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Submitted to Dr. William Page, Program Officer, Advisory Panel for the Study of Long-term Health Effects of Participation in Project SHAD (Shipboard Hazard and Defense), Institute of Medicine, the National Academies.

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SPECIAL NOTE ON PSYCHOGENIC SEQUELAE OF PERCEIVED EXPOSURE TO BIOCHEMICAL WARFARE AGENTS

This report deals primarily with the biological health challenges engendered by the agent that is the subject of the report. Nevertheless, this report also incorporates, by reference and attachment, a supplement entitled "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents".

The supplement addresses and describes a growing body of health effects research and interest centered upon the psychogenic sequelae of the stress experienced personally from actual or perceived exposure to chemical and biological weaponry. Because awareness of exposure to agents in Project SHAD logically includes the exposed person also possessing a perception of exposure to biochemical warfare agents, the psychogenic health consequences of perceived exposure may be regarded as additional health effects arising from the exposure to Project SHAD agents. This reasoning may also apply to simulants and tracers. Therefore, a general supplement has been created and submitted under this contract to address possible psychogenic effects of perceived exposure to biological and chemical weaponry.

Because such health effects are part of a recent and growing public concern, it is expected that the supplement may be revised and expanded over the course of this contract to reflect the actively evolving literature and interest in the issue.
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I. EXECUTIVE SUMMARY

Triocyl phosphate (TEHP), more commonly known as Tris(2-ethylhexyl) phosphate, bears the chemical formula C$_{24}$H$_{51}$O$_4$P, and is identified by the Chemical Abstract Service (CAS) Registry Number 78-42-2. It normally appears as a colorless viscous liquid possessing a low vapor pressure. It is soluble in alcohol, acetone, and ether but insoluble in water.

Triocyl phosphate is ordinarily used as a plasticizer or fire retardant. It is commonly employed as a component of vinyl stabilizers. More than 10 million pounds of TEHP is produced worldwide each year. In Project SHAD, TEHP was used as a simulant for the chemical warfare nerve agent VX.

A National Toxicology Program (NTP) set of studies on TEHP was performed in 1984 and serves as the main source for TEHP toxicology. Its overall profile was of a substance with little toxic risk, though with some areas of concern. Those areas related to positive carcinogenic indications from certain chronic animal tests, and to mild acute irritation effects. The report also included a subchronic dog and rhesus monkey study that suggests chronic lung injury is possible due to continuous inhalation exposure.

Mammalian acute toxicity of TEHP tends to be very low, with median lethal oral animal doses exceeding testing levels in rats and mice. Acute findings indicate TEHP induces mild temporary irritation on the skin, eye, and respiratory systems. Moderate erythema on shaved skin has been reported for rabbits. Effects on the eye are usually mild, with animal studies showing very mild irritant effects or a causing temporary and moderate conjunctivitis in rabbit (Draize) testing. Acute inhalation exposure is only harmful at high doses with continuous exposure. Wistar rats experienced no mortality at a concentration of 287-460 mg/m$^3$ for 30 minutes. Guinea pigs experienced about 30% mortality at the same concentration after 60 minutes which increased to 80% after 2 hours.

Human studies and case reports are not found in the published literature, with the exception of an NTP skin test on human volunteers which resulted in no signs of significant skin irritation. Chronic and subchronic studies in animals did show a mild chronic inflammation in dog lungs after 3 months of regular exposure to up to 85 mg/m$^3$. Other than that effect which was restricted to dogs, no dogs or rhesus monkeys (the other tested animal) showed any signs of toxic effect. Neurotoxicology testing indicates no inhibition of cholinesterase activity, and no signs of delayed neurotoxicity. Cytotoxicity and micronucleation was not found in a series of rat exposures to aerosolized trioctyl phosphate.

Triocyl phosphate is not classified anywhere as a human carcinogen. There is also no evidence of genotoxicity. Bacterial tests (Salmonella tester strains TA 98, TA 100, TA 1535, TA 1537) showed no signs of mutagenicity regardless of the presence of S9 liver fraction. Tests for sister-chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells have also been negative for genotoxicity.
Hyperplasia in thyroid follicular cells has been observed in rodents in a 2 year study. Weight loss was also reported in rats and mice after long-term exposure but it was not found to be harmful or to have resulted from toxic action.

Some evidence of possible carcinogenicity has been found in the increase of hepatocellular carcinomas in female-only B6C3F1 mice in one NTP 2 year gavage test. Equivocal evidence has also been found in the dose-related presence of pheochromocytomas appearing in some male rats. The evidence from the studies has been deemed insufficient to establish a significant risk of human carcinogenicity. Four factors were decisive in that assessment: the neoplastic tumors occurred in only one sex of one species, hepatocellular carcinoma tumors are considered rare in general, genotoxicity evidence is absent, and the background incidence of pheochromocytomas in rats is too variable to establish the significance of the tumor’s appearance in the non-control rodents

(Some studies suggest that 2-ethylhexanol, a metabolite of TEHP, as well as an ingredient of its manufacture and a characteristic component of chemicals with the 2-ethylhexyl moiety, may constitute a factor in any TEHP carcinogenic potential.)

