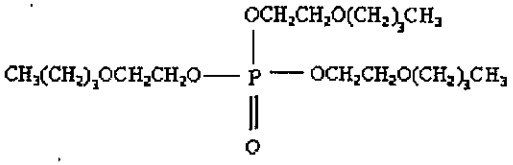
Molecular structure:



Empirical formula: $C_{18}H_{39}O_7P$

Relative molecular mass: 398.54

Common name: tris(2-butoxyethyl) phosphate

Synonyms: phosphoric acid, tris(2-butoxyethyl) ester; tri(2-butoxyethanol) phosphate; tris(2- *n*-butoxyethyl) phosphate; tributoxyethyl phosphate; TBOP; TBEP; TBXP (only in Japanese literature); 2-butoxyethanol phosphate (RTCES, 1989); tri(2-butylethylether) phosphate; tris(butylglycol) phosphate; tributyl cello solve phosphate

Trade names: Kronitex KP-140; KP-140; Phosflex T-BEP; Phosflex 176C; Amgard TBEP

CAS registry number: 78-51-3

CAS name: Ethanol, 2-butoxy, phosphate (3:1)

EINECS number: 201-122-9

RTECS number: KJ9800000

A2.2 Physical and chemical properties

TBEP is a technical product that may contain as impurities tributyl phosphate (about 3%) and traces of 2-butoxyethanol and phosphoric acid (FMC, 1990; Albright & Wilson (1999) personal communication to IPCS). There is no information on the concentration of mono- or diesters or other impurities in the technical product.

TBEP is a light-coloured, high-boiling, non-flammable viscous liquid with a butyl-like odour under normal conditions. It is more soluble in non-polar than in polar solvents.

Boiling point: 200-230°C at 5.0-5.3 hPa

Melting point: -70°C

Density: 1.02 g/ml at 20°C

Viscosity: 11-15 mPa.s at 20°C

Vapour pressure:
at 25°C 2.8×10^{-7} hPa
at 150°C 0.33 hPa (0.03 mmHg)

Refractive index: 1.434 at 25°C

Solubility: 1.1-1.3 g/litre water at 20°C; miscible in petroleum at 20°C

Acidity/alkalinity: (1 g/litre water at 20°C)	neutral
Flashpoint:	210°C (approximately); 159 ± 2°C
Ignition point:	251-52°C
Auto-ignition temperature:	322 ± 5°C; 261°C
Log K_{oc}:	4.38 (calculated)
n-Octanol/water partition coefficient:	4.78 (calculated); 3.65

References: Eldefrawi et al. (1977); Keith & Walters (1985); Laham et al. (1985b); Hoechst (1987); Watts & Moore (1988); Leo (1989); FMC (1990); Hinckley et al. (1990); Lenga (1993); Tremain & Bartlett (1994).

A2.3 Conversion factors

1 ppm = 16.53 mg/m^3 at 20°C
1 mg/m^3 = 0.0605 ppm at 20°C

A2.4 Analytical methods

TBEP is usually analysed by gas chromatography (GC) coupled with mass spectrometry (MS), infrared spectroscopy or nuclear magnetic resonance spectrometry. The detection limit is <1 ng/g (adipose tissue) using any of these methods or a nitrogen/phosphorus-selective detector (LeBel et al., 1981; Rivera et al., 1987).

A2.4.1 Air

TBEP has been found associated with particulate matter in the air of offices. Of the methods that can be used to collect the particles, Weschler (1980) used a four-stage impactor with a back-up filter and extracted with a mixture of water and methanol. Later Weschler (1984) and Weschler & Fong (1986) collected particles on Teflon(R) membranes, separating the particles according to whether the aerodynamic diameter was greater or less than 2.5 µm. The samples were analysed by GC/MS after thermal desorption of the collector membranes. Sometimes samples were desorbed or dissolved with toluene.

A2.4.2 Water

TBEP has been extracted either with dichloromethane after acidification to pH 2 or by passage through a column filled with Amberlite XAD-2 resin which is subsequently extracted with acetone and hexane. After dehydration and concentration, extracts are analysed. The concentrated extracts are determined by GC/MS, or with other detection methods, as described above (LeBel et al., 1981; Watts & Moore, 1988). LeBel et al. (1987) used large-volume resin sampling cartridges to obtain sufficient organic extracts from water for analysis. Recovery at 10 ng TBEP/litre fortification level was 103.4%.

Frimmel et al. (1987) described an analytical method to determine TBEP in water by extracting TBEP with granulated activated carbon and analysing the extract with GC/MS.

