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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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Tributyl phosphate

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CAS No. 126-73-8

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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Tributyl phosphate

This Toxicological Evaluation replaces a previously published version in volume 8.

1 Summary and assessment

Tributyl phosphate is completely absorbed from the gastrointestinal tract of the rat upon oral administration. When rats were treated with single or multiple doses of 10 mg/kg body weight, peak plasma levels were attained after 90 to 140 minutes whereas after 350 mg/kg body weight, peak levels were seen after 180 to 400 minutes. With regard to dermal absorption, there is marked interspecies variability. Under identical experimental conditions, rats absorbed 40 and 56%, respectively, of the administered doses, whereas in the Yucatan Minipig the maximum extent of absorption was 4%. No studies are available on absorption via the respiratory tract. Plasma half-life after intravenous injection into rats was found to be 1.3 hours. Irrespective of the route of administration, dose administered, duration of treatment and the species and sex of the experimental animals used, elimination essentially takes place via the urine and to a lesser extent via the faeces or as CO_2 in the exhaled air. In the rat, values for the half-life of elimination in urine following single intravenous and dermal as well as single and repeated oral administration were determined as being in the range between 16.6 and 29.9 hours. There is no bioaccumulation of tributyl phosphate or its metabolites in the body; 7 days upon intravenous, dermal and single or repeated oral administration, the bodies of rats were found to contain a maximum of 1.5% of the dose administered, the highest dose fractions being detected in the muscle, skin and fat. Tributyl phosphate undergoes almost complete metabolisation in the body, with unchanged tributyl phosphate representing less than 1% of the amounts excreted in urine and faeces. In the initial metabolic step, tributyl phosphate undergoes oxidation of the butyl chains at position 3, in particular, and to a lesser extent at positions 2 and 4, with subsequent dealkylation taking place by glutathione conjugation or hydrolytic cleavage of the oxidised butyl residues. The major metabolites identified in rat studies include dibutyl hydrogen phosphate, butyl 3-hydroxybutyl hydrogen phosphate and butyl 4-bu-

tanoic acid hydrogen phosphate, and dibutyl hydrogen phosphate, butyl dihydrogen phosphate and butyl bis(3-hydroxybutyl) phosphate as well as the mercapturic acid derivatives of the cleaved-off oxidised butyl residues, particularly (3-oxobutyl)- and (3-hydroxybutyl)mercapturic acid.

On acute oral administration and acute inhalation exposure, tributyl phosphate is found to be harmful (LD_{50} rat oral 1164 to 3350 mg/kg body weight; LD_{50} mouse oral 900 to 1240 mg/kg body weight; approximate LC_{50} rat approx. 4242 mg/m³ after 4-hour exposure to aerosol). Acute dermal toxicity is low, with LD_{50} values being > 3100 mg/kg body weight for rabbits and in the range from 9700 to 19400 mg/kg body weight for guinea pigs. Depressed general condition, lying on the side, narcosis, convulsions, blood in the areas around the nostrils, lips and eyes, as well as diarrhoea have, inter alia, been reported as signs of intoxication following acute oral administration. From concentration levels of approx. 800 mg/m³, inhalation exposure was additionally observed to cause marked dyspnoea and irritation to the eyes and nose, while there were no findings at 511 mg/m³. At necropsy of the deceased animals, findings included visceral haemorrhages, pale kidneys, spleens and livers, markings of the lobules of the liver, degeneration of the renal tubules, reddening of the gastrointestinal tract and the lungs and, following inhalation exposure, distension and liver-like appearance of the lungs. Terminal necropsy of the surviving animals was predominantly without findings.

Tributyl phosphate causes mild irritation to rabbit skin after 4-hour semi-occlusive application. Following prolonged and/or occlusive application, the chemical induces more pronounced irritation. Tributyl phosphate has a mildly irritating effect on the rabbit eye.

An epicutaneous test in the guinea pig gave no indications to suggest a skin-sensitising potential of tributyl phosphate.

Repeated oral administration of tributyl phosphate to mice and rats for ≥ 28 days resulted in damage to the urinary bladder, the liver and the kidneys. The epithelia of the urinary bladder were observed to have diffuse to focal, nodular hyperplasia. The liver findings were characterised by increased organ weights, signs of dysfunction (elevated liver enzyme activities, elevated albumin and cholesterol levels, increased thromboplastin time) and centrilobular hypertrophy. As regards the kidneys, subchronic oral admini-

stration also resulted in increased organ weight, and in a 2-generation study in rats renal pelvis epithelial hyperplasia was seen in addition. Testicular findings in terms of degenerative changes in the seminiferous tubules following subacute oral administration to rats could not be confirmed in a subsequent 18-week study. Furthermore, some studies report changes in organ weights of the brain, spleen and uterus, none of them having a histopathological correlate. The *no observed effect levels* which were ascertained in subchronic oral studies conducted in compliance with the relevant guidelines were 1000 mg/kg feed (81 mg/kg body weight/day) for female rats, 200 mg/kg feed (13.8 mg/kg body weight/day) for male rats and 500 mg/kg feed for mice (91 to 102 and 109 to 135 mg/kg body weight/day for males and females, respectively).

Tributyl phosphate is devoid of genotoxicity in numerous test systems, both in vivo and in vitro. In vitro, the chemical has been tested for genotoxicity on Salmonella typhimurium in the Salmonella/microsome assay, on Escherichia *coli* in the spot test and on Chinese hamster ovary cells in the HPRT test, the chromosome aberration test and the micronucleus test, while in-vivo studies have been carried out using the chromosome aberration test in the rat and the sex-linked recessive lethal test in *Drosophila melanogaster*.

As discussed in the foregoing, tributyl phosphate caused dose-dependent changes in the urinary bladder epithelium in subchronic studies with a *no effect level* of 200 ppm and 1000 ppm in the feed of male and female rats, respectively (see above). Following chronic administration of tributyl phosphate at levels of 700 and 3000 ppm in the feed of male and female Sprague-Dawley rats for 24 months, the only treatment-related histopathological findings consisted in corresponding changes in the form of dose-dependent epithelial hyperplasia and papillomas, with transitional cell carcinomas additionally occurring in the top dose group and squamous epithelial carcinoma being noted in one male rat. According to a mechanistic study in the male Sprague-Dawley rat, after 10-week treatment with 200, 700 or 3000 ppm administered in the feed and a subsequent 10-week observation period, the hyperplastic, proliferative and necrotic changes noted in the urinary bladder epithelium of rats from the two highest dose groups was fully reversible. The theory according to which the formation of calculi or the precipitation of microcrystalline and/or amorphous material in the urinary tract is the cause of the toxic effect of tributyl phosphate on the urinary bladder was not confirmed by this mechanistic study. No corresponding sediments

were detectable even by electron microscopy. The investigators therefore suggested that there is a causal connection between the suspected organ-specific cytotoxicity of one or several metabolites and the hyperplastic and necrotic actions of tributyl phosphate on the urinary bladder epithelium in that repeated cellular damage induces chronic repair processes and in doing so possibly causes the normal epithelium to be transformed into its metaplastic and neoplastic forms. This theory is supported, in the investigators' interpretation, by the chemical's complete lack of genotoxicity (see also above), the increase in mitotic activity as demonstrated by their own results, and the complete reversibility of the hyperplastic and proliferative changes. In chronic administration (24 months), the *no effect level* for the induction of urinary bladder changes in the Sprague-Dawley rat is 200 ppm tributyl phosphate in feed, equivalent to daily tributyl phosphate doses of approx. 9 and approx. 12 mg/kg body weight in female and male rats, respectively. In addition to the study in the Sprague-Dawley rat, the carcinogenic potential of tributyl phosphate was also investigated in a parallel study in the CD-1 mouse. In this study, which involved 18-month administration of 150, 1000 and 3500 ppm tributyl phosphate in the feed, the males and females of the mid and top dose groups were observed to have increased relative and absolute liver weights. The top-group males also exhibited a greater number of benign hepatocellular adenomas, which occurred at an incidence of 10 out of 50 (20%) as compared with 3 out of 50 (6%) in the study controls. In historical controls, this type of neoplastic change, which is very frequent particularly in the male mouse, had an incidence of 2 out of 59 (3%) to 10 out of 60 (17%), according to the investigators.

Tributyl phosphate does not affect fertility in the rat and is devoid of teratogenicity in rabbits and rats. In doses which cause marked maternal toxicity in rats, foetotoxic effects are seen in the form of reduced body weight, rudimentary ribs and delayed ossification.

Evidence from older studies indicating disturbances of the nervous system are not confirmed by acute and subchronic studies conducted in the rat and the hen in accordance with current relevant guidelines.

Tributyl phosphate penetrates the human skin, causing local irritation to the skin and mucous membranes in humans. From a patch test with repeated exposure there are no indications to suggest a skin-sensitising potential in humans. Inhalation exposure of humans results in eye and respiratory tract

irritation, but no threshold concentration is reported in the literature. Following exposure to a concentration level of 15 mg/m³, nausea and headache have been reported. In-vitro studies in human blood have demonstrated that there is a slight inhibition of cholinesterase activity in plasma and erythrocytes.

The threshold limit value in the USA is given as 2.2 mg/m³ of air. The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") has, in the List of MAK and BAT Values 2000, assigned tributyl phosphate to category 4 of carcinogenic working substances, i.e. "substances with carcinogenic potential for which genotoxicity plays no or at most a minor part. No significant contribution to human cancer risk is expected provided the MAK value is observed.". The MAK value has been established as 1 ml/m³ (ppm) (equivalent to 11 mg/m³), and the chemical has been designated with "H" because of the danger of cutaneous absorption. Furthermore, tributyl phosphate has been assigned to pregnancy risk group C, i.e. substances for which "There is no reason to fear a risk of damage to the embryo or foetus when MAK and BAT values are observed.".

2 Name of substance

2.1	Usual name	Tributyl phosphate
2.2	IUPAC name	Phosphoric acid tri-n-butyl ester
2.3	CAS No.	126-73-8
2.4	EINECS No.	204-800-2

3 Synonyms, common and trade names

Butylphosphat
Butyl phosphate
Celluphos 4
Disflamoll TB
Kronitex TBP
Phosphoric acid tributyl ester
Phosphorsäuretri-n-butylester
TBP
Tributoxyphosphine oxide

Tributyloxyphosphine oxide
Tributylphosphat
Tri-n-butyl phosphate

4 Structural and molecular formulae

4.1	Structural formula	$(CH_3–CH_2–CH_2–CH_2–O)_3P=O$	
4.2	Molecular formula	$C_{12}H_{27}O_4P$	

5 Physical and chemical properties

5.1	Molecular mass, g/mol	266.32	
5.2	Melting point, °C	< –80	(Budavari et al., 1989; Hawley, 1995)
		< –70	(EC, 1996)
5.3	Boiling point, °C	289 (decomposition)	(Budavari et al., 1989)
		292	Hawley, 1995)
		130 (at 5 hPa)	(EC, 1996)
		150 (at 1.33 kPa)	
		177–178 (at 3.6 kPa)	(IPCS, 1991)
		148–153 (at 1.33 kPa)	(Svara et al., 1991)
5.4	Vapour pressure, hPa	0.008 (at 20 °C)	
		1 (at 97 °C)	
		10 (at 144 °C)	(EC, 1996)
		170 (at 177 °C)	(ICSC, 1996)
		0.09 (at 25 °C)	
		1.33 (at 100 °C)	
		9.73 (at 150 °C)	
		667 (at 200 °C)	(IPCS, 1991)
5.5	Density, g/cm³	0.976 (at 25 °C)	(Budavari et al., 1989)
		0.97 (at 25 °C)	(EC, 1996)
		0.9727 (at 25 °C)	(Lide and Frederikse, 1996)
		0.982 (at 20 °C)	(Sax, 1995)
		0.978 (at 20 °C)	
		0.973–0.983 (at 25 °C)	(IPCS, 1991)

5.6	Solubility in water	0.4 g/l (at 20 °C) (EC, 1996) 1012 mg/l (at 4 °C) 0.422 mg/l (at 25 °C) 2.85 x 10^{-4} mg/l (at 50 °C) (IPCS, 1991)
5.7	Solubility in organic solvents	Miscible with usual solvents (Budavari et al., 1989; Hawley, 1995) Miscible with ethanol, readily soluble in benzene and diethyl ether (Lide and Frederikse, 1996)
5.8	Solubility in fat	Partition coefficient log P_{ow} 2.5 (determined by experiment) 3.5 (calculated) (EC, 1996) 4.0 (determined by experiment) (Saeger et al., 1979) 3.99–4.01 (IPCS, 1991)
5.9	pH value	–
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 10.87 mg/m³ 1 mg/m³ ≙ 0.09 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

Used as an antifoaming agent in the concrete, textile and paper industries, as a flame retardant in hydraulic fluids, as an extractant in heavy metal ore extraction and the processing of nuclear fuel, as a plasticiser for celluloid, nitrocellulose lacquers and plastics (Bayer, 1992; Falbe and Regitz, 1995).