Psychogenic effects specifically of trioctyl phosphate are not known. General psychogenic effects of perceived exposure to agents of chemical and biological warfare are examined in the supplement “Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents.” Treatment of exposure to trioctyl phosphate is the standard regimen of assistance to anyone exposed to a general or unknown toxic substance. Laboratory facilities involved in caregiving ought to monitor the affected person’s complete blood count and perform urinalysis if necessary. Liver and kidney function tests are suggested for patients with significant exposure. In cases of respiratory tract irritation or respiratory depression, the caregiver should monitor arterial blood gases, chest x-rays, and perform pulmonary function tests.

Secondary sources do not appear to contain significant errors or oversights in treating the toxicology of TEHP, although Patty’s Toxicology contains no separate monograph on trioctyl phosphate. The Hazardous Substances Data Bank of Toxnet at the National Library of Medicine contains a scattering of not clearly organized or updated information. For example, at one point it cites in all capital letters an outdated assertion that there are no long-term toxicity studies of trioctyl phosphate.
II. BACKGROUND DATA

Identification & Physical Chemistry

Project SHAD Chemical Agent Name: Trioctyl Phosphate.

Structure:

\[
\text{CH}_3\text{CH}_3\text{P(O)}\text{O}\text{O}\text{CH}_3
\]

CAS#: 78-42-2

More Commonly Appearing Names: Tris(2-ethylhexyl) phosphate, Tri(2-ethylhexyl) phosphate

Abbreviations: TEHP; TOF

Alternate Names: Phosphoric acid, tri(2-ethylhexyl) ester; Flexolreg; Kronitexreg.

Chemical Formula: C_{24}H_{51}O_4P

Molecular Weight: 434.64

Density/Specific gravity: 0.924 @ 26 deg C

Vapor Pressure: $8.25 \times 10^{-8}$ mm Hg @ 25 deg C

Boiling Point: 215 deg C @ 4 mm Hg

Melting Point: -74 deg C

Viscosity: 15 mPa sec @ 20 deg C

TEHP normally appears as a colorless viscous liquid possessing a low vapor pressure. It is soluble in alcohol, acetone, and ether but practically insoluble in water (0.6 mg/L @ 21 deg C.)
Sources: HSDB 2004, WHO 2000, NTP 1984

Use & Manufacture

Trioctyl phosphate is ordinarily used as a fire retardant and as a plasticizer for vinyl plastics and synthetic rubber compounds. It also often found as a component of vinyl stabilizers (HSDB 2004, WHO 2000, NTP 1984). In Project SHAD, trioctyl phosphate was used as a simulant for the chemical warfare nerve agent VX.

About three million pounds of trioctyl phosphate are produced per year in the United States, and as many as 10 million pounds worldwide (ECETOC Working Group 1992). TEHP is produced from phosphorus oxychloride and 2-ethylhexanol (Anonymous 2000, HSDB 2004, NTP 1984). Chief American manufacturers are Akzo Nobel Chemicals Inc. of Chicago and Rhodia Inc. of Cranbury, New Jersey (HSDB 2004).

Action

Necropsies performed after rat inhalation exposure to TEHP tagged with phosphorus-32 isotope indicate that stomach contents, brain, lungs, and liver are the primary target organs of systemic distribution, with the stomach contents receiving 64% of the dose within one hour. Elimination is mostly through feces rather than urine. 2-Ethylhexanol (which is also used in TEHP’s manufacture) is TEHP’s only confirmed metabolite (WHO 2004, NTP 1984, HSDB 2004).
III. TOXICITY PROFILES

Overview

A series of laboratory tests and reviews performed by the National Toxicology Program in 1984 remain the most comprehensive source on TEHP toxicity (NTP). With the exception of one human skin volunteer skin study, no human case reports, studies, or other clinical data exist.

The main areas of concern are mild acute local skin, respiratory, and ocular toxicity and some evidence of a carcinogenic. Subchronic dog studies suggest a possibility of mild long-term pulmonary damage. In general, however, mammalian acute toxicity tends to be very low and the absence of genotoxic effects in several tests, combined with the limited and equivocal nature of the carcinogenic effects, has led to very low level of concern about TEHP’s carcinogenic potential (NTP 1984, HSDB 2004, WHO 2000, Anonymous 2000).

Acute Effects

Acute systemic toxicity is low. Oral rat consumption of TEHP has generated NOAEL levels of >2.86 g/kg/day. Oral rat exposure over 13 weeks had no significant effect at the highest testing levels (NTP 1984, Anonymous 2000).

Rabbits receiving repeated doses of 0.1 ml to their skin showed no evidence of systemic toxicity. Moderate skin erythema in one test remains the only local effect on rabbits reported (NTP 1984). Rabbit ocular exposures to TEHP (up to 0.5ml) yielded moderate conjunctivitis (NTP 1984). The eye toxicology standard text, Grant’s Toxicology of the Eye, has rated TEHP ocular toxicity as 1 on a scale of 1 to 10 of increasing severity. (Grant 1986; HSDB 2004)

Acute inhalation testing of TEHP by Wistar rats and guinea pigs revealed no mortality in the rats at a concentration of 287-460 mg/m$^3$ for 30 minutes. A similar concentration range resulted in 30% deaths among guinea pigs after 60 minutes, which increased to 80% after 2 hours. (NTP 1984)

Key acute toxicity values are as follows:

- Rat Oral: LD$_{50}$ >36.8g/kg/day (NTP 1984)
- Rabbit Oral: LD$_{50}$ = 46g/kg/day (NTP 1984)
- Guinea Pig Inhalation: LC$_{50}$ = 450 mg/m$^3$/ 30 min (WHO 2000)
- Rat Oral: NOEL = 430 mg/kg/day. (WHO 2000)

Human studies and case reports are not found in the published literature, with the exception of a test on the arms of human volunteers which resulted in no signs of significant skin irritation. (Eight volunteers had 40% TEHP applied to 2 cm$^2$ of their arm)
for 3 days; six volunteers had a TEHP-saturated cotton swab applied to their forearm for one day (WHO 2000, NTP 1984)).

