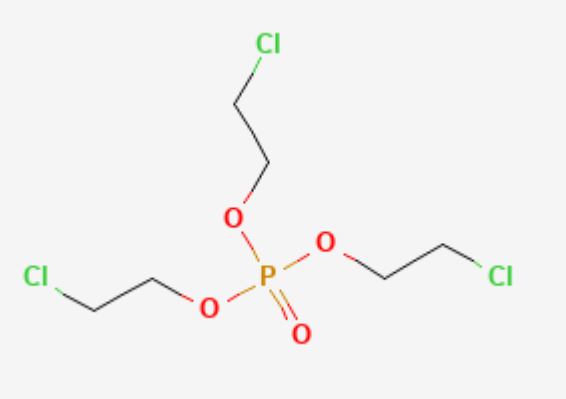
EHS Summary of Tris(2-chloroethyl) phosphate and Analogues for the MA TURA Science Advisory Board Meeting – June 20, 2024

Tris(2-chloroethyl) phosphate (TCEP)
(CAS#: 115-96-8)

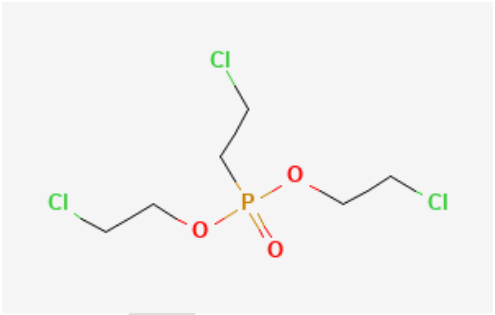


In 2023 EPA determined TCEP presents unreasonable risk to human health and the environment (EPA, 2023).

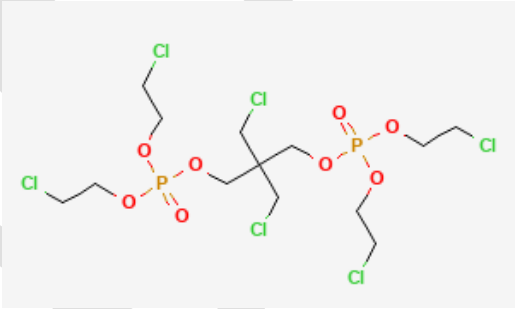
TCEP, Analogue 1, and Analogue 2 are all on the TSCA List of 30 Organophosphate Flame Retardants and all identified in the polyhalogenated organophosphate subclass of the Consumer Product Safety Commission 2018 report.

Primary hazards include cancer, kidney, reproductive, developmental and neurological toxicity, and persistence.

Analogue 1: Bis(2-chloroethyl)2-chloroethylphosphonate
(CAS#: 6294-34-4)



Analogue 2: "V6" 2,2-bis(chloromethyl)- propane-1,3-diyltetrakis(2- chloroethyl) bisphosphate
(CAS#: 38051-10-4)



PHYSICAL CHARACTERISTICS	
Primary Use	**TCEP** is used primarily as a flame retardant and plasticizer in polymers. **Analogue #2 (V6)** was only available with a purity of >90% and containing TCEP (4.5–7.5% (w/w)) (EU, 2008b). Nowadays V6 is available without the impurity of TCEP (EU, 2008b).
HEALTH HAZARDS	
Chronic or Sub-chronic Toxicity	
IARC rating	IARC has classified **TCEP** as a Category 3 carcinogen: "Not classifiable as to its carcinogenicity" based on inadequate evidence in experimental animals and no available human studies".
Carcinogenicity	**TCEP** is considered a Substance of Very High Concern (SVHC) and a Category 1B Carcinogen under REACH. **TCEP** is listed on California's Proposition 65 List of Substances Known to Cause Cancer. **TCEP:** "Under the conditions of these 2-year gavage studies, there was **clear evidence of carcinogenic activity** for male and female F344/N rats receiving TCEP as