7 Experimental results

7.1 Toxicokinetics and metabolism

Absorption, distribution and excretion

The toxicokinetics and metabolism of tributyl phosphate were investigated in the Sprague-Dawley rat and Yucatan Minipigs in accordance with the TSCA guidelines (EPA, 1989). Groups of 4 rats and 2 pigs/dose, sex and

study end point were dosed with tributyl phosphate as follows: single intravenous injection of 5 mg/kg body weight or single 6-hour occlusive dermal application of 10 or 350 mg/kg body weight; in addition, rats only were treated with 10 or 350 mg/kg body weight by oral gavage, administered as a single dose or multiple daily doses over a period of 8 days. Unlabelled tributyl phosphate (99.7% pure) and ^{14}C-tributyl phosphate (radiochemical purity > 98%, specific activity 112.6 and 206.5 µCi/mg in the rat and minipig studies, respectively) were mixed in such proportions that the radioactivity administered in each of the substudies on the kinetics of absorption, distribution and elimination was approx. 100 µCi/kg body weight in rats, approx. 20 to 30 µCi/kg body weight in minipigs and approx. 200 µCi/kg body weight in the substudies on metabolism, which were conducted only in rats. In the multiple dose studies, ^{14}C-tributyl phosphate was administered only on the last day of treatment. The vehicle used for intravenous administration consisted of a mixture of Emulphor, ethanol and water (1 : 1 : 8), while oral administration was carried out with corn oil. In the dermal studies, tributyl phosphate was applied in the undiluted form. In rats, the time course of radioactivity in blood was monitored starting 5 minutes after administration and continuing for a total period of 96 hours. Urine and faeces of the rats and minipigs were collected at intervals for a total of 7 days, and expired air trappings for 3 days. At the end of the study, the residual activity was determined in the kidneys and urinary bladder of the minipigs and the whole body of the rats. Table 1 in the appendix gives a comparative summary of the toxicokinetic data thus obtained. Practically all animals were found to have blood in their urine. In addition, the majority of the rats treated with 350 mg tributyl phosphate/kg body weight exhibited hypersalivation and red urine. Rats completely absorbed orally administered radioactivity from the gastrointestinal tract, irrespective of the size and number of doses. Upon single and repeated oral administration, peak plasma levels were reached 90 to 140 and 180 to 400 minutes after administration of the low and the high dose, respectively. Following dermal application, rats in the low dose group absorbed 40% and those in the high dose group 56% of the administered radioactivity, with peak plasma levels being reached after 4 hours, and 30 to 60 minutes, respectively. The investigators considered the rapid absorption seen in the high dermal dose group to be due to higher contact surface area as a consequence of the difference in applied volume (3 µl as opposed to 90 µl). Plasma half-life following intravenous administration was 1.3 hours. Irrespective of the route of administration,

dose administered, duration of treatment and the species and sex of the experimental animals used, elimination essentially took place via the urine and to a lesser extent via the faeces and exhaled air. Most of the administered radioactivity was excreted within the first 48 hours after administration. Following oral or intravenous administration, approx. 68 to 86% of the administered radioactivity was recovered in urine and approx. 7 to 19% in faeces within the observation period of 7 days. Unchanged tributyl phosphate accounted for less than 1% of the radioactivity excreted in urine and faeces. Excretion in the expired air, essentially in the form of CO_2, amounted to approx. 3.5 to 8% of the oral and intravenous doses within a period of 3 days. Half-life values for urinary excretion were in the range from 24.4 to 29.9 hours after single intravenous and single and multiple oral administration, while values calculated for single dermal application were 16.6 to 20 hours. After 7 days, the radioactive dose residing in the tissues did not exceed 1.5% of the administered dose, the highest percentages being found in muscle, skin and fat. The minipigs showed very low dermal absorption. It was found to be as low as 3 to 4% of the administered radioactivity in the low dose group and less than 1% in the high dose group, and thus clearly lower than the corresponding values in the rat. Similarly to the rat, the minipig excreted tributyl phosphate rapidly and predominantly via the urinary route. Of the intravenously administered radioactivity, 81 and 82%, respectively, underwent urinary excretion, essentially within the first 6 hours after administration, whereas amounts of 2 and 3%, respectively, were excreted in the faeces. The kidneys and urinary bladder contained no detectable amounts in excess of 0.03% of the administered radioactivity at the end of the study (MRI, 1992 a, b, c).

A single oral or intraperitoneal dose of 14 mg/kg body weight of ^{14}C-labelled tributyl phosphate (radiochemical purity 98%, specific activity 0.179 mCi/mmol), dissolved in corn oil, was administered to male Wistar rats. Upon oral administration, the animals excreted 50% of the administered radioactivity in urine, 10% in exhaled air and 6% in faeces within 24 hours. Total amounts excreted after 5 days amounted to 82%. Following intraperitoneal administration, 70% was excreted in urine, 7% in exhaled air and 4% in faeces within 24 hours. Total excretion over a 5-day period amounted to 90%. No data were given on the residual activity in the carcass (Suzuki et al., 1984 a).

Khalturin and Andryushkeeva (1986) treated male Wistar rats (probably 3 rats per time point of investigation) orally with single or multiple doses of 25 mg tributyl phosphate (no indication whether in absolute terms or per kilogram body weight, purity not specified) for 4 and 7 days. The blood, liver, kidneys, adrenal glands, lungs, spleen, upper leg, brain, muscle, testes and gastrointestinal tract were analysed for tributyl phosphate at 0.5, 1 and 3 hours and 1 and 3 days after administration. The limit of detection was 10 µg/sample. In contrast to the findings of Suzuki et al. (1984 a) and the MRI (1992 a, b) described above, according to which tributyl phosphate is very readily absorbed from the gastrointestinal tract, these investigators found only low absorption. They were able to detect tributyl phosphate in all tissues at 30 minutes after administration. The overall recovery of tributyl phosphate in all tissues except the gastrointestinal tract amounted to 5.75% of the administered dose at 30 minutes, 4.8% at one hour and 2.47% at 3 hours after dosing. One day after administration, 4.8% and 0.3% of the administered dose was detected in the gastrointestinal tract and the liver, respectively, and after 3 days tributyl phosphate was no longer detectable. When administered for 7 days, tributyl phosphate was detectable only in the blood, the gastrointestinal tract and the liver one hour after the last dose. On the basis of the above analytical data, the investigators concluded that elimination was rapid and that accumulation did not occur after repeated administration (Khalturin and Andryushkeeva, 1986).

The rate of tributyl phosphate penetration through the skin of pigs was reported as 1300 pmol (approx. 0.35 µg)/minute/cm². The penetration rate was not increased in areas of skin with hair follicles as compared with areas without hair follicles (no further details; Tregear, 1961).

Dermal absorption was also demonstrated in the guinea pig (no further details; Eastman Kodak, 1968).

Metabolism

When male Wistar rats were treated intraperitoneally with tributyl phosphate at 250 mg/kg body weight (> 97% pure), analysis revealed a total of 11 oxidised and partly dealkylated metabolites in the 24-hour urine. The major metabolites were dibutyl hydrogen phosphate, butyl dihydrogen phosphate and butyl bis(3-hydroxybutyl) phosphate (Suzuki et al., 1984 a).

A subsequent study, which was also carried out in male Wistar rats, more closely investigated the glutathione S-transferase-dependent metabolic fate of tributyl phosphate following intraperitoneal administration at 1 mmol (approx. 266 mg)/kg body weight. In an initial metabolic step, the butyl moieties of tributyl phosphate were oxidised by drug-metabolising enzymes, or mixed-function oxigenases. Essentially, ω-1 oxidation resulted in the formation of dibutyl 3-hydroxybutyl phosphate and dibutyl 3-oxobutyl phosphate. The oxidised butyl moieties were cleaved off in a subsequent metabolic step involving transalkylation by glutathione S-transferase and the formation of mercapturic acid derivatives. Predominantly, (3-oxobutyl)- and (3-hydroxybutyl)mercapturic acid (8.9 and 5.2% of the administered dose, respectively) and traces of (2-oxobutyl)-, (2-hydroxybutyl)- and (4-hydroxybutyl)mercapturic acid were detected. The glutathione levels in liver and kidney dropped to approx. 55 and 75% of initial levels, respectively, within 2 hours after administration due to the formation of mercapturic acid derivatives. The investigators did not preclude the possibility that dealkylation of the primary oxidised metabolites could also take place via α-hydroxylation by mixed-function oxigenases or esterases. Butylmercapturic acid, a secondary product of glutathione conjugation of the unoxidised butyl groups previously described by Jones (1970; see below), was not detected (Suzuki et al., 1984 b).

In the context of the above MRI studies (1992 a, b) in Sprague-Dawley rats analysis revealed 18 oxidised and dealkylated urinary metabolites of tributyl phosphate after oral, dermal or intravenous administration. The major metabolites were dibutyl hydrogen phosphate, butyl 3-hydroxybutyl hydrogen phosphate and butyl 4-butanoic acid hydrogen phosphate. Analysis revealed further metabolites, including dibutyl 3-hydroxybutyl phosphate, dibutyl 4-butanoic acid phosphate, butyl 3-oxobutyl 4-butanoic acid phosphate, butyl 4-hydroxybutyl and butyl 2-hydroxybutyl hydrogen phosphate, butyl 2-oxobutyl hydrogen phosphate, 2-hydroxybutyl 3-oxobutyl hydrogen phosphate, butyl ethanoic acid hydrogen phosphate, butyl dihydrogen phosphate, 2-, 3- and 4-hydroxybutyl dihydrogen phosphate, 2- and 3-oxobutyl dihydrogen phosphate and phosphoric acid. The faeces were found to contain 6 metabolites, one of which did not appear in the urine (no structures given). Though there were definite individual variations as regards the types and amounts of metabolites formed, tributyl phosphate metabolism was not found to be dependent upon sex of the animal, route

of administration, size of dose or duration of treatment. The investigators suggested that the butyl groups of tributyl phosphate are first oxidised to alcoholic, ketonic and acidic functionalities and that these oxidised butyl groups are then cleaved off by hydrolysis, yielding the corresponding monobutyl and dibutyl phosphates. The investigators provided no information on potential glutathione derivatives of the oxidised butyl groups, such as had previously been detected by Suzuki et al. (1984 a, b; see above). No glucuronic acid derivatives of the metabolites were detected by the investigators (MRI, 1992 a).

Rats and mice have been reported to metabolise tributyl phosphate to dibutyl hydrogen phosphate and butyl cysteine, a secondary product of butyl-glutathione conjugation (no further details; Jones, 1970). Later rat studies described above confirmed the formation of dibutyl hydrogen phosphate, but glutathione conjugation was demonstrated only for previously oxidised butyl groups (see Suzuki et al., 1984 a, b; MRI, 1992 a).

In vitro, rat liver homogenate in the presence of NADPH completely metabolised tributyl phosphate to dibutyl hydroxybutyl phosphate within 30 minutes. Extension of the incubation period led to the formation of butyl di(hydroxybutyl) phosphate and dibutyl hydrogen phosphate from the primary product, dibutyl hydroxybutyl phosphate. In the absence of NADPH only a small fraction (11%) of the tributyl phosphate underwent oxidation. When mixed-function oxigenase activity was inhibited by SKF-525A, tributyl phosphate ceased to be metabolised (Sasaki et al., 1984).

7.2 Acute and subacute toxicity

Acute toxicity

The results from studies investigating the acute toxicity of tributyl phosphate following oral and invasive administration, dermal application and inhalation exposure are compiled in Table 2, see appendix.

Tributyl phosphate proved to be harmful upon oral administration and inhalation exposure but was of very low toxicity following dermal application. On oral administration, the respective LD_{50} values for the rat and the mouse were in the ranges from 1164 to 3350 and 900 to 1240 mg/kg body weight (see Table 2). The acute dermal LD_{50} for the rabbit was above 3100

mg/kg body weight while for the guinea pig it ranged from 9700 to 19400 mg/kg body weight (Johannsen et al., 1977; Eastman Kodak, 1968). An approximate LC_{50} of 4242 mg/m³ was ascertained after 4-hour exposure in a rat study of tributyl phosphate aerosol conducted in accordance with OECD guideline No. 403 (Bayer, 1990). Depressed general condition, lying on the side, narcosis, convulsions, blood in the areas around the nostrils, lips and eyes, and diarrhoea were among the signs of intoxication reported after acute oral administration (see Table 2). Four-hour inhalation exposure was additionally observed to cause marked dyspnoea and irritation to the eyes and nose at concentration levels of 801 mg/m³ and above, while 511 mg/m³ was tolerated without clinical signs of toxicity (Bayer, 1990). At necropsy of the deceased animals, findings included visceral haemorrhages, pale kidneys, spleens and livers, markings of the lobules of the liver, degeneration of the renal tubules, reddening of the gastrointestinal tract and the lungs and, following inhalation exposure, distension and liver-like appearance of the lungs. Terminal necropsy of the survivors was largely without remarkable findings (Bayer, 1986 a, 1990; Food and Drug Research, 1975 a, c, 1976 a, b; Haskell, 1953; Mellon Institute, 1943; Mitomo et al., 1980).

The intraperitoneal LD_{50} values reported for the rat and the mouse were in the range from 158 to 252 mg/kg body weight (Izmerov et al., 1982; Kalinina, 1971). Two further studies in rats found that lethal intraperitoneal doses ranged from 500 to 1000 and 800 to 1600 mg/kg body weight (Dave and Lidman, 1978; Eastman Kodak, 1968). Intravenous administration to rats was lethal at 100 mg/kg body weight, whereas rats survived 80 mg/kg body weight (Vandekar, 1957).