**Long-Term Sequelae Overview**

Longer-term studies appear to confirm the generally low systemic and local toxicity of TEHP. It may bear repeating that there are no published human studies or case reports regarding TEHP (other than the acute skin testing reported above.) Nevertheless, TEHP has some areas compelling a measure of toxicological attention.

There are cases of chronic lung inflammation in dogs after subchronic exposure and there are concerns regarding carcinogenicity in the liver of female rats and perhaps in the adrenal gland medulla (pheochromatomas) in mice. Hyperplasia in thyroid follicular cells has been noted as well in both sets of rodents. Decrease in body weight of rats and inflammation of gastric mucosa was found in a two-year chronic study (gavage 5 days per week) but these were not associated with any harmful or toxic effects (NTP 1984, WHO 2000).

No studies of reproductive toxicity are known.

**Subchronic/Chronic**

Neurotoxicology testing indicates no inhibition of cholinesterase activity (in plasma or red blood cells), no evidence to date of delayed neurotoxicity, and no histologic evidence of demyelination (NTP 1984, ECETOC Working Group 1992)). Gavage administration 5 days per week to rats and mice ranging from 500 mg/kg to 4000 mg/kg for two years produced only inflammation of gastric mucosa (mice) and mild weight depression (rats). (NTP 1984, WHO 2000)

Subchronic studies in animals show mild chronic inflammation of lung parenchyma in dogs after 3 months of regular exposure to concentrations of up to 85 mg/m$^3$. Rhesus monkeys showed no toxic effects of any kind, and dogs showed no toxic effects other than the incidence of chronic parenchymal inflammation (NTP 1984).

**Carcinogenicity**

Triocyl phosphate is not classified anywhere as a human carcinogen and not conclusively considered a carcinogen to any animal. TEHP was tested for chronic toxicity and carcinogenicity in rats and mice by the NTP. Two year studies were performed on mice (B6C3F1) and rats (F-344/N) who were fed TEHP in corn oil by gavage 5 days a week for 103 weeks. Male rats were fed 2000 and 4000 mg/kg/day of administration; female rats were given 1000 and 2000 mg/kg/day of administration. Both sexes of mice were fed 500 and 1000 mg/kg/day of administration. Regular clinical observations were performed over the course of the experiments (NTP 1984).
Two results suggestive of carcinogenicity were found. The NTP concluded that there was some evidence of carcinogenicity based on an increased incidence of hepatocellular carcinomas in female mice. This occurred only at the highest-dose level, however. Another result was the increased incidence of adrenal pheochromocytomas in male rats.

The NTP concluded that neither result could be deemed sufficient to establish a significant risk of carcinogenicity for humans. The basis for that conclusion were the following:

a) the incidence of each result in only one sex of one species
b) the lack of evidence of genotoxicity
c) the variable background incidence of pheochromocytomas in rats (in the results of the NTP study, the incidence among TEHP exposed male rats was 24% (12/50) and the control incidence was 4% (2/50), but historically the incidence of pheochromocytomas in the NTP program was 18%, and in two immediately prior two studies, 25%).
d) the low incidence of hepatocellular carcinomas in general
e) the low ordinary exposure of humans to TEHP

As a result the NTP concluded that there was only some evidence of hepatocellular cancer risk for female rats and equivocal evidence of risk for pheochromocytomas in male rats.

In the NTP chronic studies, a non-neoplastic dose-related event manifested: thyroid follicular cell hyperplasia. In male and female mice, the lowest-observed-adverse-effect level (LOAEL) for thyroid follicular cell hyperplasia was 357 mg/kg body weight per day. A NOAEL in mice was not established (NTP 1984).

Rat bone marrow testing for cytotoxic effects was performed by the Mobil Oil Co in 1991. No evidence of cytotoxicity or micronucleus formation were found after 10 rats were given 9 daily exposures over two weeks of up to 0.5 mg/l for 6 hours per day (HSDB 2004).

**Genotoxicity**

There is no indication in the literature of any finding suggesting that TEHP is in any way genotoxic.

Bacterial tests (*Salmonella* tester strains TA 98, TA 100, TA 1535, TA 1537) showed no sign of mutagenicity regardless of the presence of S9 liver fraction (NTP 1984). The sister chromatid exchange (SCE) assay and the chromosome aberration assay in Chinese-hamster ovary cells have also been performed to evaluate TEHP’s *in-vitro* cytogenetic damage. No effect was seen in any assay, with to without metabolic activation (Ivett 1989).

**Special Note: 2-Ethylhexanol**
2-Ethylhexanol is a reported metabolite of TEHP and is part of the manufacturing process. Its presence as a metabolite or an impurity in the product may be of significance since some studies suggest that compounds with the 2-ethylhexyl moiety could have cancer promoting properties due to the activity of 2-ethylhexanol (Astill et al. 1996; Kluwe 1986; Kluwe 1985).
IV. PSYCHOGENIC EFFECTS

Psychogenic effects specifically of trioctyl phosphate are not known. General psychogenic effects of perceived exposure to agents of chemical and biological warfare are examined in the supplement “Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents.”
V. TREATMENT & PREVENTION

There is no known specific antidote for trioctyl phosphate toxicity.