	shown by increased incidences of renal tubule adenomas. Thyroid follicular cell neoplasms and mononuclear cell leukemia in male and female rats may have been related to chemical administration. There was **equivocal evidence of carcinogenic activity** for male B6C3F1 mice as shown by a marginally increased incidence of renal tubule cell neoplasms. There was **equivocal evidence of carcinogenic activity** for female B6C3F1 mice as shown by a marginally increased incidence of harderian gland adenomas. Renal tubule cell hyperplasia in male and female rats and gliosis, hemorrhage, pigmentation (hemosiderin accumulation), and mineralization in the brains of female rats were associated with the administration of TCEP. Karyomegaly of renal tubule epithelial cells in male and female mice was also chemical related" (NTP, 1991). **"TCEP** is likely to be carcinogenic to humans based on clear evidence of renal tubule adenomas and carcinomas in rats, equivocal evidence of kidney tumors in mice, the rarity of the kidney tumors in rodents, and equivocal evidence of several other tumors in rats or mice. Tumor incidence data are based an oral chronic bioassay in rats and mice that assessed dose levels between 44 and 350 mg/kg-day" (EPA, 2023). An epidemiological study found a statistically significant association between **TCEP** exposure and thyroid cancer. TCEP exposure was strongly associated with larger and aggressive tumors (Hoffman, 2017). **Analogue #2 (V6):** There were no carcinogenicity studies located for Phosphoric acid, P,P'-[2,2-bis(chloromethyl)-1,3-propanediyl] P,P,P',P'-tetrakis(2-chloroethyl) ester (V6), however; there was no evidence of mutagenicity from genotoxicity studies. The OncoLogic program estimated a Low-Moderate concern for carcinogenicity and there was an increase in benign tumors of the adrenal cortex and liver in a 2-year study with an analog chemical 2-Propanol, 1,3- dichloro-, phosphate (CASRN 13674-87-8). Due to concerns based on structure and analogs, a moderate hazard designation is warranted (EPA, 2014).
Neurotoxicity	**TCEP** received a moderate hazard rating based on the weight of evidence from a number of studies. TCEP produced degenerative lesions in the cerebral cortex in female rats gavaged with 88 mg/kg-day (NOAEL = 44 mg/kg-day) in a 103- week study. In addition, necrotic lesions in the hippocampus were observed in female rats following oral administration of 175 mg/kg-day TCEP (NOAEL = 88 mg/kg-day) for 16 weeks. Ataxia and convulsive movements were observed in mice administered TCEP at doses of ≥ 350 mg/kg-day (NOAEL = 175 mg/kg day) for 16 days. Convulsions were observed in female rats within 60 minutes following single oral gavage of 275 mg TCEP/kg-day. TCEP was attributed to death in dogs following ingestion of car seat cushions found to contain large amounts of the chemical. TCEP produced no evidence of neurotoxicity in white leghorn hens. TCEP promoted differentiation of the cholinergic phenotype only in an in vitro neurotoxicity study using undifferentiated and differentiating PC12 cells. There is potential for neurotoxicity based on a structural alert for organophosphates (EPA, 2015).

	TCEP: There are no epidemiological studies that evaluated neurotoxic effects, however several acute, subchronic, and chronic laboratory studies in mice and rats demonstrated neurotoxic effects in both males and females following TCEP exposure (EPA, 2023).
Developmental/Reproductive Toxicity	**TCEP** was designated a Category 1B for reproductive toxicity on the European Union List of CMR Substances. **TCEP:** "Evidence in humans is *indeterminate* based on the lack of available studies. Evidence in animals is *moderate* based on studies with decreased testes weight, sperm effects, and/or reduced fertility, and some support from histopathological changes in testes. EPA considers the mechanistic evidence (decreases in testosterone and genes expression but no direct estrogenic or androgenic agonism or antagonism) to be *slight*. Overall, EPA concluded that evidence indicates that TCEP likely causes reproductive toxicity in humans under relevant exposure circumstances. This conclusion is based on effects primarily related to fertility in the RACB study and male reproductive toxicity and is based on oral studies in rats and mice with dose levels between 22 and 700 mg/kg-day. EPA concluded that evidence indicated the TCEP likely causes developmental toxicity in humans under relevant exposure circumstances. This conclusion is based on effects primarily related to fertility in the RACB study and is based on oral studies in mice and rats that evaluated doses of 12 to 700 mg/kg-day" (EPA, 2023). **Analogue #1** may be suspected of damaging fertility or the unborn child according to REACH registrations. **Analogue #2 (V6):** V6 was given a moderate hazard rating for reproductive effects based on weight of evidence from multiple studies. "Phosphoric acid, P,P'-[2,2-bis(chloromethyl)-1,3-propanediyl] P,P,P',P'-tetrakis(2-chloroethyl) ester (V6) did not produce reproductive toxicity in an oral 2-generation reproductive study or in a 4-week gavage study in rats at doses up to 600 mg/kg-day (LOAELs were not established). Data using the analog 2-Propanol, 1,3- dichloro-, phosphate reported a LOAEL of 5 mg/kg-day (NOAEL not established) for atrophy and decreased secretory product of the seminal vesicle in an oral two-year combined chronic toxicity and carcinogenicity assay in rats. A 12-week fertility study in rabbits using the analog 2-Propanol, 1,3- dichloro-, phosphate reported a NOAEL of 200 mg/kg-day; there is uncertainty if reproductive effect could occur at a dose up to 250 mg/kg-day (the cutoff for the Moderate hazard designation criteria range)" (EPA, 2015). **Analogue #2 (V6):** Based on a NOAEL of 29 mg/kg-day (LOAEL of 86 mg/kg-day) for increased number of runts and decreased pup weight in an oral 2-generation study in rats. No developmental NOAEL/LOAEL could be established in a prenatal toxicity study in rats due to low survival of dams. There were no data located for the developmental neurotoxicity endpoint. Uncertain concern for the developmental neurotoxicity based on the potential for Cholinesterase (ChE) inhibition in dams that may result in alterations of fetal neurodevelopment (EPA, 2015).