Subacute toxicity

Groups of 10 male and 10 female Sprague-Dawley rats received by oral gavage a dose of tributyl phosphate (98.4% pure, 1.3% tributoxyethyl phosphate) of 0.14 or 0.42 ml (136 or 407 mg)/kg body weight every day for 14 consecutive days. No treatment-related clinical signs of toxicity were observed. Body weights of animals treated with tributyl phosphate and controls that were force-fed tap water were comparable. Except for one significantly lower haemoglobin value in the high-dose female group and one reduced value for mean corpuscular haemoglobin in the low-dose fe-

male group, the red and white blood cell counts of the test group animals were comparable with those of the controls. Clinical chemistry studies revealed that the plasma of females from both test groups contained elevated levels of potassium while the high dose females showed increases in amylase activity and triglyceride levels. Males of the high dose group exhibited increased amylase activity, plasma bilirubin and erythrocyte acetylcholinesterase activity, while the low-dose male group showed dose-independent increases in blood urea nitrogen, uric acid and cholesterol levels. In the high dose group, relative and absolute liver weights were increased in both sexes, while relative and absolute spleen weights were reduced in the females. In the low dose group, only the relative liver weight was increased in the males. Gross pathological examination revealed a slight enlargement of the liver and a slight decrease in spleen size in the high dose groups. Histopathologically, one out of 4 males from the high dose group was found to have degenerative changes of the seminiferous tubules (aspermatogenesis, giant cells, cells with pycnotic or karyorrhectic nuclei). There were no further histopathological findings (Laham et al., 1984 b). The low tributyl phosphate dose of 0.14 ml (136 mg)/kg body weight can be evaluated as a *no observed adverse effect level*, as there were no statistically significant or dose-dependent findings at this dose level relative to the controls, except for elevated plasma potassium levels in the females and increased relative liver weight without a histopathological correlate in the males.

When male Wistar rats were treated by oral gavage with daily tributyl phosphate doses of 140 or 200 mg/kg body weight for a period of 7 days, this resulted in diarrhoea, increased relative liver and kidney weights, elevated blood urea nitrogen levels and tubular degeneration. The daily oral administration of tributyl phosphate at 130 or 460 mg/kg body weight for one month caused marked body weight depression in addition to diarrhoea and tubular degeneration, and death in 20 or 40% of animals, respectively (no further details; Mitomo et al., 1980).

In a range-finding study for a subchronic study (see Section 7.5; Bio/dynamics, 1991 a) performed in accordance the TSCA guidelines (EPA, 1989), groups of 5 male and 5 female CD-1 mice were given tributyl phosphate (100% pure) via dietary admixture of 0 (controls), 100, 1000, 5000 and 20000 mg/kg feed every day for a period of 28 days. All animals in the top dose group receiving 20000 mg tributyl phosphate/kg feed

largely refused to eat and died within the first 10 days of the study or were killed in a moribund condition. After 10 days, the lowest dose was escalated from 100 to 10000 mg/kg feed. Administration of 1000, 5000 and 10000 mg/kg feed did not increase mortality or produce clinical signs of toxicity. At and above 5000 mg/kg feed, males and females showed depressed body weight gains and elevations of relative and absolute liver weights. Food consumption was reduced in the 10000 mg/kg dose group. Following administration of 10000 mg/kg feed, increased liver weight was noted which correlated with grossly evident liver enlargement. No histopathology or clinical chemistry studies were carried out. The *no observed effect level* for tributyl phosphate was found to be 1000 mg/kg feed. Based on food consumption, the mean tributyl phosphate intake was 23, 226 to 271, 956 to 1034 and 2090 to 2220 in females and 18, 165 to 194, 765 to 844 and 2090 to 2220 mg/kg body weight/day in males (Bio/dynamics, 1990).

A 25-percent solution of tributyl phosphate in olive oil was administered to rabbits by oral gavage or subcutaneous injection 7 times every other day. Oral doses of 100 and 500 mg/kg body weight and subcutaneously injected doses of 100 and 200 mg/kg body weight were tolerated without findings. Irrespective of the route of administration, 1000 mg/kg body weight caused transient proteinuria (no further details; Gewerbehygienisches I.G. Labor, 1936).

7.3 Skin and mucous membrane effects

The data on skin and mucous membrane effects of tributyl phosphate are summarised in Tables 3 and 4, see appendix. In a rabbit study conducted in accordance with OECD guideline No. 404, 4-hour semi-occlusive dermal exposure to tributyl phosphate was evaluated as mildly irritating to the skin (Bayer, 1986 b). Following 24-hour exposure and/or occlusive exposure, further studies demonstrated more marked irritation of the skin in guinea pigs and rabbits, and tributyl phosphate was evaluated as moderately to severely irritating (see Table 3). Mucous membrane irritation studies in accordance with OECD guideline No. 405 or the 16 CFR 1500.41 guideline showed that exposure to tributyl phosphate caused reversible irritation of the conjunctivae, iris and cornea, and thus tributyl phosphate was evaluated as mildly irritating to the eye (Bayer, 1986 b; Food and Drug Research, 1975 e).

7.4 Sensitisation

A patch test conducted in accordance with OECD guideline No. 406 in groups of 10 male and 10 female Hartley guinea pigs gave no indications of a skin-sensitising potential for tributyl phosphate. Induction was accomplished by applying a 10-percent solution of tributyl phosphate (99.7% pure) in mineral oil (maximum non-irritant concentration) to the depilated skin of the shoulder under occlusive cover 3 times for 6 hours at weekly intervals. The challenge, which also employed a 10-percent formulation and was carried out 14 days after the final induction treatment, elicited no positive skin reactions in any of the 20 guinea pigs treated (FMC, 1990).

Without providing data, it has been reported that tributyl phosphate had no sensitising effect on guinea pig skin (no further details; Haskell, 1953).

In contrast, another older, inadequately documented trial (drop-on test) found that 6 out of 14 guinea pig showed signs of skin sensitisation (no further details; Eastman Kodak, 1968).

7.5 Subchronic and chronic toxicity

Subchronic administration

In accordance with the EPA guideline for subchronic oral toxicity studies, groups of 15 Sprague-Dawley rats/dose and sex received 13 weeks' dietary treatment with tributyl phosphate (> 99% pure) at the following concentration levels: 0 (controls), 8, 40, 200, 1000 or 5000 mg/kg feed/day. Mean daily tributyl phosphate intake was 0.6, 3.4, 16.1, 81 or 423 mg/kg body weight in females and 0.55, 2.8, 13.8, 68 or 360 mg/kg body weight in males. There was no increased mortality in any of the dose groups. In the highest dose group, both sexes showed depressed body weights in conjunction with significantly reduced food consumption, increased relative and absolute liver weights without a histopathological correlate, and elevated levels of serum γ-glutamyl transpeptidase activity. Only the females exhibited elevated levels of alanine aminotransferase activity and cholesterol, whereas only the males showed increases in partial thromboplastin time, calcium and albumin. Generalised transitional-cell hyperplasia in the urinary bladders was noted in both sexes upon histopathological examination. When tributyl phosphate was administered at 1000 mg/kg feed, males

had increased platelet counts, increased relative and absolute liver weights without a histopathological correlate, and generalised transitional-cell hyperplasia in the urinary bladder upon histopathological examination. The respective *no observed effect levels* of tributyl phosphate for males and females were 200 and 1000 mg/kg feed, equivalent to 13.8 and 81 mg/kg body weight/day (Cascieri et al., 1985; FMC, 1985). Note: There are some minor differences between the published abstract of the study (Cascieri et al., 1985) and the original study report (FMC, 1985) with respect to the data reported. The findings presented above follow the data from the original study report (FMC, 1985).

Groups of 11 male JCL-Wistar rats were given feed containing 0 (controls), 0.5 or 1% tributyl phosphate (approx. 333 or 667 mg/kg body weight/day, respectively) for 10 weeks. The administered tributyl phosphate was > 97% pure. Dose-dependent findings included depression of body weight in association with reduced food consumption, reductions in absolute brain and kidney weights and increases in relative brain, kidney and liver weights, increased blood coagulation time and decreases in serum glucose levels and aspartate and alanine aminotransferase activity levels as well as elevated blood urea nitrogen levels. The high dose group additionally had elevated serum protein and cholesterol levels. Both dose groups exhibited elevated brain cholinesterase levels relative to controls, whereas the levels in liver and serum were unaltered. In vitro, there was no change in cholinesterase activity in brain and liver homogenates and in serum upon incubation with tributyl phosphate (10^{-2} to 10^{-7} µM; Oishi et al., 1980).

In a follow-up study, groups of 8 male JCL-Wistar rats were given feed containing 0 (controls), 0.5 or 1% tributyl phosphate (purity not specified; approx. 333 or 667 mg/kg body weight/day, respectively) for 9 weeks. In the animals treated with tributyl phosphate, body weight was found to be 11% lower than in the control group of 18 animals. Absolute and relative liver weights and relative kidney and testicular weights were significantly increased, whereas relative spleen weight was significantly reduced. Serum levels of urea nitrogen were significantly elevated. Cholinesterase activity levels measured in the brain, liver and serum, other clinical chemistry and haematology parameters and histopathological examination of the liver, kidneys and spleen revealed no substance-related alterations (Oishi et al., 1982).

Similarly, a subchronic study was conducted in the mouse in accordance with the TSCA guidelines (EPA, 1989). Groups of 15 male and 15 female CD-1 mice were given tributyl phosphate (99.7% pure) mixed into their feed at concentration levels of 500, 2000 and 8000 mg/kg feed/day for 90 days. Based on food consumption, the mean daily tributyl phosphate intake was 91 to 102, 355 to 418 and 1248 to 1580 in males and 109 to 135, 429 to 527 and 1514 to 2020 mg/kg body weight in females. The controls were fed the standard diet. Except for reduced faeces volume in association with reduced food consumption in the top dose group, no clinical signs of toxicity were noted. Body weight gain was depressed in the top-dose males and females and the mid-dose males. Following administration of 2000 and 8000 mg/kg feed, dose-dependent increases in relative and absolute liver weights and centrilobular hypertrophy occurred. In addition, histopathological examination revealed dose-dependent epithelial hyperplasia of the urinary bladder in those two dose groups. Only the top dose group exhibited statistically significant elevations of serum albumin, calcium and alanine aminotransferase activity in both sexes and of alkaline phosphatase activity in males, and slight decreases in haematocrit and erythrocytes in females. The low dose group receiving 500 mg tributyl phosphate/kg feed (equivalent to 91 to 102 (males) and 109 to 135 (females) mg/kg body weight) showed no treatment-related effects (Bio/dynamics, 1991 a; Auletta et al., 1997).

Tributyl phosphate was mixed into feed at concentration levels of 0 (controls), 0.05, 0.2 or 1% and fed to DDY mice and Wistar rats for 3 months (equivalent to approx. 33, 133 and 667 mg/kg body weight/day when fed to rats or 71, 286 and 1429 mg/kg body weight/day when fed to mice). Treatment dose-dependently caused diarrhoea, depression of body weight gain, increases in liver, kidney and testis weights and reduced uterus weights. Haematology parameters were unaltered, except for elevated blood urea nitrogen levels noted in the top dose group (no further details; Mitomo et al., 1980).

Chronic administration

An 18-week study was carried out in which groups of 12 male and 12 female Sprague-Dawley rats received daily doses of tributyl phosphate (98.4% pure, 1.3% tributoxyethyl phosphate) of 200 or 300 mg/kg body weight by oral gavage 5 days/week. Starting from study week 7, the dose

was increased from 300 to 350 mg. Conduct and scope of the study were largely in compliance with OECD guideline No. 408. No clinical signs of toxicity occurred during the study. Starting from the third week of the study, the high-dose males exhibited a decrease in body weight (approx. 10%) relative to the water-treated controls, lasting until the end of the study. The red and white blood cell counts showed no statistically significant changes. Clinical chemistry tests revealed that high-dose females had very low inhibition of acetylcholinesterase activity in the red blood cells. In the absence of a histopathological correlate, increases were noted in absolute and relative liver weights, absolute spleen weight and relative kidney weight in females and relative kidney weight in males at the high dose. Histopathology revealed that all examined animals in either dose group (6 animals each per dose and sex) had diffuse hyperplasia of the bladder epithelium, associated with subepithelial hyperplasia. Additionally, focal nodular hyperplasia of the epithelium was observed predominantly in males. Slight mononuclear infiltration was seen in one high-dose and one low-dose male, and slight submucosal oedema was noted in 2 low-dose males. All other histological results were comparable with the control. In contrast to the findings in a previous study by the same investigators who reported degenerative changes of the seminiferous tubules after 14 days' administration of approx. 400 mg/kg body weight/day (Laham et al., 1984 b; see Section 7.2), no such lesions were noted in this study (Laham et al., 1984 a, 1985).

Very inadequately documented chronic studies were carried out by Kalinina (1971). Over a period of 4 months, experimental animals underwent inhalation exposure to tributyl phosphate concentrations of 4.8 (rabbits), 5.1 (rats) or 13.6 mg/m³ (no indication of the species used in the studies, but probably mice, rats and/or rabbits) for 5 hours/day on 5 days/week. The top dose group was observed to have a 33% reduction in cholinesterase activity and altered physiological and biochemical parameters of hepatic function after 3 months. At the end of the observation period, cholinesterase activity was normal again. Exposure to 4.8 or 5.1 mg/m³ had no effect on cholinesterase activity. In what were presumably rats, rabbits and guinea pigs, repeated dermal application resulted in effects on the central nervous system (greatly increased excitability), the liver (decline in albumin levels, sulphobromophthalein inhibition in the blood, increased organ weights) and the kidneys (increase in nonprotein nitrogen in the blood, blood in the urine; no further details; Kalinina, 1971). Considerable inadequacies in the

conduct and/or reporting of the study (e.g. no information was given on control groups, the number, strain or sex of the animals exposed, or analytical verification of the concentrations tested), render this study unsuitable for the assessment of the systemic effects of tributyl phosphate following repeated exposure.