Rivera et al. (1987) analysed water samples with different

procedures, liquid-liquid extraction, adsorption on granular activated carbon, extraction with dichloromethane, followed by GC/MS/DS (Daughter spectral) detection. Ether-insoluble organic fractions were analysed and fractionated by high-performance liquid chromatography (HPLC) and ultraviolet absorbency detection was carried out with a 2140 diode-array detector, followed by fast atom bombardment (FAB) and FAB-collision-induced dissociation - mass analysis kinetic energy spectroscopy (CID-MIKES) mass spectrometry.

A2.4.3 Sediment

After decanting the supernatant water, the sediment samples are mixed with an equal volume of pre-extracted anhydrous sodium sulfate and transferred to a Soxhlet thimble. Soxhlet extraction is carried out overnight using dichloromethane (300 ml) (Watts & Moore, 1988).

A2.4.4 Soils and foodstuffs

There are no reports of extraction or clean-up methods for soil or food (ECETOC, 1992b).

A2.4.5 Biological media

LeBel & Williams (1983b, 1986) and LeBel et al. (1989) analysed human adipose tissue for TBEP by extraction with a mixture of acetone/hexane in the presence of anhydrous sodium sulfate. The solution was centrifuged and the supernatant filtered and evaporated. The resulting extract was dissolved in a mixture of 5% dichloromethane in cyclohexane for gel permeation chromatography (GPC) to separate residual lipids from phosphate esters. Using this method the recovery of TBEP from adipose tissue was approximately 90%.

Anderson et al. (1984) measured peaks of TBEP determined by HPLC in spiked samples of serum during the development of an analytical refinement. There was a marked inter-individual variation in peak height, which correlated with serum lipoprotein concentration.

A3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

A3.1 Natural occurrence

TBEP has not been found to occur naturally in the environment (ECETOC, 1992b).

A3.2 Anthropogenic sources

A3.2.1 Production levels and processes

TBEP is produced by reacting phosphorus oxychloride and butoxyethanol (butyl glycol) and stripping hydrochloric acid and excess of butoxyethanol. Another production method uses the sodium salt of the glycol. In this case, the by-product is sodium chloride (ECETOC, 1992b).

The world global production has been estimated to be 5000-6000 tonnes, with less than 1000 tonnes in Europe.

A3.2.2 Uses

TBEP is used mainly as a component in floor polishes, a solvent in some resins, a viscosity modifier in plastisols, an antifoam and also as a plasticizer in synthetic rubber, plastics and lacquers. TBEP is widely used as a plasticizer in rubber stoppers for vacutainer

tubes and plastic ware.

A4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION AND TRANSFORMATION

A4.1 Transport and distribution between media

All environmental TBEP derives from human activities but the input rate to the environment cannot be estimated from the available data. The input is expected to be mainly to soil, sediments and surface waters from leachates from plastics on landfills, from spillages and from effluents (ECETOC, 1992b).

The low vapour pressure, the high soil sorption coefficient (K_{oc}) and the water solubility of approximately 1 g/litre suggests that TBEP in the environment will be found mainly in water and sediment. TBEP has been detected in surface water and sediments (ECETOC, 1992b).

A4.2 Biodegradation

No data are available on mechanisms of abiotic or biotic transformation. Analogy with other phosphate esters suggests that enzymatic hydrolysis would be expected to dominate (ECETOC, 1992b).

TBEP was readily biodegradable when tested in the OECD 301B assay, achieving 87% degradation within 28 days (Mead & Handley 1998).

In a test of primary biodegradation using the semi-continuous activated sludge procedure and an addition rate of 3 mg TBEP/litre per test cycle, 88% of TBEP was eliminated. The ultimate biodegradability (using the Monsanto shake-flask procedure) was 51% of the theoretical CO_2 generated after 28 days (Monsanto, 1976).

Hattori et al. (1981) studied the degradation of TBEP in environmental water in 1979-1980. Using the molybdenum blue colorimetric method, the increase of phosphate ions was analysed in Oh and Neya river water and seawater from Osaka Bay to which 1 mg TBEP/litre had been added. The degradation depended on the source of the water (Table 1).

Table 1. Biodegradation of TBEP in water in percentages (from Hattori et al., 1981)

Test duration (days)	Oh River	Neya River	Osaka Bay	
			Tomagashima seawater	Senboku seawater
7	29.1	0	1.9[a]	0
14	100[b]	100	17.6	100

[a] Test duration 8 days
[b] Test duration 15 days

A sterilized distilled water control did not show any degradation after 15 days. TBEP was rapidly degraded in less than 14 days after an acclimatization period of several days in water containing micro-organisms. Where degradation was rapid, the phosphatase activity increased during the test period.

TBEP was eliminated from estuarine water with a half-life of

approximately 50 days (Ernst, 1988).

A4.2.1 Migration

LeBel & Williams (1983a) investigated the difficulties of obtaining representative water samples and the importance of designing suitable sampling protocols. TBEP was detected in tap water at concentrations from 11.0 to 5400 ng/litre. The authors suggested that the TBEP originated from the O-ring and seal in the tap.

