(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.

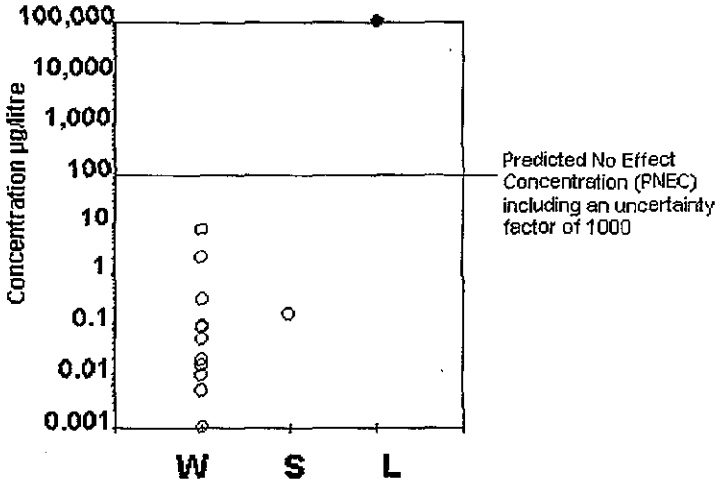

Fig. 2. Plot of measured concentrations in surface waters (W) and sewage effluents (S), and reported acute toxicity values (L) for TEHP (o= measured concentrations in the environment; ● = calculated LC_{50})

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

B2.1 Identity

Chemical structure:

リン酸(2-エチルヘキシル)ジフェニルエステルのラットを用いる28日間反復経口投与毒性試験

Twenty-eight-day Repeat Dose Oral Toxicity Test of Diphenyl 2-ethylhexyl phosphate in Rats

要約

リン酸(2-エチルヘキシル)ジフェニルエステルは，リン酸エステル系の可塑剤である[1]．今回，既存化学物質の安全性点検に係わる毒性調査事業の一環として，リン酸(2-エチルヘキシル)ジフェニルエステルを0，4，20，100および500 mg/kgの用量でSD系ラットの雌雄に28日間反復経口投与し，その毒性について検討した．対照群，100および500 mg/kg群については14日間回復群を設けた．

摂餌量の低値が500 mg/kg群の雌で認められた．血液学検査では，活性化部分トロンボプラスチン時間の延長が500 mg/kg群の雌で認められた．血液生化学検査では，血漿コリンエステラーゼの低値が500 mg/kg群の雌雄，血球コリンエステラーゼの低値が100 mg/kg群の雌と500 mg/kg群の雌雄，総コレステロールの高値が500 mg/kg群の雌，総タンパクの高値が100 mg/kg群の雄と500 mg/kg群の雌雄，アルブミンの高値が100および500 mg/kg群の雄，GOTの低値が500 mg/kg群の雌，アルカリフォスファターゼの低値が100および500 mg/kg群の雌で認められた．尿検査では，異常は認められなかった．病理学検査では，肝臓の変化として相対重量の高値が100 mg/kg群の雄，絶対重量および相対重量の高値が500 mg/kg群の雌雄，褐色化が100および500 mg/kg群の雌雄，腫大が500 mg/kg群の雌雄，小葉中心性の肝細胞肥大が100および500 mg/kg群の雌雄で認められた．腎臓の変化として，近位尿細管上皮内の硝子滴の増強が500 mg/kg群の雄で認められ，一部の動物では好酸性小体の発現も伴われていた．甲状腺の変化として，腫大が100 mg/kg群の雄と500 mg/kg群の雌雄，ろ胞上皮細胞の肥大が100および500 mg/kg群の雌雄で認められた．副腎の変化として，絶対重量と相対重量の高値が100 mg/kg群の雌と500 mg/kg群の雌雄，腫大が100および500 mg/kg群の雌雄，束状帯の脂肪滴の増加が500 mg/kg群の雌雄で認められた．これらの変化は，投与を止めることにより軽減ないし回復していた．20 mg/kg群では雌雄いずれも被験物質に起因した変化は認められなかった．