In further investigations, which were also inadequately documented, rabbits and rats treated with tributyl phosphate at 0.2 or 0.5 mg/kg and 5.0 mg/kg body weight, respectively, in a long-term study, were observed to develop necrosis of the liver (no further details; Zyabbarova and Teplyakova, 1968). Pupysheva and Peresedov (1970) reported that they observed increased liver weight, necrosis of the liver, increased kidney weight and tubular dystrophy upon repeated administration of tributyl phosphate (no further details).

7.6 Genotoxicity

7.6.1 In vitro

The in-vitro studies on the genotoxicity of tributyl phosphate are summarised in Table 5, see appendix. None of the assay systems gave any relevant indication of a genotoxic potential of tributyl phosphate.

Tests for mutagenic activity

The potential of tributyl phosphate to cause gene mutations has been investigated in a large number of *Salmonella typhimurium* strains in numerous Salmonella/microsome assays conducted as standard plate incorporation or preincubation tests, in the spot test on various *Escherichia coli* strains and in the HPRT test on Chinese hamster ovary cells (CHO-K1-BH4 cells). Tributyl phosphate did not produce any mutagenic effects either in the spot test on *Escherichia coli*, which was only carried out without metabolic activation, or in the HPRT test, carried out with and without metabolic activation. Similarly, there were no indications of mutagenic potential when tributyl phosphate was tested with and without metabolic activation in Salmonella/microsome assays using *Salmonella typhimurium* strains TA 97, TA 98, TA 100, TA 102 and TA 1537, or without metabolic activation in strains TA 1530, TA 1531, TA 1532, TA 1534, LT-2, his C117, his G46 and his

D3052 (see Table 5 in the appendix). Only strains TA 1535 and TA 1538 were reported by Gafieva and Chudin (1986) to have yielded a marked increase in revertant counts in the absence of metabolic activation but only a slight increase in the presence of metabolic activation. However, studies by other investigators who used strain TA 1535 (Bayer, 1985; Zeiger et al., 1992) or strains TA 1535 and TA 1538 (Microbiological Associates, 1977) gave clearly negative results, both with and without metabolic activation, with regard to the mutagenic potential of tributyl phosphate.

Tests for chromosome-damaging activity

Tributyl phosphate produced no clastogenic effects when investigated in the in-vitro chromosome aberration test in Chinese hamster ovary (CHO-K1) cells in the presence and absence of metabolic activation (see also Table 5; Batt et al., 1992; Microbiological Associates, 1990 b). Similarly, tributyl phosphate exhibited no chromosome-damaging potential in an in-vitro micronucleus assay which was also carried out in Chinese hamster ovary cells but only in the absence of metabolic activation (see Table 5; Brooks et al., 1996).

7.6.2 In vivo

When a cytogenetics study was carried out in the rat in accordance with the TSCA guidelines (EPA, 1989), tributyl phosphate gave no indication of a genotoxic potential. Groups of 15 male and 15 female Sprague-Dawley rats received, by gavage, a single oral dose of tributyl phosphate (99% pure) of 300, 600 or 1200 mg/kg body weight. Bone marrow examined at 12, 24 or 36 hours after dosing showed no increase in the percentage of cells with chromosome aberrations. The study comprised dose levels up to the maximum tolerated dose, the 1200 mg/kg dose of tributyl phosphate being associated with increased mortality (Microbiological Associates, 1991; Batt et al., 1992).

Tributyl phosphate (purity not specified) was also studied in male Drosophila flies with respect to its mutagenic potential. They received tributyl phosphate in the food at concentrations which had no insecticidal effect. Subsequently, they were mated with untreated females, and up to 10 emerging males of the next generation (F_1 to F_4) were assessed for infertility

and recessive lethal mutations. The males showed no such effects after oral ingestion of tributyl phosphate. Thus, this test system also gave no indication of mutagenic activity from tributyl phosphate (Hanna and Dyer, 1975).

7.7 Carcinogenicity

A carcinogenicity study was conducted in accordance with the TSCA guidelines (EPA, 1989) in groups of 50 Sprague-Dawley CD rats/dose and sex which received daily dietary treatment for 24 months with tributyl phosphate (99.7% pure) at concentrations of 0 (controls), 200, 700 or 3000 ppm. Based on mean feed consumption, this corresponded to a daily tributyl phosphate intake of 0 (controls), 8.9 ± 2.8, 32.5 ± 289.40 or 143.3 ± 39.3 mg/kg body weight in male rats and 0 (controls), 11.6 ± 2.6 42.0 ± 8.5 or 181.5 ± 32.9 mg/kg body weight in female rats. The scope of investigation, for all dose groups, encompassed clinical signs, survival rates, body weights, organ weights and macroscopic and histopathological findings. Moreover, haematology studies were carried out in the control group and the highest dose after 12 and 18 months and at study termination, and urinalyses were performed on 10 males and 10 females per group at 3 weeks and 3, 6, 12 and 18 months after study initiation. Mortality was not increased due to tributyl phosphate treatment. The high-dose males and females and the mid-dose females exhibited depressed body weight gains. The respective terminal body weights of the high-dose males and females and the mid-dose females were 19, 20 and 12% lower than those of the controls. Decreases in food consumption were seen in all dose groups, but only during the first 2 weeks of the study. The only treatment-related clinical sign of toxicity noted was red discoloration of the urine in high-dose males. Haematology evaluations and urinalyses were without abnormal findings at all time points of investigation. Macroscopic and microscopic observations revealed dose-dependent alterations in the urinary bladder which consisted in epithelial hyperplasia and papilloma in the mid- and high-dose males and females, while the high-dose animals also exhibited transitional cell carcinoma and, in the case of one male, squamous epithelial carcinoma (see Table 6). These lesions did not appear to be correlated with the presence of calcium phosphate calculi in the urinary bladder. No other treatment-related gross and/or histopathological lesions were observed. The *no observed effect level* for dietary administration of tributyl

phosphate in feed was 200 ppm, equivalent to daily doses of approx. 12 and approx. 9 mg/kg body weight for males and females, respectively (Pharmaco LSR, 1994 a; Weiner et al., 1997; Auletta et al., 1998).

Table 6. Mortality and urinary bladder lesions in the Sprague-Dawley rat after 24-month dietary administration of tributyl phosphate in the feed (after Pharmaco LSR, 1994 a; Auletta et al., 1998)

		Controls	200 ppm in feed	700 ppm in feed	3000 ppm in feed
Number of survivors*	♂	13/48	13/49	19/49	11/50
	♀	17/50	13/49	14/50	21/50
Number of animals examined	♂	50	50	49	49
	♀	50	50	49	50
Hyperplasia of the urinary bladder	♂	3	3	12	17
	♀	1	1	5	29
Neoplastic lesions of the urinary bladder	♂	0	0	2	30
	♀	0	0	1	13
including:					
– Papilloma	♂	0	0	2	23
	♀	0	0	1	11
– Squamous epithelial carcinoma	♂	0	0	0	1
	♀	0	0	0	0
– Transitional cell carcinoma	♂	0	0	0	6
	♀	0	0	0	2
Urinary bladder caluli	♂	1	1	1	3
	♀	1	0	1	1

* Total number of animals, excluding accidental deaths and animals killed in a moribund condition. Survival rates for the laboratory's own historical controls from the 1986–1992 period were given as 15 to 49% and 32 to 56% for males and females, respectively.

It was observed in the study described above (Pharmaco LSR, 1994 a; Weiner et al., 1997; Auletta et al., 1998) that Sprague-Dawley rats developed neoplastic lesions of the urinary bladder epithelium after chronic dietary administration of tributyl phosphate in the feed. It remained unclear, however, whether or not bladder lesions were a secondary effect of the chemical's potential hyperplastic activity, induced by calculi, microcrystals and/or amorphous precipitate in the urinary tract, as seen with several other chemicals. In order to investigate this point, a further, mechanistic study was carried out in the male Sprague-Dawley rat. Groups of 10 or 20 animals were given 200, 700 or 3000 ppm tributyl phosphate (99.97% pure; equivalent to approx. 15, 53 or 230 mg/kg body weight per day) in their

feed for 10 weeks. The possible effect of a lower urinary pH value was studied by including an additional treatment group receiving 3000 ppm tributyl phosphate plus 12300 ppm ammonium chloride (100% pure). The 3000 ppm groups and the control group were placed under observation for a 10-week recovery period. Control groups were given standard diet or food with an admixture of 12300 ppm ammonium chloride. At the end of the administration period and the observation period, urinary parameters were evaluated, including pH, osmolality, creatinine, total protein, calcium, phosphorous and magnesium. The urine was examined by electron microscopy for amorphous precipitate and crystalline material. The urinary bladders were examined by light and electron microscopy for histopathological and proliferative lesions, and mitotic activity (labelling index) in the epithelium was determined subsequent to bromodeoxyuridine treatment. The animals treated with tributyl phosphate showed no clinical signs of toxicity. The 3000 ppm dose groups exhibited decreased body weight gain relative to the untreated control group. Except for mild declines in osmolality and creatinine in the animals exposed to 3000 ppm and the intended decline in pH and the associated elevation in calcium levels in the groups treated with ammonium chloride, urinalyses remained essentially unchanged. In particular, no treatment-related formation of urinary calculi or amorphous or crystalline materials were detected in the urine. Relative and absolute urinary bladder weights were significantly increased in animals exposed to 3000 ppm. Noteworthy dose-dependent findings in the 700 ppm and 3000 ppm dose groups included simple hyperplasia (increase in the number of cell layers of the urothelium to 4 or more compared with the normal 3 layers), nodular and papillary hyperplasia (endophytic or exophytic proliferation, respectively, with a fibrovascular core) in association with prominent vascular dilatation in the submucosa, focal epithelial necrosis with ulcerations, acute inflammation and extensive haemorrhages, and occasional foci of squamous metaplasia. Based on their light and electron microscopic observations, the investigators considered it clear that urothelial proliferation was due to regeneration consequent to focal necrosis. The group given only ammonium chloride showed no corresponding findings while the group treated with tributyl phosphate plus ammonium chloride gave slightly less pronounced findings compared with the group given tributyl phosphate alone. The labelling index was dose-dependently increased at and above 700 ppm, with statistically significant increases being observed in the 3000 ppm groups. The 200 ppm dose group was not observed to have any gross

or histopathological changes in the urinary bladder. By the end of the 10-week observation period, all findings were completely reversible or only fibrosis in the underlying mucosa was found which was due to tissue repair or healing of erosions and ulcerations. Labelling indices were comparable with control values again. The kidneys and stomach, which were also macroscopically and histopathologically evaluated, were without remarkable findings at all time points of investigation. The investigators arrived at the final conclusion that this mechanistic study did not confirm the hypothesis according to which the formation of calculi or the precipitation of microcrystalline and/or amorphous material in the urinary tract was the cause of the toxic effect of tributyl phosphate on the urinary bladder. No such treatment-related deposits were detectable. The investigators therefore suggested that there probably was a causal connection between the suspected organ-specific cytotoxicity of one or several metabolites and the hyperplastic and necrotic actions of tributyl phosphate on the urinary bladder epithelium, in that repeated cellular damage induced chronic repair processes and in doing so possibly caused the normal epithelium to be transformed into its metaplastic and neoplastic forms. This theory is supported, in the investigators' interpretation, by the chemical's complete lack of genotoxicity (see also Section 7.6), the increase in mitotic activity as demonstrated by their own results, and the complete reversibility of the hyperplastic and proliferative changes (Bayer, 1996; Arnold et al., 1997 a, b).

The carcinogenic effect of tributyl phosphate was also investigated in the CD-1 mouse. The design and scope of the study were comparable with the rat study discussed above (Pharmaco LSR, 1994 a; Weiner et al., 1997; Auletta et al., 1998), except that no urinalyses were performed, haematology studies were carried out in all dose groups at 12 and 18 months, and the mice were only treated for 18 months with 0 (controls), 150, 1000 or 3500 ppm tributyl phosphate in the feed. Based on mean food consumption, the mean tributyl phosphate intake was 18 to 32, 128 to 215 and 402 to 1580 in males and 22 to 42, 146 to 263 and 506 to 2020 mg/kg body weight/day in females. Relative to controls, the animals in the high dose group exhibited body weight loss during a brief period at the beginning of the study, followed by depressed body weight gain, with the respective terminal body weights of males and females being 7% and 4% lower than controls. No effect on food consumption was noted, except that food consumption in the high-dose males was repeatedly found to be lower than in

the controls for short periods of time. Survival was slightly, statistically non-significantly reduced in high-dose males relative to controls while it was slightly increased in the high-dose females. No treatment-related clinical signs of toxicity or changes in haematological parameters were observed. Dose-dependent elevations of relative and absolute liver weights were seen in the mid- and high-dose males and females. The only statistically significant treatment-related finding revealed by macroscopic and microscopic pathology consisted in an increase in the incidence of benign proliferative changes in the liver in the form of adenomas in high-dose males (incidence: control 3/50 (6%), low dose group 6/50 (12%), mid dose group 7/50 (14%), high dose group 10/50 (20%)). In historical controls, this type of neoplastic change, which is very frequent particularly in the male mouse, was reported to have an incidence of 2 out of 59 (3%) to 10 out of 60 (17%). From these data, the investigators derived the following *no effect levels*: for males and females, 150 ppm in feed (18 to 32 and 22 to 42 mg/kg body weight/day, respectively), based on increases in liver weights, or 1000 ppm (402 to 773 mg/kg body weight/day) for males and 3500 ppm (506 to 918 mg/kg body weight/day) for females, based on proliferative lesions of the liver (Pharmaco LSR, 1994 b; Kotkoskie et al., 1997; Auletta et al., 1998).

7.8 Reproductive toxicity

The reproductive toxicity of tributyl phosphate was investigated, inter alia, in a two-generation feeding study in the rat which was carried out according to the TSCA guidelines (EPA, 1989) and in teratogenicity studies conducted in two species, the rabbit and the rat. Tributyl phosphate did not affect fertility nor did it produce any teratogenic effects. Only in the maternally toxic dose range did foetuses exhibit reduced body weights, rudimentary ribs, delayed ossification and, though not statistically significant, increased resorption rates.

Two-generation study

When a two-generation feeding study was performed in Sprague-Dawley rats in accordance with the TSCA guidelines (EPA, 1989), no adverse effects were noted with regard to mating behaviour, fertility and pup development following administration of tributyl phosphate. Starting at the age of

8 weeks, groups of 30 males and 30 females of the F_0 and F_1 generations were treated with tributyl phosphate (99.7% pure) which was mixed into their feed at concentration levels of 0 (controls), 200, 700 or 3000 mg/kg feed (equivalent to a daily intake of approx. 10 to 31, 36 to 107 or 160 to 502 mg/kg body weight). The animals were mated at the age of 18 weeks, and the males were subsequently sacrificed and subjected to gross necropsy and histopathological examination. The females continued treatment with tributyl phosphate until the end of lactation. Mortality was unchanged, and no clinical signs of toxicity occurred. When tributyl phosphate was administered at levels of 700 and 3000 mg/kg feed, the F_0 and F_1 generations exhibited reductions in body weight, weight gain and food consumption, epithelial hyperplasia of the urinary bladder and, in female livers only, centrilobular hypertrophy. Males dosed with 3000 mg/kg feed were additionally noted to have renal pelvis epithelial hyperplasia. The 200 mg/kg dose group exhibited transient reductions in food consumption and body weight of dams and urinary bladder epithelial hyperplasia in some males and females. Effects of tributyl phosphate on pups were limited to reduced foetal weight in the F_1 generation at the 3000 mg/kg feed level and in the F_2 generation at the 200 (only for postnatal day 14), 700 and 3000 mg/kg feed levels. The investigators considered the reduction in pup weight to be the consequence of maternally mediated toxicity. There were no effects of treatment at any dose level on mating behaviour, fertility or development of pups. Gross necropsy and histopathological examination of the reproductive organs yielded no remarkable findings (Gerhart et al., 1993; Research Triangle Institute, 1992; Tyl et al., 1997).

The dose levels for the above study were determined in a preliminary study in which groups of 10 animals/dose and sex received tributyl phosphate at levels of 0 (controls), 100, 300, 1500 or 5000 mg/kg feed from 2 weeks prior to mating until postnatal days 21 or 35. The parental animals in the 5000 mg/kg dose group exhibited profoundly reduced body weight and weight gain, epithelial hyperplasia of the urinary bladder and hyperaemia. F_1 mortality was increased in the 5000 mg/kg dose group while F_1 pup weights in the 1500 mg/kg group were reduced. The F_1 animals in the 1500 mg/kg (females only) and 5000 mg/kg dose groups were found to have dose-related epithelial hyperplasia of the urinary bladder when they were examined on postnatal day 21. Males had no lesions in the testes (SOCMA, 1991; Tyl et al., 1997).

Teratogenicity studies

Tributyl phosphate, 99.7% pure, dissolved in corn oil, was administered to groups of 5 rabbits (New Zealand White) by oral gavage at daily dose levels of 0 (controls), 50, 250, 412, 775, 1137 and 1500 mg/kg body weight on days 6 to 18 of gestation in a range-finding study performed in accordance with TSCA guidelines (EPA, 1989). Clinical signs of toxicity, food consumption and body weights were recorded regularly. The survivors were sacrificed on day 30 of pregnancy and the gravid uteri removed. Starting from a daily dose of 775 mg/kg body weight, all animals consumed little or no food and died within the study period, exhibiting considerable body weight loss. One rabbit per group died in the 250 and 412 mg/kg dose groups, again showing considerably reduced body weight following little or no food consumption. Administration of 50, 250 and 412 mg/kg body weight did not result in significant changes in body weight gain or food consumption relative to the control group. No treatment-related clinical signs of toxicity were observed. Dose-dependent embryotoxicity, foetotoxicity or externally visible teratogenic alterations did not occur (Bio/dynamics, 1991 b).

On the basis of these findings, the main study was carried out under the same experimental conditions in groups of 18 rabbits which were treated with tributyl phosphate at dose levels of 0 (controls), 50, 150 and 400 mg/kg body weight/day. Maternal toxicity was encountered only in the highest dose group receiving 400 mg/kg body weight, the dams exhibiting retarded body weight gain in association with slight but not statistically significant reduction in food consumption and a slight increase in mortality. At this maternally toxic dose level, there was a statistically nonsignificant increase in resorption rate but no foetotoxic or teratogenic alterations. In the 50 and 150 mg/kg dose groups, no maternal toxicity, embryotoxicity, foetotoxicity or teratogenicity was encountered (Bio/dynamics, 1991 c; Schroeder et al., 1991).

The same group of investigators performed another study in accordance with the TSCA guidelines (EPA, 1989) in order to assess the teratogenic and embryotoxic effects of tributyl phosphate (99.7 and 100% pure) in the rat. Again, the main study was preceded by a range-finding study in which groups of 5, 6 or 11 pregnant CD rats received tributyl phosphate, dissolved in corn oil, at daily dose levels of 0 (controls), 80, 435, 600, 790,

1145 and 1500 mg/kg body weight by oral gavage on days 6 to 15 of gestation. The survivors were sacrificed on day 20 of gestation and the gravid uteri removed. Doses of 435 mg/kg body weight and above resulted in dose-dependent maternal toxicity. Mortality in the dose group receiving 790 mg/kg body weight was 5 out of 11 dams. Administration of 1145 and 1500 mg/kg body weight was lethal to all dams. Dose-dependent and statistically significant effects included reduced body weight associated with reduced food consumption, increased relative liver weights, salivation, lacrimation and yellow staining and wetness of the fur. Marked maternal toxicity was encountered in the dose group treated with 790 mg/kg body weight, one dam showing an increased number of resorptions and reduced foetal weights. No further embryotoxicity, foetotoxicity or externally visible teratogenic alterations occurred (Bio/dynamics, 1991 b).

The main study was carried out under the same experimental conditions in groups of 24 CD rats that were given tributyl phosphate at 0 (controls), 188, 375 or 750 mg/kg body weight/day by oral gavage. Tributyl phosphate caused maternal toxicity in all dose groups. The same clinical signs of toxicity were observed as in the range-finding study described above. Mortality in the highest dose group receiving 750 mg/kg body weight was 7 out of 24. Foetal weights were markedly reduced in this dose group. Foetal ossification was slightly delayed on administration of 188 and 375 mg/kg body weight whereas it was a markedly delayed on administration of 750 mg/kg body weight. No embryotoxicity or teratogenicity was observed (Bio/dynamics, 1991 e; Schroeder et al., 1991).

In view of the maternal toxicity encountered in all dose groups of the above teratogenicity study in the CD rat (Bio/dynamics, 1991 e; Schroeder et al., 1991), a Japanese research group conducted a further teratogenicity study in the Wistar rat, the scope of which additionally encompassed the maternally nontoxic dose range. However, this study also failed to provide any significant indications of teratogenicity due to tributyl phosphate. For dose-finding purposes, tributyl phosphate (purity not specified), formulated in olive oil, was administered to groups of 5 rats by stomach tube at dose levels of 0 (controls), 100, 200, 400 and 800 mg/kg body weight on days 7 to 17 of gestation. Clinical signs of toxicity, body weight and food consumption were recorded on a daily basis. The survivors underwent removal of the gravid uterus and necropsy on day 20 of gestation. The top dose group receiving 800 mg/kg body weight exhibited marked reduction of maternal

body weight gain and food consumption, piloerection, wetting of abdominal hair with urine, and salivation. All animals in that dose group died on day 12 or 13 of gestation. Depression of body weight gain and food consumption also occurred in the 200 and 400 mg/kg dose groups, transient piloerection and salivation in the 400 mg/kg dose group. The lowest dose level of 100 mg/kg body weight did not cause maternal toxicity. Uterus weights and the numbers of resorptions and dead and live foetuses were comparable with controls at all dose levels. The only exception was the 200 mg/kg dose, at which level the female foetuses exhibited increased body weights. One foetus from the 400 mg/kg dose group showed general oedema, deformity of the fore- and hindlimbs, agnathia and maxillary micrognathia, omphalocele and open eyelids. This finding lacked statistical significance and was not reproducible in the main study. The same experimental conditions were used in the main study in which groups of 20 pregnant females were treated with tributyl phosphate at dose levels of 0 (controls), 62.5, 125, 250 and 500 mg/kg body weight/day. Body weight and food consumption were depressed in the dams of the two high dose groups. The graphical presentation of the data suggests that body weight in the 125 mg/kg dose group was depressed in the absence of reduced food consumption. However, according to the data as presented in the tables, terminal body weight and corrected body weight gain (body weight gain during gestation corrected for uterus weight) was comparable with the control data. The dams in the top dose group exhibited increased absolute liver weight and reduced absolute spleen weight. Clinical signs of toxicity (salivation, piloerection and urine-soiled fur), with the exception transient salivation in one 250 mg/kg female, were seen only in the highest dose group. Numbers of corpora lutea, implantations, resorptions, dead and live foetuses, sex ratio and body weight of the live foetuses and examination of the foetuses for external, visceral and skeletal malformations gave no indications of embryotoxicity or teratogenicity for tributyl phosphate. Foetotoxic effects in the form of an increased incidence of rudimentary lumbar ribs were only observed at the markedly maternally toxic top dose level of 500 mg/kg body weight/day. The investigators considered 62.5 and 250 mg/kg body weight to be the respective *no observed adverse effect levels* for maternal toxicity and foetotoxicity (Noda et al., 1994).

Other studies

As discussed in Section 7.2, 14-day treatment with tributyl phosphate at 0.42 ml/kg body weight was observed to cause degeneration of the seminiferous tubules in 1 out of 4 male rats (Laham et al., 1984 b). However, this finding was not confirmed in a subsequent 18-week study carried out by the same investigators (Laham et al., 1984 a, 1985).

A 5 mg dose of tributyl phosphate was injected into the yolk sac of 10 fertile Leghorn hens' eggs on day 4 of incubation. The 18 to 21-day-old embryos or hatched chicks were examined for malformations (beak and leg anomalies), weight, body length and leg length, poor feathering and hatching rate. Treatment was found to cause slight foetotoxicity in the form of reduced hatching rate (20% as compared with 95% in the controls treated with physiological saline solution) and reductions in body weight gain, body length and leg length. No malformations were noted (Roger et al., 1969). It is hardly possible to extrapolate these findings to mammalian systems, however, because such comparison would fail to take into account the absorption barriers and detoxification mechanisms which are present in mammals. Furthermore, this test system is excessively sensitive and does not permit the distinction between teratogenic and embryolethal effects (Skofitsch, 1988; Neubert et al., 1992; Neubert, 1993; Heinrich-Hirsch, 1992).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

The neurotoxic potential of tributyl phosphate was investigated in accordance with the TSCA guidelines (EPA, 1989) in an acute study that was carried out in rats. Groups of 12 Sprague-Dawley rats per dose and sex were treated by oral gavage with tributyl phosphate (> 99% pure) in corn oil on a single occasion at dose levels of 100, 325 and 1000 mg/kg body weight. Clinical signs of toxicity and neurotoxic changes were recorded 1, 6 and 24 hours after dosing and on observation days 7 and 14. In the top dose group, one female died, and there was body weight depression. The

animals displayed staining at the muzzle and/or nares and the abdominal region. The results of functional observational battery assessments included significant urine staining, salivation and reduced grip strength of the forelimbs, but not the hindlimbs. Motor activity was reduced. Administration of 325 mg/kg body weight also resulted in staining at the muzzle and/or nares and the abdominal region. The functional observational battery evaluations revealed no differences compared with the control, other than significant urine staining. The 100 mg/kg body weight dose was tolerated without significant toxicological changes. The functional observational battery evaluations were negative. Seven days after administration, none of the animals showed any signs of toxicity. There were no gross pathological findings at necropsy. The investigators evaluated their observations as indicative of acute unspecific toxicity rather than a neurotoxic potential of tributyl phosphate (Bio-Research, 1990 a; Healy et al., 1992, 1995).

In order to identify the time point at which tributyl phosphate reached its peak effect on motor activity, a preliminary study was conducted in groups of 4 male and 4 female Sprague-Dawley rats/dose which were dosed with 1000 or 2000 mg/kg body weight. Motor activity was assessed by observing the animals in the figure-eight maze for 23 hours after dosing. The high-dose animals died within 24 hours after administration or were sacrificed due to poor clinical condition. The clinical signs of toxicity following administration of 1000 mg/kg body weight were largely the same as those observed in the main study described above. One female in that dose group died 2 days after dosing. Motor activity was reduced 7 to 20 hours after dosing, with the peak effect being reached after 11 hours (Bio-Research, 1990 b; Healy et al., 1995).

A subchronic study was also carried out in the rat in order to investigate the neuromorphological changes and the behavioural effects induced by tributyl phosphate. The study, which was conducted in accordance with the TSCA guidelines (EPA, 1989), also failed to provide any indication of neurotoxic potential on the part of tributyl phosphate. Groups of 12 male and 12 female Sprague-Dawley rats/dose were treated by oral gavage for 13 weeks with tributyl phosphate (> 99% pure) at daily dose levels of 32.5, 100 and 325 mg/kg body weight. The control group was treated with the vehicle, corn oil. Dose-dependent effects occurred at and above the intermediate dose level of 100 mg/kg body weight and included increased mortality, salivation, staining of the fur at the muzzle and urogenital re-

gions, and hair loss. The high dose group additionally displayed reduced body weights and food intake. There were no gross pathological findings at necropsy. The 32.5 mg/kg dose was tolerated without any harmful effects. All dose groups had normal results with regard to motor activity, functional observational battery evaluations, hindlimb grip strength and hindlimb splay and comprehensive neuropathological assessment of the central and peripheral nervous system (Bio-Research, 1991; Healy et al., 1992, 1995).

Groups of 10 male and 10 female Sprague-Dawley rats received, by oral gavage, a dose of tributyl phosphate (98.4% pure, 1.3% tributoxyethyl phosphate) of 0.28 or 0.42 ml (274 or 407 mg)/kg body weight every day for 14 consecutive days. The controls were given 0.42 ml water/kg body weight by oral gavage. The animals were assessed for clinical signs of toxicity on a daily basis, and body weights were recorded on a weekly basis. Electrophysiological recordings were carried out in the caudal nerve in 4 animals/dose 24 hours after the last dose in order to determine nerve conduction velocity and absolute and relative refractory periods. Two weeks after the last treatment, both sciatic nerves were removed from the controls and high-dose animals (0.42 ml) after perfusion, fixed and embedded appropriately and cut into transverse and longitudinal sections for examination by light microscopy and electron microscopy. The 14-day treatment was tolerated without clinical signs of toxicity. After 7 days, all animals exhibited body weights lower than controls while after 14 days this effect was only seen in the females of the low dose group. The electrophysiological measurements revealed a significant reduction in nerve conduction velocity in high-dose males. The absolute refractory period was significantly increased in all treated females and in low-dose males whereas the relative refractory period was increased in both sexes at the low dose level. When examined by light microscopy, the sciatic nerve appeared unchanged, whilst examination by electron microscopy revealed retraction of Schwann cell processes in unmyelinated nerve fibres. In the discussion of their work, the authors proposed that these findings represented an early response to a chemical insult. As the overall outcome of the study, it was concluded that, in the absence of overt neurological signs and considering the equivocal electron microscopy findings, tributyl phosphate did not induce axonal degeneration (Laham et al., 1983).

Kalinina (1971) reported that oral and intraperitoneal administration of lethal doses to rats and rabbits led to reductions in cholinesterase activity

levels in serum, erythrocytes and liver by up to 35% (no further details; Kalinina, 1971).

Male Swiss albino mice (weighing 14 to 17 g) were injected with a single intraperitoneal tributyl phosphate dose of 850 to 1000 mg/kg body weight (in corn oil solution). Treatment induced flaccid paralysis and respiratory failure in the animals. Mean knockdown time (number of minutes after treatment until the animal could no longer right itself when placed on its back) was 11.9 (± 0.8) minutes. Experiments with various different nicotinic acid and pyridine derivatives conducted to assess their protective effect led the to the conclusion that tributyl phosphate exerts its action by blocking impulse transmission at the neuromuscular junctions (Chambers and Casida, 1967).

Single oral, intraperitoneal or intramuscular administration of 0.1 or 0.2 ml technical or reagent-grade tributyl phosphate to Sprague-Dawley rats (one rat/dose and mode of administration) resulted in pallor, laboured breathing and hypersalivation, irrespective of the grade of test substance administered. Intramuscular and intraperitoneal injection caused paralysis, with intraperitoneal injection additionally resulting in coma and, after 0.2 ml, death. Occlusive dermal application of 20 drops of tributyl phosphate to the depilated abdominal skin for 5 days was tolerated without adverse effects other than local irritation at the application site. The investigators evaluated tributyl phosphate as a very weak inhibitor of cholinesterase (Sabine and Hayes, 1952).

Older acute studies describe tributyl phosphate as being a cholinesterase inhibitor of low activity (no further details; Eastman Kodak, 1968).

Intraperitoneal injection of tributyl phosphate at 0.062 to 1.0 mmol (approx. 17 to 266 mg)/kg body weight into female Wistar rats was not found to cause dose-dependent inhibition of serum cholinesterase activity. Serum β-glucuronidase activity was increased in a dose-dependent manner (Suzuki et al., 1977).

The majority of findings from neurotoxicity studies in hens gave no indication of a neurotoxic potential on the part of tributyl phosphate.

When hens were given a single oral dose of tributyl phosphate at 1000 mg/kg body weight, there were no indications at 24 hours after dosing that

the activity of the brain enzyme referred to as “neurotoxic esterase” was inhibited nor were any clinical signs of toxicity observed which would have been indicative of cholinesterase inhibition (no further details; Cascieri, 1982; FMC, 1977).

Likewise, when hens (white Leghorn) were dosed with tributyl phosphate (purity not specified) by oral gavage for 2 days at 1840 mg/kg body weight (LD_{50} 1800 mg/kg body weight), there were no indications of neurotoxicity. The animals were placed under observation for a 42-day period at the end of which the brain, sciatic nerve and spinal cord were removed, appropriately stained and subjected to histopathological evaluation. None of the 9 hens studies exhibited clinical signs of neurotoxicity or histopathological changes in the removed neural tissues (Johannsen et al., 1977).

In contrast, a review article described tributyl phosphate as neurotoxic in the hen when administered orally at 100 mg/kg body weight (no further details; Abou-Donia, 1981). However, later work by the same research group, in which hens were dosed twice with tributyl phosphate at the oral LD_{50} (1500 mg/kg body weight, 98.37% pure, dosing interval 21 days), on the other hand failed to provide any indications that the chemical produced neurotoxic effects in that species. Out of a total of 20 hens, 4 died after the first dose and another 2 after the second. No clinical signs of neurotoxicity were observed nor were any treatment-related histopathological lesions found in the peripheral nerves, the spinal cord or the medulla oblongata at the end of the observation period, 21 days after the last dose. Upon single administration of 1500 mg/kg body weight, the activity of acetylcholinesterase and that of an enzyme referred to as “neurotoxic esterase” were not inhibited in the brain. Plasma cholinesterase activity was markedly increased (Carrington et al., 1989, 1996).

Similarly, when 1500 mg of tributyl phosphate was applied to the skin twice at an interval of 21 days, followed by an observation period of 21 days, the 10 exposed hens exhibited no signs of neurotoxicity due to tributyl phosphate (no further details; Monsanto, 1986).

7.11 Other effects

Tributyl phosphate was studied for cytotoxicity to HeLa cells in the MIT-24 test system (metabolic inhibition test with microscopic evaluation at 24

hours after incubation). The chemical was suspended in medium and added to HeLa cells (5 x 10^4 cells/ml) on microtitre plates, the wells were sealed with liquid paraffin, and the plates were incubated at 37 °C for 7 days. Twenty-four hours after the start of incubation the cells were examined under the microscope for viability. Seven days later, the phenol red method was used in order to determine the concentration causing 50% inhibition (IC_{50}). Tributyl phosphate was found to produce complete inhibition after 24 hours at a concentration level of 4 mg/ml. The IC_{50} for 7-day exposure was ascertained as 1.5 mg/ml. Taking into consideration that a high concentration was employed, the investigators interpreted their findings as indicative of a low cytotoxic potential on the part of tributyl phosphate (Ekwall et al., 1982).

Tributyl phosphate inhibited viruses (influenza, hepatitis and other viruses) in plasma samples without, however, altering the function of plasma proteins such as immunoglobulins and clotting factor VIII. On the basis of this property, it was proposed to utilise tributyl phosphate for sterilisation of plasma and plasma products (Claeys et al., 1988; Danihelkova and Zavadova, 1984; Edwards et al., 1987; Guillaume, 1991; Michalski et al., 1988; Piet et al., 1990; Tocci et al., 1993).

8 Experience in humans

Studies in human skin specimens, forearm stratum corneum from three subjects, demonstrated a high dermal penetration rate for tributyl phosphate (0.18 µg tributyl phosphate/cm²/minute; no further details; Marzulli et al., 1965).

Six human subjects had cotton-wool pads that were soaked with concentrated tributyl phosphate affixed to the arm by means of an occlusive bandage. All individuals immediately felt a strong burning sensation at the application site. Further tests were carried out with tributyl phosphate in lanolin ointment. One subject was exposed to a 75-percent formulation which was applied to the skin of the upper arm under occlusive cover. The treatment caused a strong burning sensation on the skin so that the cotton-wool pad with the ointment had to be removed after 3 hours. The application site and the area surrounding it were slightly red. The application site was normal again after 20 hours. When a 50-percent formulation was applied to

the skin of one subject for 24 hours it caused persistent burning and mild reddening that was reversible within 24 hours, while a 10-percent formulation caused no dermal irritation in 6 subjects tested under the same experimental conditions (Gewerbehygienisches I.G. Labor, 1936).

Without giving further details, it was reported that exposure to tributyl phosphate caused severe irritation to the human skin, eyes and respiratory tract (no further details; Eastman Kodak, 1968; Pestic. Toxic. Chem. News, 1986; Stauffer, 1984).

Formulations containing 1 or 0.1% tributyl phosphate caused no skin lesions in human subjects. Formulated in petrolatum, tributyl phosphate (purity not specified) was applied under occlusive cover to the skin of the upper arms or backs of 25 subjects for 3 days. The subjects were assessed after 72 and 96 hours (Monsanto, 1989).

A study in human subjects gave no relevant indications of a skin-sensitising potential for tributyl phosphate. Fifty-three human subjects were exposed, on alternate days, to a total of 15 applications of 0.2 ml of the hydraulic fluid Skydrol 500B-4, a formulation containing less than 25% tributyl phosphate. None of the individuals exhibited treatment-related local reactions when assessed 24 to 72 hours after the last application (no further details; Monsanto, 1980).

Workers who were exposed to tributyl phosphate at levels of 15 mg/m³ complained of headache and nausea (no further details; Mastromatteo, 1964).

Russian workers who were exposed to tributyl phosphate, and other chemicals, in connection with the production of scandium oxide were observed to have an increased incidence of respiratory disorders and damage to the skeletal muscles. The workers were employed in an only part-mechanised, partly open production plant and, in addition to tributyl phosphate, they were also exposed to large quantities of dust, polyacrylamide, hydrochloric acid vapours, sodium carbonate, kerosine and scandium oxide aerosol (no further details; Fejgin, 1985).

When 12 workers with exposure to tributyl phosphate were assessed by means of three different techniques, no significant difference was noted in the monocyte counts in comparison with unexposed plant employees and

the general population. A fourth method, by which monocytes were identified with the aid of a colour reaction for nonspecific esterases, yielded reduced counts when compared with workers who had not been exposed to tributyl phosphate. The investigators suspected that inhibition of monocyte nonspecific esterases could have been due to tributyl phosphate exposure but stated that the clinical significance of their finding was unknown (Mandel et al., 1989).

In vitro, dose-dependent inhibition of cholinesterase activity has been demonstrated in human but not in bovine serum (no further details; Oishi et al., 1980).

In-vitro studies in which tributyl phosphate was added to human plasma or human red blood cell haemolysate found a slight decrease in acetylcholinesterase activity. The investigators concluded that tributyl phosphate was not a selective inhibitor of plasma enzyme (no further details; Sabine and Hayes, 1952).

The activity of human blood monocyte carboxylesterase was not inhibited by tributyl phosphate (> 99% pure) in vitro (Saboori et al., 1991).

9 Classifications and threshold limit values

The threshold limit value in the USA is given as 0.2 ppm (equivalent to 2.2 mg/m³; ACGIH, 2000).

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") has, in the List of MAK and BAT Values 2000, assigned tributyl phosphate to category 4 of carcinogenic working substances, i.e. "substances with carcinogenic potential for which genotoxicity plays no or at most a minor part. No significant contribution to human cancer risk is expected provided the MAK value is observed". The MAK value has been established as 1 ml/m³ (ppm) (equivalent to 11 mg/m³), and the chemical has been designated with "H" because of the danger of cutaneous absorption. Furthermore, tributyl phosphate has been assigned to pregnancy risk group C, i.e. substances for which "there is no reason to fear a risk of damage to the embryo or foetus when MAK and BAT values are observed" (DFG, 2000; Greim, 2000).

Appendix	
Table 1	Comparative studies on the toxicokinetics and metabolism of tributyl phosphate in accordance with the TSCA guidelines (EPA, 1989; MRI, 1992 a, b, c)
Table 2	Acute toxicity of tributyl phosphate
Table 3	Skin irritancy of tributyl phosphate
Table 4	Mucous membrane effects of tributyl phosphate
Table 5	In-vitro genotoxicity of tributyl phosphate

Beginning of Table 1

Table 1. Comparative studies on the toxicokinetics and metabolism of tributyl phosphate in accordance with the TSCA guidelines (EPA, 1989; MRI, 1992 a, b, c)

a) single intravenous or dermal administration to Sprague-Dawley rats (4 rats/dose and sex)

	5 mg/kg body weight		10 mg/kg body weight		350 mg/kg body weight			
	single intravenous dose		single dermal application		single dermal application			
			occlusive 6-hour exposure		occlusive 6-hour exposure			
	males	females	males	females	males	females		
Peak plasma concentration (µg/g) C_{max}	3.67	3.45	10.58	7.52	230.7	235.4		
Time of peak plasma concentration (minutes) t_{max}	0	0	240	240	53	45		
Plasma clearance (g/minute x kg body weight) Cl_p	11.3	14	–	–	–	–		
Half-life of elimination from plasma (hours) $t_{1/2,\ plasma}$	1.3	1.3	–	–	–	–		
Half-life for urinary elimination (hours) $t_{1/2,\ urine}$	29.9	29.4	16.6	19.7	19.2	20.0		
Absorption	100%	100%	42%	40%	57%	55%		
	(% of radioactivity administered, relative to intravenous administration)							
Excreted in urine within 6 hours	56.27%	65.45%	8.35%	12.77%	6.86%	6.20%		
24 hours	67.32%	76.91%	19.38%	23.03%	21.81%	26.13%		
48 hours	68.22%	78.32%	25.28%	26.48%	32.15%	36.94%		
Total excretion at 168 hours - in urine	69.07%	79.56%	28.92%	31.54%	39.56%	44.08%		
- in faeces	16.59%	7.24%	4.04%	3.16%	7.30%	5.79%		
Total excretion in expired air at 96 hours, as - volatile metabolites	0.07%	0.18%	0.00%	0.00%	0.23%	0.09%		
- CO_2	6.13%	4.30%	1.63%	1.56%	1.91%	1.64%		
- volatile metabolites + CO_2	6.20%	4.48%	1.63%	1.56%	2.13%	1.73%		
Residual activity at study termination - in the body	1.05%	0.75%	0.96%	0.94%	1.22%	1.05%		
- bound at the site of application	–	–	0.43%	0.48%	0.11%	0.06%		
- removable dose wash	–	–	30.36%	43.01%	27.44%	23.84%		
Total recovery (% of radioactivity administered)	92.92%	92.02%	65.90%	80.21%	77.64%	76.49%		

Table 1. Comparative studies on the toxicokinetics and metabolism of tributyl phosphate in accordance with the TSCA guidelines (EPA, 1989; MRI, 1992 a, b, c)

b) single and repeated oral administration to Sprague-Dawley rats (4 rats/dose and sex)

	10 mg/kg body weight, single oral dose		350 mg/kg body weight, single oral dose		10 mg/kg body weight, multiple oral doses (8 days)[1)]		350 mg/kg body weight, multiple oral doses (8 days)[1)]	
	males	females	males	females	males	females	males	females
Peak plasma concentration (µg/g) C_{max}	1.08	1.42	48.7	41.4	7.94	7.44	185.8	197.1
Time of peak plasma concentration (minutes) t_{max}	90	105	180	330	140	90	400	300
Half-life for urinary elimination (hours) $t_{1/2,\ urine}$	24.4	25.7	24.8	25.8	24.8	25.9	25.9	24.4
Absorption	101%	100%	98%	103%	109%	103%	94%	94%
	(% of radioactivity administered, relative to intravenous administration)							
Excreted in urine within 6 hours	37.63%	41.45%	7.55%	16.00%	47.99%	59.40%	25.27%	21.70%
24 hours	67.21%	75.49%	43.71%	62.22%	76.58%	82.38%	63.70%	77.22%
48 hours	69.00%	78.00%	62.42%	78.51%	78.26%	84.48%	67.92%	81.98%
Total excretion at 168 hours - in urine	70.10%	79.65%	67.93%	82.23%	79.39%	85.86%	69.76%	83.84%
- in faeces	18.61%	9.17%	13.52%	7.06%	12.34%	8.53%	12.45%	5.90%
Total excretion in exhaled air at 96 hours, as - volatile metabolites	0.05%	0.23%	2.56%	1.23%	0.13%	0.07%	0.19%	0.16%
- CO_2	5.45%	4.07%	5.75%	3.25%	6.04%	4.72%	4.69%	3.42%
- volatile metabolites + CO_2	5.50%	4.30%	8.30%	4.48%	6.17%	4.78%	4.88%	3.58%
Residual activity at study termination - in the body	1.36%	1.22%	1.35%	1.06%	1.07%	0.86%	0.83%	0.58%
Total recovery (% of radioactivity administered)	95.56%	94.34%	91.11%	94.82%	98.96%	100.04%	87.91%	93.90%

Table 1. Comparative studies on the toxicokinetics and metabolism of tributyl phosphate in accordance with the TSCA guidelines (EPA, 1989; MRI, 1992 a, b, c)

c) single intravenous or dermal administration to Yucatan Minipigs (2 pigs/dose and sex)

	5 mg/kg body weight		10 mg/kg body weight		350 mg/kg body weight			
	single intravenous dose		single dermal application		single dermal application			
			occlusive 6-hour exposure		occlusive 6-hour exposure			
	males	females	males	females	males	females		
Absorption	100%	100%	3.6%	5.39%	0.8%	0.6%		
(% of radioactivity administered, relative to intravenous administration)	(combined urinary and faecal excretion)							
Total excretion at 168 hours - in urine	81%	82%	2.5%	4%	0.2%	0.2%		
- in faeces	3%	2%	0.5%	0.9%	0.4%	0.3%		
Residual dose-site activity (includes dose site, dose wash and dose wrappings)	–	–	57%	64%	87%	92%		
Residual activity in the kidney and urinary bladder	0.01%	0.00%	0.03%	0.025%	0.00%	0.00%		
Total recovery (% of radioactivity administered)	85%	84%	60%	69%	88%	92%		

[1)] Daily treatment with unlabelled tributyl phosphate for 7 days, followed by a corresponding dose consisting of unlabelled tributyl phosphate and ^{14}C-tributyl phosphate and on day 8.

End of Table 1

Beginning of Table 2

Table 2. Acute toxicity of tributyl phosphate

Species, strain, sex[a)]	Route	Dose (mg/kg body weight or mg/m³)	Purity	Effects	Observa-tion period	Reference
Rat, Wistar, male, female	oral	20000	no data	mortality: 10/10 within one day; autopsy: visceral haemorrhage	14 days	Food and Drug Research, 1975 a
Test substance was the commercial product Kronitex TBP.						
Rat	oral	1000–4000	no data	1000 mg: mortality was 0/2; 2000 mg: mortality was 2/2; 4000 mg: no mortality data given; cholinergic signs; autopsy: dehydration and congestion of organs	no data	Dow Chemical, 1956
Rat	oral	3350	no data	LD_{50}	no data	Izmerov et al., 1982; Kalinina, 1971
Rat	oral	1600–3200	no data	LD_{50}, 3200 mg was lethal; blood around the nostrils, mouth and eyes, laboured breathing and convulsions	no data	Eastman Kodak, 1968
Rat	oral	3200	no data	LD_{50}, 10000 mg was lethal to 10/10 animals, 1000 mg was survived by 10/10 animals; no clinical signs; autopsy of deceased animals: pale kidney, spleen and liver; terminal autopsy: no abnormal findings	14 days	Mellon Institute, 1943
Rat	oral	3000	no data	LD_{50}	no data	Eastman Kodak, 1968
Rat, Wistar, male	oral	3000	no data	LD_{50}	14 days	Smyth and Carpenter, 1944
Rat	oral	2250	no data	approximate lethal dose; laboured respiration, weakness; autopsy of deceased animals was without abnormal findings; terminal autopsy of survivors revealed evidence of injury to the liver	no data	Haskell, 1953
Rat, Wistar, male, female	oral	1552 (1.6 ml)	no data	LD_{50}; poor general condition, narcosis, bloody edges of the eyelids, lying on the abdomen or side, rough coats; autopsy of deceased animals: reddening of the gastrointestinal tract and lungs; terminal autopsy was without abnormal findings	14 days	Bayer, 1986 a
Rat, Wistar, female	oral	1530	no data	LD_{50}; diarrhoea; elevated blood urea nitrogen levels; degeneration of the renal tubules	no data	Mitomo et al., 1980
Rat, Sprague-Dawley, male, female	oral	1400	no data	LD_{50}	14 days	Johannsen et al., 1977
Rat, Wistar, male	oral	1390	no data	LD_{50}; diarrhoea; elevated blood urea nitrogen levels; degeneration of the renal tubules	no data	Mitomo et al., 1980

Table 2. Acute toxicity of tributyl phosphate

Species, strain, sex[a)]	Route	Dose (mg/kg body weight or mg/m³)	Purity	Effects	Observation period	Reference
Rat, Wistar, male, female	oral	1164 (1.2 ml)	no data	LD_{50}; diarrhoea, chromorhinorrhoea; autopsy: visceral haemorrhage	14 days	Food and Drug Research, 1976 a
Test substance was the commercial product Kronitex TBP.						
Mouse, DDY, male	oral	1240	no data	LD_{50}; diarrhoea; elevated blood urea nitrogen levels; degeneration of the renal tubules	no data	Mitomo et al., 1980
Mouse	oral	1189	no data	LD_{50}	no data	Izmerov et al., 1982; Kalinina, 1971
Mouse, DDY, female	oral	900	no data	LD_{50}; diarrhoea; elevated blood urea nitrogen levels; degeneration of the renal tubules	no data	Mitomo et al., 1980
Mouse	oral	400–800	no data	LD_{50}, 800 mg was lethal; blood around the nostrils, mouth and eyes, laboured breathing and convulsions	no data	Eastman Kodak, 1968
Hen, white Leghorn	oral	1800	no data	LD_{50}	14 days	Johannsen et al., 1977
Hen	oral	1500	98.37%	LD_{50}; salivation, diarrhoea, impaired respiration	no data	Carrington et al., 1989, 1996
Rat	dermal	< 670	no data	LD_{50}	no data	Izmerov et al., 1982
(This value is considerably lower than the oral LD_{50} values reported for the rat and far lower than the dermal LD_{50} values for the rabbit and the guinea pig. Moreover, there are no indications from other dermal studies that tributyl phosphate is severely toxic when administered via this route. Hence, it is considered likely that the authors of this review article, who presented the data in tabular format, erroneously gave an incorrect LD_{50} value for tributyl phosphate.)						
Rabbit	dermal (intact and abraded skin)	10000	no data	mortality: 0/10	14 days	Food and Drug Research, 1975 b
Test substance was the commercial product Kronitex TBP.						
Rabbit, New Zealand, male, female	dermal (24 hours, occlusive)	> 3100	no data	LD_{50}	14 days	Johannsen et al., 1977
Guinea pig	dermal (24 hours, occlusive)	9700–19400 (10–20 ml)	no data	LD_{50}, 19400 mg was lethal within 3 days; severe irritation	no data	Eastman Kodak, 1968
Rat, Wistar, male, female	inhalation (one-hour whole-body exposure, aerosol)	200000 (200 mg/l)	no data	mortality: 10/10 within one day; autopsy: visceral haemorrhage	14 days	Food and Drug Research, 1975 c
Test substance was the commercial product Kronitex TBP.						

Table 2. Acute toxicity of tributyl phosphate						
Species, strain, sex[a)]	Route	Dose (mg/kg body weight or mg/m³)	Purity	Effects	Observa-tion period	Reference
Rat	inhalation (6 hours)	41990 (3800 ppm; calculated)	no data	mortality: 1/3; irritating	no data	Eastman Kodak, 1968
Rat, Wistar, male, female	inhalation (one-hour whole-body exposure, aerosol)	28000	no data	approximate LC_{50}, mortality at 42000 mg/m³ was 10/10 within 3 days, mortality at 20000 mg/m³ was 0/10; loss of co-ordination; autopsy of the deceased animals and terminal autopsy of survivors were without noteworthy findings	no data	Food and Drug Research, 1976 b
Test substance was the commercial product Kronitex TBP.						
Rat	inhalation (4 hours)	< 22000	no data	LC_{50}	no data	Izmerov et al., 1982
Rat, Wistar, male, female (study in accordance with OECD guideline No. 403)	inhalation (4 hours, breathable aerosol, maximum technically feasible concentration, head-nose exposure)	4242	99.62%	approximate LC_{50}; mortality: 2/5 males, 0/5 females; concentration-dependently reduced motility from 801 mg; ataxia, weakness of the hind paw, prostration, piloerection, unkempt fur, serous nasal discharge, red lacrimation, sneezing noises, bradypnoea, difficulty in breathing, breath sounds, red-coloured urine, distended abdomen, bloody snout, loss of reflexes, body weight loss; 511 mg was without clinical findings; necropsy of animals that died intermittently: nose with red encrustations, distension and liver-like appearance of the lungs, pale spleen and kidneys, lobular markings in the liver, reddening of the glandular stomach, gastrointestinal tract with yellowish mucous-like content; necropsy of the survivors from the highest dose group (4242 mg): distension of the lungs, gelatinous or with gelatinous foci, the spleen was pale, dark, smaller or enlarged, lobular markings in the liver, colon distension; necropsy of survivors from groups receiving ≤ 4242 mg was largely without remarkable findings	14 or, at the highest dose, 28 days	Bayer, 1990
Rat	inhalation (6 hours)	3868 (350 ppm, calcu-lated)	no data	not lethal, irritating	no data	Eastman Kodak, 1968

Table 2. Acute toxicity of tributyl phosphate						
Species, strain, sex[a)]	Route	Dose (mg/kg body weight or mg/m³)	Purity	Effects	Observation period	Reference
Rat	inhalation (6 hours)	1359 (123 ppm)	no data	mortality: 0/3; severely irritating	no data	Patty, 1963
Rat	inhalation (6 hours, vapour)	206 (18.6 ppm)	no data	no findings	no data	Eastman Kodak, 1968
Rat	inhalation hazard test (a tributyl phosphate aerosol was generated by bubbling air through the fluid at 170 °C and subsequently cooling the mist to room temperature)		no data	exposure for one hour was lethal to 6/6 animals, exposure for 30 minutes was lethal to 4/6 animals, exposure for 15 minutes was not lethal; irritation of the respiratory tract, injury to the liver	no data	Mellon Institute, 1943
Rat	inhalation	15000	no data	no changes in haematology parameters (erythrocytes, haemoglobin, reticulocytes, leukocytes, myelocytes)	no data	Kalmykova et al., 1990
Rat	inhalation	1/80 of the $LD_{50/30}$	no data	significant decrease, or increase, in cytotoxic activity of natural killer cells in the lung without a time-dependent trend	240	Kirillova et al., 1990
Mouse	inhalation (2 hours)	< 22000	no data	LC_{50}	no data	Izmerov et al., 1982
Cat	inhalation, 5 or 2.5 or 6.5 hours	24510 or 22400 or 2530	purified	lethal in 2 animals per group after 5.5 hours, 2 days and 8 days; irritation, dyspnoea, ataxia; autopsy: pulmonary emphysema, bronchopneumonic foci, inflammation of the upper airways	8 days	Eller, 1937
Rat	intraperitoneal	800–1600	no data	LD_{50}, 1600 mg was lethal; blood around the nostrils, mouth and eyes, laboured breathing and convulsions	no data	Eastman Kodak, 1968
Rat, Sprague-Dawley, male, female	intraperitoneal	50–5000	no data	1000 and 5000 mg: lethal to 2/2 animals within 4 hours; 500 mg: not lethal; coma, salivation; terminal autopsy: abdominal adhesions; 100 and 50 mg: no findings	14 days	Dave and Lidman, 1978
Rat	intraperitoneal	251	no data	LD_{50}	no data	Kalinina, 1971
Rat	intraperitoneal	215	no data	LD_{50}	no data	Izmerov et al., 1982
Mouse	intraperitoneal	100–200	no data	LD_{50}, 200 mg was lethal; blood around the nostrils, mouth and eyes, laboured breathing and convulsions	no data	Eastman Kodak, 1968
Mouse	intraperitoneal	158	no data	LD_{50}	no data	Izmerov et al., 1982; Kalinina, 1971

Table 2. Acute toxicity of tributyl phosphate

Species, strain, sex[a)]	Route	Dose (mg/kg body weight or mg/m³)	Purity	Effects	Observation period	Reference
Rabbit (1 animal/dose)	intraperitoneal	100–200	no data	100 mg: no findings; 200 mg: lethal after 11 days; autopsy: oedema and bronchopneumonia, reddening and mucus in the trachea, indistinct marks on the spleen, hyperaemia of the internal organs	11 days	Gewerbehygienisches I.G. Labor, 1936
Mouse	subcutaneous	2000–12000	purified	2000 mg: not lethal; dyspnoea, cyanosis; ≥ 3000 mg: lethal within 7 to 24 hours to the 1 or 2 animals tested	no data	Eller, 1937
Rat, female	intravenous	80–100	no data	80 mg: not lethal; incoordination, mild anaesthesia, weakness; 100 mg: lethal; anaesthesia, dyspnoea, respiratory failure	no data	Vandekar, 1957
Mouse	probably by intravenous injection	602	no data	LD_{50}	no data	Octapharm, year not given
Rat	no data	1400	no data	LD_{50}	no data	Zyabbarova and Teplyakova, 1968
Mouse	no data	1200	no data	LD_{50}	no data	Zyabbarova and Teplyakova, 1968
Guinea pig	no data	ca. 970 (1 ml) once daily for 4 days	no data	lethal to 2/3 animals	no data	Patty, 1963

[a)] where specified

End of Table 2

Beginning of Table 3

Table 3. Skin irritancy of tributyl phosphate

Species	Guideline and/or dose, mode, duration*	Findings	Reversibility	Evaluation (by the investigators)	Reference
Rabbit	OECD guideline No. 404 (test substance 99.8% pure)	mild to moderate erythema, slight oedema formation, necrotic skin lesions in one animal	completely reversible in 4/6 animals, one animal still had mild, hardly noticeable erythema after 14 days, one animal had necrotic skin lesions	slightly irritating[a)]	Bayer, 1986 b
[a)] The investigators attributed the necrotic skin lesions seen in one rabbit to the animal's idiosyncratic higher sensitivity and excluded it from evaluation.					
Rabbit	0.5 ml, intact depilated dorsal skin, 4 hours, occlusive exposure (guideline 49 CFR 173.1200)	mild erythema, slight oedema formation in 1/6 animals	not reversible within 48 hours	not corrosive	Consumer Product Testing, 1979
Rabbit	0.5 ml, intact and abraded depilated dorsal skin, 24 hours, occlusive exposure (guideline 16 CFR 1500.41)	severe erythema and oedema formation	not reversible within 72 hours	severely irritating	Food and Drug Research, 1975 d
Test substance was the commercial product Kronitex TBP, purity not specified.					
Rabbit	0.5 ml, intact and abraded depilated dorsal skin, 24 hours, occlusive exposure	moderate erythema and severe oedema formation	not reversible within 7 days, necrotic skin lesions and/or eschar formation in 6/6 animals	moderately irritating	Stillmeadow, 1981
Rabbit	0.1 g undiluted substance, intact depilated dorsal skin, 24 hours, occlusive exposure	slight erythema formation	reversible in 4/6 animals within 2 days and in 1/6 animals within 5 days, 1/6 animals showed slight erythema even at the end of the observation period	mildly to moderately irritating	Haskell, 1971
Rabbit	undiluted substance, ear, 24 hours, occlusive exposure or painted on once	swelling and severe reddening, oozing spots, encrustation	reversible within 21 days	irritant	Gewerbehygienisches I.G. Labor, 1936
Rabbit	50% test substance in lanolin, ear, 24 hours, occlusive exposure or painted on once	swelling and reddening, encrustation	reversible within 13 days	irritant	Gewerbehygienisches I.G. Labor, 1936
Rabbit	10% test substance in lanolin, ear, 24 hours, semi-occlusive exposure or painted on once	no findings	–	not irritating	Gewerbehygienisches I.G. Labor, 1936

Table 3. Skin irritancy of tributyl phosphate					
Species	Guideline and/or dose, mode, duration*	Findings	Reversibility	Evaluation (by the investigators)	Reference
Rabbit	undiluted substance or 10% aqueous emulsion, 3 to 10 applications to the intact skin of the ear or to the intact or abraded abdominal skin	hyperaemia, exfoliation and/or necrosis	reversible	irritation upon repeated or prolonged skin contact	Dow Chemical, 1956
Rabbit	application of undiluted substance to the abdominal skin	mild erythema in 2/5 animals	no data	mildly irritating	Mellon Institute, 1943
Rat	20 drops, 5 days, occlusive application to the depilated abdominal skin	superficial necrosis of the skin	reversible	no data	Sabine and Hayes, 1952
Guinea pig	undiluted substance, occlusive exposure, 24 hours	severe irritant effects	no data	severely irritating	Eastman Kodak, 1968; Haskell, 1953
Guinea pig	10% test substance in dimethyl phthalate, intact skin	no data	no data	mildly irritating	Haskell, 1953
Guinea pig	10% test substance in dimethyl phthalate, broken skin	no data	no data	moderately irritating	Haskell, 1953
Guinea pig	2% test substance in dimethyl phthalate, intact skin	no data	no data	not irritating	Haskell, 1953
Rabbit, rat and guinea pig	single and multiple treatment	single application caused oedema, discoloration, local increase in temperature, scaly scabs; multiple application caused purulent, necrotic skin, poor healing	no data	no data	Kalinina, 1971, 1973
* where specified					

End of Table 3

Table 4. Mucous membrane effects of tributyl phosphate					
Species	Guideline and/or dose, mode, duration*	Findings	Reversibility	Evaluation (by the investigators)	Reference
Rabbit	OECD guideline No. 405 (test substance 99.8% pure)	slight clouding of the cornea, mild to moderate conjunctivitis, mild iritis	iritis within 24 hours, clouding of the cornea within 7 days, conjunctivitis completely reversible within 14 days	mildly irritating	Bayer, 1986 b
Rabbit	0.1 ml, unwashed (guideline 16 CFR 1500.41)	mild to moderate conjunctivitis, iridial effects in 1/6 animals	reversible within 7 days	mildly irritating	Food and Drug Research, 1975 e
Test substance was the commercial product Kronitex TBP, purity not specified.					
Rabbit	0.1 ml, eyes washed 4 seconds following instillation	mild conjunctivitis in 1/3 animals	reversible within 7 days	not irritating	Food and Drug Research, 1975 e
Test substance was the commercial product Kronitex TBP, purity not specified.					
Rabbit	0.1–0.5 ml	eye injury of grade 2 on a 10-point scale	no data	no data	Carpenter and Smyth, 1946; Mellon Institute, 1943
Rabbit	undiluted substance, eyes washed and unwashed	slight conjunctival irritation and questionable to slight iritis in 1/2 animals	reversible within 24 hours	questionable to mild irritating	Dow Chemical, 1956
Unspecified	no information	irritant	reversible	no data	Eastman Kodak, 1968
* where specified					

Beginning of Table 5

Table 5. In-vitro genotoxicity of tributyl phosphate

Test system	Concentration range tested (µg/ml)	Metabolic activation	Result		Reference
			without metabolic activation	with metabolic activation	
1 Gene mutation					
1.1 Salmonella/microsome assay systems					
Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535 (preincubation test)	1–3333 µg/plate, toxicity tested	S9 mix from Aroclor-induced rat and hamster liver	negative	negative	Zeiger et al., 1992
The tributyl phosphate tested was > 99% pure.					
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 (plate incorporation test)	20–12500 µg/plate, bacteriotoxic from 240 µg/plate	S9 mix from Aroclor-induced rat liver	negative	negative	Bayer, 1985
The tributyl phosphate tested was > 99.8% pure.					
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538 (plate incorporation test)	0.1–100 µl/plate (ca. 97–97000 µg/plate), no data on bacteriotoxicity	S9 mix from Aroclor-induced rat liver	negative	negative	Microbiological Associates, 1977
Test substance was the commercial product Kronitex, 100% pure.					
Salmonella typhimurium TA 1535, TA 1538	10–1000 µg/plate	no data	positive	weakly positive	Gafieva and Chudin, 1986
Salmonella typhimurium LT-2, his C117, his G46, TA 1530, his D3052, TA 1531, TA 1532, TA 1534 (plate incorporation test)	5–10 µl/plate (ca. 4850–9700 µg/plate)	not tested	negative	not tested	Hanna and Dyer, 1975
Purity of the test substance unspecified.					
Salmonella typhimurium TA 102 (plate incorporation test)	9.8 ng–98 mg/plate, bacteriotoxic from 0.98 mg/plate	S9 mix from Aroclor-induced rat liver	negative	negative	Pancorbo et al., 1987
The tributyl phosphate tested was 100% pure.					
Salmonella typhimurium TA 98 (no further details)	no information	S9 mix from PCB-induced liver	no data	negative	Ishidate, 1991
Salmonella typhimurium TA 97, TA 98, TA 100, TA 102	0–5000 µg/plate, bacteriotoxic from 250 µg/plate	S9 mix	negative	negative	Watabe et al., 1993
The publication, written mostly in Japanese, provides no further details.					

Table 5. In-vitro genotoxicity of tributyl phosphate

Test system	Concentration range tested (µg/ml)	Metabolic activation	Result		Reference
			without metabolic activation	with metabolic activation	
Salmonella typhimurium TA 102, TA 2638 (plate incorporation test)	31.3–5000 µg/plate, bacteriotoxic from 500 µg/plate	not tested	negative	not tested	Watanabe et al., 1996
1.2 Further gene mutation tests in bacteria					
Escherichia coli WP2, WP2uvrA, CM561, CM571, CM611, WP67, WP12 (spot test)	no data	not tested	negative	not tested	Hanna and Dyer, 1975
Purity of the test substance unspecified.					
Escherichia coli WP2/pKM101, WP2uvrA/pKM101	125–5000 µg/plate, bacteriotoxic from 2000 µg/plate	not tested	negative	not tested	Watanabe et al., 1996
1.3 Gene mutation tests in mammalian cells					
CHO/HPRT test, 6-thioguanine resistance, Chinese hamster ovary cells, (CHO-K1-BH4)	0.05–0.11 (without S9 mix), 0.06–0.15 (with S9 mix), toxicity tested	S9 mix from Aroclor-induced rat liver	negative	negative	Microbiological Associates, 1990 a; Batt et al., 1992
Study in accordance with the TSCA guidelines (EPA, 1989). The tributyl phosphate used was 99.7% pure.					
2 Tests for clastogenic potential in mammalian cells					
Chromosome aberration test, Chinese hamster ovary cells (CHO-K1)	0.013–0.15 (without S9 mix), 0.01–0.15 (with S9 mix), toxicity tested	S9 mix from Aroclor-induced rat liver	negative	negative	Microbiological Associates, 1990 b; Batt et al., 1992
Study in accordance with the TSCA guidelines (EPA, 1989). The tributyl phosphate used was 99.7% pure.					
Micronucleus test, Chinese hamster ovary (CHO) cells, 1000 cells/concentration were scored with at least two independent repeats	ca. 27–80 (0.1–0.3 mM), cytotoxicity 10 to 50%	not tested	negative	not tested	Brooks et al., 1996
The purity of the tributyl phosphate used was not specified.					
3 Other tests					
Induction of micronuclei*	mouse embryos at the two-cell stage	5.15–40 µM for 18 hours, examination of the embryos at the eight-cell stage	–	negative*	Müller et al., 1987
* The publication reports no data on the frequency of micronuclei. It is only stated that no micronuclei were induced in the cells of the embryos. The study was not conducted as a micronucleus test in accordance with current relevant guidelines, and hence the result is not evaluable.					

End of Table 5

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