A5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

A5.1 Environmental levels

A5.1.1 Air

An indoor aerosol sample was collected in a large building in New Jersey, USA. The abundance of TBEP was greatest both for particles larger than 7.0 μm diameter and for those smaller than 1.1 μm; there was considerably less material present in the intermediate size ranges. This pattern is consistent with its use in floor polish. Buffing operations generate relatively large particles which are likely to contain TBEP. However, this compound may also migrate from the floor polish and be attached to particles. In this case the majority of the adsorbed TBEP would accumulate in the submicron size range (Weschler, 1980). The mean concentrations measured in representative samples of dust from air in 7 offices in the USA was reported to be 15 ng/m^3 (Weschler & Shields, 1986). The significance of floor polish, which may contain 1% TBEP (Nakashima et al., 1993), as a source of these particulates is suggested by the fact that the highest concentration measured (25 ng/m^3) was found immediately following floor polishing work by a night crew.

Airborne concentrations of fine (2.5 μm) and coarse aerosol (2.5-15 μm) particles were simultaneously measured outside and inside two buildings, one in Wichita, Kansas, USA, during the fall and early winter (1981-1982) and the second one in Lubbock, Texas, USA, during late winter and spring 1982. The average indoor concentrations of TBEP in Wichita and Lubbock were 4 and 25 ng/m^3, mainly in fine aerosol particles. TBEP was not found in outdoor aerosol particles (Weschler, 1984).

Yasuda (1980) reported the results of a study of 19 outdoor air samples from 7 locations in 1976. Two samples from Kawauchi Town contained 149.1 and 176.8 ng TBEP/m^3 and one from Ehime University 9.6 ng TBEP/m^3. TBEP was not detected in the other 16 samples.

A5.1.2 Water (drinking-water and surface water)

Levels of TBEP have been determined in rivers, sewage, tap water, lakes and estuaries. The investigations have been carried out in the Great Lakes area of Canada, USA, Japan, Germany and the United Kingdom.

The lower part of the River Weser (over 33 km), Germany, was examined for the presence of TBEP during the period May 1985 to April 1987. TBEP was found at a mean concentration of 125 ng/litre. Systematic measurements of effluent samples from five sewage treatment plants in the Bremen region showed concentrations of TBEP ranging from 800 to 34 900 ng/litre (Bohlen et al., 1989).

Ernst (1988) analysed water of the estuary of the Rivers Elbe and Weser, Germany, for the presence of TBEP during the period 1983-1985.

The concentrations that were found ranged from 5 to 70 ng/litre.

One hundred samples of surface water were collected from various locations throughout Japan in 1975 and analysed for the presence of TBEP. TBEP was identified in none of the samples (the limit of determination ranged from 0.02 to 0.5 µg/litre). In 1978, 114 samples were analysed in Japan and TBEP was not identified (the limit of determination ranged from 0.005 to 1.5 µg/litre) (Environmental Agency Japan, 1978, 1983, 1987).

In a survey conducted between 1989 and 1990, Fukushima et al. (1992) identified TBEP in Lake Biwa, Yodo River and also in the Yamato Osaka Rivers and Osaka Bay at levels of about 0.2-2.5 µg/litre.

Drinking-water was collected in Japan over a 12-month period and analysed. Concentrations ranging up to 0.0585 ng/litre were found (Adachi et al., 1984).

Two samples of drinking-water collected from six Eastern Ontario water treatment plants in the period June-October 1978 contained 0.9-75.4 ng/litre (LeBel et al., 1981). In another study two samples of drinking-water were collected from five Great Lakes water treatment plants of Eastern Ontario and analysed for TBEP. The concentration found in surface water samples ranged from 9.8 to 54.4 ng/litre as determined by GC/MS. When determined by GC/NPD, concentrations of 0.4 to 73.8 ng/litre were found (LeBel et al., 1987).

Williams et al. (1982) collected samples of drinking-water from 12 Ontario municipal water treatment plants which draw their water from the Great Lakes system in January and August 1980. All samples contained TBEP at concentrations ranging from 1.6 to 271.6 µg/litre. The authors noted that TBEP is a common constituent of rubber gaskets and washers and can be introduced into water from components of the tap used for sampling.

In 1983, LeBel et al. (1983a) found up to 5400 ng/litre in a sample of drinking-water taken after non-use of the tap for 65 h.

In the period August 1976 to March 1977, 16 grab samples of river water were collected from the Delaware River, USA (between river mile 78 and 132). In addition to other compounds, TBEP was identified in all samples. The concentrations ranged from 0.3 to 3.0 µg/litre in the winter and from 0.4 to 2.0 µg/litre in the summer (Sheldon & Hites, 1978).

