min/day, and body weight (BW) is 7.5 kg for infants and 15.5 for toddlers (Health Canada 2009).

For dermal exposure to dust, Health Canada used a dermal adherence rate of 0.05 mg/cm^2 -day for infants and toddlers and 0.07 mg/cm^2 -day for older children and adults. The dermal absorption factor was set to 1 since it is not known for TCEP (Health Canada 2009).

According to a report on toys from the Scientific Committee on Health and Environmental Risks (SCHER):

> "It is not possible to give an adequate estimate of the TCEP exposure of children sucking on toys containing TCEP, due to the scant representativeness and reliability of the available data." (SCHER 2012)

The European Union Risk Assessment Report for TCEP (2009) also states that there are no data available for estimating TCEP exposure from children sucking on toys (ECHA 2009).

In 2000, The National Academy of Sciences published Toxicological Risks of Selected Flame-Retardant Chemicals. While TCEP was not one of the flame retardants included in this report, the report included trismonochloropropyl phosphate, another chloroalkyl phosphate. For the dermal and oral assessments, the following factors were used: application rate to the upholstery of 5 mg/cm^2, extraction rate of 0.038, and a release rate of 0.06/day. For particulate inhalation, the following values were used: application rate to the upholstery of 5 mg/cm^2 and release rate of $2.3x10^{-7}$/day. For vapor inhalation, the following values were used: application rate to the upholstery of 5 mg/cm^2 and saturated vapor concentration of 35,300 mg/m^3 (NRC 2000).

Food

Average daily intakes for TCEP are presented in the ATSDR document. However, these values are calculated from 1979 and 1980 and are based solely on fruit consumption and calculated only for infants and toddlers.[6] Average daily intakes for infants were 0.016 µg/kg (1979) and 0.004

[6] It should be noted that multiple approaches exist to calculate the average daily intake, each utilizing different values, institutional practices and accepted assumptions about many factors (e.g., safety factors, using high end or average values for intake estimates, assumptions about food intake, derivation of those values for subpopulations

µg/kg (1980). For toddlers, the 1979 average daily intake was 0.009 µg/kg and in 1980 was not detected (Gartrell et al. 1985 as cited by ATSDR 2012).

Worker Exposures

Makinen et al. (2009) evaluated respiratory and dermal exposures to flame retardants in five work places. Inhalation exposures were measured using both personal air samplers and stationary devices. Detection frequency of TCEP in personal air monitors depended on the type of work place and ranged from 50% to 100%. TCEP was detected in personal air monitors at four of the five work places. The geometric means from the personal air monitors ranged from 5 to 450 ng/m^3. Dermal exposure potential was measured using patch samples attached to the workers' outer clothes (i.e., chest, arms and thigh). The detection frequency of TCEP in the patch samples was 67% at three of the work places and 100% at the fourth work place. The geometric means of the patch samples ranged from 0.1 to 0.4 ng/cm^2 (Mäkinen et al. 2009).

6.7 Summary

TCEP has been detected in several media including outdoor and indoor air, surface water, groundwater, house dust, food, and consumer products. V6, another flame retardant, contains a significant level of TCEP as an impurity and may be an additional source of exposure to TCEP.

The primary sources of exposure to TCEP for consumers appear to be dust and indoor air. The exposure routes are dust ingestion and inhalation of vapors and particulates in indoor air. For toddlers and infants, mouthing of foam is a significant exposure route (Health Canada 2009). For infants the upper bound estimate of daily intake from dust was 0.2 ug/kg-day for infants and 0.3 ug/kg-day for 0.5 to 4 year olds. The upper bound estimate for mouthing was 39 ug/kg-day for infants and 19 ug/kg-day for 0.5-4 year olds (Health Canada 2009). However, there are limited US data for these sources. There are data from other countries, but use of these data may introduce uncertainty into exposure estimates because TCEP levels in other countries may not be representative of US levels. Due to California's stringent flame retardant regulations, levels of flame retardants in dust are higher than in other parts of the country. Using California data may result in conservative estimates for other areas of the country where TCEP levels may be lower. A separate assessment for California residents may be warranted. There are US foam data available for use in calculating exposures from mouthing (see Table 6-5).