The Hazardous Substances Data Bank at the National Library of Medicine recommends that caregivers offer the same treatment one would normally give for exposure to a general or unknown toxic chemical. (HSDB 2004):

In case of inhalation exposure, one should ensure a stable airway, watching for signs of respiratory insufficiency, and assisting ventilations when needed. (Oxygen should be administered by nonrebreather mask at 10 to 15 L/min.) Monitoring for and treating pulmonary edema, shock and seizures are important as well. In the case of eye contamination, eyes should be flushed immediately with water and irrigated continuously during transport to a care facility.

Emetics should be avoided. In cases of ingestion, the mouth should be rinsed and 5 ml/kg (up to 200 ml) of water swallowed for dilution purposes, if the patient is able to swallow. Dermal exposure requires treating any skin burns with dry sterile dressings after decontamination.

For advanced treatment, orotracheal or nasotracheal intubation for airway control may be necessary for the patient who is unconscious, has severe pulmonary edema, or is in respiratory arrest. Positive pressure ventilation techniques with a bag valve mask device may be beneficial. Cardiac rhythm should be monitored and arrhythmias treated as appropriate.

Further recommendations for advanced treatment include watching for signs of fluid overload and considering drug therapy for pulmonary edema. Seizures should be treated with diazepam (Valium). Eye irrigation may be assisted with proparacaine hydrochloride.

Recommended post-exposure laboratory steps for a “general or unknown” chemical also include monitoring the complete blood count and performing urinalysis. Liver and kidney function tests are suggested for patients with significant exposure. In cases of respiratory tract irritation or respiratory depression, the caregiver should monitor arterial blood gases, chest x-rays, and perform pulmonary function tests.
VI. SECONDARY SOURCE COMMENT

Secondary sources do not appear to contain significant errors or oversights in their reviews of TEHP. Patty’s Toxicology nevertheless contains no separate discussion or monograph on trioctyl phosphate (Bingham 2001). The Hazardous Substances Data Bank of TOXNET at the National Library of Medicine contains a scattering of not clearly organized information (HSDB 2004). At one point, it cites in all capital letters an outdated assertion that there exist no long-term toxicity studies of trioctyl phosphate.
Tris(2-ethylhexyl) phosphate (TEHP) is a nonflammable, colourless liquid with low water solubility and very low vapour pressure, which is used as a flame retardant and plasticizer for PVC and cellulose acetate and as a solvent. It is produced from phosphorus oxychloride and 2-ethylhexanol. Figures for current worldwide production are not available. Approximately 1000 tonnes are currently produced in Germany. TEHP has not been detected in outdoor air; it has been detected in indoor air at concentrations of less than 10 ng/m3, in river water at concentrations of up to 7500 ng/litre and in sediments at 2-70 ng/g. TEHP was detected in a single sample of drinking-water at 0.3 ng/litre. Reported daily dietary intake from market basket studies, from a range of age groups, was less than 0.05 ug/kg body weight per day. TEHP is rapidly biodegraded in natural waters, but in laboratory tests with activated sludge the results were equivocal. There is no significant abiotic degradation. TEHP has a low acute toxicity for mammals, the oral LD50 being > 10 000 mg/kg body weight for rats. TEHP is a skin irritant but not an eye irritant. Repeated application of 0.1 ml (93 mg) TEHP to the skin of rabbits produced no signs of systemic intoxication. Thirteen-week gavage studies in rats and mice revealed no significant toxic effects. The no-observed-adverse-effect level (NOAEL) in rats was 2860 mg/kg body weight per day and in mice was 5710 mg/kg body weight per day, the highest dose tested in each species. In a 3-month inhalation study at concentrations up to 85.0 mg TEHP/m3, the lungs of dogs showed mild chronic inflammatory changes, and conditioned avoidance performance deteriorated in relation to the concentration administered. No studies on reproductive toxicity were available. TEHP gave negative results in several in vivo and in vitro tests for mutagenicity. TEHP was tested for chronic toxicity and carcinogenicity in rats and mice. The NOAEL for chronic toxicity in male rats was 2857 mg/kg body weight per day and in female rats was 1428 mg/kg body weight per day. In male and female mice, the lowest-observed-adverse-effect level (LOAEL) for thyroid follicular cell hyperplasia was 357 mg/kg body weight per day. A NOAEL in mice was not established. The authors concluded there was some evidence of carcinogenicity based on an increased incidence of hepatocellular carcinomas in female mice at the high-dose level and equivocal evidence of carcinogenicity based on the increased incidence of adrenal pheochromocytomas in male rats in both dose levels. Although there were increases in adrenal pheochromocytomas in both dose groups of male rats and in hepatocellular carcinomas in female mice in the high-dose group, these results are not considered to indicate that TEHP presents a significant carcinogenic risk to humans. Pheochromocytomas show a variable background incidence in rats. The incidences of these tumours in two previous National Toxicology Programme (NTP) bioassays were equal to the incidence observed in the TEHP bioassay. The only other significant neoplastic finding was hepatocellular carcinomas in the high-dose group of
female mice. Considering the low incidence of this tumour, its occurrence in only one sex of one species, the lack of evidence of genetic toxicity, and the low exposure of humans to TEHP, it is unlikely that TEHP poses a significant carcinogenic risk to humans. Neurotoxicity studies have been conducted in several species. TEHP causes no alteration in activity of plasma or red blood cell cholinesterase. No studies on delayed neurotoxicity have been reported. In a study on human volunteers, no skin irritation was reported. The few data available indicate a low acute aquatic toxicity of TEHP. The IC50 for bacteria is greater than 100 mg/litre and the 96-h LC50, for zebra fish (Brachydanio rerio) is greater than 100 mg/litre, which is the solubility limit of TEHP in water. Evaluation. Occupational exposure to TEHP is likely to be by the dermal route during manufacture (accidental exposure) and from the use of some products. The compound is absorbed dermally in experimental animals but no information is available on its kinetics or metabolism via this route. Dermal exposure cannot, therefore, be quantified but is expected to be low. Inhalation exposure in the office environment has been measured to be 10 ng/m3 or less. Exposure of the general population is principally via food and drinking-water. Exposure from both sources is very low (estimated to be <0.05 ug/kg body weight per day from the diet; a single measured concentration in drinking-water was 0.3 ng/litre). Given the reported LOAEL for thyroid hyperplasia of 357 mg/kg body weight per day in mice, the risk to the general population is very low. The risk to those exposed occupationally is also considered to be very low, although this cannot be quantified. TEHP is not considered to be carcinogenic in humans. In the environment, TEHP is expected (from its low volatility, high adsorption coefficient and low water solubility) to partition to sediment. Measured data are too few to confirm this. Degradation in environmental media is expected, although laboratory data on degradation in sewage sludges are equivocal. No information is available on breakdown products; phosphate released during breakdown is not expected to contribute significantly to environmental nutrient levels. Fig. 2 plots measured environmental concentrations in environmental media against reported acute toxicity values (the latter indicating no toxic effects at the limit of water solubility). The margin of safety between highest reported concentrations and lowest reported toxicity values is several orders of magnitude, indicating low risk to organisms in the aquatic environment. No assessment of risk can be made for the terrestrial compartment.