以上の結果より，本試験条件下におけるリン酸(2-エチルヘキシル)ジフェニルエステルの無影響量は雌雄いずれも20 mg/kg/dayであると判断した．

方法

1. 被験物質

リン酸(2-エチルヘキシル)ジフェニルエステル(大八化学工業㈱，製品名＃41，CAS No. 1241-94-7，Lot No. K70801，純度91.4％)は，凝固点-54℃，融点239℃/13.3 hPa，油溶性の無色透明液体である．本ロットは投与期間中安定であることが確認された．投与液は被験物質を0.1%Tween 80水溶液に乳化させ調製し，冷蔵保存した．投与液中の被験物質は冷蔵保存条件下で8日間安定であり，使用した投与液にはほぼ所定量の被験物質が均一に含有されていることを確認した．

2. 試験動物および飼育条件

日本チャールス・リバー㈱より入手したSD系ラット(Crj:CD，SPF)の雌雄を7～8日間検疫・馴化し，試験に使用した．投与開始前日に体重別層化無作為抽出法により群分けした．1群の動物数は雌雄各6匹とし，対照群，100および500 mg/kg群については雌雄各6匹の14日間回復群を設けた．投与開始時の週齢は5週齢，体重範囲は雄が156～177 g，雌が137～158 gであった．

検疫・馴化期間を含めた全飼育期間中，温度20～25℃，湿度40～70％，換気約12回／時，照明12時間(7:00～19:00)に自動調節された飼育室を使用した．動物は，実験動物用床敷(ベータチップ：日本チャールス・リバー㈱)を敷いたポリカーボネート製ケージに1ケージあたり2匹で収容し飼育した．

動物には，実験動物用固型飼料(MF：オリエンタル酵母工業㈱)および5 μmのフィルター濾過後，紫外線照射した水道水を，それぞれ自由に摂取させた．

3. 投与量および投与方法

被験物質を0，100，500および1000 mg/kgの各用量でSD系ラットに14日間反復経口投与した結果，全投与群で流涎が発現し，体重増加抑制とヘモグロビン濃度およびヘマトクリット値の低値が1000 mg/kg群の雄で認められた．また，肝臓の絶対重量および相対重量の高値が全投与群の雌雄で認められ，1000 mg/kg群の雌雄では肝肥大が顕著であった．その他，副腎の絶対重量および相対重量の高値が500 mg/kg以上の投与群の雌，副腎の相対重量の高値が1000 mg/kg群の雄，腎臓の相対重量の高値が1000 mg/kg群の雌雄で認められた．従って，本試験では高用量を500 mg/kgとし，以下公比5で中間量を100および20 mg/kg，低用量を4 mg/kgとした．

被験物質は28日間毎日1回，午前中に胃ゾンデを用いて強制経口投与した．投与液量は10 mL/kgとし，至近測定日の体重を基に算出した．対照群には同様に溶媒を投与した．

4．観察および検査方法

1） 一般状態，体重および摂餌量

全例について一般状態を毎日観察した．体重および摂餌量は投与開始日およびその後週1回測定した．摂餌量については，各期間毎の1匹あたりの1日平均摂取量を算出した．

2） 血液学検査

各計画殺時の全例について，チオペンタールナトリウムの腹腔内投与による麻酔下で後大静脈より採血し，赤血球数(シースフローDCインピーダンス検出法)，白血球数(RF/DCインピーダンス検出法)，血小板数(シースフローDCインピーダンス検出法)，ヘモグロビン濃度(SLSヘモグロビン法)，ヘマトクリット値(赤血球パルス波高値検出法)を多項目自動血球分析装置(NE-4500：東亜医用電子)，白血球百分率(Wright染色塗抹標本)を血液細胞自動分析装置(MICROX HEG-70A：立石電機)，網状赤血球数(アルゴンレーザーを用いたフローサイトメトリー法)を自動網赤血球測定装置(R-2000：東亜医用電子)，プロトロンビン時間(PT；Quick一段法)，活性化部分トロンボプラスチン時間(APTT；活性化セファロプラスチン法)を血液凝固自動測定装置(KC10A：アメルング社)により測定した．また，検査の結果から平均赤血球容積(MCV)，平均赤血球血色素量(MCH)，平均赤血球血色素濃度(MCHC)を算出した．凝固阻止剤として，プロトロンビン時間および活性化部分トロンボプラスチン時間測定には3.13%クエン酸ナトリウム水溶液を，それ以外の項目の測定にはEDTA-2Kを用いた．

