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Tricresyl phosphate, sum of all ortho isomers

MAK Value Documentation – Translation of the German version from 2020

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Keywords

tricresyl phosphate; o-isomers; neurotoxicity; OPIDN; maximum workplace concentration; MAK value; toxicity; peak limitation; carcinogenicity; reproductive toxicity

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated tricresyl phosphate, sum of all ortho isomers [78-30-8], considering all toxicological end points. The ortho isomers of tricresyl phosphate can cause organophosphate-induced delayed neuropathy (OPIDN), while the para and meta isomers do not severely affect the central nervous system. In the past, thousands of people have suffered from delayed neuropathy in the legs caused by the consumption of food or beverages contaminated with ortho-tricresyl phosphate. In spite of the numerous reports of human poisoning, these data are insufficient to derive a dose-response relationship and to determine a maximum concentration at the workplace (MAK value). The neurotoxic effects of ortho-tricresyl phosphates were demonstrated also in hens and cats, while rodents showed only slight effects with a different mechanism of action. Dermal subchronic treatment with triortho-cresyl phosphate produced hindlimb weakness in cats with a no observed adverse effect level (NOAEL) of 0.5 mg/kg body weight and day, resulting in a MAK value of 0.01 ml/m^3. Investigations in hens show that the relative potency of monoortho-tricresyl phosphate, di-ortho-tricresyl phosphate and tri-ortho-tricresyl phosphate to induce OPIDN is 10 : 5 : 1. Therefore, a MAK value of 0.001 ml/m^3 has been established for the sum of all ortho-tricresyl phosphate isomers, as all three ortho isomers are assumed to be present in technical tricresyl phosphate. Tricresyl phosphate, sum of all ortho isomers, is classified in Pregnancy Risk Group D because sufficient data for developmental toxicity are not available. Carcinogenicity studies are not available. In rats, DNA adducts were detected in the liver, kidneys, lung and heart after oral application of tri-ortho-cresyl phosphate. Therefore, ortho-tricresyl phosphates are classified in Carcinogen Category 3 B for suspected carcinogens. The ortho-tricresyl phosphates are not regarded as mutagenic in germ cells. Skin contact is suspected to contribute to systemic toxicity and ortho-tricresyl phosphates are designated with “H”. Studies of the sensitization potential are not available.

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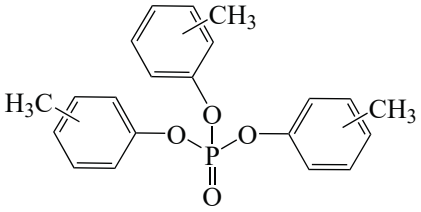
MAK value (2019)	**0.001 ml/m³ (ppm) ≙ 0.015 mg/m³ a)**
Peak limitation (2019)	**Category II, excursion factor 8**
Absorption through the skin (2019)	**H**
Sensitization	–
Carcinogenicity (2019)	**Category 3 B**
Prenatal toxicity (2019)	**Pregnancy Risk Group D**
Germ cell mutagenicity	–
BAT value	–
Synonyms	*o*-tricresyl phosphates *o*-tritolyl phosphates
Chemical name	tris(2-methylphenyl) phosphate for the tri-*o*-isomer
CAS number	78-30-8 for the tri-*o*-isomer
Structural formula	 o-o-o, o-o-m, o-o-p, o-m-m, o-m-p, o-p-p
Molecular formula	$C_{21}H_{21}O_4P$
Molar mass	368.37 g/mol
Melting point	tri-*o*-isomer: 11 °C (IFA 2018)
Boiling point at 1013 hPa	tri-*o*-isomer: 410 °C (IFA 2018)
Density at 20 °C	tri-*o*-isomer: 1.18 g/cm³ (IFA 2018)
Vapour pressure at 25 °C	tri-*o*-isomer: 0.0000026 hPa (NCBI 2020 a)
log K_{OW}	tri-*o*-isomer: 6.34 (calculated; NCBI 2020 b)
Solubility	tri-*o*-isomer: 0.102 mg/l water (calculated; NCBI 2020 b)
1 ml/m³ (ppm) ≙ 15.286 mg/m³	**1 mg/m³ ≙ 0.0654 ml/m³ (ppm)**

a) for the sum of all ortho isomers

Note: The substance can occur simultaneously as vapour and aerosol.

In this documentation, the term *o*-tricresyl phosphates is used for tricresyl phosphates containing at least one cresyl moiety methylated at the *ortho* position.

Tri-*o*-cresyl phosphate (commonly abbreviated as TOCP) is the tri-*o*-isomer of tricresyl phosphate. Besides tri-*o*-cresyl phosphate, there are two di-*o*-tricresyl phosphates (*o-o-m* and *o-o-p*) and three mono-*o*-tricresyl phosphates (*o-m-m*, *o-m-p* and *o-p-p*) (Winder and Balouet 2002).

Technical tricresyl phosphate (TCP) formerly contained up to 30% *o*-tricresyl phosphate isomers (ACGIH 2016), primarily mono-*o*-isomers and di-*o*-isomers (De Nola et al. 2008; Henschler 1958). These tricresyl phosphates lead more frequently to signs of paralysis in exposed chickens and there are symptomatic and histological differences compared with the effects induced by pure tri-*o*-cresyl phosphate (Henschler 1958). The mono-*o*-isomers and di-*o*-isomers are more neurotoxic than the tri-*o*-isomer.

The *o*-tricresyl phosphates are formed from phosphorus oxychloride and technical cresol during the synthesis of tricresyl phosphate (CAS number: 1330-78-5). As a result of the production process, technical cresol is a mixture of different fractions of *o*-cresol, *m*-cresol and *p*-cresol. Cresols containing the lowest possible amounts of *o*-isomer are used to ensure that the fraction of neurotoxic *o*-tricresyl phosphates is kept to a minimum (De Nola et al. 2008).

In the following, the term "tri-*o*-cresyl phosphate" is given for the composition description if the authors of the respective publication used this name for the test substance, even if this isomer was not determined by analysis and, in some cases, it is likely that other *o*-isomers were present. Furthermore, the test substance may have contained only mono-*o*-isomers or di-*o*-isomers, and not the tri-*o*-isomer. This is because the method for the determination of the isomers was based on the saponification of TCP to form phosphate and cresol followed by an analysis of the *o*-cresol produced. This method cannot be used to determine the number of *o*-cresyl moieties in one molecule of tricresyl phosphate. If any exact data for the di-*o*-isomers and the mono-*o*-isomers were given, these are included in the documentation.

Tricresyl phosphates with only low levels of *o*-isomers are primarily made up of mono-*o*-isomers (Henschler and Bayer 1958). There are few data available for the levels of mono-*o*-tricresyl phosphate and di-*o*-tricresyl phosphate.

Today, commercially available tricresyl phosphate mixtures contain the tri-*o*-isomer in maximum concentrations of 0.07 percent by weight (ECHA 2018). Other sources report tri-*o*-cresyl phosphate levels of < 0.2% in commercial products available on the market today (Wolkoff et al. 2016). However, a different source reported much higher levels of up to 2% tri-*o*-cresyl phosphate in commercial mixtures of tricresyl phosphate (Duarte et al. 2017).

An analysis of the tricresyl phosphate isomer mixture contained in aircraft engine oil found that the *o*-tricresyl phosphates were present only as mono-*o*-tricresyl phosphates in concentrations of 13 to 150 mg/l oil (De Nola et al. 2008).

Lubricating oils such as aircraft engine oils contain up to 3% tricresyl phosphates; however, less than 1% of these are *o*-isomers (Mackerer et al. 1999; van Netten 1998).

The tricresyl phosphate isomer mixture contains different isomers of tricresyl phosphate and may include 1 to 3 *m*-cresyl residues (for example, tri-*m*-cresyl phosphate, CAS number 563-04-2), 1 to 3 *p*-cresyl residues (for example, tri-*p*-cresyl phosphate, CAS number 78-32-0) and traces of *o*-cresyl phosphates (for example, tri-*o*-cresyl phosphate, CAS number 78-30-8). Phosphotriesters, which contain only *m*-isomers or *p*-isomers, do not induce neurotoxic effects (Hartwig and MAK Commission 2023; NTP 1994).

A large number of reviews have been published on the toxicological profile of tri-*o*-cresyl phosphate: reports and reviews are available from the ACGIH (2016), Nordic Expert Group (Sjögren et al. 2010), ECETOC (1998) and WHO (1990).

Tri-*o*-cresyl phosphate is odourless (Wolkoff et al. 2016).

Tricresyl phosphate is used as a flame retardant, plasticizer, in coatings and paints, as a photographic chemical, and in lubricants, greases and metal-working fluids (ECHA 2018). Tri-*o*-cresyl phosphate is not an insecticide. In the past, it was added to polyvinyl chloride ("Igelit", a soft PVC material) as a plasticizer.

1 Toxic Effects and Mode of Action

o-Tricresyl phosphate induces neurotoxic effects in humans. The syndrome observed is an organophosphate-induced neuropathy which develops as a delayed reaction (OPIDN) and may be irreversible depending upon the severity of the poisoning.

This neuropathy occurs also in various animal species. The symptoms, its progression and the degree of sensitivity observed in humans are most similar to the effects induced in chickens and cats. Rodents are much less sensitive.

Oral exposure of chickens to tri-*o*-cresyl phosphate for 90 days induced neurotoxic symptoms at doses of 2.5 mg/kg body weight and day and above. An oral dose of 5 mg/kg body weight and day given over a period of 180 days led to a weakening of the hindlimbs in sheep. In both species, axonal degeneration was observed before clinical symptoms became noticeable. After oral exposure of rats for 24 weeks, neurotoxic changes were observed only at markedly higher doses of 116 mg/kg body weight and above; these were not accompanied by functional disorders.

Dermal application of tri-*o*-cresyl phosphate to cats for 90 days induced weakness of the legs and ataxia at doses of 1 mg/kg body weight and day and above in addition to histopathological changes in the spinal cord and the peripheral nervous system at doses of 5 mg/kg body weight and day and above.

Aircraft engine oil containing 3% tricresyl phosphate given to chickens at 2 g/kg body weight and day was equivalent to the uptake of an *o*-tricresyl phosphate dose of 0.24 mg/kg body weight. After 10 weeks, axonal degeneration and ataxia were observed in almost all of the animals.

Tri-*o*-cresyl phosphate given orally to rats and mice induced effects on the testes and sperm.

In a prenatal developmental toxicity study with treatment of Long Evans hooded rats on gestation days 6 to 18, no effects on development were observed up to the highest tri-*o*-cresyl phosphate dose tested of 350 mg/kg body weight and day. Increased mortality was observed in the dams at this dose. Two-generation studies found the offspring to be less sensitive than the dams with respect to the inhibition of acetylcholinesterase (AChE) activity.

There are no findings in humans or animal studies relevant for the evaluation of skin sensitizing effects of tri-*o*-cresyl phosphate. No data are available for sensitizing effects on the respiratory tract induced by tri-*o*-cresyl phosphate.

Tri-*o*-cresyl phosphate was mutagenic in the Salmonella typhimurium strain TA100. The formation of DNA adducts was observed in vitro and in vivo, but further investigations are required.

There are no carcinogenicity studies available.

2 Mechanism of Action

The toxic effects of organophosphates such as tri-*o*-cresyl phosphate are attributed to the inhibition of esterases, particularly the neurotoxic esterase (NTE), via phosphorylation (Winder and Balouet 2002). Only tricresyl phosphate with a substitution at the ortho position can induce OPIDN (NTP 1994).

2.1 Organophosphate-induced delayed neuropathy (OPIDN)

Delayed neurotoxicity is defined as persistent locomotor ataxia that develops as a delayed reaction after exposure to organophosphates. A single exposure may suffice, but generally the reaction develops after repeated exposure. Both the sensory and motor nerve fibres in the central and peripheral nervous systems are affected, resulting in distal neuropathy. The symptoms may develop after a delay of up to 4 weeks (Abou-Donia and Lapadula 1990).

The initial signs of OPIDN are a tingling, burning sensation followed by numbness and weakness in the distal extremities. Neuromuscular blockades appear as the disorder progresses; these cause symptoms ranging from muscle weakness to paralysis of the extremities (complete paralysis of the motor nerves). Regeneration is possible only if the damage is slight (Winder and Balouet 2002).

After acute poisoning, the initial symptoms of OPIDN are nausea, vomiting, diarrhoea and stomach pains. After an asymptomatic period of 3 to 28 days, sharp, cramping pains develop in the calf muscles, leading first to numbness and tingling in the feet (and hands) and then, within a few hours, to muscle weakness and paralysis in both feet. About 10 days later, this is followed by weakness in the muscles of the hands and wrists, which, however, is not as severe

as that experienced in the feet. Paralysis above the elbow has not been observed. Sensory changes occur only rarely (König 1969; NIOSH 1978).

In several cases of tri-*o*-cresyl phosphate poisoning in South Africa caused by the consumption of contaminated cooking oil, symptoms of OPIDN were still noticeable 18 years later. This demonstrates that tri-*o*-cresyl phosphate can lead to irreversible nerve damage (Susser and Stein 1957).

Irreversible damage after tri-*o*-cresyl phosphate poisoning was found mainly in the lower limbs; the symptoms were still noticeable decades later (WHO 1990).

Autopsies performed in humans found uniform degeneration in anterolateral spinal nerves, particularly along the long pathways. In addition, skeletal muscle atrophy was a secondary manifestation of peripheral neuropathies (ACGIH 2016). Degeneration of the myelin sheath of the peripheral nerves was found in humans who had died of tri-*o*-cresyl phosphate poisoning (Susser and Stein 1957). Degeneration of the peripheral nerves (spinal nerves and the sciatic nerve) was found also in dogs, monkeys, cats and chickens after exposure to tri-*o*-cresyl phosphate. Wallerian degeneration of the axons and the myelin in the distal parts of the major neural pathways was observed (Abou-Donia 1993; Cavanagh 1954, 1964; Henschler 1958; WHO 1990).

In chickens and cats given tri-*o*-cresyl phosphate, degeneration of the long supraspinal ascending and descending fiber pathways was found in the lumbar spinal cord (Beresford and Glees 1963).

The involvement of Ca^{2+} in nerve degeneration has been suggested because calcium/calmodulin-dependent protein kinase II (CaM-kinase II) appears to be responsible for the hyperphosphorylation of neurofilaments in hens given organophosphates (ECETOC 1998; Gupta and Abou-Donia 1995).

Studies in chickens found that hyperphosphorylation of the cytoskeletal proteins, an increase in cyclin-dependent kinase 5 in the spinal cord and a decrease in neurofilaments in the spinal cord and the sciatic nerve are involved in the development of OPIDN. At a constant rate of entry into the axon, hyperphosphorylation of cytoskeletal proteins decreases their transport rate down the axon, leading to the accumulation of phosphorylated neurofilament aggregates in the central and peripheral axons (Gupta and Abou-Donia 1993, 1995; Sjögren et al. 2010; Zhao et al. 2004).

The activation of the ion channel TRPA1 (transient receptor potential cation channel, member A1) and subsequent increase in intracellular calcium levels caused by tri-*o*-cresyl phosphate is also regarded as a mediator of OPIDN (Ding et al. 2017). Lipids may be involved in the development of OPIDN induced by tri-*o*-cresyl phosphate, because an increase in cholesterol levels and a 50% decrease in the triglycerides in the sciatic nerve were observed after chickens were given oral doses of tri-*o*-cresyl phosphate. The distribution of phospholipids in the brain and sciatic nerve of the animals remained unchanged (Morazain and Rosenberg 1970; WHO 1990).

2.2 Neurotoxic esterase (neuropathy target esterase, NTE)

The symptoms of OPIDN are attributed also to the inhibition of NTE (Emerick et al. 2010).

Animal studies have demonstrated that the irreversible inhibition of NTE in nerve tissue can be regarded as an indicator of OPIDN. The NTE activity in the nerve tissue correlates with that in the lymphocytes, also in humans. However, the inhibition of AChE and NTE are not related. NTE activity is inhibited within hours of exposure to organophosphates. By contrast, with a regeneration half-life of 3 to 5 days, lymphocytic NTE activity may have returned to pre-exposure levels by the time the first clinical symptoms are observed (Johnson 1974; Richardson 1992).

NTE is a 150 kDa transmembrane protein. It is anchored to the cytoplasmic side of the endoplasmic reticulum. There, it catalyses the deacylation of phosphatidylcholine and lysophosphatidylcholine to glycerophosphocholine in its function as a serine hydrolase. NTE is a member of the 9-protein family called patatin-like phospholipase domain-containing proteins (PNPLA1-9) and is involved in membrane lipid homeostasis, maintaining nervous system integrity, cell differentiation and embryonic development. The enzyme has a catalytic and a regulatory domain which is similar to protein kinase A (Akassoglou et al. 2004; Glynn 2005; Sogorb et al. 2016; Zhu et al. 2016).

The phospholipase activity of NTE reduces phosphatidylcholine levels by degrading phosphatidylcholine to glycerophosphocholine and free fatty acids. This facilitates the constitutive secretory pathway of the neurons, thereby optimizing the export of material from the neuronal soma. This relationship between NTE activity and phosphatidylcholine synthesis occurs not only in the neuronal soma, but also in the distal axons. Local phosphatidylcholine homoeostasis is probably essential for efficient vesicular transport within the axon (Glynn 2013).

NTE has an active site serine which is used as a binding site by organophosphates. Covalent phosphorylation of the serine residue at the active site inhibits NTE, thereby disrupting axoplasmic transport. This leads to axon swelling and neurite degeneration. This effect may occur in sensory or motor nerves both in the peripheral nervous system (PNS) and in the central nervous system (CNS). In primary cultures of cortical neurons from mouse embryos, tri-*o*-cresyl phosphate reduced neurite growth even at micromolar concentrations (see Section 5.8; Glynn 1999; Hausherr et al. 2014; Winder and Balouet 2002).

Many authors consider the inhibition of NTE to be the most sensitive end point for OPIDN (ECETOC 1998). However, the involvement of NTE inhibition in the development of OPIDN remains controversial. As the enzyme NTE occurs in many other types of tissues besides neurons, it is difficult to explain the mechanism of OPIDN based only on the inhibition of NTE (Henschler et al. 1992).