2-Ethylhexanol (2EH) is a weak nongenotoxic hepatic peroxisome proliferator in the rat. It is a high-volume chemical intermediate in the preparation of the plasticizers bis-(2-ethylhexyl) adipate (DEHA), bis-(2-ethylhexyl) phthalate (DEHP), and tris-(2-ethylhexyl) phosphate (TEHP), which are weak hepatocellular tumorigens in female mice. In consequence, the oncogenic potential of 2EH was evaluated in male (M) and female (F) rats and mice (50 animals/sex/group). Oral gavage doses of 2EH in 0.005% aqueous Cremophor EL (poloxyl-35 castor oil) were given five times a week to rats: 0 (water), 0 (vehicle), 50, 150, and 500 mg/kg for 24 months, and to mice: 0 (water), 0 (vehicle), 50, 200, and 750 mg/kg for 18 months. Statistical comparisons of data were made between vehicle controls and treatment groups. There were no differences of biological significance between data from vehicle and water control groups. In rats, there
there were no dose-related changes at 50 mg/kg. There was reduced body weight gain at 150 mg/kg (M, 16; F, 12%) and 500 mg/kg (M, 33; F, 31%) and an increased incidence of lethargy and unkemptness. There were dose-related increases in relative liver, stomach, brain, kidney, and testis weights at sacrifice. Female rat mortality was markedly increased at 500 mg/kg. There was marked aspiration-induced bronchopneumonia in rats at 500 mg/kg; hematologic, gross, and microscopic changes, including tumors, were otherwise comparable among all rat groups. In mice at 50 and 200 mg/kg there were no dose-related changes and essentially no time-dependent or time-independent adverse trends in liver tumor incidence at the 5% significance level. At 750 mg/kg mouse body weight gain was reduced (M, 26; F, 24%), and mortality increased (M and F, 30%) versus vehicle controls. At 750 mg/kg there was a slight increase in nonneoplastic focal hyperplasia in the forestomach of mice (M 5/50, F 4/50) versus vehicle controls (M 1/50, F 1/50). There were increases in mouse relative liver (F, 21%) and stomach (M, 13%; F, 19%) weights at 750 mg/kg. There was a 12% incidence of hepatic basophilic foci and an 18% incidence of hepatocellular carcinomas in male mice at 750 mg/kg, not statistically significant compared with either control by Fisher's exact test. There was a 12% incidence of hepatic basophilic foci and a 10% incidence of hepatocellular carcinomas in female mice at 750 mg/kg, statistically significant (p < 0.05) compared with vehicle but not with water controls by Fisher's exact test. There were no metastases. Time-dependent and -independent statistical analyses showed an adverse trend in the incidence of hepatocellular carcinomas in male and female mice, correlated with toxicity (expressed as mortality) at 750 mg/kg. The time-adjusted incidence of hepatocellular carcinomas in male mice (18.8%) was within the historical normal range at the testing facility (0-22%), but that in females (13.1%) lay outside the normal range (0-2%). Under the conditions of these studies 2EH was not oncogenic in rats, but there were weak adverse trends in hepatocellular carcinoma incidence in mice at high dose levels which may have been associated with toxicity. The major effects of chronic dosing were mortality in female rats at 500 mg/kg and in male and female mice at 750 mg/kg, accompanied by reductions in body weight gain in rats at 150 and 500 mg/kg and in mice at 750 mg/kg. Direct comparison of any tumorogenic effects of 2EH given alone to female mice with those due to 2EH formed in vivo from DEHA, DEHP, or TEHP is limited by the high mortality caused by 2ER in female mice at equivalent doses of 2EH. While 2EH may be a contributing factor in the hepatocellular carcinogenesis in female mice associated with the chronic administration of DEHA and DEHP, it is unlikely to be the entire proximate carcinogen.