There are limited data available to perform an exposure assessment. Most notably, information on migration and degradation from indoor media and dermal exposure factors are lacking. However, reasonable worst case estimates of exposure can be made using the media

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such as children, toddlers). In an exposure assessment, choices for those values and the assumptions and approaches should be discussed and defended.

concentrations presented herein along with age-specific estimates of inhalation or ingestion rates of these media.

7 Discussion

Exposure

For consumers, the three main sources of exposure are indoor air, dust, and polyurethane foam. TCEP levels in indoor air range from non-detect to 7230 ng/m^3. The main exposure route appears to be incidental ingestion, primarily of dust. Studies of house dust in Massachusetts and California indicate that TCEP levels range from <20 to 1350 ng/g (Massachusetts) and in California from 330 to 160,000 ng/g. TCEP levels in polyurethane foam range from 1.01 mg/g to 5.94 mg/g. No suggested average daily intake has been proposed. In 2009, Health Canada conducted an assessment on TCEP and estimated total daily intakes that ranged from 0.09 µg/kg-day (60 and older) to 0.5 µg/kg-day (0.5-4 years) (Health Canada 2009). These intakes may represent upper bounds, as the maximum concentrations in each medium were used in the calculations.

There are several uncertainties/data gaps associated with the exposure data:

- lack of US-specific data for TCEP levels in consumer products and other media;
- lack of data on migration rates from products, dermal exposure factors, and emission rates from products;
- lack of degradation rates;
- uncertainty regarding the representativeness of California data for the United States as a whole;
- Uncertainty regarding the representativeness of international TCEP data for the United States.

Data may be available for some of the factors listed above for chemicals similar to TCEP, such as tris monochloropropylphosphate (TMCPP) and TDCPP, which can be used as a proxy for TCEP. However, use of such data will likely introduce a level of uncertainty in the exposure estimate.

Toxicity under FHSA

Animal data were sufficient to support the conclusion that **TCEP fits the designation of "acutely toxic" under the Federal Hazardous Substances Act (FHSA) (16**

CFR§1500.3(c)(2)(i)(A)) following single oral exposures. Acute oral toxicities (LD50's) for TCEP in rats ranged from 46<LD_{50}<100 mg/kg (USEPA 1989) to 1410 mg/kg (Smyth et al. 1951 as cited in WHO 1998; ATSDR 2012; NTP 1991b). Considering the more reliable studies where data was reported, the oral LD_{50} range was 430-790 mg/kg (Ulsamer et al. 1980; USEPA 1989), which is in the oral LD_{50} range (50 to 5000 mg/kg) necessary to be termed acutely toxic.

No studies adequately evaluated the acute toxicity of TCEP by the inhalation or dermal routes or its sensitization potential. Very limited animal data were available with limited documentation addressed whether TCEP is primary skin or ocular irritant (USEPA 1989), but the available data suggest that TCEP does not cause skin or eye irritation.

Sufficient animal data exist to support the conclusion that TCEP can be considered "toxic" under the FHSA due to its toxicity following short-term, subchronic, and chronic exposures. TCEP caused adverse effects in a variety of organs (NTP 1991b) and is a rodent reproductive toxicant (NTP 1991a) and carcinogen (NTP 1991b; Mathews et al. 1993). TCEP induced alterations in male rodent reproductive organs, the brain, kidney, and liver. It produced tumors in the kidney of male and female rats. The evidence is weaker for increases in thyroid, liver, and forestomach tumors and leukemia in rats exposed orally, and for increases in kidney and Harderian gland tumors in mice exposed orally. Developmental toxicity has not been observed following testing up to maternally toxic doses of TCEP (Hardin et al. 1987; Kawashima et al. 1983), although standardized testing that can evaluate a dose-response has not been conducted. There is inadequate evidence to determine the systemic toxicity and carcinogenicity following the inhalation or dermal routes.

There is sufficient evidence to support the conclusion that TCEP is not a direct acting genotoxicant (ATSDR 2012; WHO 1998; IARC 1999).

Data are adequate to set exposure limits for acute (2-week), subchronic, and chronic exposures, although the data are weaker for the acute limit.