A5.1.3 Soils and sediment

TBEP was detected in 7 out of 80 samples of sediment collected at different locations in Japan in 1975. The concentrations ranged from 0.22 to 0.54 mg/kg and the limit of determination was 0.002-0.1 mg/kg. In 1978, none of the 114 sediment samples collected at different places in Japan contained TBEP (limit of determination 0.0005-0.12 mg/kg) (Environmental Agency Japan, 1978, 1983).

Watts & Moore (1988) did not detect TBEP in suspended particles or bottom sediments in a river in the United Kingdom, even though TBEP was found in corresponding water columns.

A5.1.4 Aquatic organisms

No TBEP could be detected in 74 samples of fish from numerous locations throughout Japan (limit of determination 0.005-0.1 mg/kg). Another report from the same agency stated that TBEP was not found in 93 fish samples (limit of determination 0.0005-0.15 mg/kg)

(Environmental Agency Japan, 1978).

A5.2 Human tissue levels

LeBel & Williams (1983b) analysed 16 samples of human adipose tissue for TBEP. Four of sixteen samples contained TBEP at concentrations of 4.0-26.8 µg/kg. LeBel & Williams (1986) reported the results of 115 human adipose tissue (omentum) samples for TBEP, obtained at autopsy of humans from the Eastern Ontario cities, Kingston and Ottawa, Canada. TBEP was detectable in 21 out of 68 male adipose tissue samples and in 20 out of 47 female samples. Although the frequency of detection was similar in the two cities, mean concentrations in Ottawa were about 2.5 times those in Kingston. In both cities the concentrations in women were 2-3 times greater than in men. The arithmetic mean concentration of TBEP in the 41 detectable samples was 11.3 µg/kg in extracted fat (in males 6.3 µg/kg and in females 16.6 µg/kg). The mean concentration overall was 4.2 µg/kg in extracted fat. In a different study, LeBel et al. (1989) showed the presence of TBEP in human adipose tissue autopsy samples from 3 out of 6 Ontario (Canada) municipalities (based on a detection limit of 20 ng/g). No statistical difference between sexes was found, the mean concentration being 396 ± 56 ng/g in Toronto and 173 ± 32 ng/g in Cornwall.

A5.3 Food

In a series of articles Gunderson (1988, 1995a,b) reported data on daily intake of TBEP for a range of age groups and for a period between 1982 and 1991 from the USA FDA Total Diet Study (see Table 2).

Similar data were collected in a parallel study on ready-to-eat food from 1982 to 1991. TBEP was found in 5 out of 230 food items (baby food, ketchup, grapefruit juice, strawberries, tomatoes) and in 5 out of 17 050 chemical or pesticide samples, with an average concentration per residue of 0.28 µg/g (Kan-Do Office and Pesticides Team, 1995).

A5.4 Occupational exposure

The only data on occupational exposure to TBEP is from an office environment. Weschler & Shields (1986) measured a mean concentration of 15 ng/m^3 in dust samples from some offices in the USA. NIOSH (USA) has estimated that the number of workers exposed to TBEP is more than 200 000.

Table 2. Mean daily intake of TBEP per unit body weight (µg/kg body weight p according to age and gender

	6-11 months old	2 years old	14-16 years old		25-30 years o	
			females	males	females	ma
1982-1984	0.0029	0.0144	0.0084	0.0077	0.0129	0.
1984-1986	0.0002	0.0015	0.0007	0.0011	0.0004	0.
1986-1991	0.0052	0.0037	0.0012	0.0011	0.0020	0.

A6. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

No data are available on the kinetics or metabolism of TBEP either in animals or humans.

The Task Group considered that 2-butoxyethanol is a metabolite. Information on the toxicity of 2-butoxyethanol is given in IPCS (1998).

A7. EFFECTS ON LABORATORY MAMMALS AND IN VITRO TEST SYSTEMS

A7.1 Single exposure

A7.1.1 Oral and dermal

The acute toxicity of TBEP following oral or dermal administration is low (Table 3).

Table 3. Acute toxicity of TBEP

Species	Route	LD_{50} (mg/kg body weight)	Reference
Rat	oral	3000	Eldefrawi et al. (1977)
Rat	oral	4700	Monsanto (1984c)
Rabbit	dermal	>5000	Gabriel (1980c)
Rabbit	dermal	>10 000	Report ICD/T.76.019 by FMC Corporation, Princeton, NJ, USA (1976)

An acute oral toxicity study was conducted according to the "fixed dose" procedure. Two out of three male rats but no females died at 5000 mg/kg body weight; no rats died at 500 mg/kg body weight. Signs of toxicity included chromorhinorrhoea, dyspnoea and decreased locomotion (Freeman, 1991a).