Genotoxicity/Mutagenicity	**TCEP** was given a moderate hazard rating based on weight of evidence from multiple studies. Results were positive in in vitro gene mutation and chromosomal aberrations tests. TCEP was cytotoxic in a neutral read uptake assay in Chinese hamster V79 cells, produced sister chromatid exchanges in Chinese hamster V79 cells and mouse lymphoma cells, and was positive in a cellular transformation study in mouse BALB/3t3 cells. TCEP was not mutagenic in bacteria or yeast, and did not produce chromosomal aberrations in any available in vivo studies. In addition, TCEP was negative in an Unscheduled DNA synthesis study in human WI-38 cells. There is potential for genetic toxicity based on a structural alert for aliphatic substituted alkyl halides (EPA, 2015).
Endocrine Disruption	**TCEP** is listed on the TEDx List of potential endocrine disruptors. **TCEP** increased 17-estradiol (E2) and testosterone (T) concentrations following exposure for 48 hours in human H295R cells and inhibited luciferase expression induced by dihydrotestosterone in a reporter gene assay. TCEP was negative for estrogenic activity in a yeast two-hybrid assay and was not an estrogen receptor antagonist in human MVLN cells following a 72-hour incubation period. There were no adverse effects on endocrine glands of rats and mice administered TCEP via oral gavage for up to 103 weeks (EPA, 2015). **Analogue #2 (V6):** There were thyroid weight changes and associated histopathology in an oral 2-generation study in rats and there was an increase in benign tumors of the adrenal cortex and liver in 2-year study with an analog chemical 2-Propanol, 1,3-dichloro-, phosphate (CASRN 13674-87-8) (EPA, 2015).
Thyroid	(Liu, 2022) Reported a significantly high-risk association between exposure of organophosphate esters including, **tris (1,3-dichloro-2-propyl) phosphate, TCEP,** and others and thyroid cancer in both males and females. In the females of the control group, **TCEP** levels exhibited a significantly positive association with thyroid-stimulating hormone and a negative association with triiodothyronine (T3), free triiodothyronine (FT3), and free thyroxine (FT4) levels. **Analogue #2 (V6):** Increase in thyroid weight in males and females (EU, 2008b).
Immunotoxicity	**TCEP** produced a dose-dependent growth inhibition in B cells but not T cells in a mouse lymphocyte mitogenesis test. The IC_{50} was $1.0x10^{-5}$ mol/L (EPA, 2015). **Analogue #2 (V6):** Decreased absolute and relative spleen weights and decreased absolute thymus weights were observed in pups in an oral 2-generation reproductive toxicity study in rats (EPA, 2015).
Kidney	The kidney was identified as the target organ in animal toxicity studies of rats and mice of both sexes after **TCEP** exposure. Health effects noted included increased indications of histopathological lesions in rats and mice, and changes in kidney