ECETOC Working Group. 1992. Tris(2-ethylhexyl)phosphate, bis(2-ethylhexyl)phosphate, mono(2-ethylhexyl)phosphate. ECETOC Joint Assessment of Commodity Chemicals. 20Tris (2-ethylhexyl) phosphate (TEHP) is a non-flammable, colourless liquid with low water solubility and very low vapour pressure which is used as a flame retarder/plasticizer for PVC and cellulose acetate and as a solvent. It is produced from phosphorus oxychloride and 2-ethylhexanol. The worldwide production is estimated to be 1,000 - 5,000 tons a year. Only small amounts of TEHP are expected to enter the environment during manufacturing and use. TEHP has not been detected in ambient air; it
has been detected in indoor air in concentrations of less than 10 ng/m³, in polluted river water at concentrations of up to 2,000 ng/l and in sediments at 2-70 ng/g. TEHP is rapidly biodegradable in natural waters but in laboratory tests with activated sludge the results are equivocal. The few data available indicate a low acute aquatic toxicity of TEHP. The IC₅₀ for bacteria is greater than 100 mg/l and the 96 h LC₅₀ for zebra fish Brachydanio rerio is greater than 100 mg/l. TEHP has a low acute toxicity for mammals, the oral LD₅₀ being > 10,000 mg/kgbw and it causes only moderate skin erythema and moderate conjunctivitis in rabbits. Thirteen week feeding studies in which rats received up to 4,000 mg/kgbw and mice 8,000 mg/kgbw revealed no toxic effects other than a slight-moderate depression in bodyweight gain. Twenty daily doses of 0.1 ml TEHP to the skin of rabbits produced no signs of systemic intoxication. In a 3-month inhalation study at concentrations up to 85.0 mg TEHP/m³ dogs and monkeys showed no treatment-related alterations in any biochemical or haematological measurements. The lungs of dogs showed mild inflammatory changes and the performance of dogs trained in a conditional avoidance deteriorated in relation to the concentration administered. Microscopic examination of guinea pigs exposed to 1.6 or 9.6 mg TEHP/m³ showed inconsistent and reversible changes in the renal parenchyma at the 9.6 mg/m³ TEHP concentration. Neurotoxicity studies on TEHP revealed no alteration of cholinesterase levels nor histological evidence of demyelination. TEHP does not induce gene mutation in bacteria, or chromosomal aberration and sister chromatid exchange induction in Chinese hamster ovarian cells and mutagenic response in the mouse lymphoma L 5178Y cell assays. TEHP was tested for chronic toxic and carcinogenic effects in rats and mice. There was some evidence of a treatment related increase in hepatocellular carcinoma in female mice, with a significant increase in the high dose (1,000 mg/kg). In addition the incidence of carcinomas was not significant at the low dose (500 mg/kg). The findings in male rats are not regarded as being clearly related to administration of TEHP. As a consequence the validity of extrapolating the carcinogenicity data derived from female mice administered TEHP to the assessment of cancer in man is doubtful. Bis (2-ethylhexyl) phosphate (BEHP) is a colourless liquid with a low water solubility and low vapour pressure which is used as a solvent in liquid-liquid extractions. It is produced from phosphorus oxychloride and 2-ethylhexanol. The worldwide production is estimated to be about 1,000 tons per year. Data on environmental levels are not available. Laboratory tests demonstrate that BEHP is biodegradable. BEHP is practically nontoxic to bacteria. The EC₅₀ for growth inhibition of Chlorella emersonii was > 100 mg/l. The 96 h LC₅₀ for Daphnia magna was 16.5 mg/l and the LCO (48 h) to Leusiscus idus was 20 mg/l. The acute oral toxicity to rats is low; the rat oral LD₅₀ was 5,000 mg/kgbw and the dermal LD₅₀ in rabbits was > 1,250 mg/kgbw. BEHP is corrosive to rabbit skin and eyes. Diet containing up to 3% of BEHP when fed to rats for 5 days resulted in no obvious signs of toxicity. BEHP does not induce gene initiation in bacteria. Mono (2-ethylhexyl) phosphate (MEHP) is not produced in commercial scale. Toxicological and environmental information are not available.


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Health Effects of Trioctyl Phosphate
The toxicity to HeLa cells of 29 plasticizers was determined in the MIT (Metabolic Inhibition Test)-24 test system. The 7-day IC50 (median inhibitory concentration) for HeLa cells varied from 260 to 1.5 g/l. Phthalates, adipates, sebacates, azelates and phosphates with long carbon chain alcohols were very non-toxic to the cells, probably due to insolubility in water of the compounds, while the citrates, some phosphates and the 2 polymer plasticizers had a higher toxicity to the cells. A comparison of the HeLa cytotoxicity with the toxicity in vitro to other cells for 7 plasticizers showed a similarity of the cytotoxicity to all cell types. A comparison of the HeLa cytotoxicity for 20 plasticizers with i.p. lethal dosage in rodents demonstrated a rough similarity of values, suggesting a toxicity in rodents of the compounds by toxic interference of the agents with basal functions and structures of tissues (basal cytotoxicity). Tissue culture studies of the cytotoxic mechanisms of the plasticizers therefore could reveal modes of toxic action in vivo.