A7.1.2 Inhalation

The median lethal concentration in air has been investigated in a 4-h aerosol inhalation test (Hoechst, 1989). Groups of five male and five female Wistar rats were exposed to measured TBEP concentrations of 3.3, 3.4 or 6.4 mg/litre. No animal died but at all concentrations the animals exhibited depressed and irregular respiration, increased salivation, sneezing, unsteadiness and tremor, but these symptoms had cleared in most animals 9 days later. There were no body weight changes and gross necropsy revealed no abnormality. The 4-h LC_{50} was thus >6.4 mg/litre.

The 4-h LC_{50} in rats was reported to be greater than 4.43 mg/litre determined gravimetrically (particle size 2.46 ± 2.52 µm) (Mount 1991).

A7.2 Short-term repeated exposure

A7.2.1 Oral

In a 14-day oral dosing regime using male and female rats, where the highest dose was 100 mg/kg body weight per day, a comprehensive

biochemical, haematological and histopathological evaluation showed no changes (Komsta et al., 1989).

In a 4-week study, diets containing 0, 500, 2000, 7500 or 15 000 mg TBEP/kg were fed to male and female Sprague-Dawley rats. No signs of toxicity were found in male rats of any group whereas there was a slight decrease in body weight and food consumption in females receiving diets containing 7500 or 15 000 mg/kg diet. No compound-related changes were observed at necropsy (Monsanto, 1985a).

In a 14-week oral toxicity study with TBEP, Wistar rats (5 weeks old, male and female, 15 rats/group) were given a diet containing 0, 0.3, 3 or 30 g TBEP/kg. Suppression of body weight gain was observed in both sexes at 30 g/kg. Serum cholinesterase activity was significantly decreased in both sexes at 3 and 30 g/kg, and serum gammaglutamyl transferase activity was significantly increased in both sexes at 30 g/kg. Examination of the liver in both sexes revealed moderate periportal hepatocyte swelling in male rats at 30 g/kg after 14 weeks of exposure but this change was not found in male rats given 3 g/kg or less. The no-observed-effect level (NOEL) of TBEP in the diet was 0.3 g/kg diet (for males 20 mg/kg body weight per day and for females 22 mg/kg body weight per day. The Task Group considered the NOAEL of this study to be 3 g/kg diet (Tsuda et al., 1993; Saitoh et al., 1994).

In a gavage study, groups of 12 male and 12 female Sprague-Dawley rats were administered 0, 0.25 or 0.5 ml/kg body weight undiluted TBEP on 5 days/week for 18 weeks. During the first week, two high-dose females showed muscular weakness and ataxia which had disappeared by the end of the fourth week. After about 7 weeks, nearly all animals exhibited some signs of toxicity, which seemed to be treatment related. All treated animals appeared less active, and one female died during week 13. Breathing difficulties and ataxia were present in several males and females in both treatment groups, though the low-dose group was affected to a lesser extent. Tremors, piloerection, lacrimation and increased urination were observed in both males and females of the high-dose group. After the last dose, the clinical signs observed in the high-dose group decreased in intensity. High-dose females had significantly elevated level of serum gamma-glutamyltransferase. Red cell acetylcholinesterase (AchE) activity was significantly reduced in males at both doses. There were no haematological changes. Animals were necropsied one week after the last dose. Liver weight was significantly increased (about 20%) in both high- and low-dose groups. Kidney weight was increased by about 20% in both groups and the increase was statistically significant in high-dose groups. Histopathological changes were confined to the heart of male rats of both groups. Three of six high-dose and two of six low-dose animals had multiple foci of mononuclear cell infiltration, haemorrhages and/or myocardial fibre degeneration. Two of six high-dose, three of six low-dose and one of six control rats demonstrated multifocal interstitial fibrosis with or without macrophage containing haemosiderin pigment. The authors concluded that TBEP may have accelerated the development of focal myocarditis, which is a normal feature of older male Sprague-Dawley rats. A NOAEL was not ascertained in this study (Laham et al., 1984a, 1985a).

In an 18-week study, four groups of 20 male and 20 female Sprague-Dawley rats were fed diets containing 0, 300, 3000 or 10 000 mg TBEP/kg. Body weight, food intake and clinical observations were similar in treated and control rats. Haematological and clinical chemistry parameters were normal except for increased platelet counts in the 10 000 mg/kg group, and increased serum gamma-glutamyl-transpeptidase and decreased plasma cholinesterase

activity in the 3000 and 10 000 mg/kg groups. Liver weight was increased in the 10 000 mg/kg group. Microscopic examination showed mild periportal hepatocellular hypertrophy and periportal vacuolization in males receiving 3000 and 10 000 mg/kg in the diet. The NOEL was 300 mg/kg diet, equivalent to 15 mg/kg body weight per day (Monsanto, 1987a).