3） 血液生化学検査

採取した血液の一部をヘパリン(リチウム塩)処理後，3000 r.p.m.，10分間遠心分離し，得られた血漿を用いてGOT(SSCC改良法)，GPT(SSCC改良法)，ALP(GSCC改良法)，γ-GTP(SSCC改良法)，尿素窒素(Urease-GLDH法)，グルコース(GK-G6PDH法)，総コレステロール(CES-CO-POD法)，トリグリセライド(LPL-GK-G3PO-POD法)，クレアチニン(Jaffé法)，総蛋白(Biuret法)，アルブミン(BCG法)，A/G比(総蛋白およびアルブミンより算出)，カルシウム(O-CPC法)，無機リン(UV法)，ナトリウム，カリウム，クロール(イオン選択電極法)を自動分析装置(日立736-10形：㈱日立製作所)により測定した．

全例の血漿コリンエステラーゼ(アセチルチオコリン-DTNB法)を自動分析装置(日立736-10形：㈱日立製作所)，血球コリンエステラーゼ(アセチルチオコリン-DTNB法)を自記分光光度計(日立U-3200形：㈱日立製作所)および脳コリンエステラーゼ(アセチルチオコリン-DTNB法)を自動分析装置(COBAS FARA：F. Hoffmann La Roche & Co.)により測定した．

4） 尿検査

雄は投与開始後26日，雌は投与開始後25日に各群雌雄6匹の新鮮尿を採取して，pH，潜血，蛋白，糖，ケトン体，ビリルビン，ウロビリノーゲン(試験紙法，マルティスティックス，マイルス・三共㈱)を尿分析器(クリニテック100：マイルス・三共㈱)により測定した．

5） 病理学検査

全例について採血後に腹大動脈を切断して放血致死させ剖検した．剖検後，全例の脳，肝臓，腎臓，副腎，胸腺，脾臓，甲状腺(上皮小体を含む)，精巣および卵巣の重量を測定した．また，全例の上記器官に加え，下垂体，眼球，ハーダー腺，肺，胃，心臓，膀胱，骨髄(大腿骨)，坐骨神経，脊髄を採取し，10%中性リン酸緩衝ホルマリン液で固定，保存した．ただし，眼球およびハーダー腺はDavidson液で固定した．

投与期間終了時解剖動物の対照群および500 mg/kg群の雌雄の心臓，肝臓，脾臓，腎臓，副腎，脳，坐骨神経，脊髄，甲状腺，下垂体および全動物の肉眼的異常部位を対象に，常法に従いヘマトキシリン・エオジン染色標本を作製し鏡検した．その結果，雌雄の肝臓，副腎，甲状腺と雄の腎臓に被験物質投与に起因すると考えられる変化が認められたため，投与期間終了時解剖動物の他の群と回復試験終了時解剖動物の当該器官(腎臓は雄のみ)を検査した．また，代表例の肝臓および副腎についてオイルレッドO染色と甲状腺の渡辺鍍銀染色を行い，鏡検した．

6） 統計解析

計量データについては，Bartlett法による等分散の検定を行い，分散が一様の場合は一元配置分散分析を行った後，Dunnett法またはScheffé法により検定した．分散が一様でない場合はKruskal-Wallisの検定を行い，Dunnett型またはScheffé型の順位和検定を行った．計数データおよび病理組織所見については，Armitageのχ^2検定を行った．有意水準は5%未満とした．

結果

1．一般状態

投与後の流涎が100 mg/kg群の雄で投与開始後6日以降，雌で投与開始後5日以降，500 mg/kg群の雄で投与開始後1日以降，雌で投与開始後4日以降に発現した．また，投与前の流涎が100 mg/kg群の雌で投与開始後8日に，500 mg/kg群の雄で投与開始後7日以降，雌で投与開始後13日以降に発現した．投与後の流涎は，投与直後に発現する一過性の変化であり，投与前の流涎は動物の体に触れることにより発現した．回復期間にはこれらの変化は認められなかった．