Gesellschaft Deutscher Chemiker (GDCh) - Advisory Committee on Existing Chemicals of Environmental Relevance (BUA). 1997. Di(2-ethylhexyl)phosphate, tri(2-ethylhexyl)phosphate. S.Hirzel Verlag, P.O.Box 10 10 61, 70009 Stuttgart, Germany. xviii: 117p.

Conclusions of this criteria document, translation of a report finalized in August 1995: no data are available on the effects of bis(2-ethylhexyl)phosphate (BEHP) in humans; skin tests with tris(2-ethylhexyl)phosphate (TEHP) produced no irritation; both substances exhibit a low acute toxicity in animals; in direct contact with the skin and mucous membranes, BEHP is corrosive and TEHP causes moderate irritation; no mutagenic effects have been observed.


Fifteen chemicals were tested, with and without exogenous metabolic activation, by the sister chromatid exchange (SCE) assay and the chromosome aberration assay in Chinese-hamster ovary cells, to evaluate their in-vitro cytogenetic damage. These chemicals included bisphenol-A (80057), 2-chloroethanol (107073), C-I-acid-orange-10 (1936158), C-I-disperse-yellow-3 (2832408), C-I-solvent-yellow-14 (842079), cytembena (21739913), D-and-C-red-9 (5160021), 1,2-dibromoethane (106934), F-D-and-C-yellow-6 (2783940), malaoxon (1634782), D,L-menthol (15356704), phenol (108952), sulfisoxazole (127695), titanium-dioxide (13463677), and tris(2-ethylhexyl)-phosphate (78422). These compounds were tested at doses up to 5mg/ml or as limited by solubility and/or toxicity. Solvent and positive controls were run concurrently with each trial. Descriptions of the SCE and chromosome aberration assays used and of the data analysis
were provided. Results indicated that 2-chloroethanol, cytembena, 1,2-dibromoethane, and phenol gave positive responses in both assays; whereas bisphenol-A, D-and-C-red-9, F-D-and-C-yellow-6, D,L-menthol, titanium-dioxide, and tris(2-ethylhexyl)-phosphate gave negative responses in both assays. Seven of the eight chemicals that were positive for SCE were positive both with and without activation; the exception was disperse-yellow-3, which was positive for SCE only without activation. Solvent-yellow-14 and phenol did not induce SCE without activation using standard harvest times, but were positive after delayed harvest times.


Weanling inbred Fischer-344-rats and weanling hybrid B6C3F1-mice were used to study the carcinogenic properties of phthalic-anhydride (85449) (PAn), butyl-benzyl-phthalate (85687) (BBP), diallyl-phthalate (131179) (DAP), di(2-ethylhexyl)-phthalate (117817) (DEHP), di(2-ethylhexyl)-adipate (103231) (DEHA), tris(2-ethylhexyl)-phosphate (78422) (TEHP) and 2-ethylhexyl-sulfate (126921) (EHS). Animals were chronically exposed for 2 years. The evidence of toxicity for these compounds correlated with their ester substituents. Many of the phthalic-acid esters (PAEs) showed some carcinogenic activity, but the sites where the activity occurred differed with each compound, negating the theory of a common mode of action for these chemicals. DEHP induced tumors in the liver, while BBP and DAP were carcinogenic in the hematopoietic system and DAP the forestomach. The increased tumor occurrences were in general restricted to one sex and species. Each compound, which contained a 2-ethylhexyl moiety, DEHP, DEHA, TEHP, and EHS, caused hepatocarcinogenesis, particularly in female mice. The strongest hepatocarcinogenic activity was demonstrated by DEHP, which displayed the same effect in rats. The author suggests that the release of 2-ethylhexanol (104767) may be responsible for the hepatocarcinogenic effect, although sufficient data is not yet available to support or refute this conclusion.


Four compounds containing a 2-ethylhexyl moiety [di(2-ethylhexyl)phthalate (DEHP), di(2-ethylhexyl)adipate (DEHA), tris(2-ethylhexyl)phosphate (TEHP), and 2-ethylhexyl sulfate (EHS)] were tested for carcinogenic and other chronic and subchronic toxic effects in 90-day and 2-year studies in male and female Fischer 344 rats and B6C3F1 mice. The low generalized toxic potencies of the test chemicals allowed relatively high doses of all of these compounds to be administered. Despite differences in chemical structure, all four chemicals were related to increased occurrences of hepatocellular neoplasms, principally carcinomas, in female mice. DEHA and DEHP also induced hepatocellular neoplasms in male mice, while DEHP caused hepatocellular neoplasms in both male and female rats. No other neoplasms were considered to be unequivocally related to compound administration in these studies. There was a positive correlation between the magnitude of the hepatocarcinogenic response in female mice and the probability of a hepatocarcinogenic response in male mice and in male and female rats, suggesting quantitative differences in the carcinogenic potentials of these agents. These
results suggest that compounds containing a 2-ethylhexyl moiety (and 2-ethylhexanol, by implication) may possess some carcinogenic potential, especially for the rodent liver. No other organ-specific toxic effects common to two or more test chemicals were observed in these studies.