A7.2.2 Dermal

In a 21-day dermal toxicity study on New Zealand White rabbits, groups of 6 male and 6 female animals were treated with TBEP applications of 0, 10, 100 or 1000 mg/kg body weight per day, 5 days/week for 3 weeks. The unabraded dorsal clipped skin was used. The tests sites were occluded for 6 h after each exposure. No animals died and no adverse clinical signs of pharmacological/toxicological effects were observed. There was no indication that dermal exposure to 1000 mg/kg body weight per day resulted in any adverse systemic effect, but local irritation, oedema, atonia and desquamation occurred at all dose levels (Monsanto, 1985b).

A7.3 Skin and eye irritation; sensitization

In three studies TBEP was non-irritating to intact and abraded skin when applied topically to albino rabbits. (Gabriel 1980b; Monsanto, 1984c; Freeman, 1991b).

In the 21-day dermal toxicity study on New Zealand White rabbits, slight to moderate erythema was noted. The skin irritation was dose-related and severity progressed over time. Microscopic observations of the skin (of the 1000 mg/kg group) showed squamous cell hyperplasia, hyperkeratosis, hair follicles distended with keratin and surface accumulation of keratin and cellular debris, erosions ulcers, acute/subacute inflammation and congestion and haemorrhages in various combinations (Monsanto, 1985b) (see also section A7.2.2).

In four studies TBEP was non-irritating to the eyes of albino rabbits (Gabriel 1980a; Monsanto, 1984c; Freeman, 1991c; personal communication from Hoechst AG, Frankfurt, Germany entitled: Eye irritation test on New Zealand rabbit with TBEP, 1988).

No animal data are available on skin sensitization potential.

A7.4 Reproductive toxicity, embryotoxicity and teratogenicity

TBEP was administered by gavage in corn oil to three groups of 25 mated Charles River CD female rats at dose levels of 0 (corn oil), 250, 500 or 1500 mg/kg body weight per day on days 6 to 15 of gestation. The treatment had no effect at any dose level on fetal resorption, fetal viability, post-implantation loss, total implantations or the incidence of fetal malformations. The NOEL was the highest dose level tested, 1500 mg/kg body weight (Monsanto, 1985e). In an earlier range-finding study maternal weight loss was observed in animals receiving 2000 mg/kg but not 1000 mg/kg body weight per day (Monsanto, 1985d).

A7.5 Mutagenicity and related end-points

A mutagenicity test was carried out with *Salmonella typhimurium* strains TA1535, TA1538, TA1537, TA98 and TA100, with and without metabolic activation. Liver S9 fractions were used from male Sprague-Dawley rats or from male Syrian hamsters induced by Aroclor 1254. TBEP was non-mutagenic (MacKeller, 1978)

TBEP was tested for mutagenic activity with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, in the presence and absence of rat liver metabolic system, in comparison with positive controls. The concentrations tested were 0, 50, 100, 500, 1000, 5000 and 10 000 µg/plate with and without S9. Toxicity to strain TA100 was observed at 5000 and 10 000 µg/plate in the presence and absence of metabolic activation. The same effect was seen at 10 000 µg/plate with TA1535 and TA98 in the absence of S9 mix. TBEP did not cause any mutagenic response either with or without metabolic activation (Monsanto, 1984d).

A CHO/HGPRT mammalian cell forward gene mutation assay with TBEP was carried out. The tests were conducted at 50, 100, 150, 225 and 300 µg/ml with S9 and at 5, 50, 75, 100 and 130 µg/ml without S9. TBEP was not mutagenic (Monsanto, 1985c).

A7.6 Carcinogenicity

No data on the carcinogenicity of TBEP are available.

A7.7 Special studies

A7.7.1 Neurotoxicity

A7.7.1.1 Acute administration

An acute delayed neurotoxicity study was carried out using groups of 20 hens. Dermal or oral (in gelatin capsules) TBEP doses of 5000 mg/kg body weight were administered at the start of the study and again 21 days later. Positive control hens were given 750 mg/kg body weight of tri- *ortho*-cresyl phosphate (TOCP) at the same time intervals. Negative controls were either untreated (dermal study) or given empty capsules (oral study). All hens were treated with 15 mg/kg body weight of atropine sulfate three times a day for 5 days following each dosing. Hens were killed 21 days after being given the final dose, and histological preparations were made from brain, spinal cord and peripheral nerves. No treatment-related lesions were detected in the nerves of TBEP-treated hens. TBEP had no effect on neuropathy target esterase (NTE). Brain and plasma cholinesterases were inhibited in treated hens (Carrington et al., 1990).