For acute exposure, increases in absolute and relative liver and kidney weights were observed in rats exposed via gavage for 12 doses over 16 (NTP 1991b) or 14 days (Mathews et al. 1990). (The primary sources are inconsistent regarding the dosing protocol.) Although these weight increases were not supported by histopathological changes and so may have been adaptive, changes of >10% relative to control were observed, and similar changes were considered adverse in the subchronic and chronic studies. A marginal LOAEL of 125 mg/kg/day was identified for kidney effects in males. This dose was associated with a 12% increase in absolute kidney weight, but only an 8% increase in relative kidney weight. The corresponding NOAEL would be 62.9 mg/kg/day. A clearer effect was seen at 250 mg/kg/day, which caused 10% increases in both absolute and relative kidney weight in males, and increased liver weight (17% absolute and 14% relative) in females. Thus, an alternative approach would be to consider 250 mg/kg/day a

minimal (low adversity) LOAEL, and 125 mg/kg/day as the study NOAEL. This was a well-conducted study that included full histopathology of all high-dose animals.

For the subchronic duration, the most sensitive endpoint was brain lesions (cerebral gliosis) in female rats exposed for 3 months (NTP, 1991b), for which ATSDR (2012) developed a $BMDL_{10}$ of 60.76 mg/kg/day. This $BMDL_{10}$ is based on data from a well-conducted study of the appropriate duration, where the endpoint is clearly adverse.

For the chronic duration, the most sensitive endpoint was renal lesions (focal hyperplasia of the tubular epithelium) in female rats exposed via gavage for 2 years (NTP 1991b). ATSDR (2012) estimated a $BMDL_{10}$ of 23.4 mg/kg/day. This $BMDL_{10}$ is based on data from a well-conducted study of the appropriate duration, where the endpoint is clearly adverse. This lesion was seen in both male and female rats (with males less sensitive than females), and both sexes developed renal tubular tumors, suggesting that the renal hyperplasia was a preneoplastic lesion. The most sensitive noncancer effect that is clearly not preneoplastic was degenerative lesions in the brain of female rats (NTP 1991b). Based on the ATSDR (2012) analysis, the $BMDL_{10}$ for this endpoint is 42.8 mg/kg/day.

Data gaps

As discussed under confidence in the database, the overall database is strong, with several well-done studies in animals. Reproductive and developmental studies are available, and do not indicate that these endpoints are the most sensitive effects. Thus, although there are data gaps, it does not appear that filling these data gaps would change the identification of the critical target(s).

Inadequacies in the database for TCEP affect the scientific interpretation of the toxicity of TCEP. There are no studies in humans. Toxicokinetic and epidemiologic studies as well as studies comparing likely effects in children and adults are lacking.

Data are lacking on immunotoxicity, quantitative information on absorption by the inhalation and dermal routes, and whether the ultimate toxicant is the parent compound or a metabolite of TCEP.

Oral developmental and reproductive studies identified reproductive effects at the lowest dose tested in rodents, 175 mg/kg/day (NTP 1991a). ATSDR (2012) derived a $BMDL_{10}$ 167.83 mg/kg/day for the decrease in live male F2 pups (altered sex ratio) in this study. However, it appears that ATSDR did not use the models for nested data that are designed for developmental toxicity studies for this modeling. Developmental studies found no effect at the highest dose tested (940 mg/kg/day), although maternal toxicity was observed. There is only one inhalation

study, which reported severe effects at the lowest concentration tested (0.5 mg/m^3). Additional studies to elucidate the dose-response and mechanism(s) for these endpoints are needed.

There are no neurotoxicity studies by the inhalation or dermal routes. Only limited evaluations have been conducted of the potential for neurobehavioral deficits associated with observed histopathology. An acute oral study suggests that there may be an impact on learning and memory. A full neurotoxicity assessment would be useful. Information is also incomplete regarding the mechanism of development of brain lesions, convulsions, and possible neurobehavioral outcomes.

There are no inhalation studies for carcinogenicity or well-designed and reported inhalation investigating systemic toxicity.

Confidence/uncertainty

Confidence in the database for systemic toxicity and carcinogenicity is medium-high, as it includes well-conducted subchronic and chronic studies in both sexes of two species of rodents by the oral route. While the database includes reproductive studies in mice and a developmental study in rats, study designs for many of the endpoints did not include multiple doses, and no NOAEL is available for the reproductive endpoints for which multiple doses were tested. The database is missing a full neurotoxicity assessment, and mechanistic and toxicokinetic information is incomplete, making confidence in the database for other endpoints low-medium.

Confidence in the database for inhalation and dermal routes is low, as there are large data gaps and inadequacies.

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