**National Toxicology Program. 1984.** NTP Toxicology and Carcinogenesis Studies of Tris(2-ethylhexyl)phosphate (CAS No. 78-42-2) In F344/N Rats and B6C3F1 Mice (Gavage Studies). *Natl Toxicol Program Tech Rep Ser.* 1984 Aug;274:1-178

Tris(2-ethylhexyl)phosphate is one of a family of trialkyl phosphates that have been widely used as fire retardants and plasticizers. Another trialkyl phosphate, tris(2,3-dibromopropyl)phosphate (Tris-BP), once used as a flame retardant in children's sleepwear, has been shown to be carcinogenic, but tris(2-ethylhexyl)phosphate has not been previously studied. Tris(2-ethylhexyl)phosphate, a clear, viscous liquid, is used as a component of vinyl stabilizers, grease additives, and flame-proofing compositions; however, it is used primarily as a plasticizer for vinyl plastic and synthetic rubber compounds. In 1974, approximately 3 million pounds of tris(2-ethylhexyl)phosphate was produced in the United States; imports during that year were negligible. Substantial human exposure probably occurs during production of tris(2-ethylhexyl)phosphate and during the manufacture and use of products containing it, but data on the magnitude of exposure are not available. Two-year toxicology and carcinogenesis studies of tris(2-ethylhexyl)phosphate were conducted by administering the test chemical in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 male and 50 female F344/N rats and B6C3F1 mice. Male rats received doses of 2,000 or 4,000 mg/kg body weight, female rats received 1,000 or 2,000 mg/kg, and male and female mice received 500 or 1,000 mg/kg. Fifty vehicle control animals of each sex and species received 10 ml/kg body weight (rats) or 3.3 ml/kg (mice) corn oil by gavage on the same schedule. Inflammation of the gastric mucosa in mice and mild weight depression in rats and mice were the only dose-related effects observed in the preliminary studies. In the 2-year studies, survival rates and mean body weight gains of dosed female rats and dosed mice were comparable to those of their perspective controls. Survival rates of dosed male rats were comparable to that of the vehicle controls, but body weight gains were depressed. One nonneoplastic lesion, follicular cell hyperplasia of the thyroid, was observed at increased incidences in dosed male and female mice. Two compound-related increased incidences of neoplasms could not be discounted. In male rats, the incidence of pheochromocytoma of adrenal glands increased with dose (2/50, 4%; 9/50, 18%; 12/50, 24%). There were also two additional malignant pheochromocytomas in the high dose group. However, the incidence of adrenal pheochromocytoma in vehicle controls of this study (2/50, 4%) was low compared with the 25% incidence observed in two previous studies in this laboratory or the overall historical incidence of 18% observed throughout the Program, and thus the evidence of carcinogenicity was considered to be equivocal. In female mice, the incidence of hepatocellular carcinoma (0/48; 4/50; 7/50) in high dose animals (1,000 mg/kg) was significantly increased relative to that of the vehicle controls.
Decreased incidences were observed for acinar cell adenomas of the pancreas in dosed male rats (14/50, 28%; 5/48, 10%; 2/49, 4%) and for fibroadenomas of the mammary glands in low dose female rats (11/50, 22%; 2/50, 4%; 7/50, 14%). Hemangiosarcomas of the circulatory system in male mice (7/50, 14%; 0/50; 1/49, 2%) and lymphomas of the hematopoietic system in female mice (14/49, 29%; 10/50, 20%; 6/50, 12%) were decreased compared with vehicle controls. A decrease in the incidence of lymphomas and an increased incidence of carcinomas of the liver in female mice (both seen in this study) were observed in studies of di(2-ethylhexyl) adipate. Increased incidences of liver carcinomas and decreased incidences of mammary fibroadenomas were observed also in female rats in the di(2-ethylhexyl) phthalate studies. A possible link among these three chemicals may be metabolic conversion to 2-ethylhexanol. Tris(2-ethylhexyl)phosphate was not mutagenic in Salmonella typhimurium strains TA98, TA100, TA1535, or TA1537 in the presence or absence of 9000 x g (S9) fractions from Aroclor 1254-induced Sprague-Dawley rat or Syrian hamster liver. An audit of the experimental data from these carcinogenesis studies was conducted by the National Toxicology Program. No data discrepancies were found that significantly influenced the final interpretations of these experiments. Under the conditions of these studies, a comparison of concurrent and historical controls indicated that there was equivocal evidence of carcinogenicity in male F344/N rats receiving 2,000 and 4,000 mg/kg tris(2-ethylhexyl)phosphate, as evidenced by increased incidences of pheochromocytomas of the adrenal glands. There was no evidence of carcinogenicity in female F344/N rats or in male B6C3F1 mice receiving tris(2-ethylhexyl)phosphate. There was some evidence of carcinogenicity in female B6C3F1 mice that received 1,000 mg/kg tris(2-ethylhexyl)phosphate, as shown by an increased incidence of hepatocellular carcinoma. Tris(2-ethylhexyl)phosphate was associated with increased incidences of follicular cell hyperplasia of the thyroid gland in male and female B6C3F1 mice. Synonyms and Trade Names: TOF; trioctyl phosphate; phosphoric acid tri(2-ethylhexyl) ester; Flexolreg. TOF; Kronitexreg.