In another study, groups of five hens were treated orally with TBEP (5000 mg/kg), with TOCP (750 mg/kg) as positive control group, or with the capsules alone. The animals were killed 24 h after treatment. Brain AChE, brain neuropathy target esterase (NTE) and plasma butyrylcholinesterase (BuChE) activity was measured. No differences were seen between control and TBEP-treated brain NTE activity, although plasma BuChE and brain AChE levels in TBEP-treated hens were depressed to 5% and 13% of the control group, respectively (Monsanto, 1986).

Laham et al. (1985b) reported the results of the administration by gavage to Sprague-Dawley rats of a single dose of TBEP (98.2%). Groups of randomized female and male rats (10 rats of each sex per dose level) were used. The doses were 1.0, 1.5, 1.75, 2.0 and 3.2 g/kg for females and 1.0, 3.2, 6.8, 8.0 and 9.0 g/kg body weight for males. Three weeks after the administration of TBEP, electrophysiological parameters were determined in four or less surviving animals for each group, selected from survivors showing overt clinical signs. Reductions in caudal nerve conduction velocity and increases in refractory period (in males) were observed. Sciatic nerve sections showed degenerative changes in some myelinated and unmyelinated fibres. It should be noticed that the doses were in the region of or greater than the LD_{50}. There was a high mortality. Survivors were ill

and had marked weight loss.

The Task Group considered this study of inadequate quality for use in risk evaluation.

A study of similar design as the oral study of Monsanto (1986) but with dermal application of 5000 mg/kg body weight both on day 0 and on day 21 showed no clinical signs of toxicity in chickens (Monsanto, 1986).

A7.7.1.2 Repeated oral administration

In a 14-day repeated-dose study on Sprague-Dawley rats dosed at 0.8 to 2.24 ml/kg body weight (08-2.28 g/kg), electro-physiological measurements were made on days 15 and 28. Apart from a significant decrease in the body weight of low-dose females at 7 days, there were no clinical signs or significant differences between dosed groups and controls in the 14-day study. Minor and inconsistent changes in electro-physiological parameters were reported. No morphological changes were found using light or electron microscopy (Laham et al., 1984b).

A second study (Lahman et al., 1984a) involved dosing on 5 days per week for 18 weeks at dose levels of 0 (0.5 ml water), 0.25 and 0.5 ml/kg body weight (0.25-0.51 g/kg) with observations at 6, 12 and 18 weeks. There were no significant body weight differences between exposed groups and their controls at any stage. A few females (2/12) from the high-dose group showed, at the beginning of the experiment, transient muscular weakness and ataxia which disappeared 4 weeks later. In the second half of the study almost all treated animals exhibited tremors, piloerection, lacrimation and increased urination. Males were less affected than females.

Electro-physiological changes were observed at 18 weeks in all test animals (Table 4) and included a statistically significant reduction in nerve conduction velocity and a significant increase of both relative and absolute refractory periods. The increased refractory period and the decreased conduction velocity were dose-related in females, but in males the maximum effect appears to have been reached by the low dose, suggesting that the magnitude of the maximum attainable neurophysiological changes is modest. Three animals of each sex at each dose level were examined for neurohistological abnormalities by light and electron microscopy of the sciatic nerve. Most of the treated animals showed the presence of some degenerative myelin sheaths accompanied by axonal swelling and an advanced stage of degeneration, indicated by the presence of lamellated electron-dense inclusions in unmyelinated nerve fibres (Laham et al., 1984a).

In the 18-week studies of Monsanto (1987a,b), TBEP was administered to four groups of 20 male and 20 female Sprague-Dawley rats at concentrations of 0, 300, 3000 and 10 000 mg/kg diet for approximately 18 weeks. No clinical signs of neurotoxicity were observed. The only neurophysiological alteration observed was reduced caudal nerve conduction velocity in high-dose females, and there were no treatment-related changes in peripheral nerve or spinal cord histopathology.

Table 4. Electro-physiological parameters at 18 weeks in rats treated wi (Laham et al., 1984a)[a]

	Control (water)	Low-dose TBEP

	Males	Females	Males	Females	Ma
Number of animals	12	12	12	12	12
Dose (ml/kg per day)	-	-	0.25	0.25	0.
Nerve conduction velocity (m/s)	36.3	36.3	30.7[b]	32.0[b]	30.
Absolute refractory period in caudal nerve (ms)	1.02	0.95	1.24[b]	1.26[b]	1.2
Relative refractory period in caudal nerve (ms)	2.06	1.93	2.39[b]	2.33[b]	2.3

[a] results at 6 and 12 weeks were quantitatively similar to those at 18 weeks
[b] $P<0.001$

A7.7.1.3 Effects on esterase activity

Laham et al. (1984b) reported a 5-7% reduction in red cell cholinesterase activity at 18 weeks in male rats dosed by gavage with 0.25 or 0.5 ml TBEP/kg body weight per day but no reductions in female rats.