	weights in multiple species. Markers of apoptosis and cell proliferation in kidneys of rats was also included as supporting evidence (EPA, 2023).
Other organ toxicity	**Analogue #2 (V6)**: "When mixed with nanopolystyrene particles (NPs), V6 could exhibit significant toxicity to HeLa cells. The enhanced toxicity was much higher than the toxicity of nanopolystyrene particles (NPs) alone and was related to the size of NPs. The mixture of V6 and small polystyrene NPs (10 nm and 15 nm in radius) showed obvious toxicity to HeLa cells. The toxicity increased with the concentrations of both V6 and NPs. On the contrary, the mixture of V6 and larger NPs (25 nm, 50 nm, 100 nm, and 500 nm in radius) showed almost no toxicity even at extremely high concentrations (NPs: 100 mg/L; V6: 50 mg/L). The small NPs could enter the cells and accumulated in cytoplasm. However, the larger NPs did not distribute inside the cells. NPs efficiently adsorbed V6 on the surface. The mechanism of the enhanced toxicity was attributed to the increased intracellular reactive oxygen species (ROS) production and the regulation of gene expression concerning apoptosis and ROS scavenging. Our study not only showed that a safe chemical V6 could be turned to be toxic by NPs, but also pointed out a potential risk caused by the joint toxicity of 'safe' chemicals and plastic particles with small size" (Zhong, 2023).
ENVIRONMENTAL & ECO-SYSTEM HAZARDS	
PBT	**"TCEP** has been detected in surface water, air, and snow in remote locations with no known source of releases but is known to undergo long-range transport through atmospheric, plastic debris, and other natural processes. Overall, TCEP appears to be a persistent mobile organic compound (PMOC). PMOCs can dissolve in water or bind to particles, resulting in longer environmental half-lives and greater potential for long-range transport—especially in the air, water, and sediment compartments" (EPA, 2023). **TCEP** was designated a Priority Pollutant in 2009 under the EU to reduce its use to various materials and then ultimately banned in 2015. In the 2009 risk assessment they concluded that TCEP is considered non-biodegradable (EU, 2009). **Analogue #2 (V6)** is an OSPAR Priority PBT **Analogue #2 (V6):** The persistence hazard designation for Phosphoric acid, P,P`-[2,2-bis(chloromethyl)-1,3- propanediyl] P,P,P`,P`-tetrakis(2-chloroethyl) ester (V6) is based on guideline biodegradation studies. There is evidence for biodegradation to occur, at rates resulting in a high hazard designation. 37% removal was found in 28 days with an OECD 302C guideline study. Under aerobic conditions in ready biodegradability test OECD 301B, 5% biodegradation occurred after 28 days. This compound is relatively stable to hydrolysis, with experimental half-lives of >1 year at pH 4, pH 7, and pH 9. This compound is not expected to be susceptible to direct photolysis by sunlight, since it does not absorb light at wavelengths >290 nm. It is expected to be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 0.14 days (EPA, 2015).

	Analogue #2 (V6): According to the SCHER V6 is not a PBT substance (SCHER, 2007b), but in a risk assessment report of V6 of the EU (2008b) it was concluded that V6 does meet the criteria of being persistent or very persistent in the environment. Only a limited number of studies have been found in the literature on V6 levels in the environment (van der Veen, 2012).
Bioaccumulation	**TCEP** can accumulate in fish if they are living in a body of water with high concentrations of TCEP. EPA found unreasonable risk to people eating fish from TCEP-contaminated water (EPA, 2023).
Aquatic Toxicity: LC_{50}, EC_{50}, ErC_{50}, NOAEC/NOEC	**TCEP** was given a high hazard rating based on experimental LC50 values of 6.3 and 4.9 mg/L for fish and daphnia, respectively and an acute EC50 of 1.1 mg/L for algae (EPA, 2015). **Analogue #1** may be harmful to aquatic life with long lasting effects according to REACH registrations. **Analogue #2 (V6)** is more toxic to fish and invertebrates than TCEP, and it may cause long term effects in the aquatic environment (Van der Veen, 2012).

Though limited, No Observable Effect Level (NOAEL) and Lethal Dose 50 (LD50) values were available on EPA's CompTox for TCEP, Analogue #1 and Analogue #2. See these values here.

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