The initial inhibition of NTE by various organophosphate compounds is not sufficient to induce OPIDN. A negative charge first needs to be generated on the terminal side of the phosphate group, which binds to the enzyme, leading to a secondary reaction known as "ageing". In this step, the hydrolysis of 1 of the 2 ester bonds leads to the formation of a negatively charged, monosubstituted phosphoric acid ester which remains attached to the enzyme (Emerick et al. 2012; Glynn 2000). The reaction is shown in Figure 1.

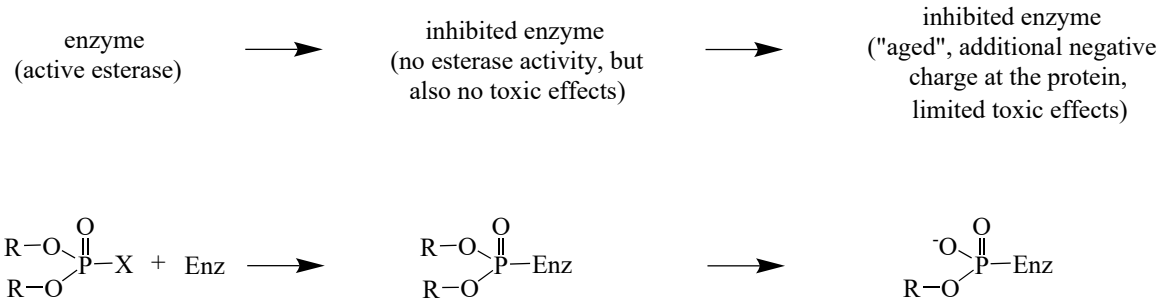


Fig. 1 Mechanism of the ageing reaction induced by organophosphates, R = alkyl, aryl (according to Johnson 1974)

A comparison of the clinical symptoms linked with NTE activity in sheep, chickens and rats revealed that a 50% to 70% inhibition of NTE activity in the brain or spinal cord leads to clinical symptoms of neurotoxicity. The inhibition of NTE activity can be used as an indirect measure of tri-*o*-cresyl phosphate toxicity; neurotoxic effects do not necessarily have to occur (Craig and Barth 1999; Daughtrey et al. 1996; Soliman 1983). An analogy can be drawn to the use of AChE inhibition in erythrocytes as a biomarker. Even if the function of AChE in erythrocytes is still unknown, AChE activity in erythrocytes remains a suitable parameter for synaptic AChE activity (Richardson 1992).

In hens given a single oral dose of tri-*o*-cresyl phosphate of 164 mg/kg body weight, NTE activity in the brain was inhibited by 70%. By contrast, in Carey's Nick 320 Leghorn hens given orally 2000 mg/kg body weight of aircraft engine oil containing an isomer mixture of tricresyl phosphate with low levels of *o*-isomers, NTE activity in the brain was inhibited by only 19% to 39% (no other details; Mackerer et al. 1999).

Chronic, low-level inhibition of NTE is not expected to cause neuropathy. OPIDN is a threshold event (ECETOC 1998).

A study with Drosophila found evidence that NTE is an enzyme with protein kinase A activity, which is inhibited by organophosphates (Wentzell et al. 2014).

NTE-deficient (*nte*$^{-/-}$) mice die on embryonic day 8, meaning they are not viable. In comparison with wild type mice, *nte*$^{+/-}$ mice have lower NTE activity in the brain and higher mortality when exposed to the NTE-inhibiting compound ethyl octylphosphonofluoridate. OPIDN was not observed because mice do not develop this syndrome (Winrow et al. 2003).

Mutations in the NTE gene in humans cause autosomal recessive motor neuron disease. Family members with this mutation exhibit progressive spastic weakness of the distal extremities that begins in childhood and is later associated with atrophy of the lower leg muscles and the intrinsic hand muscles. The deficits in the upper and lower motor neurons observed in the patients correspond to the symptoms of OPIDN (Rainier et al. 2011).

NTE activity was reduced in the lymphocytes 24 hours after a single oral tri-*o*-cresyl phosphate dose of 500 mg/kg body weight was given to laying hens. The activity had returned to normal levels after 8 days (see Section 5.1.2; Emerick et al. 2010).

After groups of 8 adult Beijing laying hens were given a single tri-*o*-cresyl phosphate dose of 0 or 750 mg/kg body weight in gelatine capsules, paralysis, and thus clear signs of OPIDN, were observed on day 21 in the animals treated with tri-*o*-cresyl phosphate. Another group of 8 animals was injected subcutaneously with the NTE inhibitor phenylmethylsulfonyl fluoride at a dose of 60 mg/kg body weight 24 hours before being given tri-*o*-cresyl phosphate. The animals did not show signs suggestive of neurotoxicity. The phospholipid profile in the spinal cord of the animals treated with tri-*o*-cresyl phosphate was markedly changed in comparison with the profile of the control animals or with that of the animals pre-treated with phenylmethylsulfonyl fluoride. Thus, phospholipid homoeostasis in the endoplasmic reticulum appears to be involved in the induction of neurotoxicity by tri-*o*-cresyl phosphate (see Section 5.1.2; Zhu et al. 2016).

2.3 Cholinesterases

Plasma cholinesterase (also called pseudocholinesterase or butyrylcholinesterase) is a non-specific cholinesterase, while erythrocyte cholinesterase is identical with the enzyme that hydrolyses acetylcholine in the central and peripheral nervous systems (AChE). A decrease in erythrocyte cholinesterase activity (by about 50%), but a slight increase in plasma cholinesterase activity was found in patients that exhibited symptoms of OPIDN after exposure to tri-*o*-cresyl phosphate (Vora et al. 1962).

Blood analyses carried out in 124 employees of a company that produced plasticizers containing tri-aryl phosphate did not find a statistically significant decrease in plasma cholinesterase activity in exposed persons in comparison with the levels determined in the controls. The cholinesterase activities in the erythrocytes and in the plasma were not related. The mean maximum daily variation in the activities of the two enzymes was 15% to 20%. The authors assumed that the clinical signs and symptoms of poisoning/exposure occur only after a reduction in cholinesterase activity by more than 80% (no other details; Morgan and Hughes 1981). The BAT value for inhibition of the erythrocytic AChE activity is set at 70% of the reference value (Lewalter 1995).

The plasma cholinesterase activity was inhibited by 40% to 50% in laying hens given tri-*o*-cresyl phosphate for 6 weeks at a dose of 0.24 mg/kg body weight in aircraft engine oil with TCP, 10 mg/kg body weight in aircraft engine oil or 10 mg/kg body weight in corn oil. Only a slight effect on AChE activity was observed (inhibition by 12% to 22%) (see Section 5.2.2; Freudenthal et al. 1993).

When the brains of laying hens given a single oral dose of tri-*o*-cresyl phosphate of 500 mg/kg body weight were examined 24 hours after exposure, NTE activity was found to be inhibited by 80% and AChE activity only by about 20%. OPIDN was observed beginning on day 12 after treatment (see Section 5.1.2; Emerick et al. 2012).

The activities of AChE in the spinal cord and brain were inhibited by 38% and 15%, respectively, in laying hens given a single oral dose of tri-*o*-cresyl phosphate of 500 mg/kg body weight and above; the activities were not inhibited at 50 or 200 mg/kg body weight. The pseudocholinesterase activity was markedly inhibited in the plasma, but not in the

blood; the level of inhibition was concentration-dependent. The effects were observed 24 hours after administration (see Section 5.1.2; Classen et al. 1996).

The pseudocholinesterase activities in the plasma, brain and spinal cord were markedly decreased in laying hens only a day after the administration of a tri-*o*-cresyl phosphate dose of 1 ml/kg body weight and remained at this low level for the next 10 days. The AChE activity remained unchanged in these tissues. The authors suggested that these changes in enzyme activity may lead to a loss of myelin (Earl and Thompson 1952).

The pseudocholinesterase activities were determined in 2 laying hens given a single oral tri-*o*-cresyl phosphate dose of 300 mg/kg body weight (dissolved in arachis oil). After 24 hours, the activities in the blood and in the brain were inhibited by 100% in 1 animal and by 75% in the other. After 14 to 21 days, no inhibition was observed in the blood and the inhibition in the brain had been reduced by about a third. Tri-*o*-cresyl phosphate inhibited the cholinesterase activity in the blood by about 83% (500 mg/kg body weight, n = 7), while the mono-*o*-isomers inhibited the activity by 77% to 87% (500 mg/kg body weight, n = 2). Signs of ataxia were observed in the hens; the effects were the same as those determined in hens given triethyl phenyl phosphate. However, pseudocholinesterase was not inhibited in the latter case. The 83% inhibition of the cholinesterase activity in the blood following treatment with triethyl phenyl phosphate at doses of 200 to 1000 mg did not lead to neurotoxic effects (see Section 5.1.2; Aldridge and Barnes 1961).

Of 9 phosphoric acid esters, only tri-*o*-cresyl phosphate and two other compounds caused paralysis in chickens, even though pseudocholinesterase activity was inhibited to a statistically significant degree by all substances (Barnes and Denz 1953; Henschler 1958).

After 18 to 24 hours, triaryl phosphates with low neurotoxic potential led to a marked decrease in plasma cholinesterase activity by 64% to 90% in poultry (Bondy et al. 1973).

In three different chicken strains given tri-*o*-cresyl phosphate, the inhibition of plasma cholinesterase and AChE activities was statistically significant 24 hours and 28 days after exposure. However, symptoms of OPIDN such as unsteady gait, paresis and total paralysis were observed only in Isabrown chickens and not in the strains Babcock and Hy-line-w36 (see Section 5.1.2; de Oliveira et al. 2002).

In vitro, erythrocytic and plasma cholinesterase activities in humans and the plasma cholinesterase activity in hens were not inhibited by tri-*o*-cresyl phosphate and other aromatic phenyl phosphates (Hine et al. 1956).

Summary: In the studies of Aldridge and Barnes (1961), Barnes and Denz (1953), Bondy et al. (1973), Emerick et al. (2012) and de Oliveira et al. (2002) a clear relationship between the inhibition of cholinesterase activities and the neurotoxic effects of OPIDN could not be established. Various phenyl phosphates inhibit cholinesterase activity in chickens, but only a small number of compounds induce OPIDN or ataxia. However, OPIDN developed after exposure to tri-*o*-cresyl phosphate even without marked inhibition of cholinesterase.

2.4 *o*-Cresyl saligenin phosphate (CBDP)

The critical metabolite of tri-*o*-cresyl phosphate is *o*-cresyl saligenin phosphate (2-(2-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide or 2-(*o*-tolyloxy)-4H-1,3,2-benzodioxaphosphorin-2-oxide, CBDP), a potent NTE inhibitor (Casida et al. 1961; Hausherr et al. 2017). Given in oral doses to hens, the neurotoxic effects (ataxia) induced by CBDP were 5 times stronger than those induced by tri-*o*-cresyl phosphate (WHO 1990). CBDP is likewise a strong irreversible inhibitor of butyrylcholinesterase and other serine esterases and a weak inhibitor of AChE (Baker et al. 2013; de Boer et al. 2015; ECETOC 1998). The human recombinant butyrylcholinesterase and the human recombinant AChE were irreversibly inhibited by CBDP in vitro (Carletti et al. 2011, 2013). A schematic depiction of the binding of CBDP to serine 198 at the active site of butyrylcholinesterase is shown in Figure 2 (Liyasova et al. 2013).

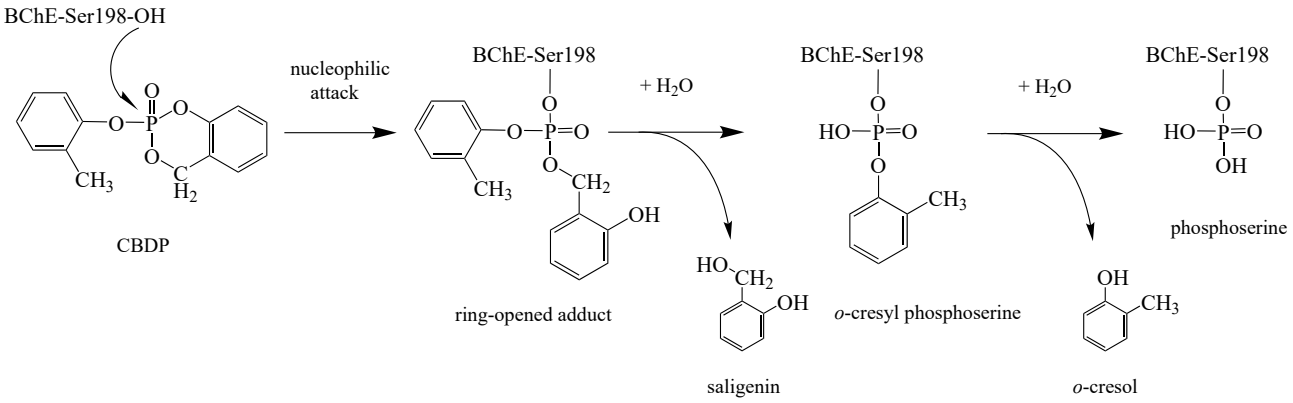


Fig. 2 Reaction of CBDP with butyrylcholinesterase (BChE) (according to Liyasova et al. 2013)

Mass spectral analysis revealed CBDP–albumin adducts at tyrosine via phosphorylation and at histidine and lysine via alkylation (Liyasova et al. 2012).

The demethylated analogue of CBDP, phenyl saligenin phosphate (2-phenoxy-4H-1,3,2-benzodioxaphosphorin 2-oxide), is a reactive benzyl ester. Phenyl saligenin phosphate forms *o*-hydroxybenzyl ether and DNA adducts with sulfur-containing nucleophiles such as cysteine and glutathione and with several oximes. The same DNA adducts as those determined for phenyl saligenin phosphate in vitro were observed also with tri-*o*-cresyl phosphate in vivo (Mentzschel et al. 1993 b).

2.5 Other action-related effects

After a single oral tri-*o*-cresyl phosphate dose of 500 mg/kg body weight was given to laying hens, enzyme activities and Ca^{2+} levels were examined in the sciatic nerve and in the calf muscle. A marked increase in calcium activated neutral protease (CANP) activity was found, beginning after 12 hours and reaching its maximum after 14 days. Reduced Ca^{2+} levels were determined in the muscle and nerve tissues, but not in the plasma (see Section 5.1.2; Emerick et al. 2010).

The impairment of glutamate signalling by tri-*o*-cresyl phosphate in the primary cultures of cortical neurons isolated from mouse embryos is regarded as evidence of an additional mechanism of action. Reduced expression of the glutamate receptor and increased intracellular Ca^{2+} levels were observed in the primary cultures after treatment with tri-*o*-cresyl phosphate (see Section 5.8; Hausherr et al. 2014).

A reduced expression of glutamine synthetase and lower glutamine levels were observed in the brains of chickens 5 days after a single tri-*o*-cresyl phosphate dose (1000 mg/kg body weight). Glutamate and cytosolic Ca^{2+} levels were increased. After 21 days, all levels had returned to the range of the controls (Jiang et al. 2014).

In chickens, a tri-*o*-cresyl phosphate dose of 500 mg/kg body weight increased calpain levels in the brain by 40% and 20% after 24 hours and 21 days, respectively. When treatment with tri-*o*-cresyl phosphate was preceded by 1 mg/kg body weight (intramuscular) of the calcium channel blocker nimodipine and followed after 18 hours by a calcium gluconate dose of 5 mg/kg body weight, the paralysis induced was considerably less severe than the effects caused by exposure to tri-*o*-cresyl phosphate alone (see Section 5.1.2; Emerick et al. 2012).

In chickens given gavage doses of tri-*o*-cresyl phosphate, an increase in autophagosomes in the axons of the spinal nerves in addition to several marker enzymes are regarded as evidence of autophagy inhibition (Song et al. 2014).

Besides OPIDN, another condition that was observed was the "intermediate syndrome". This syndrome is characterized by paralysis of the proximal extremities, weakness of the neck muscles, respiratory paralysis and effects on the cranial nerves. The symptoms improve after about 18 days. A down-regulation of the AChE receptor has been

suggested as the cause of these effects, as the half-life of the receptor is 10 days. Another condition that may be induced is the chronic organophosphate neuropsychological disorder (COPIND). This disorder does not involve the inhibition of esterase (Sedgwick and Senanayake 1997; Winder and Balouet 2002).

2.6 The so-called "aerotoxic syndrome"

The so-called "aerotoxic syndrome" refers to adverse health effects caused by substances in the air of cockpits and the passenger cabins of aeroplanes. It is characterized by an acute restriction of CNS functions such as poor concentration, balance disorders, numbness, mood swings and further cognitive impairments. These symptoms cannot sufficiently be explained by the mechanism of OPIDN. Further symptoms attributed to the so-called "aerotoxic syndrome" are irritation of the mucous membranes, dyspnoea, cardiac arrhythmia, headaches, stomach cramps and flulike symptoms (Liyasova et al. 2011; Michaelis et al. 2017; de Ree et al. 2014; Winder et al. 2002). The specificity of the symptom complex of the so-called "aerotoxic syndrome" requires further characterization.

The symptoms of tri-*o*-cresyl phosphate poisoning are OPIDN and acute gastrointestinal symptoms (nausea, vomiting, diarrhoea) which are not the same as those of the so-called "aerotoxic syndrome".

2.7 Effects on the male reproductive organs

After the exposure of rats to tri-*o*-cresyl phosphate, a higher concentration of the active metabolite CBDP was found in the testes than in the other organs or the blood (Chapin et al. 1990). However, this study did not investigate the reproductive toxicity of the substance.

In male Kunming mice, exposure to tri-*o*-cresyl phosphate at doses of 100 mg/kg body weight and day and above impaired spermatogenesis, inhibited the growth of spermatogonial stem cells and the NTE activity in the testes. The metabolite CBDP markedly inhibited NTE activity and cell proliferation in the spermatogonial stem cells. Additional evidence for the involvement of NTE in the viability of spermatogonial stem cells was provided by the reduced expression of NTE facilitated by NTE-shRNA (small hairpin RNA), and the associated decrease in cell proliferation (see Section 5.5.1; Chen et al. 2012).

As the testosterone levels in the testes of rats remained unaffected by treatment with tri-*o*-cresyl phosphate, Sertoli cells are assumed to be the target (Somkuti et al. 1987 a). In a primary culture of rat Sertoli cells, the activity of non-specific esterase (NSE) was inhibited by about 50% after treatment with 20 µM of CBDP, but not with tri-*o*-cresyl phosphate (Chapin et al. 1991). In vitro studies of radioactively labelled tri-*o*-cresyl phosphate in co-cultures of rat Sertoli and Leydig cells demonstrated that Leydig cells activate tri-*o*-cresyl phosphate to CBDP, resulting in a decrease in NSE activity in Sertoli cells (Chapin et al. 1990).

In vitro, tri-*o*-cresyl phosphate inhibited the viability of spermatogonial stem cells of rats in a concentration-dependent manner at 0.25 mM and above. The substance did not lead to cell cycle arrest up to 1 mM. Proteins that are responsible for autophagy were formed. Autophagic vesicles in the cytoplasm containing extensively degraded materials such as mitochondria and endoplasmic reticulum were determined by electron microscope (Liu et al. 2015). A follow-up study carried out by this research group found that oxidative stress is involved in the autophagy induced by tri-*o*-cresyl phosphate (Liu et al. 2016). The mechanistic relationship between the inhibition of NTE or NSE activities and the inhibition of cell proliferation or autophagy in spermatogonial cells remains unclear.

2.8 Mono-*o*-tricresyl phosphates and di-*o*-tricresyl phosphates

In chickens treated with mono-*o*-isomers and di-*o*-isomers, lesions developed mainly in the cervical and lumbar marrow, with hardly any forming peripherally in the sciatic nerve. The same pattern was observed following exposure to the technical tricresyl phosphate product (26% *o*-tricresyl phosphate). By contrast, pure tri-*o*-cresyl phosphate induced lesions mainly in the sciatic nerve. The mono-*o*-isomers caused more severe neurotoxic effects than the di-*o*-isomers and tri-*o*-isomer at a relative potency of 10 : 5 : 1 (Henschler 1958).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

There are no data available for absorption by inhalation.

In hens given a single oral dose of ^{14}C-tri-*o*-cresyl phosphate (purity 98%) of 50 mg/kg body weight, the highest concentration was observed in the plasma 0.5 to 1 day after application. The degradation of tri-*o*-cresyl phosphate in plasma proceeded monoexponentially with a half-life of 2.2 days. Likewise, accumulation in the liver, kidneys and lungs was highest after 12 to 24 hours. The AUC values (area under the concentration–time curve) in these organs were greater than those in the plasma. Appreciable concentrations of the metabolite CBDP were determined in the plasma, liver, kidneys and lungs with half-lives of 2.06, 1.36, 1.11 and 4.4 days, respectively (ACGIH 2016; Sjögren et al. 2010; Suwita and Abou-Donia 1990). The highest level of radioactivity was found in the gastrointestinal tract and only a very small fraction of the applied radioactivity was found in the egg yolk (0.24%) and albumen (0.12%). About 47% of the radioactivity had been excreted with the urine and faeces after 12 hours and 99% had been excreted after 5 days (Abou-Donia et al. 1990 b). As poultry excrete urine and faeces together, the uptake of the substance cannot be determined based on the levels recovered separately in the urine and faeces.

In hens given an oral dose of ^{32}P-tri-*o*-cresyl phosphate of 770 mg/kg body weight, CBDP accumulated in the liver. After 72 hours, 26.5% of the dose had been excreted, mainly in the form of unmetabolized tri-*o*-cresyl phosphate (Sharma and Watanabe 1974).

To investigate distribution and elimination, male F344/N rats were given ^{14}C-tri-*o*-cresyl phosphate in corn oil by gavage in doses of 0.5, 2, 20 or 200 mg/kg body weight and by intravenous injection in doses of 2 or 20 mg/kg body weight. Tri-*o*-cresyl phosphate was readily absorbed after oral administration. At all doses, 70% of the dose was excreted with the urine and 20% with the faeces within a period of 24 hours. *o*-Hydroxybenzoic acid (salicylic acid) formed during the metabolism of tri-*o*-cresyl phosphate was excreted with the urine either as free salicylic acid or in the form of a conjugate. Within the first 6 hours after intravenous injection of a ^{14}C-tri-*o*-cresyl phosphate dose of 2 or 20 mg/kg body weight, 40% to 60% of the radioactivity was excreted with the bile. Enterohepatic circulation is assumed because less radioactivity was recovered in the faeces than in the bile. All of the radioactivity had been excreted within 3 days. The substance was rapidly distributed to the muscles and liver and then redistributed to adipose tissue and the skin. The parent substance was rapidly metabolized and did not accumulate in the organs (NTP 1994).

After an oral dose of ^{14}C-tri-*o*-cresyl phosphate (purity 98%) of 50 mg/kg body weight was given to male Sprague Dawley rats, 63% of the radioactivity was excreted with the urine and 36% with the faeces. The highest levels of radioactivity were found in the gastrointestinal tract, the bladder, the liver and the kidneys. Noteworthy levels of radioactivity were detected in the plasma, the erythrocytes, the lungs and the adipose tissue. In the nerve tissues, the highest concentrations were found in the spinal cord. The terminal half-life in the plasma was 46 hours (Abou-Donia et al. 1990 a).

After treatment with a single oral dose of 50 mg/kg body weight, the concentration of tri-*o*-cresyl phosphate in the plasma of hens was 6 to 13 times higher than the level in the plasma of rats. The concentration of CBDP in the plasma was 13 to 65 times higher in hens. Furthermore, the half-life of CBDP in the plasma was 49 hours in hens and thus considerably longer than the half-life of 18 hours determined in rats (Suwita and Abou-Donia 1990). Therefore, metabolic clearance prevents the cumulative inhibition of NTE activity in rats (Mackerer et al. 1999).

In an earlier study in humans with the application of 0.22 g and 0.11 g of radioactively labelled ^{32}P-tri-*o*-cresyl phosphate per person to the skin of both hands (according to the authors, on an area of 200 cm^2) for 3.5 hours, only 797 and 143 µg (0.1%–0.4% of the dose) was excreted via the kidneys within 3 days. Excretion with the faeces was not investigated (Hodge and Sterner 1943; IFA 2018). Assuming that, as in cats (see below), the same fraction of the dose is also excreted with the faeces, an amount of up to 1.6 mg per 200 cm^2, or 8 µg/cm^2, would have been absorbed. Assuming exposure for only 1 hour, the absorbed amount would be 2.3 µg/cm^2 and hour. Extrapolated to a surface area of skin of 2000 cm^2, this would be equivalent to an absorbed amount of 4.6 mg.

After open application of a ^{14}C-tri-*o*-cresyl phosphate dose of 50 mg/kg body weight to 10 cm^2 of skin on the back of the neck of groups of 3 male cats for a period of 0.5, 1, 2, 5 or 10 days, 73% of the substance disappeared in the first 12 hours. After 10 days, there was hardly any trace of the substance. Within 10 days, 28% of the radioactivity was recovered in the urine and 20% in the faeces. Complete excretion had not yet been reached; 3.3% was recovered in the examined tissues of the animals. The amounts determined in the rest of the body were not specified. No radioactivity was recovered in the exhaled air. The highest levels of radioactivity were found in the gall bladder, bladder, liver and kidneys and the lowest levels in the nerve tissue, spinal cord, brain, muscles and spleen. A chemical analysis revealed that unchanged tri-*o*-cresyl phosphate was found mainly in the brain, spinal cord, sciatic nerve and in the faeces, while the metabolites were detected in the liver, kidneys, lungs and in the urine (Nomeir and Abou-Donia 1986; Sjögren et al. 2010). As the substance almost completely disappeared from the application site, this is regarded as evidence of very good absorption through the skin.

After dermal application of tri-*o*-cresyl phosphate in dogs, the levels of tri-*o*-cresyl phosphate recovered in the examined tissues were, in decreasing order: liver > kidneys > lungs > nerve tissue > bones (Hodge and Sterner 1943).

Undiluted tri-*o*-cresyl phosphate was absorbed at a rate of 0.18 µg/cm^2 and hour using an in vitro human skin model (Marzulli et al. 1965). Extrapolated to 1 hour and a surface area of skin of 2000 cm^2, this would be equivalent to 0.36 mg.

When ^{14}C-tri-*o*-cresyl phosphate was given by intravenous injection to pregnant mice (gestation day 18) and mice that were not pregnant, 55% and 50% of the radioactivity, respectively, was excreted with the urine and 9% with the faeces (both groups). Accumulation was detected in the lungs, spleen, gall bladder and liver in the dams and foetuses. The lowest levels of radioactivity were determined in the brain and spinal cord. This demonstrates that other organs are the targets of tri-*o*-cresyl phosphate toxicity in mice. The authors suggest that this may be the reason why mice are not susceptible to the neurotoxicity observed in other species (Ahmed et al. 1993). The radioactivity detected in the foetuses demonstrates that the substance or its metabolites pass through the placenta.

3.2 Metabolism

3.2.1 Animals

Nine metabolites were identified in the excreta and bile of hens treated with a single oral dose of ^{14}C-tri-*o*-cresyl phosphate of 50 mg/kg body weight (purity 98%). About half of the tri-*o*-cresyl phosphate dose was recovered unmetabolized. Following acid hydrolysis, the main metabolites in the excreta were *o*-hydroxybenzyl alcohol and *o*-hydroxybenzoic acid. The amount of the metabolite CBDP recovered in the excreta was < 1% of the administered dose. However, CBDP was the main metabolite found in the bile after 0.5 to 1 day (Abou-Donia et al. 1990 b). Dicresyl phosphates were the main metabolites recovered in the urine in studies with rats (Schindler et al. 2013).

The metabolism follows similar pathways in different species and differs only in the amounts of certain metabolites produced and in the rate of their formation. Metabolism occurs by hydroxylation at one or several methyl groups. In some cases, this is followed by further oxidation reactions to form aldehydes and carboxylic acids or by dearylation. Possible metabolites are mono-hydroxymethyl tri-*o*-cresyl phosphate and di-hydroxymethyl tri-*o*-cresyl phosphate and hydroxybenzyl alcohol, cresol, dicresyl phosphate, monocresyl phosphate and phosphoric acid, mono and dicresyldi(or mono)carboxyphenyl phosphate and hydroxybenzoic acid. No $^{14}CO_2$ was found in the exhaled air when the cresol ring was radioactively labelled; this suggests that the ring is not opened and degraded during metabolism (NTP 1994).

Tri-*o*-cresyl phosphate is metabolized by various enzymes in the liver. Products such as CBDP are formed by oxidation catalysed by cytochrome P450 and cyclization catalysed by serum albumin. Studies in human liver microsomes demonstrated that cytochrome P450 3A4 and 3A5 play a significant role in CBDP formation. The recombinant cytochrome P450 enzymes 2B6, 2C18, 2D6, 3A4 and 3A5 were responsible for the highest levels of tri-*o*-cresyl phosphate bioactivation (Hausherr et al. 2017; Reinen et al. 2015).

Besides tri-*o*-cresyl phosphate and CBDP, 8 other metabolites were identified in the plasma, liver, kidneys and lungs of laying hens given an oral dose of tri-*o*-cresyl phosphate. These metabolites were present in almost equal amounts. After 12 hours, tri-*o*-cresyl phosphate made up 4.3% of the total radioactivity in the plasma. The authors regarded this as evidence that tri-*o*-cresyl phosphate is rapidly metabolized (Suwita and Abou-Donia 1990).

In rats and hens given oral doses of tri-*o*-cresyl phosphate, lower levels of the substance and its metabolite CBDP were found in the plasma of rats than in the plasma of hens. In addition, the plasma half-life of tri-*o*-cresyl phosphate was considerably shorter in rats than in hens. The authors assume that this is why rats are much less susceptible to OPIDN induced by tri-*o*-cresyl phosphate (Sjögren et al. 2010; Suwita and Abou-Donia 1990).

After dermal application of tri-*o*-cresyl phosphate to the shaved skin on the back of the neck of male cats, low levels of hydroxymethyl tri-*o*-cresyl phosphate, dihydroxymethyl tri-*o*-cresyl phosphate and CBDP were found in numerous tissues (see Section 3.1; Nomeir and Abou-Donia 1986).

3.2.2 Humans

Cytochrome P450 is involved in the metabolism of tri-*o*-cresyl phosphate. The evidence in humans shows that the cytochrome P450 activities vary considerably from individual to individual. Therefore, large differences in sensitivity to tri-*o*-cresyl phosphate are assumed (de Boer et al. 2015). However, these differences only become relevant at tri-*o*-cresyl phosphate concentrations that are high enough for the metabolism of tri-*o*-cresyl phosphate to reach the range of saturation.

4 Effects in Humans

The signs of tri-*o*-cresyl phosphate poisoning are peripheral paraesthesia, ataxia and flaccid paralysis. In severe cases of poisoning following ingestion, spasms and specific neurologic symptoms arising from lesions in the pyramidal tract (pyramidal signs) were observed in the lower extremities. It is estimated that the minimal oral dose of tri-*o*-cresyl phosphate required to induce paralysis in humans is 10 to 30 mg/kg body weight (ACGIH 2016).

In another publication, severe paralysis in humans was induced at doses of 6 to 7 mg/kg body weight and an oral dose of about 1000 mg/kg body weight caused death (NCBI 2020 a).

Several studies determined that the dose level at which even a single exposure can lead to toxic effects is 100 to 500 mg per person. It is estimated that lethal effects may be induced at doses of 100 mg/kg body weight and above (Glees and Janzik 1965).

The symptoms of neuropathy observed after ingestion of tri-*o*-cresyl phosphate correspond to those of OPIDN. These include clinical signs such as ataxia, flaccid paralysis and paralysis which develop only after a delay of 7 to 28 days (see Section 2).

o-Tricresyl phosphate levels were determined in the air of aircraft cabins and cockpits and biomonitoring methods were applied to collect data from aircraft crew members and passengers (de Boer et al. 2015; Crump et al. 2011; De Nola et al. 2011; Heutelbeck et al. 2016; Houtzager et al. 2013; de Ree et al. 2014; Rosenberger et al. 2013; Schindler et al. 2013; Schuchardt et al. 2019; Solbu et al. 2011; Weiss et al. 2015; Wolkoff et al. 2016). The concentrations determined (detection limit for *o*-tricresyl phosphates: 0.3–100 ng/m^3) and the biomonitoring data (detection limit for the metabolite *o,o*-dicresyl phosphate: 0.5 µg/l urine) indicate either no or very low levels of exposure and do not provide evidence of a causal relationship with the so-called “aerotoxic syndrome” described by aircraft crew members.

4.1 Single exposures

Blood samples were collected from 12 healthy, adult airline passengers (non-smokers) 24 or 48 hours after the end of a flight. In 6 of them, exposure to tri-*o*-cresyl phosphate was detected based on the irreversible modification of butyrylcholinesterase by the metabolite CBDP. Exposure to the *p*-isomers and *m*-isomers of tricresyl phosphate or to

other organophosphates does not lead to the modification of butyrylcholinesterase. Symptoms of toxicity were not observed in any of the persons. However, on the basis of their findings, the authors concluded that only very low levels of exposure to tri-*o*-cresyl phosphate occur in the air of aircraft cabins (Liyasova et al. 2011).

No CBDP-butyrylcholinesterase adducts were determined in 15 pilots of F16 fighter planes and all values were below the detection limit of 0.1% adducts in plasma (Tacal and Schopfer 2014).

After ingestion of an unknown amount of a liquid containing tri-*o*-cresyl phosphate, the clinical findings in a 4-year-old child were acute gastrointestinal symptoms, a delayed cholinergic crisis and neurological toxicity (WHO 1990).

4.2 Repeated exposure

A report from as long ago as 1899 described 6 cases of polyneuropathy following the administration of "phospho-creosote" containing 15% tri-*o*-cresyl phosphate, which was prescribed as a treatment for tuberculosis. Reports describe numerous cases of poisoning with the symptoms of OPIDN after the ingestion of foods to which TCP containing tri-*o*-cresyl phosphate had accidentally or intentionally (criminal cases) been added. Another source of poisoning were plastics (such as "Igelit", a soft PVC material) to which TCP containing tri-*o*-cresyl phosphate was added as a plasticizer. In this case, uptake occured via absorption through the skin. Additional cases of poisoning were observed after inhalation exposure in workers (Henschler 1958; Inoue et al. 1988; WHO 1990).

4.2.1 Uptake at the workplace

Polyneuritis with paralysis of both legs was observed in a worker who had cleaned a boiler with an acetone/TCP mixture and inhaled vapours while monitoring a chemical production process. The syndrome typically begins with diarrhoea, vomiting and headaches (interval of 2–3 weeks), progresses to pain in the calf muscles, flaccid paralysis in the distal extremities, particularly in the legs, reduction of sensory activity and, after another interval of 2 years, the development of spastic symptoms (pyramidal tract impairment). In the case of the worker, the poisoning was attributed to the use of TCP as a plasticizer in the production process. The primary route of absorption was probably percutaneous. The authors estimate that the minimum dose required for the development of the entire spectrum of clinical symptoms is 150 to 300 mg per person (Gärtner and Elsasser 1943).

Signs of polyneuritis were observed in 3 factory workers. The workers worked in a room in which TCP (impure, containing hydrochloric acid), heated to 60 °C, was mixed with cold water in open tanks. The finished product contained 60% *o*-isomers. Inhalation exposure to *o*-tricresyl phosphates was therefore possible. The workers were exposed to TCP concentrations of 0.55 to 1.7 mg/m^3, which is equivalent to *o*-tricresyl phosphate levels of about 0.33 to 1.0 mg/m^3. The workers were exposed for 2.5 years, 8 or 6 months (ECETOC 1998; Hunter et al. 1944). Absorption by inhalation can be assumed. The polyneuritis described in this study corresponds in its symptoms and progression to OPIDN.

After exposure to tri-*o*-cresyl phosphate for about 3 years, a worker at a chemical plant developed complete flaccid paralysis of the lower legs and feet. The authors assume that absorption was via the skin (no other details; Parnitzke 1946).

A worker in a plant producing a formulation of a mixture of TCP isomers containing 6% to 10% tri-*o*-cresyl phosphate suffered irreversible paralysis of the legs. The symptoms occurred after exposure for 6 months. The cholinesterase activities in the plasma and in the erythrocytes were not determined until 20 months after the symptoms were first observed. A change in activity was not observed (no other details; ACGIH 2016; Bidstrup and Bonnell 1954; ECETOC 1998).

In a plant producing TCP, workers were divided into high and low exposure groups depending upon their function. The high exposure group consisted of 28 production workers who were exposed to TCP both by inhalation and through the skin. The low exposure group was made up of 6 workers (laboratory or office workers). However, it was assumed that all workers were exposed by inhalation to low levels of the substance. The average age of the workers was 42.4 years. The average length of employment was 8.9 years; 16 persons were employed for longer than 10 years. TCP concentrations of 0.27 to 3.4 mg/m^3 were determined in the ambient air. It was noted that aerosols were present;

however, the method of determination used in the study did not yield reliable data. Additional absorption of the substance is assumed to have occurred via the skin. The observed effects were divided into symptoms or clinical findings. In the high exposure group, the effects were irritation of the eyes, ears, nose and pharynx (symptoms: 14, clinical findings: 17), effects on the respiratory tract (symptoms: 25, clinical findings: 9), effects on the skin (symptoms: 14, clinical findings: 9), neuromuscular effects (symptoms: 5, clinical findings: 5) and gastrointestinal effects (symptoms: 14, clinical findings: 3). In the low exposure group, clinical findings (irritation: 2, neuromuscular effects: 1) were observed in only 3 persons. In 16 workers, the cholinesterase activities in plasma were found to be only 70% of the normal activity or lower. However, a statistically significant correlation between the decrease in cholinesterase activity, the length of employment or the level of exposure, and neuromuscular or gastrointestinal effects was not found. No effects on the cholinesterase activity in red blood cells were observed. The authors note that the neuromuscular effects (reduced sensitivity to irritation, slowed reflexes and hypoesthesia) and gastrointestinal effects (nausea, heartburn, vomiting) may have been caused by co-exposure to phosphorous oxychloride (no other details; ACGIH 2016; Tabershaw et al. 1957; Tabershaw and Kleinfeld 1957). Assuming that the TCP isomer mixture was made up of 20% tri-o-cresyl phosphate, this would be equivalent to exposure to tri-*o*-cresyl phosphate concentrations of 0.05 to 0.68 mg/m^3 (Sjögren et al. 2010).

In a shoe factory in Italy, 37 female workers were diagnosed with polyneuropathy (in some cases with spastic paresis, n = 8) involving the upper motor neurons. In all symptomatic persons, the neurotoxic effects were preceded by symptoms such as headaches, loss of appetite, cramp-like pains and weight loss. The activity of AChE in the erythrocytes were decreased, but not the levels of serum cholinesterase activity. According to the authors, the symptoms were suggestive of TCP poisoning. As the factory had ceased operations, the exposure concentrations could not be determined. The artificial leather and adhesives used in the factory probably contained tri-*o*-cresyl phosphate (Cavalleri and Cosi 1978). Other authors consider it likely that these effects were induced by tri-*o*-cresyl phosphate in combination with other organic solvents (Leveque 1985; WHO 1990).

4.2.2 Ingestion

The ingestion of tri-*o*-cresyl phosphate has led to a large number of poisonings worldwide. It is estimated that there have been more than 50 000 cases (ECETOC 1998; WHO 1990).

Approved medicines such as Jamaica Ginger Extract ("Jake") contained about 2% tri-*o*-cresyl phosphate as a flavour enhancer and as a substitute for ginger extract. These medicines were once consumed in large quantities because they had an alcohol content of about 80%. The first signs of poisoning were sensations of numbness and weakness in the hands and feet. As the disease progressed, this was followed by a loss of muscle control and the development of a specific gait which was known as the "Jake leg". The weakness in the muscles developed over several weeks and then persisted unchanged. Most of the persons who developed this syndrome suffered permanent nerve damage. It has been estimated that up to 50 000 persons living in the US in the 1930s were affected by this disease (ACGIH 2016; Bidstrup and Bonnell 1954). The syndrome described here has the same symptoms and progression as OPIDN.

In 1959, more than 10 000 cases of tri-*o*-cresyl phosphate poisoning occurred in Morocco. Aircraft engine oil had illegally been mixed into cooking oil. The disease in about 60% of the children was more severe than that that developed in adults; many of the children had marked, early-onset lesions along the pyramidal tract (Section 5.1; Inoue et al. 1988; König 1969; Smith and Spalding 1959). An analysis of the contaminated cooking oils found not only the *o*-isomers of tricresyl phosphate, but also xylenols and phenols (Henschler and Neumann 1967).

In Europe, a large number of cases described as "polyneuritis" occurred after the ingestion of specific apiol preparations containing tri-*o*-cresyl phosphate (Henschler 1958). The reported symptoms of polyneuritis are similar to those of OPIDN.

Eleven persons in Durban, South Africa, developed OPIDN. These persons had used drums to collect water that had previously been used to store tri-*o*-cresyl phosphate. In all of these cases, the cholinesterase activity remained at the same level as the control values (Susser and Stein 1957).

There are reports of the consumption of homemade schnapps containing tri-*o*-cresyl phosphate. The tri-*o*-cresyl phosphate had passed into the schnapps from Igelit tubes used during distillation. Five persons developed OPIDN. After a latency period of 2 to 3 weeks, flaccid motor paralysis was observed in the distal extremities (Parnitzke 1946). No data are available for the amounts ingested.

Persons who had consumed a "Stollen" (German cake) contaminated with 0.07% tri-*o*-cresyl phosphate (about 70 mg tri-*o*-cresyl phosphate/100 g Stollen) developed OPIDN (no other details; König 1969).

In 1941, 48 persons in Dresden developed symptoms after consuming foods contaminated with tri-*o*-cresyl phosphate. Two to 4 years later, follow-up examinations were carried out in 31 of these persons. Symptoms were no longer noticeable in 5 of the 31 persons. Effects that were still evident were: severe paralysis in 4 cases, slight paralysis in another 4 cases, clonus of the feet in 6 cases, an absent or weak Achilles tendon reflex in 7 and 5 cases, respectively, a loss of strength in the hands in 10 cases, the inability to walk on the heels in 14 cases, the inability to stand on the toes in 11 cases and atrophy of the muscles in the legs or hands in 10 and 8 cases, respectively. Also a number of other clinical findings were reported. Six of the 31 persons were unable to work 2 to 4 years later. A second follow-up study carried out after 20 years reported marked paraparesis of the legs in 11 of 32 persons. There were hardly any cases of muscle atrophy. The patients reported that they no longer perceived any further improvement in the complaints after an average of 2 to 8 years. The most severe effects were determined in patients who had been adolescents at the time of exposure to tri-*o*-cresyl phosphate (König 1969).

In several villages in China, the consumption of flour containing tri-*o*-cresyl phosphate induced OPIDN in 74 persons from 26 families. A follow-up study of 61 of these patients carried out 13 years later found that 35 patients did not have persisting symptoms, 23 persons were only able to move with "bilateral support" and 3 persons were completely reliant upon assistance from other persons. Moderate to severe sequelae were observed in 8 of formerly 32 persons in the group of adults and in 18 of 29 persons in the group of children/adolescents. The examination of 15 persons with sequelae revealed intact cranial nerve functions and normal cortical functions such as speech, articulation, memory, mental state and cognition. However, the examined persons had a spastic gait. No cholinergic effects were observed. Three of the persons had died by the time of the follow-up study (cerebral haemorrhage, lung cancer, depression – suicide) (Wang et al. 2009). The amount of tri-*o*-cresyl phosphate ingested by each individual was not specified.

4.2.3 Summary

In summary, a clear exposure–effect relationship cannot be derived from the reported cases. However, as a general guide, absorption of tri-*o*-cresyl phosphate in the gram range may lead to the induction of the complete range of paralysis symptoms. Most of the severe cases involved oral poisoning. Individual cases at the workplace were caused by absorption though the skin or exposure by inhalation to tri-*o*-cresyl phosphate concentrations in an order of magnitude of about 1 mg/m^3, often over long periods of time.

4.3 Local effects on skin and mucous membranes

Tri-*o*-cresyl phosphate applied to the skin on the back of the hands of 2 persons in amounts of 0.22 g and 0.11 g did not cause symptoms of irritation (ACGIH 2016; Hodge and Sterner 1943).

Tri-*o*-cresyl phosphate is not irritating to the skin (ACGIH 2016).

4.4 Allergenic effects

There are no data available.

There are only few clinical findings available for mixtures of tricresyl phosphates, free of *o*-isomers (see Hartwig and MAK Commission 2023); they are not regarded as reliable or sufficient to determine whether tricresyl phosphate or tricresyl phosphates have contact sensitizing potential.

4.5 Reproductive and developmental toxicity

There are no data available.

4.6 Genotoxicity

There are no data available.

4.7 Carcinogenicity

There are no data available.

5 Animal Experiments and in vitro Studies

Paralysis of the legs was observed in chickens, cats, dogs, sheep, monkeys, ferrets, cows and even in tigers treated with tri-*o*-cresyl phosphate (WHO 1990).

In studies with chickens and cats, the clinical manifestations induced by the mono-*o*-isomers and di-*o*-isomers (paresis and spastic paralysis) often differed from those induced by tri-*o*-cresyl phosphate poisoning (most commonly flaccid, peripheral paralysis) (Henschler 1958).

5.1 Acute toxicity

The symptoms observed in rodents after exposure to tri-*o*-cresyl phosphate were quite uncharacteristic. However, the clinical manifestations in cats and chickens were very similar to those in humans (Henschler 1958; Smith et al. 1932). Different strains of chicken demonstrated varying levels of sensitivity to tri-*o*-cresyl phosphate (de Oliveira et al. 2002). Rats reacted with less sensitivity to tri-*o*-cresyl phosphate, but effects on the immune system were observed (de Boer et al. 2015). Disorders of the digestive tract were observed in mice and rabbits, but only mild neurotoxic effects (Henschler 1958). Table 1 provides an overview of the sensitivity of different species to tri-*o*-cresyl phosphate neurotoxicity (ECETOC 1998).

Tab. 1 Overview of the sensitivity of different species following exposure to tri-*o*-cresyl phosphate (ECETOC 1998)

Species	OPIDN		Route of administration
	induced	not induced	
chicken	0.59–1.18 mg/kg body weight		oral
rat		2000–10 000 mg/kg body weight	oral, subcutaneous
rabbit		50–100 mg/kg body weight	oral
guinea pig		100 mg/kg body weight	oral
cat	0.59–0.89 mg/kg body weight		intramuscular
dog		0.47–4.7 mg/kg body weight	oral
dog	0.47–1.89 mg/kg body weight		subcutaneous
calf	200 mg/kg body weight		oral, intramuscular
rhesus monkey		4.18–17.7 mg/kg body weight	oral
rhesus monkey	0.59–1.18 mg/kg body weight		subcutaneous

5.1.1 Inhalation

There are no data available.

5.1.2 Oral administration

The LD_{50} values of tri-*o*-cresyl phosphate for different species are listed in Table 2.

Tab. 2 Tri-*o*-cresyl phosphate: LD_{50} values for different species (WHO 1990)

Species	LD_{50} (mg/kg body weight)
rat	8400
rat	1160
rabbit	3700
chicken	100–200
chicken	> 500

5.1.2.1 Chicken

Tri-o-cresyl phosphate

Groups of 7 hens (in 3 test series) were given tri-*o*-cresyl phosphate into the crop in single doses of 0.1, 0.2, 0.4, 0.5 or 0.8 ml/kg body weight in olive oil (about 118, 236, 472, 590 or 696 mg/kg body weight). The tri-*o*-cresyl phosphate had a purity of 98% (1.7% phenol, 0.5% *p*-isomer, 0 to 0.2% *m*-isomer). At 0.2 ml/kg body weight and above, a slight, but clearly noticeable, unsteadiness in the gait and weakness in the legs were observed (1 animal). Complete paralysis was observed in all animals at doses of 0.4 ml/kg body weight (about 472 mg/kg body weight) and above. Two animals died of progressive respiratory paralysis. Other studies were carried out with 3 different technical products containing *o*-isomers (reported as *o*-cresol) in percentages of 26.7%, 23.3% and 22.7%. A single dose of technical tricresyl phosphate of 0.01, 0.02, 0.05, 0.1, 0.2 or 0.4 ml/kg body weight (about 11.8, 24, 59, 118, 236 or 472 mg/kg body weight) given into the crop induced slight but evident unsteadiness in the gait in 2 of 4 animals of the low dose group. The weakness in the legs increased with the dose. One animal died of respiratory paralysis (1/10) after a dose of 0.05 ml/kg body weight (about 59 mg/kg body weight). Four of 8 animals died after a dose of 0.2 ml/kg body weight (about 236 mg/kg body weight). Using the same test method, exposure to technical tricresyl phosphates (doses: 0.25 to 5.0 ml/kg body weight; about 295 to 5900 mg/kg body weight) with a lower fraction of *o*-isomers (3.3%, 2.8%, 2.5%; 3 animals in each group) induced weakness in 1 animal (1/9) after administration of a TCP dose of 0.5 ml/kg body weight and no effects after administration of a dose of 0.25 ml/kg body weight. After doses of 0.8 ml/kg body weight and above, marked weakness and paralysis were observed in almost all of the animals (7/9). These effects increased with the dose. At 5 ml/kg body weight, all of the animals were completely paralysed. In all test series, the onset of the first signs varied between days 8 and 15 after substance administration (Henschler 1958). It is noteworthy that the technical product containing about 25% *o*-isomers induced much stronger neurotoxic effects than the pure tri-*o*-isomer.

Chickens were fed aircraft engine oils following incidents of mass poisoning in Morocco caused by cooking oils contaminated with aircraft engine oil. In order to compare toxicity levels, also tri-*o*-cresyl phosphate and a mono-*o*-tricresyl phosphate (isomer *o*-*m*-*p*) were administered. Groups of 2 Italian chickens were given a single dose of one of the substances in olive oil. The threshold doses that were just toxic enough to induce the first signs of paralysis were determined. The threshold dose of the aircraft engine oil containing a higher fraction of *o*-tricresyl phosphate (reported as *o*-cresol) (5% triaryl phosphate, 13% by weight *o*-cresol) was 1000 mg/kg body weight, which is equivalent to a triaryl phosphate dose of 50 mg/kg body weight. The threshold dose of the other aircraft engine oil (4% triaryl phosphate, 10% by weight *o*-cresol) was 4000 mg/kg body weight, which is equivalent to a triaryl phosphate dose of 160 mg/kg body weight. The LOAEL (lowest observed adverse effect level) for pure tri-*o*-cresyl phosphate was 400 mg/kg body weight. The mono-*o*-isomer (*o*-*m*-*p*) caused unsteadiness in gait at a dose as low as 25 mg/kg body weight (Henschler and Neumann 1967).

Chickens (group size not specified, 37 animals in total) were given a single gavage dose of tri-*o*-cresyl phosphate of 0, 50, 200 or 500 mg/kg body weight. After 21 days, ataxia and reduced body weights were observed only in the high

dose group; the effects became noticeable after days 12 to 15. After 24 and 48 hours, the NTE activity in the brain and spinal cord was markedly reduced in all dose groups in comparison with the levels of activity determined in the control group. The same was observed with respect to the pseudocholinesterase activity in the blood plasma. Neuropathy was observed in the distal tibial nerves, the medulla oblongata and the cerebellum; these effects were dependent on the dose. The occurrence of ataxia correlated with marked neuropathy of the PNS. Degeneration of the nerve fibres in the cerebellum was the most sensitive histological indicator of OPIDN (Classen et al. 1996).

Groups of 5 White Leghorn chickens were exposed once to 1 ml of corn oil containing tri-*o*-cresyl phosphate at doses of 0, 50, 90 or 500 mg/kg body weight (purity 98%). After 48 hours, the NTE activity in the brain was clearly inhibited with a 65% decrease in activity even in the low dose group. The AChE activity in the brain was not affected. At the two higher tri-*o*-cresyl phosphate doses, NTE inhibition in the brain increased with the dose, reaching levels of 83% and 93%. The inhibition of NTE in the spinal cord was slightly less pronounced at 40%, about 44% and about 84%, respectively. After 14 to 21 days, gait changes were observed in all treated animals, even those of the low dose group; this effect increased in severity with the dose. No mortality was observed (Ehrich et al. 1995).

Nine laying hens were given a single dose of tri-*o*-cresyl phosphate of 0.2 ml/kg body weight (about 236 mg/kg body weight) and 1 hen was given 0.1 ml/kg body weight (about 118 mg/kg body weight). Weakness ("first motor symptoms") was observed in all animals after 9 to 14 days. Signs of ataxia ("unable to stand") were noticeable in 5 animals from day 13 onwards (Beresford and Glees 1963).

The NOAELs (no observed adverse effect level) and LOAELs derived from single-exposure studies with chickens are shown in Table 3.

Tab. 3 NOAELs and LOAELs after single oral exposure of chickens

Substance	NOAEL	LOAEL	References
TOCP (purity 98%)	118 mg/kg body weight	236 mg/kg body weight	Henschler 1958
TCP containing about 25% *o*-isomers		11.8 mg/kg body weight	
TCP containing 2.5%–3.3% *o*-isomers	295 mg/kg body weight	590 mg/kg body weight	
pure TOCP		400 mg/kg body weight	Henschler and Neumann 1967
Aircraft engine oil B containing TAP		1000 mg/kg body weight (about 50 mg TAP/kg body weight, 13% *o*-isomers as *o*-cresol)	
Aircraft engine oil C containing TAP		4000 mg/kg body weight (about 160 mg TAP/kg body weight, 10% *o*-isomers as *o*-cresol)	
TOCP (purity 98%)		50 mg/kg body weight	Ehrich et al. 1995
TOCP (purity not specified)	200 mg/kg body weight	500 mg/kg body weight	Classen et al. 1996
TOCP (purity not specified)		118 mg/kg body weight	Beresford and Glees 1963

TAP: triaryl phosphate (phenyl, cresyl, xylyl); TCP: tricresyl phosphate; TOCP: tri-*o*-cresyl phosphate

In the studies below, very high doses were administered to investigate symptoms of paralysis.

In White Leghorn hens given a single dose of 500 mg/kg body weight, ataxia was observed after 13 days, which progressively worsened until the end of the observation period on day 21. NTE activity decreased in the brain by 90% and in the spinal cord by 96%. Lesions in the spinal cord, the sciatic nerve and the tibial nerve were observed (FMC Corp 1995).

In Isabrown Leghorn hens given a single gavage dose of tri-*o*-cresyl phosphate of 500 mg/kg body weight (28 animals), symptoms of OPIDN developed after 12 days (2/28) which intensified over a period of 28 days in a time-dependent manner (11/28). No symptoms were observed in the control group (4 animals) (see Section 2; Emerick et al. 2010).

Groups of 6 Isabrown Leghorn hens were given tri-*o*-cresyl phosphate doses of 0 or 500 mg/kg body weight. Three animals of each group were sacrificed after 24 hours and 3 after 21 days. After 11 days, 2 animals yielded a score for

ataxia of 2, and 1 animal a score of 4 (scale: 0–4). The symptoms were much weaker (1 animal asymptomatic, 2 animals with a score of 1) when the tri-*o*-cresyl phosphate dose was followed 18 hours later by the administration of the calcium channel inhibitor nimodipine at a dose of 1 mg/kg body weight (intramuscular) and then by calcium gluconate at a dose of 5 mg/kg body weight. After 24 hours, the NTE activity in the brain of chickens was inhibited by 80%; this inhibition was no longer observed after 21 days. However, after 24 hours, the AChE activity in the brain was inhibited by only about 20%. After 24 hours and 21 days, the increase in calpain levels in the brain was statistically significant at 40% and 20%, respectively. Axonal (Wallerian) degeneration was observed in the spinal cord after 21 days (Emerick et al. 2012).

Groups of 4 laying hens were given tri-*o*-cresyl phosphate doses of 0.5 or 1 ml/kg body weight (about 590 or 1180 mg/kg body weight). There were no acute symptoms, but weakness in the legs and an unsteady gait became noticeable after 9 to 12 days (Barnes and Denz 1953).

A single gavage dose of tri-*o*-cresyl phosphate of 0, 750 or 1000 mg/kg body weight given to Roman hens (6 animals per group) induced symptoms of OPIDN in 4 animals of the 750 mg/kg group and paralysis in all 6 animals of the high dose group after 21 days (no other details; Jiang et al. 2014).

A single dose of tri-*o*-cresyl phosphate of 750 mg/kg body weight given to 12 Leghorn chickens induced slight ataxia after 7 days, severe ataxia and a 10% loss in body weight after 15 days and paralysis after 21 days. Swollen axons with an aggregation of phosphorylated neurofilaments were observed in the sciatic nerve and in the tracts of the spinal cord (Jensen et al. 1992).

Groups of 8 adult Beijing laying hens were given a single tri-*o*-cresyl phosphate dose of 0 or 750 mg/kg body weight in gelatine capsules and observed for signs of OPIDN up to day 21. Neurotoxic effects were noted from day 7 onwards (score of 1 on a scale with a maximum of 8) and the animals developed complete paralysis (score of 7.8 on a scale with a maximum of 8) after 21 days. Two days after substance administration, the NTE activity in the animals treated with tri-*o*-cresyl phosphate had decreased to only 11% (no other details; Zhu et al. 2016).

Tri-*o*-cresyl phosphate given to adult laying hens at doses of 0, 0.8 or 1.0 ml/kg body weight (about 0, 994, 1180 mg/kg body weight) induced ataxia in all treated animals after 11 to 14 days (see Section 2; Morazain and Rosenberg 1970).

Laying hens (n = 24) given a single dose of tri-*o*-cresyl phosphate of 1 ml/kg body weight (about 1180 mg/kg body weight) developed an unsteady gait from day 10 onwards. In the further course, the paralysis in the legs grew progressively more severe and ataxia and weakness in the wing muscles were observed on day 21 (see Section 2; Earl and Thompson 1952).

A single dose of tri-*o*-cresyl phosphate of 50 mg/kg body weight (purity 99%) given to laying hens did not induce neurotoxic effects after 0.5, 1 or 5 days (Suwita and Abou-Donia 1990).

A comparison of different chicken strains revealed varying levels of sensitivity following exposure to single gavage doses of tri-*o*-cresyl phosphate. Half of the treated animals were sacrificed after 24 hours and the activities of a number of enzymes in the brain determined. The development of OPIDN was investigated over a period of 28 days. Under these test conditions, no signs of OPIDN were observed in 8 Babcock hens given doses of 800 mg/kg body weight. After 24 hours and 28 days, the NTE activities were determined to be 18% and 62%, respectively, the plasma cholinesterase levels were 60% and 95%, respectively, and the AChE levels were 100% and 85%, respectively. In Hy-line-w36 hens, a dose of 1600 mg/kg body weight resulted in comparable esterase activity and, again, no signs of OPIDN were observed in spite of the markedly higher dose. An unsteady gait (4/4; 9 to 13 days), paralysis (4/4) and total paralysis (2/4; 28 days) developed in 4 Isabrown hens at 1600 mg/kg body weight. The esterase activities of NTE were about 15% (24 hours) and about 70% (28 days). However, the cholinesterase activities did not differ markedly from those determined in the two other chicken strains. All enzyme activities were compared with the levels determined in the control groups that were made up of an equal number of chickens of the respective strain (de Oliveira et al. 2002).

A single tri-*o*-cresyl phosphate dose of 500 mg/kg body weight given to 10 male broilers induced atrophy of the testes. The authors regarded this as evidence that tri-*o*-cresyl phosphate induces sterility (Fathy and Bursian 1991), a conclusion that is supported by the findings of the long-term studies (see Section 5.5.1).

Mono-o-tricresyl phosphate and di-o-tricresyl phosphate isomers

Different *o*-tricresyl phosphate isomers in olive oil were given into the crop of hens (2 animals per group) in single doses of 0.025, 0.05, 0.1, 0.2 or 0.4 ml/kg body weight (about 29.5, 59, 118, 236 or 472 mg/kg body weight). The mono-*o*-isomers *o*-*m*-*p* and *o*-*m*-*m* and the technical product (26.7% *o*-isomers) caused an unsteady gait and weakness in the legs at the lowest dose tested of 0.025 ml/kg body weight. The effects intensified with the dose. Marked effects were induced by the di-*o*-isomers at 0.1 ml/kg body weight and by the tri-*o*-isomers at 0.4 ml/kg body weight. The *para*-isomers and *meta*-isomers were also investigated, but did not lead to any effects in hens at doses up to 2.5 ml/kg body weight. Using statistical probability, the author calculated the fractions of monoesters, diesters and triesters based on the total *o*-cresol levels in hydrolyzed TCP and then plotted a toxicity curve on the basis of their different levels of toxicity. An *o*-cresol fraction of 43% was found to have the highest possible level of toxicity; it was estimated to be 6 times more toxic than pure tri-*o*-cresyl phosphate. However, in actuality, its toxicity level was higher by a factor of 10. According to the author, the *ortho*-cresol isomer binds less readily during esterification than *meta*-cresol or *para*-cresol because of steric hindrance. This favours the formation of the more toxic mono-*o*-esters over the formation of di-*o*-cresyl esters and tri-*o*-cresyl esters. The technical cresols may also contain dimethyl phenols and ethyl phenols, which form the corresponding phosphates and are even more toxic than the mono-*o*-tricresyl ester (Henschler 1958).

When the mono-*o*-tricresyl phosphate isomer (*o*-*m*-*p*) was given to groups of 2 chickens in single oral doses of 0, 0.025, 0.05 or 0.1 ml/kg body weight (about 0, 29.5, 59, 118 mg/kg body weight), slight unsteadiness and weakness in the legs were observed in 1 animal of the low dose group after 14 days. The effects intensified with the dose. The most severe effects were observed in 1 animal of the high dose group; after 8 days, the animal exhibited severe weakness and its feet repeatedly buckled while walking (Henschler 1959).

Groups of 2 Italian chickens were given mono-o-tricresyl phosphate (*o*-*m*-*p*) in olive oil in a single dose of 25, 50 or 100 mg/kg body weight. After 12 days, unsteadiness in the gait was observed in 1 animal at the low dose of 25 mg/kg body weight. Weakness in the legs and repeated buckling of the feet while walking was observed at the dose of 50 mg/kg body weight, and full paralysis at 100 mg/kg body weight. The 2 animals of the high dose group died after 29 and 32 days (Henschler and Neumann 1967).

White Leghorn roosters given a single oral dose of different tricresyl phosphate isomers developed paralysis. The findings are shown in Table 4 (Hine et al. 1956).

Tab. 4 Paralysis in roosters given different TCP isomers (Hine et al. 1956)

Isomer	Dose (mg/kg body weight)	Paralysis/total number animals
TOCP pure	800	4/4
	400	4/4
	200	2/4
	100	4/4
	50	2/4
	25	1/4
TOCP 90%	800	3/3
	400	3/3
	200	1/2
o-*o*-*p*-TCP	400	4/4
	200	4/4
	100	3/4
	50	2/4
	25	2/4

Tab. 4 *(continued)*

Isomer	Dose (mg/kg body weight)	Paralysis/total number animals
o-p-p-TCP	200	4/4
	100	4/4
	50	1/4
	25	0/4
m-m-m-TCP	5000	0/2
p-p-p-TCP	5000	0/2

TOCP: tri-*o*-cresyl phosphate

Hens were given a single dose of differently substituted phenyl phosphates and observed for 21 days. The test substances were dissolved in arachis oil. The individual doses and findings of neurotoxicity are shown in Table 5. In all cases, ataxia was first observed after 14 days. A histopathological examination was not performed (Aldridge and Barnes 1961).

Tab. 5 Neurotoxicity of differently substituted triphenyl phosphates (Aldridge and Barnes 1961)

TCP isomer in arachis oil	Dose (mg/kg body weight)	Neurotoxicity
control (arachis oil)	500	0/4
TOCP	200–250	4/6
	100	0/9
o-o-m-TCP	250	2/2
	50	0/2
o-p-p-TCP	250	2/2
	50	3/4
m-m-m-TCP	2000	0/2
p-p-p-TCP	500 (15 times)	0/2

TOCP: tri-*o*-cresyl phosphate

The study confirms the order of toxicity given by Henschler (1958): mono-*o*-isomers > di-*o*-isomers > tri-*o*-cresyl phosphate.

5.1.2.2 Other species

Groups of 5 cats were given a single dose of tricresyl phosphate containing 26.7% *o*-cresol or the same amount of a commercial TCP product containing 3.3% *o*-cresol. In each case, the substance was dissolved in olive oil in a 1:1 ratio. Technical TCP containing the high *o*-cresol fraction was assumed to have a relative toxicity of 100%. The 3.3% *o*-cresol product had a relative toxicity of 3% in cats, which is similar to the degree of toxicity determined in chickens (no other details; Henschler and Bayer 1958).

A single tri-*o*-cresyl phosphate dose of 2000 mg/kg body weight led in Long Evans rats to the almost complete inhibition of brain NTE (around 93.4%) and cholinesterase (around 98%) in blood serum after 24 hours. Formulations with a mixture of isomers of tricresyl phosphate produced by different companies inhibited brain NTE by 0 to 78% at a dose level of 2000 mg/kg body weight (Mackerer et al. 1999).

Male Long Evans rats were given single doses of tri-*o*-cresyl phosphate of 0, 300, 600 or 1000 mg/kg body weight. After 48 hours, this led to the inhibition of NTE and AChE in the brain by 37% and 0%, 57% and 12%, and 88% and 78%, respectively. The effects in the spinal cord were more severe than those in the brain with a 47%, 67% and 85% inhibition of NTE and a 54%, 74% and 81% inhibition of AChE. In comparison with the control group, changes in gait were observed

only at dose levels of 600 mg/kg body weight and above after 14 to 21 days. Over the course of the study, 1 animal treated with 600 mg/kg body weight and 3 animals treated with 1000 mg/kg body weight died (Ehrich et al. 1995).

In mice given 2 doses of 1000 mg/kg body weight, no adverse effects were observed after 270 days. In comparison with the controls (untreated animals), no changes were determined in the activities of NTE and AChE (see also Section 5.2.2; Lapadula et al. 1985).

A single oral dose of tri-*o*-cresyl phosphate of 100 to 1600 mg/kg body weight given to swine (Yorkshire) caused only minimal acute symptoms; however, severe neuropathy developed within 15 days. A subcutaneous injection of 800 mg/kg body weight induced the same symptoms (Wilson et al. 1982).

The acute symptoms observed in ewes given oral doses of tri-*o*-cresyl phosphate of 100, 200 or 400 mg/kg body weight were diarrhoea, dehydration and metabolic acidosis. After 6 days, almost all animals had died or had developed ataxia. Also a subcutaneous injection of 40 or 80 mg/kg body weight (5 to 8 doses) was either lethal to the animals or induced neurotoxicosis (Wilson et al. 1982).

Mild ataxia developed in European ferrets given a single oral dose of tri-*o*-cresyl phosphate of 1000 mg/kg body weight. Neurologic effects were not observed at doses of 0, 25 or 500 mg/kg body weight, even after 54 days (WHO 1990).

5.1.3 Dermal application

Within 1 day, undiluted tri-*o*-cresyl phosphate applied twice to the comb of 32 adult chickens (male and female) at a dose of 0.1 ml/kg body weight (about 118 mg/kg body weight) induced the paralysis of the leg muscles typical for tri-*o*-cresyl phosphate with degeneration of myelin and axons in the spinal cord. Tri-*o*-cresyl phosphate led to slight degeneration of the nerve fibres in the anterior medial tract of the cord even at the low dose of 0.05 ml/kg body weight (about 59 mg/kg body weight) (Glees and White 1961).

After the application of tri-*o*-cresyl phosphate (purity 99%) to 15 cm^2 of skin on the back of the neck of adult male cats (3 animals per group) at doses of 0, 100, 250, 500, 1000, 1500 or 2000 mg/kg body weight, followed by 1 to 3 injections of 0.8 mg atropine sulfate and 1000 mg pyridine-2-aldoxime methyl chloride (only given to animals in the 1000 mg/kg or higher dose groups), neurotoxic symptoms were observed at doses of 250 mg/kg body weight and above. Clinical symptoms were weakness of the forelegs and, more markedly, of the hindlimbs, ataxia and paralysis. All effects correlated strongly with the amount applied and the length of the observation period. Histopathological changes in the spinal cord and unusual electromyographic findings were observed at doses of 250 mg/kg body weight and above and histopathological changes in the PNS at doses of 1000 mg/kg body weight and above. Other acute effects found at doses of 1000 mg/kg body weight and above were general weakness, increased salivation, contraction of the pupils, diarrhoea, vomiting and weight loss. Two of 3 animals of the 2000 mg/kg body weight dose group died 6 and 7 days after treatment (Abou-Donia et al. 1986).

OPIDN developed in European ferrets after dermal application of tri-*o*-cresyl phosphate at doses of 250, 500 or 1000 mg/kg body weight. The severity of the effects of paralysis, ataxia or the varying degrees of weakness of the hindlimbs was dose-dependent. Axonal degeneration was observed at a dose of 1000 mg/kg body weight. The NTE activity in the brain was inhibited by 46%. The authors concluded that, in ferrets, dermal application induces stronger effects than oral exposure (see Section 5.1.2; WHO 1990).

5.1.4 Intramuscular and subcutaneous injection

After intramuscular injection of tri-*o*-cresyl phosphate in cats (1 animal/dose), no effects were induced at doses of 0.05 and 0.1 ml/kg body weight (about 118 mg/kg body weight), severe weakness at doses of 0.2 ml/kg body weight (about 236 mg/kg body weight) and above and death at a dose of 0.3 ml/kg body weight (about 354 mg/kg body weight). Initial effects were induced by TCP mixtures containing high fractions of *o*-cresol (26.7%) at doses as low as 0.01 ml/kg body weight (11.8 mg/kg body weight) and above and by mixtures containing low fractions of *o*-cresol (3.3%) at 0.5 ml/kg body weight (about 590 mg/kg body weight) and above (Henschler 1958).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

There are no studies available that investigated inhalation exposure to pure tri-*o*-cresyl phosphate.

Repeated exposure to a mixture of tricresyl phosphates, trixylyl phosphates and other trialkyl esters containing less than 1.5% tri-*o*-cresyl phosphate caused neurotoxic effects in hens after 90 days at concentrations of 23 mg/m^3 and above and in rabbits at concentrations of 102 mg/m^3 and above. Dogs, monkeys and rats did not exhibit any symptoms up to a concentration of 110 mg/m^3. In hens, but not in the other species, neurotoxicity was observed after 30 exposures (8 hours/day, 5 days/week) to 50 mg/m^3 (Craig and Barth 1999; Siegel et al. 1965).

5.2.2 Oral administration

5.2.2.1 Chicken

The chicken is considered the best animal model for the investigation of neurotoxic effects (Roberts et al. 1983).

Detailed data for the studies described below are shown in Table 6.

Tab. 6 Effects of tri-*o*-cresyl phosphate or *o*-TCP after repeated oral exposure of chickens

Species, strain, number per group	Exposure	Findings	References
chicken, Italian, groups of 2 ♀	**2–6 days**, 0.15 ml *o*-TCP/kg body weight and day (about 177 mg/kg body weight, with 3.3% *o*-cresol in the aromatic fraction)	**177 mg/kg body weight**: weakness in the legs: 2 days: slight (only 1 animal), 3 days: severe (2 animals), 4 days: severe (1 animal), paralysis (1 animal), from day 5 onwards: complete paralysis	Henschler 1959
chicken, Carey's Nick 320 Leghorn, groups of 4 ♀	**5 days**, 0, 60 mg TOCP/kg body weight and day (2 g aircraft engine oil or corn oil containing 0 or 3% TOCP/kg body weight and day)	**60 mg/kg body weight (aircraft engine oil)**: brain NTE activity ↓* (by 80%); **60 mg/kg body weight (corn oil)**: brain NTE activity ↓* (by 87%)	Mackerer et al. 1999
	observation period: 30 days	**60 mg/kg body weight (aircraft engine oil)**: ataxia (2/4); **60 mg/kg body weight (corn oil)**: ataxia (2/4)	
	0, 0.24 mg TOCP/kg body weight and day (2 g aircraft engine oil with 0 or 3% TCP isomer mixture/kg body weight and day, TCP with 0.4% TOCP), gavage	**0.24 mg TOCP/kg body weight**: brain NTE activity ↓* (by 32%)	
	observation period: 30 days	**0.24 mg TOCP/kg body weight**: **no ataxia**	
chicken, Leghorn, 10 ♂	**18 days**, 0, 100 mg TOCP/kg body weight and day	**100 mg/kg body weight:** 7–10 days: paralysis of the legs (OPIDN), 18 days: brain NTE activity ↓* (by 88%), AChE activity ↓* (by 30%), plasma: ChE activity ↓* (by 72%), testes: NTE activity ↓* (by 86%)	Somkuti et al. 1987 b

Tab. 6 *(continued)*

Species, strain, number per group	Exposure	Findings	References
chicken, no data	**4 weeks**, 0, 0.25, 0.5, 0.75, 1 ml TOCP/kg body weight and day (about 0, 30, 590, 890, 1180 mg/kg body weight), administered into the oesophagus	**30 mg/kg body weight**: minimal neurotoxic findings (no other data); **1180 mg/kg body weight**: from day 8 onwards: body weights ↓, unsteady gait, from day 14 onwards: paralysis of the legs, from day 15 onwards: mortality ↑	Cavanagh 1954
chicken, Italian, groups of 2 ♀	**5 weeks**, 5, 10 mg TAP/kg body weight with an *o*-cresol fraction of 13% by weight in the TAP (100, 200 mg aircraft engine oil B/kg body weight and day)	**5 mg TAP (13% *o*-cresol fraction)/kg body weight**: weakness with a latency period of 37 or 41 days; **10 mg TAP (13% o-cresol fraction)/kg body weight**: complete paralysis with a latency period of 14 or 17 days	Henschler and Neumann 1967
	5 weeks, 4, 8 mg TAP/kg body weight with an *o*-cresol fraction of 10% by weight in the TAP (100, 200 mg aircraft engine oil C/kg body weight and day)	**4 mg TAP (10% *o*-cresol fraction)/kg body weight: NOAEL**; **8 mg TAP (10% *o*-cresol fraction)/kg body weight**: weakness with a latency period of 35 or 39 days	
chicken, White Leghorn, groups of 13–15 ♀	**6, 8, 10 weeks**, 0, 0.04, 0.08 mg TOCP/kg body weight and day (2 g aircraft engine oil with 0, 0.5% or 1% TCP isomer mixture/kg body weight and day, TCP including 0.4% TOCP)	**0.04 mg/kg body weight**: enzyme activities: plasma ChE ↓* (by 29.4%), brain ChE ↓ (by 13.9%), brain NTE activity ↓* (by 29.9%), NTE activity in the spinal cord ↓* (by 46.4%); **0.08 mg/kg body weight**: enzyme activities: plasma ChE ↓* (by 28.8%), brain ChE ↓ (by 11.1%), brain NTE activity ↓* (by 39.9%), NTE activity in the spinal cord ↓* (by 52.6%), **no** ataxia, no time dependency	Mackerer et al. 1999
chicken, White Leghorn, groups of 30 ♀, groups of 5 of these animals examined after weeks 6, 7 and 8	**10 weeks**, 0, 10 mg TOCP/kg body weight and day (2 g aircraft engine oil with 0 or 0.5% TOCP/kg body weight and day), gavage, 5 days/week	**10 mg/kg body weight**: 6 weeks: axonal degeneration (1/5), 10 weeks: axonal degeneration (3/10), enzyme activities: plasma ChE ↓* (by 41%), brain NTE activity ↓* (by 68%), NTE activity in the spinal cord ↓* (by 52%)	Freudenthal et al. 1993; Mackerer et al. 1999
	0, 10 mg TOCP/kg body weight and day (2 g **corn oil** with 0 or 0.5% TOCP/kg body weight and day), gavage, 5 days/week	**10 mg/kg body weight**: 6 weeks: axonal degeneration (5/5), 10 weeks: axonal degeneration (7/10), body weights ↓*, ataxia (3/30, from week 7 onwards), atrophy of the skeletal muscles (1/30), enzyme activities: plasma ChE ↓* (by 44%), brain NTE activity ↓* (by 71%), NTE activity in the spinal cord ↓* (by 55%)	
	0, 0.24 mg TOCP/kg body weight and day (2 g aircraft engine oil with 0 or 3% TCP isomer mixture/kg body weight and day, TCP including 0.4% TOCP), gavage, 5 days/week	**0.24 mg/kg body weight**: 6 weeks: axonal degeneration (4/5), 10 weeks: axonal degeneration (10/11), 4 animals died, body weights ↓*, ataxia (22/30, from week 3 onwards), atrophy of the skeletal muscles (2/30), enzyme activities: plasma ChE ↓* (by 49%), brain NTE activity ↓* (by 77%), NTE activity in the spinal cord ↓* (by 62%)	
chicken, groups of 5 ♀, no data	**13 weeks**, 0, 10 mg TOCP/kg body weight and day, 7 days/week	**10 mg/kg body weight**: delayed onset neuropathy, ataxia	Abou-Donia et al. 1980

Tab. 6 *(continued)*

Species, strain, number per group	Exposure	Findings	References
chicken, White Leghorn, groups of 17–20 ♀	**13 weeks**, 0, 7.5 mg TOCP/kg body weight and day and a single dose of 500 mg TOCP/kg body weight on day 76 (in corn oil), 5 days/week	**7.5 mg/kg body weight**: OPIDN from day 39 onwards, 6 weeks: brain NTE activity ↓* (by 50%), NTE activity in the spinal cord ↓* (by 43%), 13 weeks: brain NTE activity ↓* (by 76%), NTE activity in the spinal cord ↓* (by 50%)	Daughtrey et al. 1996
	about 0, 0.15, 0.3 mg *o*-TCP/kg body weight (1 g aircraft engine oil with 3% TCP isomer mixture/kg body weight and day, TCP including < 0.5% or 1% *o*-isomers), 5 days/week	**0.15 mg *o*-TCP/kg body weight**: brain NTE activity ↓* (by 32%); **0.3 mg *o*-TCP/kg body weight**: brain NTE activity ↓* (by 34%), **no** signs of OPIDN	
chicken, "heavy hybrids", groups of 12 ♀	**13 weeks**, 0, 1, 1.25, 2.5, 5, 7.5, 10, 20 mg/kg body weight, TOCP (purity 97%) in corn oil, gavage, 7 days/week	**1.25 mg/kg body weight**: **NOAEL**; **2.5 mg/kg body weight and above**: degeneration CNS and PNS; **5 mg/kg body weight and above**: ataxia (from day 28 onwards)	Prentice and Majeed 1983; Roberts et al. 1983

*$p < 0.05$; AChE: acetylcholinesterase; ChE: cholinesterase; CNS: central nervous system; NTE: neurotoxic esterase; OPIDN: organophosphate-induced delayed neuropathy; *o*-TCP: *o*-isomers of tricresyl phosphate; PNS: peripheral nervous system; TAP: triaryl phosphate; TCP: tricresyl phosphate; TOCP: tri-*o*-cresyl phosphate

A daily *o*-tricresyl phosphate dose of 0.15 ml/kg body weight (about 177 mg/kg body weight, with 3.3% *o*-cresol in the aromatic fraction) given to 2 chickens (Italian) led to slight unsteadiness when standing and walking even as early as after the second dose. After 4 doses, 1 animal was completely paralysed. The animals died 19 to 20 days after the beginning of treatment after receiving 3 to 6 doses (Henschler 1959).

Groups of 4 Carey's Nick 320 Leghorn hens were given phosphate esters in oils for 5 days at a dose of 60 mg/kg body weight and day. Aircraft engine oil containing 3% TCP (about 0.24 mg tri-*o*-cresyl phosphate/kg body weight; probably not tri-*o*-cresyl phosphate but *o*-TCP, see Section 6), aircraft engine oil containing 3% tri-*o*-cresyl phosphate or corn oil containing 3% tri-*o*-cresyl phosphate inhibited brain NTE by 32%, 80% and 67%, respectively, compared with the values in the controls exposed only to corn oil. During the 30-day observation period, ataxia developed in 2 of the 4 animals in each group that were given the mixtures containing 3% tri-*o*-cresyl phosphate. The TCP was made up of two thirds tricresyl phosphate and one third triethyl phosphate or trixylyl phosphate (Mackerer et al. 1999).

Tri-*o*-cresyl phosphate given to 10 adult roosters for 18 days in gavage doses of 0 or 100 mg/kg body weight induced paralysis of the legs and decreasing esterase activities in the testes and brains of the treated animals (see Section 5.5.1; Somkuti et al. 1987 b).

After tri-*o*-cresyl phosphate was given into the oesophagus of chickens for 4 weeks, neurotoxic symptoms were observed from day 8 onwards (no other details) at the dose of 1 ml/kg body weight and day (about 1180 mg/kg body weight). No effects were noticeable at lower doses (Cavanagh 1954).

After cases of poisoning in humans, the 3 aircraft engine oils detected in the cooking oils they had used were given to groups of 2 Italian chickens in formulations with olive oil every day for 5 weeks. Oil B contained about 5% triaryl phosphate (TAP) with an *o*-cresol fraction of 13% by weight and Oil C about 4% TAP with an *o*-cresol fraction of 10% by weight. Oil A did not contain triaryl phosphates. Weakness in the legs was observed at TAP doses of 5 mg/kg body weight (*o*-cresol fraction: 13% by weight) and above with a latency period of 37 or 41 days. TAP doses of 10 mg/kg body weight (*o*-cresol fraction: 13% by weight) caused complete paralysis after a latency period of 14 or 17 days. Weaker effects were induced by the aircraft engine oil C containing about 4% TAP (*o*-cresol fraction: 10% by weight) (Henschler and Neumann 1967). The fractions of mono-*o*-aryl phosphates, di-*o*-aryl phosphates and tri-*o*-aryl phosphates contained in the oils were not analysed.

Aircraft engine oil with different contents of a TCP isomer mixture were given to 13 to 15 White Leghorn hens at an oral dose of 2000 mg/kg body weight over periods of 6, 8 or 10 weeks. The TCP isomer mixture contained 0.4% tri-*o*-cresyl phosphate. The aircraft engine oil was formulated with 0.5% TCP (equivalent to a tri-*o*-cresyl phosphate dose of 0.04 mg/kg body weight), 1.0% TCP (equivalent to a tri-*o*-cresyl phosphate dose of 0.08 mg/kg body weight), 3% TCP (equivalent to a tri-*o*-cresyl phosphate dose of 0.24 mg/kg body weight) or 0.5% tri-*o*-cresyl phosphate (pure). Another group of hens was given corn oil containing 0.5% tri-*o*-cresyl phosphate. Plasma cholinesterase activity and, to a greater extent, NTE activity in the brain and spinal cord were inhibited in all animals. However, the changes in the activity of the enzymes were not statistically significant after 6, 8 or 10 weeks. The control animals were given TCP-free aircraft engine oil at a dose of 2000 mg/kg body weight (Mackerer et al. 1999). Some of the findings of this study were also included in the study report published by Freudenthal et al. (1993).

In a 10-week gavage study, groups of 30 White Leghorn hens were given aircraft engine oil or corn oil, each containing 0.5% tri-*o*-cresyl phosphate (10 mg tri-*o*-cresyl phosphate/kg body weight), at a dose of 2000 mg/kg body weight and day on 5 days a week. Another group was given aircraft engine oil with 3% of a TCP isomer mixture (60 mg TCP/kg body weight) at a dose of 2000 mg/kg body weight and day. The isomer mixture contained 0.4% tri-*o*-cresyl phosphate. The hens in this group were thus treated with a tri-*o*-cresyl phosphate dose of 0.24 mg/kg body weight and day. A control group was given 2000 mg/kg body weight of aircraft engine oil that did not contain tri-*o*-cresyl phosphate and another control group did not receive treatment. The clinical and histopathological findings are shown in Table 6. The effects observed in the animals treated with aircraft engine oil containing a tri-*o*-cresyl phosphate dose of 0.24 mg/kg body weight and day (from the formulation with the isomer mixture) were more severe than the effects induced in the animals treated with aircraft engine oil containing a dose of (pure) tri-*o*-cresyl phosphate of 10 mg/kg body weight and day. This applies to the incidence of ataxia and the severity of the axonal degeneration in the lumbar spinal cord. Ataxia developed after treatment with 0.5% tri-*o*-cresyl phosphate in corn oil, but not after treatment with aircraft engine oil containing 0.5% tri-*o*-cresyl phosphate (pure). The incidence and severity of the effects induced were found to be stronger in the animals that ingested tri-*o*-cresyl phosphate (in the formulation with the isomer mixture) in aircraft engine oil at a dose of 0.24 mg/kg body weight and day than in the animals given a tri-*o*-cresyl phosphate dose of 10 mg/kg body weight and day in corn oil. On the basis of these findings, the authors concluded that other substances contained in the TCP isomer mixture had neurotoxic effects. Corn oil is absorbed almost completely, while aircraft engine oil is eliminated almost completely. This suggests that the hydrophobic tri-*o*-cresyl phosphate in the aircraft engine oil was not fully bioavailable (Freudenthal et al. 1993). The TCP administered in the study was made up of two thirds tricresyl phosphate and one third triethyl or trixylyl phosphate (Mackerer et al. 1999). A MAK value cannot be derived from this study because the TCP used for treatment contained trixylyl and triethyl phosphate isomers.

Delayed neuropathy was observed in hens given tri-*o*-cresyl phosphate doses of 10 mg/kg body weight for 13 weeks (Abou-Donia et al. 1980).

Groups of 17 to 20 adult White Leghorn hens were given synthetic aircraft engine oil (hydrocarbon polyolester with additives) containing 3% TCP in an oral dose of 1000 mg/kg body weight on 5 days a week for 13 weeks. The TCP was acquired commercially from two different companies and contained *o*-isomer levels of < 0.5% and < 1%, respectively. The positive control group was given tri-*o*-cresyl phosphate in corn oil (purity 98%) for 13 weeks at a dose of 7.5 mg/kg body weight in addition to a single high dose of 500 mg/kg body weight 12 days before the end of the study. The control group was treated with a saline solution. A total of 12 chickens died before the end of the study; the test substance was not considered the cause of death. A comparison of the body weights of the treated animals and those of the control animals did not reveal statistically significant differences over the course of the study. Signs of OPIDN were observed from day 39 of the study onwards only in the animals of the positive control group treated with tri-*o*-cresyl phosphate. While the level of inhibition of NTE activity in the brain was statistically significant in all treated animals after 13 weeks, the inhibition of AChE in the brain and spinal cord did not reach statistically significant levels in any of the animals. On the basis of these findings, the authors concluded that the inhibition of NTE by about 30% is not sufficient to induce functional neurotoxic effects (Daughtrey et al. 1996).

In a 90-day study, groups of 12 hens were given daily oral doses of tri-*o*-cresyl phosphate ranging from 0 to 20 mg/kg body weight. Histopathological effects were determined at doses of 2.5 mg/kg body weight and above and clinical

symptoms at doses of 5 mg/kg body weight and above. The substance had a purity of 97% (Prentice and Majeed 1983; Roberts et al. 1983). A NOAEL of 1.25 mg/kg body weight has been derived from this study.

Two inadequately described studies in chickens with the administration of tri-*o*-cresyl phosphate either in daily doses of 5 or 10 mg/kg body weight (12 or 26 doses) or in a concentration of 400 mg/kg diet with the feed for 21 days reported severe ataxia and nerve damage, respectively. No effects were observed when tri-*o*-cresyl phosphate was administered at lower doses and for longer periods of exposure (76 doses at 2.5 mg/kg body weight; 146 doses at 1.3 mg/kg body weight; administration of tri-*o*-cresyl phosphate for 140 days with the feed at 100 mg/kg diet) (Barnes 1975).

A comparison of the two studies (Table 7) in which hens were given aircraft engine oil with TCP containing tri-*o*-cresyl phosphate in amounts of 0.5% to 1% found that only strong inhibition of NTE correlated with axonal degeneration.

Tab. 7 Comparison of the toxicity induced in chickens given oral doses of the o-isomers of tricresyl phosphate in aircraft engine oil for 10 and 13 weeks

Substance	Administration	Dose	Effects	References
aircraft engine oil with 3% TCP isomer mixture containing 0.4% TOCP	2 g aircraft engine oil/kg body weight, 10 weeks	0.24 mg/kg body weight	axonal degeneration 10/11 animals (77% NTE inhibition)	Freudenthal et al. 1993; Mackerer et al. 1999
aircraft engine oil with 3% TCP isomer mixture containing < 0.5% *o*-TCP	1 g aircraft engine oil/kg body weight, 13 weeks	< 0.15 mg/kg body weight	no neurotoxicity (32% NTE inhibition)	Daughtrey et al. 1996
aircraft engine oil with 3% TCP isomer mixture containing < 1.0% *o*-TCP	1 g aircraft engine oil/kg body weight, 13 weeks	< 0.3 mg/kg body weight	no neurotoxicity (34% NTE inhibition)	

o-TCP: *o*-isomers of tricresyl phosphate; TOCP: tri-*o*-cresyl phosphate

Summary: A comparison of oral dose studies with exposure of chickens to TCP (isomer mixture) in aircraft engine oil containing tri-*o*-cresyl phosphate at doses of 0.04, 0.08 and 0.24 mg/kg body weight found a dose-dependent, cumulative intensification of the effects on the nerves (Freudenthal et al. 1993; Mackerer et al. 1999).

The TCP isomer mixture containing 0.24 mg tri-*o*-cresyl phosphate was found to be more potent than pure tri-*o*-cresyl phosphate (10 mg/kg body weight). More animals developed ataxia and, even though the level of NTE inhibition was about the same after 10 weeks, the effects were observed earlier (Freudenthal et al. 1993). This shows that the isomer mixture contains other, more highly toxic *o*-isomers or *o*-aryl phosphates.

5.2.2.2 Sheep

Tri-*o*-cresyl phosphate given to groups of 4 male Suffolk sheep in oral doses of 0 or 5 mg/kg body weight and day in corn oil for 6 months induced mild leg weakness in 1 of the treated animals and lameness in another animal at the end of the 6 months. No clinical symptoms were observed in the 2 other animals. All animals were sacrificed 24 hours after the last treatment. The histopathological examination found slight axonal degenerative lesions in the brain and spinal cord of 3 animals, but no changes in the sciatic nerve tissue. In the treated animals, the cholinesterase activity in the plasma was inhibited to about 70% of the normal level and the AChE activity in the brain and spinal cord to about 50%. The NTE activity in the brain and spinal cord was reduced to about 20% in the two symptomatic animals, while the NTE activities in the asymptomatic animals were 35% to 50% (Soliman et al. 1983).

5.2.2.3 Rat

For 5 days, groups of 5 Long Evans rats were given 2 g/kg body weight and day of either aircraft engine oil containing 60 mg of phosphate esters (3% TCP or tri-*o*-cresyl phosphate) or corn oil containing 3% tri-*o*-cresyl phosphate. Aircraft engine oil containing 3% TCP or 3% tri-*o*-cresyl phosphate and corn oil containing 3% tri-*o*-cresyl phosphate inhibited brain NTE activity by 5%, 11% and 22%, respectively, in comparison with the level of activity in a control group given only corn oil. The animals did not develop ataxia. The TCP test product was found to have only one third of the

potency of tri-*o*-cresyl phosphate. The TCP used in the test contained two thirds tricresyl phosphate and one third triethyl or trixylyl phosphate (Mackerer et al. 1999).

When another organophosphate (chlorpyrifos) was concurrently administered or the animals were stressed (corticosteroid-mediated), the administration of 14 tri-*o*-cresyl phosphate doses of 0, 75, 150 or 300 mg/kg body weight to male Long Evans rats over a period of 63 days resulted in a higher incidence of nerve damage (axonal and myelin degeneration). After administration of 7 tri-*o*-cresyl phosphate doses of 75, 150 or 300 mg/kg body weight over a period of 14 days, lower levels of AChE were determined in the erythrocytes and different regions of the brain and the NTE and carboxylesterase activities were inhibited (ACGIH 2016; Sjögren et al. 2010).

A daily tri-*o*-cresyl phosphate dose of 150 mg/kg body weight given by gavage to male Fischer 344 rats (8 animals per group) over a period of 21 days caused irreversible testicular lesions. A decrease in the activities of NTE and other non-specific esterases in the testicular tissue was observed. The beta-glucuronidase activity was unchanged. No neurotoxic effects were noted during the 98-day observation period. Acute cholinergic toxicity with lacrimation and diarrhoea was observed in 30% of the animals during the first 5 days of treatment. No unusual findings were noted in other organs (see Section 5.5.1; Somkuti et al. 1987 c).

Male Fischer 344 rats were given daily gavage doses of tri-*o*-cresyl phosphate of 0, 10, 25, 50, 75 or 100 mg/kg body weight for 63 days. Histopathological changes (structures that can be stained with periodic acid-Schiff reagent, immature germ cells and multinucleated giant cells in the lumen) in the testicular tissue were observed at doses of 25 mg/kg body weight and above. Tests for neurological changes in behaviour such as the grip strength in the forelegs and hindlimbs, motor activity, tremor or delayed reactions to thermal stimuli did not reveal any effects. In addition, there were no lesions in the nerve tissue. By contrast, ataxia and paralysis developed in the group of chickens included as the positive control group and given daily tri-*o*-cresyl phosphate doses of 100 mg/kg body weight in corn oil (see Section 5.5.1; Somkuti et al. 1987 a, 1988).

In Long Evans rats, gavage doses of tri-*o*-cresyl phosphate of 1160 mg/kg body weight (every 2 weeks, 12 doses in total over 24 weeks, 70 animals) or daily doses of 116 mg/kg body weight (5 days per week, 40 animals) over a period of 24 weeks led to marked lesions in the spinal cord and in the peripheral nervous system. In spite of the severe neurological effects, hardly any functional impairment, such as ataxia, was observed. The tri-*o*-cresyl phosphate had a purity of 97%. A control group (n = 30) was treated with corn oil (ECETOC 1998; Veronesi 1984).

Changes in the course of the immune response were observed in rats given daily doses of tri-*o*-cresyl phosphate of about 10 mg/kg body weight for 6 months. A concurrent injection of the tetanus toxoid reduced the number of leukocytes and decreased macrophage migration (ACGIH 2016).

Oral doses of an isomer mixture containing 79% tricresyl phosphate ester (< 0.1% tri-*o*-cresyl phosphate) and 18% dicresyl phosphate ester given to rats reduced the grip strength of the hindlimbs and inhibited serum cholinesterase at doses of 13 mg/kg body weight and above (NTP 1994).

5.2.2.4 Mouse

Male Kunming mice were given tri-*o*-cresyl phosphate in gavage doses of 0, 100, 200 or 400 mg/kg body weight and day for 28 days. The alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST) and the malondialdehyde concentration were increased and the glutathione concentration, superoxide dismutase activity and glutathione peroxidase activity were decreased. The authors concluded that lesions were caused by oxidative stress induced in the liver by tri-*o*-cresyl phosphate (Xu et al. 2016). The study report does not provide information on group sizes.

Groups of 5 ICR mice were given tri-*o*-cresyl phosphate in gavage doses of 0 or 225 mg/kg body weight and day for 270 days. In the treated animals, the body weights were reduced and muscle weakness, ataxia and paralysis of the distal extremities were observed. The NTE and AChE activities in the brain were only 35% and 10%, respectively, of the levels determined in the control animals; the butyrylcholinesterase activity was 12%. A statistically significant

increase in liver enzyme activity was determined. The histopathological examination found degeneration of the axons and myelin in the spinal cord and sciatic nerve (Lapadula et al. 1985).

After tri-*o*-cresyl phosphate was given to male mice in gavage doses of 0, 5, 50 or 500 mg/kg body weight once a week for 13 weeks, the immunoglobulin levels remained unchanged. Lymphocyte proliferation was reduced, but without dose dependency (Sjögren et al. 2010).

In several studies with oral doses of a formulation with a mixture of isomers (79% tricresyl phosphate ester (< 0.1% tri-*o*-cresyl phosphate) and 18% dicresyl phosphate ester) given to mice, the grip strength of the hindlimbs was reduced and serum cholinesterase was inhibited (NTP 1994).

5.2.2.5 Rabbit

No signs of paralysis were observed after continuous exposure of rabbits to a tri-*o*-cresyl phosphate dose of 50 mg/kg body weight and day (no other details; Barnes and Denz 1953).

5.2.3 Dermal application

When 0.1 or 0.2 ml of tri-*o*-cresyl phosphate was applied epicutaneously to 2 cm^2 of skin on the back of the neck of 2 cats twice a week, the first signs of ataxia became noticeable in the animals of the higher dose group after 3 to 4 weeks (Beresford and Glees 1963).

Tri-*o*-cresyl phosphate doses of 0, 0.5, 1, 5 or 100 mg/kg body weight and day (purity 99%) were applied to 15 cm^2 of skin on the back of the neck of groups of 3 young, adult cats. Another group of 6 cats was given a daily dose of 10 mg/kg body weight. After a treatment period of 90 days, the animals were observed for another 30 days. Animals that showed signs of acute toxicity were injected with 0.4 mg of atropine sulfate and 50 mg of pyridine-2-aldoxime methyl chloride (directly after administration of the tri-*o*-cresyl phosphate until the acute cholinergic effects subsided). At doses of 1 mg/kg body weight and above, weakness in the legs was observed, but without visible histopathological changes in the spinal cord and the peripheral nervous system. Full remission of the symptoms occurred during the recovery phase. At a dose of 5 mg/kg body weight and above, additionally mild ataxia and in 1 animal histopathological changes in the spinal cord were observed. The symptoms intensified at 10 mg/kg body weight and above with severe ataxia developing in all animals in addition to paralysis (1 animal) and changes in the spinal cord and the peripheral nervous system (Abou-Donia et al. 1986). On the basis of the study findings, the NOAEL for cats is 0.5 mg/kg body weight and day.

5.2.4 Subcutaneous injection

Tri-*o*-cresyl phosphate doses of 0.05, 0.1, 0.25, 0.5 or 0.75 ml/kg body weight (purity 95%) (about 59, 118, 295, 590 or 885 mg/kg body weight) were administered subcutaneously to cats at varying frequencies and intervals. Ataxia developed in all treated animals. After 9 injections of the low dose of 0.05 ml/kg body weight, signs of ataxia were observed after 32 days; after a single injection of the high doses of 0.75 and 0.5 ml/kg body weight, signs of ataxia developed after 13 and 14 days. In the peripheral nervous system, the lesions selectively affected the large diameter and long fibres. In the central nervous system, lesions were observed also in the long nerve fibres of the spinal cord, but no changes in the brain (Cavanagh 1964; Cavanagh and Patangia 1965).

5.3 Local effects on skin and mucous membranes

There are no data available.

As the formulation with a mixture of tricresyl phosphate isomers was not irritating to the skin and eyes of rabbits (Hartwig and MAK Commission 2023), the *o*-isomers of tricresyl phosphate are not assumed to cause irritation.

5.4 Allergenic effects

There are no data available.

A local lymph node assay carried out with a mixture of tricresyl phosphate, free of *o*-isomers (Hartwig and MAK Commission 2023), yielded a just positive result that was not possible to clearly interpret. At most, this can be regarded as evidence of a very slight skin sensitizing potential. The same can be assumed for the *o*-isomers of tricresyl phosphate.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

No fertility studies are available for tri-*o*-cresyl phosphate itself. Two studies investigated tricresyl phosphate mixtures, one of which is a continuous mating study (Chapin et al. 1988) and the other a one-generation study (Carlton et al. 1987).

In the continuous mating study, groups of 20 CD1 mice were fed a tricresyl phosphate mixture with the diet at doses of 0, 62.5, 124 or 250 mg/kg body weight and day. Sperm motility was reduced and atrophy was found in the seminiferous tubules of the males of the F0 and F1 generations at doses of 62.5 mg/kg body weight and day and above. At doses of 124 mg/kg body weight and day and above, the number of living offspring per litter was reduced in the F0 and F1 generations and, in the F1 generation, mating of treated female animals with untreated male animals led to a reduced fertility index. The latter effect was observed in the F0 generation at a dose of 250 mg/kg body weight and day. The tricresyl phosphate mixture contained 20.6% tri-*m*-cresyl isomer, 3.9% tri-*p*-isomer and about 0.1% tri-*o*-isomer. In total, 74.9% of the mixture was made up of pure and mixed *o*-cresyl isomers, *m*-cresyl isomers, and *p*-cresyl isomers. Other constituents were dicresyl phenyl phosphates, dicresyl xylyl phosphates and tricresyl xylyl phosphates (Chapin et al. 1988). The NOAEL for the effects of the tricresyl phosphate mixture on fertility and for perinatal toxicity was 62.5 mg/kg body weight and day. It was not possible to derive a NOAEL for maternal and paternal toxicity because histological effects were found in the adrenal glands in the low dose groups of the F0 and F1 generations (Hartwig and MAK Commission 2023). The Nordic Expert Group suggested that the effects on sperm parameters induced by the tricresyl phosphate mixture may be attributed to tri-*o*-cresyl phosphate (Sjögren et al. 2010) because tri-*o*-cresyl phosphate was found to induce such effects in a number of studies in rats (Hoshino et al. 1999; Somkuti et al. 1987 a, c) and mice (Chen et al. 2012).

In a one-generation study with administration of a tricresyl phosphate mixture to Long Evans rats by gavage, the females were treated with doses of 0, 200 or 400 mg/kg body weight and day and the males with 0, 100 or 200 mg/kg body weight and day (12 females and 24 males per group). The animals of the low and of the high dose groups were then mated with other animals of the same dose group. In the low dose groups (females: 200 mg/kg body weight and day, males: 100 mg/kg body weight and day), changes in sperm parameters were determined (lower concentration, reduced progressive motility) and the number of litters was decreased. At the higher doses, litter sizes were reduced and the survival of the offspring was decreased. The tri-*o*-cresyl phosphate fraction in the isomer mixture was below 9% (Carlton et al. 1987).

The studies of repeated exposure to tri-*o*-cresyl phosphate and the effects on the reproductive organs are shown in Table 8.

Tab. 8 Studies with repeated exposure to tri-*o*-cresyl phosphate and effects on the reproductive organs

Species, strain, number per group	Exposure	Findings	References
rat, F344, 8 ♂	**14 days**, 0, 100, 200, 400, 800, 1600 mg TOCP/kg body weight and day, gavage, vehicle: corn oil, purity: 99%	**100 mg/kg body weight and above**: epididymis: sperm density ↓; testes: degeneration of the seminiferous tubules (dose-dependent increase in severity); **200 mg/kg body weight and above**: mortality ↑, body weight gains ↓, cholinergic symptoms	Somkuti et al. 1987 a
rat, F344, 10 ♂	**63 days**, 0, 10, 25, 50, 75, 100 mg TOCP/kg body weight and day, pair-fed control group: feed consumption based on the preceding day of the 100 mg/kg body weight group, gavage, vehicle: corn oil, purity: 99%	**10 mg/kg body weight and above**: testes: NSE activity ↓, NTE activity ↓; **25 mg/kg body weight and above**: testes: disorganization of the germ cells, PAS-positive bodies in the lumen of the seminiferous tubules; **50 mg/kg body weight and above**: body weight gains ↓; epididymis: sperm density ↓, sperm motility ↓; **75 mg/kg body weight and above**: testes: relative testis weights ↓ (not in comparison with the pair-fed control group), sperm motility ↓, number of germ cells ↓, immature germ cells, multinucleated giant cells in the lumen of the seminiferous tubules; **100 mg/kg body weight**: epididymis: no sperm detected, no effects on testosterone concentration in the interstitial fluid of the testes	Somkuti et al. 1987 a
rat, F344, 10 ♂	**3, 7, 10, 14 or 21 days**, 0, 150 mg TOCP/kg body weight and day, recovery group: 98 days without exposure, gavage, vehicle: corn oil, purity: 99%	**150 mg/kg body weight**: from day 3 onwards: testes: NSE activity ↓, NTE activity ↓; from day 10 onwards: sperm motility ↓, number of sperm per mg of tail of the epididymis ↓; from day 21 onwards: relative testis weights ↓; recovery: irreversible effects on spermatogenesis and histological effects on the testes (complete absence of germ cells, epididymis without sperm, decreased diameter of the seminiferous tubules, intratubular region: only Sertoli cells and Sertoli cell processes); no noticeable changes in: β-glucuronidase, LH, FSH, testosterone in serum and interstitial fluid, histology of spleen, liver, kidneys, pancreas, small intestines, adrenal glands, pituitary gland	Somkuti et al. 1987 c
rat, Sprague Dawley, ♂, number not specified	**single**, 500 mg TOCP/kg body weight, no data for control group, oral, vehicle: no data, purity: no data, examination: 1, 3, 7, 10, 14, 21 days after substance administration	**500 mg/kg body weight**: morphological abnormalities in the sperm from day 7 after treatment onwards; study only available as summary	Hoshino et al. 1999
mouse, Kunming, 9 ♂	**14 days**, 0, 100, 200, 400 mg TOCP/kg body weight and day, probably gavage, vehicle: DMSO, purity: 97%	**100 mg/kg body weight**: number of sperm/epididymis ↓; **200 mg/kg body weight**: seminiferous tubules: slightly disorganized histoarchitecture; **400 mg/kg body weight**: seminiferous tubules: dissolution of the germinal epithelium, loss of germ cells, decreased number of round sperm	Chen et al. 2012

DMSO: dimethyl sulfoxide; FSH: follicle stimulating hormone; LH: luteinizing hormone; NSE: non-specific esterase; NTE: neurotoxic esterase; PAS: periodic acid-Schiff; TOCP: tri-*o*-cresyl phosphate

Tri-*o*-cresyl phosphate given to F344 rats by gavage for 14 days led to decreased sperm density in the epididymis and degeneration of the seminiferous tubules at dose levels of 100 mg/kg body weight and day and above (Somkuti et al. 1987 a).

Histological changes in the testes were observed in F344 rats given tri-*o*-cresyl phosphate for 63 days at doses of 25 mg/kg body weight and day and above. The sperm density in the epididymis was decreased and the sperm motility reduced at doses of 50 mg/kg body weight and day and above. Body weight gains may have contributed to the effects on sperm count and sperm motility, but not to the histological changes in the testes (Somkuti et al. 1987 a). A study carried out by the same research group on the time course found that exposure to tri-*o*-cresyl phosphate at a dose level of 150 mg/kg body weight and day led to a reduction in the activities of NSE and NTE after 3 days, effects on the sperm after 10 days and irreversible effects on the germ cells from day 21 onwards (Somkuti et al. 1987 c).

Tri-*o*-cresyl phosphate given to male Sprague Dawley rats in a single oral dose of 500 mg/kg body weight induced morphological abnormalities in the sperm during the last stage of spermatogenesis from day 7 after treatment onwards (Hoshino et al. 1999). The study is available only in the form of a summary.

Oral doses of tri-*o*-cresyl phosphate given to male Kunming mice for 14 days decreased the number of sperm per epididymis at dose levels of 100 mg/kg body weight and day and above; this effect was dependent on the dose. Histological changes in the testes were evident at dose levels of 200 mg/kg body weight and day and above (Chen et al. 2012).

5.5.2 Developmental toxicity

Studies of prenatal exposure to tri-*o*-cresyl phosphate are shown in Table 9.

Tab. 9 Studies of prenatal exposure to tri-*o*-cresyl phosphate

Species, strain, number per group	Exposure	Findings	References
rat			
Long Evans hooded, high dose: 18 ♀, control: 14 ♀, other groups: 10 ♀	**GD 6–18**, similar to OECD Test Guideline 414, method sufficiently valid in the year the study was performed, 0, 87.5, 175, 350 mg TOCP/kg body weight and day, gavage, vehicle: corn oil, purity: > 99%, examination: GD 21	**175 mg/kg body weight: NOAEL maternal toxicity**; **350 mg/kg body weight: NOAEL developmental toxicity**; **350 mg/kg body weight**: dams: mortality 5/18 (28%); no unusual findings: dams: body weights, number of implantations/litter, percentage of pre-implantation losses, percentage of resorptions, sex ratio; foetuses: body weights, malformations and variations	Tocco et al. 1987
no data for strain and number	**GD 18–19**, 0, 500, 750 mg TOCP/kg body weight and day, single dose of 1500 mg/kg body weight, gavage, vehicle: corn oil, purity: no data, examination: GD 22	**up to 750 mg/kg body weight**: dams: placental weight unchanged, no other data; foetuses: no unusual findings for mortality, body weights, litter sizes, incidence of malformations; **1500 mg/kg body weight**: embryotoxic; studies available only in summary form	Mele and Jensh 1977
guinea pig			
Ssc:AL,Mol:DHF, no data for number	**GD 41 and 43**, 0, 100 mg TOCP/kg body weight and day, probably oral, vehicle: no data, purity: 90%–95%, examination: day of birth	no changes in the weights of brain, cerebellum, medulla, diencephalon, hippocampus, quadrigemina, cortex; no other examinations	Mehl et al. 1994

GD: gestation day; TOCP: tri-*o*-cresyl phosphate

In a prenatal developmental toxicity study, tri-*o*-cresyl phosphate was given to Long Evans hooded rats in doses up to 350 mg/kg body weight and day from gestation days 6 to 18. No toxic effects on development were observed up to the high dose. At the high dose, mortality was increased in the dams (Tocco et al. 1987). The study was carried out using a method similar to OECD Test Guideline 414; deviations from the guideline can be found in the number of animals tested and the presentation of the results. However, at the time, the method was regarded as valid and the data adequate for inclusion in the evaluation. A NOAEL for developmental toxicity of 350 mg/kg body weight and day, the high dose, has been derived from these findings. The NOAEL for maternal toxicity was 175 mg/kg body weight and day.

Embryotoxic effects did not develop in the foetuses of rats given tri-*o*-cresyl phosphate at a dose of 750 mg/kg body weight and day on gestation days 18 and 19. A single, higher dose of 1500 mg/kg body weight was found to be embryotoxic (Mele and Jensh 1977). The study is available only in the form of a summary and is therefore not suitable for the evaluation of the developmental toxicity induced by tri-*o*-cresyl phosphate.

Tri-*o*-cresyl phosphate given to Ssc:AL,Mol:DHF guinea pigs at a dose of 100 mg/kg body weight on gestation days 41 and 43, probably via an oral route of administration, did not lead to changes in the weight of the whole brain and of parts of the brain (Mehl et al. 1994). As only one end point was investigated, and tests for developmental toxicity were not performed, the study cannot be used for the evaluation of the developmental toxicity induced by tri-*o*-cresyl phosphate.

5.6 Genotoxicity

5.6.1 In vitro

In Salmonella mutagenicity tests, tri-*o*-cresyl phosphate was found to be mutagenic in the strain TA100 with metabolic activation (695 revertants/µmol), but not without activation. 2-Phenoxy-4H-1,3,2-benzodioxaphosphorin-2-oxide, a demethylated analogue of the toxic tri-*o*-cresyl phosphate metabolite CBDP, was found to have higher mutagenic potency without metabolic activation (1452 revertants/µmol) (Mentzschel et al. 1993 a).

In ^{32}P-postlabelling assays, DNA adducts were found in the Salmonella typhimurium strain TA100 after incubation with 2-phenoxy-4H-1,3,2-benzodioxaphosphorin-2-oxide. Two different DNA adducts were detected (^{32}P-postlabelling) in the human hepatoma cell line HepG2 after incubation with the metabolite CBDP, but not after incubation with tri-*o*-cresyl phosphate. The evaluation of the cytotoxicity in hepatoma cells revealed an about 50% loss of viability after incubation for 8 hours with 5×10^{-7} M 2-phenoxy-4H-1,3,2-benzodioxaphosphorin-2-oxide; the effects were dependent on time and the concentration. Tri-*o*-cresyl phosphate was markedly less cytotoxic at a concentration of 2×10^{-4} M with a 24% reduction in viability after 24 hours (Mentzschel et al. 1993 b). As no DNA adducts were detected after incubation with tri-*o*-cresyl phosphate and the cytotoxicity was less severe, it is assumed that the substance is not metabolized by this cell line.

In bacterial mutagenicity tests with the Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537, tricresyl phosphate was not found to have mutagenic potential up to a concentration of 10 000 µg/plate both with and without the addition of metabolic activation (Haworth et al. 1983; NTP 1994). The tricresyl phosphate used for testing contained < 0.1% tri-*o*-cresyl phosphate. The levels of di-*o*-cresyl phosphate and mono-*o*-cresyl phosphate were not given.

Tricresyl phosphate (tri-*o*-cresyl phosphate < 0.1%) did not induce chromosomal aberrations and did not increase sister chromatid exchange in CHO cells (a cell line derived from Chinese hamster ovary) up to a concentration of 5000 µg/ml (NTP 1994).

5.6.2 In vivo

Male Fischer 344 rats were given tri-*o*-cresyl phosphate in corn oil in an oral dose of 50 mg/kg body weight for 10 days. DNA adducts formed in the kidneys, the liver, the heart and the lungs (^{32}P-postlabelling). No DNA adducts were found in the brain and in the testes. The autoradiogram pattern of the DNA adducts was identical with the pattern that was observed in in vitro studies with bacteria and a hepatoma cell line treated with

2-phenoxy-4H-1,3,2-benzodioxaphosphorin-2-oxide. The major DNA adduct was a cytidine adduct. Also the uridine adduct was detected, but to a lesser extent. Both adducts were still present in the lungs 28 days after the end of exposure to tri-*o*-cresyl phosphate, but only the cytidine adduct was still found in the kidneys. In the liver and heart, the adducts were detected only on the first day after treatment (Mentzschel et al. 1993 b).

5.7 Carcinogenicity

A formulation with a mixture of isomers containing 79% tricresyl phosphate ester (< 0.1% tri-*o*-cresyl phosphate) and 18% dicresyl phosphate ester was fed to F344 rats with the diet for up to 24 months. Male F344 rats were given doses of 0, 3, 6, 13 or 26 mg/kg body weight and day and female F344 rats doses of 0, 4, 7, 15 or 30 mg/kg body weight and day. In the same time period, B6C3F1 mice were given 0, 7, 13 or 27 mg/kg body weight and day (males) and 0, 8, 18 or 37 mg/kg body weight and day (females). Survival, body weight gains and feed consumption were similar in all groups. The dicresyl phosphate and tricresyl phosphate mixture did not lead to an increased incidence of tumours up to the highest dose of 30 mg/kg body weight and day (rats) and 37 mg/kg body weight and day (mice) (NTP 1994).

5.8 Other effects

The neuregulin 1/epidermal growth factor receptor (ErbB) signalling pathway is crucial for axonal myelination. In Beijing laying hens (n = 28) treated with the ErbB inhibitor lapatinib, the severity of the ataxia induced by a single oral dose of tri-*o*-cresyl phosphate of 750 mg/kg body weight was considerably lower in comparison with that observed in the control group that was not given lapatinib (n = 7) (Xu et al. 2018).

A single dose of 0.3 µmol tri-*o*-cresyl phosphate was injected into the vitreous fluid of the eyes of rats, leading to marked inhibition of the rapid axoplasmic transport of the optic nerve (Reichert and Abou-Donia 1980).

Behavioural changes (transitions to light) and neuronal degeneration were noted 14 days after Drosophila were fed 0, 8 or 16 mg of tri-*o*-cresyl phosphate per ml of glucose solution for 1 day. After incubation with tri-*o*-cresyl phosphate for 6 hours, axonal degeneration was observed in primary neuron cultures of Drosophila larvae (Wentzell et al. 2014).

Primary cortical neurons isolated from mouse embryos were cultured in vitro for 24 hours or 6 days and then incubated with tri-*o*-cresyl phosphate. After incubation for 24 hours, the EC_{50} was determined to be 90 µM tri-*o*-cresyl phosphate under both cultivation conditions. The impairment of neurite outgrowth was statistically significant at a tri-*o*-cresyl phosphate concentration of 10 µM, but not after incubation with the metabolite CBDP, which was more cytotoxic (IC_{50} 15 µM). Also, treatment with 10 µM tri-*o*-cresyl phosphate led to a statistically significant increase in intracellular Ca^{2+} levels. The response to glutamatergic signals was reduced at 100 nM tri-*o*-cresyl phosphate. No effects were noted after incubation with CBDP. The authors concluded that tri-*o*-cresyl phosphate inhibits the signalling of the neurotransmitter glutamate in the brain. As CBDP had no effect on the neurotoxic end points investigated in this study, the authors consider the specific affinity of CBDP for NTE and the general cytotoxicity to possibly be the most relevant mechanism of action of this metabolite in the context of neurotoxicity and OPIDN induced by tri-*o*-cresyl phosphate (Hausherr et al. 2014, 2017).

Primary cortical neurons from newborn rats (postnatal days 0 to 1) exhibited increased mitochondrial activity and limited effects on neuronal electrical activity and neurite length after treatment for 24 or 28 hours with 10 µM of different isomer mixtures. Cytotoxicity occurred only at 100 µM. A comparison of the isomers tri-*o*-cresyl phosphate, tri-*m*-cresyl phosphate and tri-*p*-cresyl phosphate revealed that the tri-*o*-cresyl phosphate isomer increases mitochondrial activity and neuronal electrical activity at exposure levels of 10 µM and above. After 48 hours, the inhibition of neurite outgrowth (length) was statistically significant at 10 µM tri-*m*-cresyl phosphate. On the basis of a NOEC (no observed effect concentration) of 1 µM TCP mixture and assuming 100% bioavailability and distribution in 5 l of body fluids, the authors estimated that the amount in humans would be 1.75 mg TCP. From this amount and assuming a body weight of 70 kg, a NOAEL of about 25 µg/kg has been determined. The commercial mixtures contained 0 to 2% tri-*o*-cresyl phosphate (Duarte et al. 2017).

In differentiated mouse N2a neuroblastoma cells, axon outgrowth was inhibited by tri-*o*-cresyl phosphate in vitro at an IC_{50} of 0.7 mg/l (Fowler et al. 2001; Sjögren et al. 2010).

In the cell lines N18 (neuroblastoma, mouse) and C-6 (glioma, rat), neurite outgrowth was inhibited after 14-day incubation with 0.1 to 10 µM *o*-tricresyl phosphate isomers; this effect was dependent on the concentration. Complete inhibition of neurite outgrowth was observed in both cell lines following incubation with 10 µM of the mono-*o*-tricresyl phosphate isomer (*o*-*m*-*p*). The two di-*o*-tricresyl phosphate isomers inhibited neurite outgrowth by about 60% to 80%, even at the lowest concentration tested of 0.1 µM, while the level of inhibition did not reach statistical significance after incubation with tri-*o*-cresyl phosphate and the mono-isomer. In a comparison of the effects induced by tri-*o*-cresyl phosphate with those of the di-isomers and the mono-isomers, tri-*o*-cresyl phosphate induced the weakest effects. According to the IC_{50} values, the mono-isomer was more potent than the two di-isomers and these in turn, were more potent than tri-*o*-cresyl phosphate. This agrees with the order of potency determined in vivo (Henschler et al. 1992).

After incubation for 24 hours with 0.5 or 1.0 mM tri-*o*-cresyl phosphate, the number of autophagic vesicles in neuroblastoma cells (SH-SY5Y) was increased. After incubation of the cells with 0.2 to 1.0 mM tri-*o*-cresyl phosphate, the MTT cytotoxicity assay did not reveal a decrease in cell viability, but the decreased lengths of the fully developed axons and reduced levels of the neurofilaments NF-H and NF-L and β-tubulin were statistically significant (Chen et al. 2013). The signalling pathways of autophagy were not investigated.

Following incubation of human neuroblastoma cells (SK-N-SH) with 5 mM tri-*o*-cresyl phosphate for 12 hours, only 50% of the cells were still viable and the expression of the microtubule-associated protein 2c and tau protein, but not β-actin, was inhibited. The level of inhibition of NTE and AChE was statistically significant. The neurofilament NF-H was already inhibited to a statistically significant degree by 1 mM tri-*o*-cresyl phosphate (Chang and Wu 2006).

6 Manifesto (MAK value/classification)

The critical effects after oral or dermal exposure to *o*-tricresyl phosphate are neuropathy in humans and effects on the testes and sperm in male rats and mice.

MAK value. The large number of poisonings in humans clearly shows the relevance of neurotoxicity for humans. However, as the data needed to establish a dose–response relationship are not available, it is not possible to derive a MAK value. Therefore, the value has been derived on the basis of the findings from animal studies.

After the application of 99% tri-*o*-cresyl phosphate to the skin of cats every day for 90 days, a NOAEL for tri-*o*-cresyl phosphate of 0.5 mg/kg body weight and day and a LOAEL of 1 mg/kg body weight and day was derived for clinically observed muscle weakness (Abou-Donia et al. 1986).

The following toxicokinetic data are taken into consideration for the extrapolation of this NOAEL to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the corresponding toxicokinetic species-specific correction value for the cat (1:2), the assumed dermal absorption (100%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person and the assumed 100% absorption by inhalation. The concentration calculated from this is 2.5 mg/m^3. At a dose of 1 mg/kg body weight and day, the effects developed only after 74 days; for this reason, the NOAEC may decrease further in the case of chronic exposure (1:6, life expectancy of the cat: 10 years). This would result in a concentration of 0.42 mg/m^3. As this value was calculated based on a NOAEL from animal studies (1:2), a tri-*o*-cresyl phosphate concentration of 0.21 mg/m^3 ≙ 0.0137 ml/m^3 can be derived. The saturation concentration is 0.28 mg/m^3; therefore, the MAK value can be set in ml/m^3. Taking the concentration of 0.0137 ml/m^3 as a starting point and applying the preferred value approach, a value of 0.01 ml/m^3 has been calculated from these data.

In chickens given daily oral doses of tri-*o*-cresyl phosphate, degeneration in the CNS and PNS was observed after 90 days at doses of 2.5 mg/kg body weight and day and above. A NOAEL of 1.25 mg/kg body weight was determined from the study findings (Prentice and Majeed 1983; Roberts et al. 1983).

The following toxicokinetic data are taken into consideration for the extrapolation of this NOAEL to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the corresponding toxicokinetic species-specific correction values for the chicken (1:3), the assumed oral absorption (100%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person and the assumed 100% absorption by inhalation. The concentration calculated from this is 4 mg/m^3. As the symptoms may intensify over a longer period of exposure (1:3, life expectancy of the chicken: 5 years) and as this value is based on a NOAEL derived from the findings of animal studies (1:2), a concentration of 0.67 mg/m^3 (about 0.04 ml/m^3) has been determined from the study data.

Besides tri-*o*-cresyl phosphate, the formulations with a mixture of isomers contain also other *o*-isomers. The higher toxicity of these mono-*o*-isomers and di-*o*-isomers is a possible explanation for the noticeably higher neurotoxicity induced by formulations with a mixture of isomers of tricresyl phosphate in comparison with pure tri-*o*-cresyl phosphate. For this reason, the MAK value is derived for the sum of all *o*-isomers of tricresyl phosphate.

On the basis of the toxicity ratio of 10 : 5 : 1 for mono-*o*-cresyl phosphate, di-*o*-cresyl phosphate and tri-*o*-cresyl phosphate, according to which the mono-*o*-isomers are 10 times more toxic than tri-*o*-cresyl phosphate (Henschler 1959), a MAK value of 0.001 ml/m^3 has been derived for tricresyl phosphate, sum of all *ortho*-isomers. This is a tenth of the value determined for tri-*o*-cresyl phosphate based on the findings from the study in cats.

In the few cases in which symptoms arising at the workplace could clearly be attributed to exposure by inhalation to tricresyl phosphate, sum of all *o*-isomers, it is likely that exposure occurred at concentrations markedly above the MAK value and there was additional dermal exposure. Therefore, the human data are compatible with the MAK value.

Peak limitation. As the MAK value was derived based on systemic effects, tricresyl phosphate, sum of all *o*-isomers, has been classified in Peak Limitation Category II. In chickens, both tri-*o*-cresyl phosphate and its critical metabolite CBDP have a half-life in plasma of about 2 days (Suwita and Abou-Donia 1990). In addition, NTE was irreversibly inhibited with a regeneration half-life of 3 to 5 days (Richardson 1992). As its half-life exceeds 8 hours, an excursion factor of 8 has been set for tricresyl phosphate, sum of all *o*-isomers.

Prenatal toxicity. The NOAEL for developmental toxicity in rats was 350 mg/kg body weight and day, the highest dose tested.

o-Tricresyl phosphates are neurotoxic, inhibit NTE and lead to OPIDN (ACGIH 2016). For this reason, developmental neurotoxicity needs to be evaluated. A generation study of *o*-tricresyl phosphates investigating neurotoxic end points in offspring for the evaluation of central and peripheral neuropathy has yet to be carried out.

As the data available are as yet incomplete, tricresyl phosphate, sum of all *o*-isomers, has been classified in Pregnancy Risk Group D.

Carcinogenicity. In vitro, tri-*o*-cresyl phosphate was mutagenic in Salmonella typhimurium TA100, the only strain tested, with the addition of metabolic activation. The formation of DNA adducts in the liver, kidneys, lungs and heart after oral exposure of rats may be regarded as evidence of genotoxic effects. A carcinogenicity study was carried out with an isomer mixture that did not contain tri-*o*-cresyl phosphate in detectable amounts (< 0.1% tri-*o*-cresyl phosphate). The tumour incidence was not increased in this study.

On the basis of the formation of DNA adducts in vivo and the resulting potential for genotoxic effects, tricresyl phosphate, sum of all *o*-isomers, has been classified in Carcinogen Category 3 B.

Germ cell mutagenicity. No DNA adducts were found in the testes after oral exposure of rats to tri-*o*-cresyl phosphate. The germ cells were not investigated. Other data are not available. Therefore, tricresyl phosphate, sum of all *o*-isomers, does not require classification in a category for germ cell mutagens.

Absorption through the skin. Evidence that systemically toxic amounts are absorbed through the skin was provided by a study in cats that established a LOAEL of 1 mg/kg body weight for tri-*o*-cresyl phosphate (Abou-Donia et al. 1986). In addition, a study in workers whose jobs required them to handle tri-*o*-cresyl phosphate reported findings of neurotoxic effects after probable dermal exposure. On the basis of the findings of an in vivo study, the maximum amount dermally absorbed by humans is estimated to be 4.6 mg after exposure to undiluted tri-*o*-cresyl phosphate under standard conditions (2000 cm^2 area of skin, exposure for 1 hour). After exposure at the level of the MAK value and assuming inhalation absorption of 100% and a respiratory volume of 10 m^3, an amount of 0.15 mg would be absorbed. Therefore, the amount that can potentially be absorbed through the skin is much higher than the amount taken up after inhalation exposure at the level of the MAK value. A similar level of dermal absorption is assumed for both the mono-*o*-isomers and the di-*o*-isomers. For this reason, tricresyl phosphate, sum of all *o*-isomers, has been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. There are no data available for sensitizing effects of tri-*o*-cresyl phosphate on the skin or respiratory tract. Therefore, tricresyl phosphate, sum of all *o*-isomers, has not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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