A study was made of the effect of TBEP on NTE, brain AChE and plasma BuChE in three groups of five hens. Each was administered a single oral dose of 5000 mg TBEP/kg body weight. All animals were killed 24 h after treatment. The NTE activity was unchanged but plasma BuChE and brain AChE levels were depressed to 5% and 13%, respectively, of control levels (Monsanto, 1986).

In an acute delayed neurotoxicity study in hens, two doses of 5000 mg TBEP/kg body weight were given 21 days apart, each followed by antidote treatment with atropine. There was no effect on NTE activity, whereas brain AChE and serum BuChE were inhibited (Carrington et al., 1990).

A8. EFFECTS ON HUMANS

A repeat human insult patch test on a panel of 209 volunteers was undertaken by Monsanto (1984e). In the 3-week induction period, four applications per week of 0.2 ml of the test material were applied for 24 h to occluded skin. During the fourth week, four similar applications were made to previously untreated sites. During induction, minimal irritation was observed in 9 of the individuals. The irritation was only seen once or twice during the 12 applications. There was no dermal reaction to challenge applications. The results indicate minimal skin irritation and do not indicate any sensitizing potential.

A9. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

A9.1 Laboratory experiments

A9.1.1 Aquatic organisms

A9.1.1.1 Invertebrates

The 24-h and 48-h LC_{50} values for TBEP in *Daphnia magna* were 84 mg/litre and 75 mg/litre, respectively. The no-observed-effect concentration (NOEC) was 32 mg/litre (Monsanto, 1984a).

A9.1.1.2 Vertebrates

The 96-h LC_{50} in fathead minnow *(Pimephales promelas)* was 16 mg/litre (95% confidence interval 13-22 mg/litre) at 22°C (Monsanto, 1984b). The 48-h LC_{50} values in killifish *(Oryzias latipes)* at 10, 20 and 30°C were 44 mg, 27 mg and 6.8 mg/litre, respectively (Tsuji et al., 1986).

In goldfish *(Carassius auratus)* Eldefrawi et al. (1977) reported no death at 5 mg/litre after 168 h (temperature 20°C).

In rainbow trout *(Oncorhynchus mykiss)*, a 96-h LC_{50} of 24 mg/litre and a NOEC of 10 mg/litre were reported in a test conducted under OECD guideline 203 (Wetton & Handley, 1998).

PART B

TRIS(2-ETHYLHEXYL) PHOSPHATE
(TEHP)

B. SUMMARY, EVALUATION AND RECOMMENDATIONS

B1. Tris (2-ethylhexyl) phosphate (TEHP)

B1.1 Summary

Tris(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 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/m^3, 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 µg/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 LD_{50} 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/m^3, 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 phaeochromocytomas in male rats in both dose levels. Although there were increases in adrenal phaeochromocytomas 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. Phaeochromocytomas 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 IC_{50} for bacteria is greater than 100 mg/litre and the 96-h LC_{50} for zebra fish *(Brachydanio rerio)* is greater than 100 mg/litre, which is the solubility limit of TEHP in water.

B1.2 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/m^3 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 µg/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.

B1.3 Recommendations

For full scientific evaluation of the compound, identification and assessment of metabolites in mammals would be required, given the toxicological profile of one of the suggested metabolites, 2-ethylhexanol.

Reproductive toxicity needs to be investigated, in particular the potential for developmental effects.

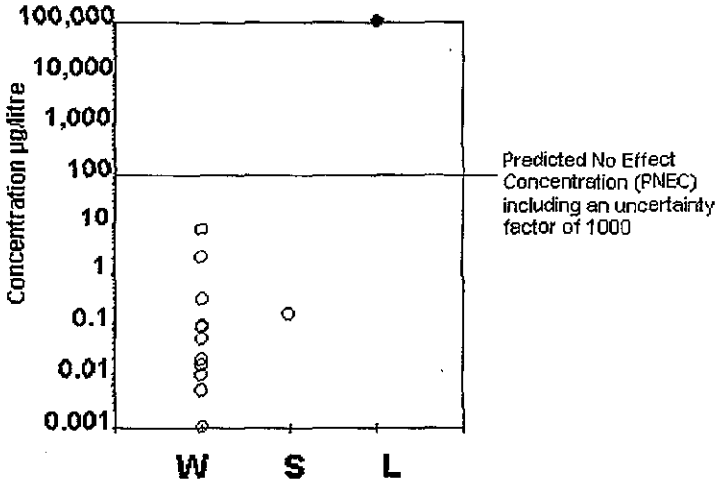


Fig. 2. Plot of measured concentrations in surface waters (W) and sewage effluents (S), and reported acute toxicity values (L) for TEHP (o= measured concentrations in the environment; ● = calculated LC_{50})

B2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, AND ANALYTICAL METHODS

B2.1 Identity

Chemical structure: