

TRANSLATION:

Henschler, D. and H.-H. Bayer: TOXICOLOGICAL STUDIES OF TRIPHENYL PHOSPHATE, TRIXYLENYL PHOSPHATES, AND TRIARYL PHOSPHATES FROM MIXTURES OF HOMOLOGOUS PHENOLS. (Toxikologische Untersuchungen über Triphenylphosphat, Trixylenylphosphate und Triarylphosphate aus Mischungen homologer Phenole.) Arch. exper. Path. u. Pharmacol., Vol. 233, pp. 512-517, 1958. Department of Pharmacology, University of Würzburg.

Triaryl phosphates are used in large amounts in industry primarily as additives to paints and lubricants. In the past, technical tricresyl phosphates contained about 30% *o*-cresol in the phenolic component. In the meantime, so-called "low-ortho" preparations, sometimes also called "ortho-free" preparations, have been made available, in which the *o*-cresol content is reduced as far as technically possible to 2-3% of the total amount of cresol.

The neurotoxic activity of industrial tricresyl phosphates is not based, as has long been assumed, on its content of tri-*ortho*-cresyl phosphate. It is determined, as we have been able to show, by the content of *ortho*-cresyl-containing mixed esters, among which those with only one *o*-cresyl group show comparatively the greatest toxicity. Because the *ortho*-cresol in low-ortho tricresyl phosphates is present primarily in the form of these mono-*ortho* esters, such modern products are not free of a paralyzing action; the intensity of this action, however, is reduced to about 30% of that of the ester mixtures commonly used in the past, which caused a great number of poisonings [Neumann and Henschler; Henschler (1, 2)].

We report here on a toxicological investigation of several triaryl phosphates, which are similar to tricresyl phosphate with respect to their technical properties, but in which the cresol is partially or completely replaced by homologs.

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## METHODOLOGY

We have already given a critical discussion of the criteria for selecting suitable experimental animals and experimental techniques [Henschler (2)]. As a standard of comparison for the paralyzing action, we used threshold doses which were just sufficient to cause paralytic phenomena in chickens. Because triaryl phosphates have different forms of activity in different animal species, we also tested the most important representatives of the group of substances in cats as well for the sake of orientation.

As the standard substance with "100% toxicity," we chose the previously used tricresyl phosphate with 26.7% *o*-cresol (preparation 1 of Table 1). The mixed triaryl phosphates\* nos. 3-5 of Table 1 were produced by adding the appropriate equivalents of phenol or its homolog in each case; they are therefore mixtures of a large number of mixed esters of phosphoric acid. For the pure triesters (nos. 8-13 of Table 2), we used chemically pure dimethylphenols and pure phenol (no. 7). The *o*-cresol contents listed in Table 1 were determined by chromatography according to the method of Stoltenberg and calculated as a percentage of tricresyl phosphate (molecular weight, 368). Tetra-*o*-cresyl pyrophosphate (preparation 14) was synthesized by the method described by Todd.

Liquid preparations (nos. 1-6, 8, 9, and 14) were mixed in a 1:1 ratio with olive oil; triphenyl phosphate was mixed in a 1:4 ratio. All other solid substances were rubbed in a 1:4 ratio with lactose. The preparations were administered in gelatin capsules.

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\*We thank Farbwerke Hoechst AG for kindly providing us with the study preparations and for the performance of the analyses.

RESULTS

TABLE 1. TOXICITY OF TECHNICAL ESTERS AFTER A SINGLE ADMINISTRATION. KEY: (a) preparation no.; (b) name; (c) o-cresol; (d) percent relative toxicity; (e) chickens; (f) cats; (g) tricresyl phosphate from crude cresol, DAB 4 M; (h) tricresyl phosphate, commercial product; (i) diphenylmonocresyl phosphate, commercial product; (j) diphenylmonoxylenyl phosphate, commercial product; and (k) trixylenyl phosphate, commercial product.

Präp. (a)	Bezeichnung (b)	O-Kresol (c) %	Prozent relative Toxizität (d)	
			Hühner (e)	Katzen (f)
1	Trikresylphosphat aus Rohkresol (g) DAB 4 M	26.7	100	100
2	Trikresylphosphat. Handelsprodukt (h)	3.3	3	3
3	Diphenylmonokresylphosphat. (i) Handelsprodukt	1.7	5	5-10
4	Diphenylmonokresylphosphat. (i) Handelsprodukt	1.1	2.5	—
5	Diphenylmonoxylenylphosphat. (j) Handelsprodukt	0.8	1.2-2.5 (1-3)*	2.5-5 1
6	Trixylenylphosphat. Handelsprodukt (k)	1.5		

\*See text.

Table 1 shows the results of the comparative study in 136 chickens and 29 cats.

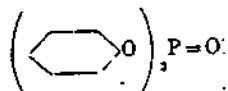
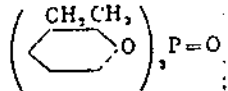
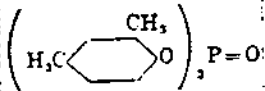
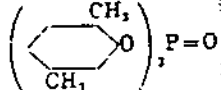
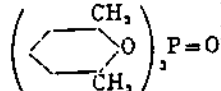
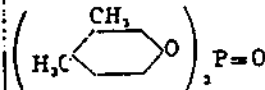
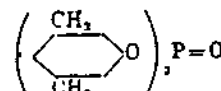
The value for technical trixylenyl phosphate (preparation no. 6) in chickens is estimated from several subchronic experiments at daily doses of 0.1-2.5 g/kg, because such a large amount would have to have been given in a single administration that it could not have been completely absorbed.

Because technical mixed preparations nos. 3-5 and also trixylenyl phosphate (no. 6) still showed paralyzing effects, we tested the pure triesters of the phenol (no. 7, Table 2) and all 6 isomers of dimethylphenol (nos. 8-13), none of which contained any detectable o-cresol. After a single administration (large amounts were distributed over 2-3 days), chickens were free of symptoms at doses of up to 10 g/kg. The results of a long-term test series

are summarized in Table 2. According to these results, 2,3-trixylenyl phosphate has, comparatively speaking, a very weak paralyzing action.

2,4-Trixylenyl phosphate is somewhat more effective; large amounts of this ester caused the animals to die after progressive paralysis.

TABLE 2. ACTION OF TRIARYL PHOSPHATES (2.5 g/kg PER DAY) IN 2 CHICKENS IN EACH CASE. KEY: (a) preparation no.; (b) formula; (c) name; (d) first symptoms appeared after \_\_\_ days; (e) total dose until appearance of first symptoms, g/kg; (f) total dose overall, g/kg; (g) triphenyl phosphate; and (h) . . . trixylenyl phosphate.

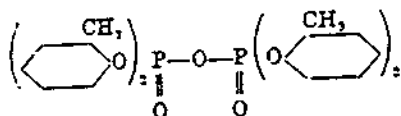
Präp. Nr. (a)	Formel (b)	Bezeichnung (c)	Erste Sympt. nach Tagen	Gesamtdosis bis zu ersten Symptomen g/kg	Gesamtdosis überausp. g/kg
			(d)	(e)	(f)
7	 P=O	Triphenyl-phosphat (g)	—	—	30
8	 P=O	2,3-Trixylenyl-phosphat (h)	20	42.5	50*
9	 P=O	2,4-Trixylenyl-phosphat	12	20	45**
10	 P=O	2,5-Trixylenyl-phosphat	—	—	45
11	 P=O	2,6-Trixylenyl-phosphat	—	—	45
12	 P=O	3,4-Trixylenyl-phosphat	—	—	45
13	 P=O	3,5-Trixylenyl-phosphat	—	—	45

\*Weakness in walking, etc., not complete paralysis.

\*\*One died after 21, the other after 40 days.

Triphenyl phosphate had no effect on chickens, in confirmation of older data [Smith et al.].

Technical mixed esters, including tricresyl phosphates, showed a higher toxicity than would be expected on the basis of the investigation of the pure esters. In the search for an explanation for this, we considered impurities, which appear in technical but not in pure ester mixtures. To come to a basic conclusion as to whether derivatives of pyrophosphoric acid might have a paralyzing effect, we treated chickens with tetra-*o*-cresyl pyrophosphate (no. 14):



in increasing single doses per os and i.m. in a subchronic experiment over the course of 4 weeks. There was no discernible effect at doses of up to 0.1 ccm/kg. Higher doses produced the intoxication picture familiar from insecticidal organic phosphoric acid esters and also from analogous tetraethyl pyrophosphate (TEPP); from 0.4 ccm/kg, the animals died in a few hours in spite of the administration of atropine. No paralysis was observed in any of the surviving chickens.

## DISCUSSION

The experiments show that technical mixed triaryl phosphates such as the diphenylmonocresyl esters have paralyzing effects to the extent that they still contain *o*-cresol (See Table 1). Their toxicity is clearly dependent on the percentage of *o*-cresol (Table 1). In spite of their lower *o*-cresol content, they still have about the same activity as a modern tricresyl phosphate (preparation no. 2) containing about 3% *o*-cresol.

Technical diphenylmonoxylenyl phosphate and trixylenyl phosphate, which still contain some cresol, are also toxic in the sense of a paralyzing action, probably as a result primarily of the presence of a small amount of o-cresol (compare Table 1). Of the pure triesters from dimethylphenols (see Table 2), however, two (2,3-trixylenyl phosphate and 2,4-trixylenyl phosphate) have a slight paralyzing action; it is therefore probable that xylenyl radicals in technical preparations are also involved in the overall toxicity. The two weakly toxic pure triesters, the weak paralyzing action of which escaped other investigators [Hine et al.], are the only liquid members of the group and presumably are therefore also absorbed easily. In liquid mixed preparations, therefore, the radicals of the two other dimethylphenols with the ortho-configuration, namely, 2,5-xylenol and 2,6-xylenol, could also play a role. Among the tricresyl phosphate mixed esters, those with only one o-cresyl group have the highest toxicity on a comparative basis; in a similar way, among the large number of conceivable mixed esters in technical mixed preparations from various phenol homologs, those with one of these "toxophoric ortho groups" could have a stronger action than the corresponding pure triesters. To what extent these possibilities are actually in play cannot be determined at present. Our comparative studies show in any case that the technical mixed triaryl phosphates we studied are in toxicological terms to be put in the same category as the low-ortho tricresyl phosphates.

Triphenyl phosphate produced paralysis only in cats, although of a somewhat different type than that induced by tricresyl phosphate [Smith et al.]; in no other species, in particular not in chickens, can a set of symptoms comparable to that of tricresyl phosphate poisoning in humans be induced. Nothing is known about the action of triphenyl phosphate in humans or about any

poisonings which may have occurred, nor can it be derived at present from animal experiments. Estimating the dose of triphenyl phosphate toxic or even fatal to humans [Hierholzer, Noetzel, and Schmidt] by analogy to the toxic limit dose of tricresyl phosphate therefore remains in the realm of speculation.

It is also true that mistaken ideas exist concerning the minimum toxic limit dose of tricresyl phosphate in humans. According to observations of Staehelin, this limit dose is 0.5 g of a technical tricresyl phosphate with about 30-40% *o*-cresol in the cresol component. The earlier calculation of a limit dose of 0.12-0.15 g of tri-*o*-cresyl phosphate, which was based on this figure, is incorrect; the toxicity of these technical cresyl phosphates is based primarily on mixed esters with only one *o*-cresyl group [Neumann and Henschler; Henschler (1, 2)].

Nothing is known about the lethal dose of tricresyl phosphate or even of tri-*o*-cresyl phosphate in humans. In the literature two deaths are described [Vonderahe; Schmitt] in which a relationship with tricresyl phosphate absorption is probable but not confirmed; no information is available concerning the amounts of tricresyl phosphate involved in this reaction. Van Esveld pointed out very early that, although the lethal dose of tri-*o*-cresyl phosphate is 4-5 times greater than the minimum toxic dose in animal experiments, clinical findings have shown that a human being can ingest at least 10-20 times the minimum dose capable of inducing paralysis without life-threatening consequences.

In the case of technical triphenyl phosphates, however, we must expect paralyzing effects if they contain *o*-cresol; diphenylmono-*o*-cresyl phosphoric acid ester causes paralysis in chickens [Hine et al.].

Pyrophosphoric acid derivatives can be assumed not to exist in technical aryl phosphates because of the method of production and the high boiling range of the pyrophosphates, but their presence would be theoretically conceivable, because the phosphorus oxychloride ( $\text{POCl}_3$ ) used as a starting material for synthesis can be converted into, for example, the chloride of pyrophosphoric acid ( $\text{P}_2\text{O}_3\text{Cl}_4$ ) on contact with air and moisture [Besson], from which the corresponding esters could then form by reactions with phenols. In addition, the suspicion that aryl esters of pyrophosphoric acid might have greater activity than the corresponding compounds of orthophosphoric acid is justified *a priori* because it is known that alkyl derivatives of these acids show an analogous phenomenon. In the case of tetraethyl pyrophosphate (TEPP), both the general toxicity and the insecticidal action are orders of magnitude greater than those of triethyl phosphate [Schrader].

Our findings for tetra-*o*-cresyl phosphate show that it has a parasymphathetic action similar to that of TEPP, but they also make it improbable that pyrophosphoric acid esters are involved in the paralyzing effects of triaryl phosphates. In spite of the insolubility of tetra-*o*-cresyl pyrophosphate in water, the pyro bridge is presumably saponified in the organism. The cleavage product di-*ortho*-cresyl-*ortho*-phosphoric acid has no paralyzing action [Smith and Stohlman].

#### SUMMARY

After it had been shown in the case of tricresyl phosphate that the extent of the characteristic paralysis depended in a certain way on the *o*-cresol content, studies in chickens and cats were carried out to provide further

clarification of the relationships between the structure and action of triaryl phosphates.

Technical triaryl phosphates, the aromatic component of which consists partially or completely of phenol and/or xylenols, have a paralyzing action if they still contain some *o*-cresyl. It is probable, however, that xylenyl groups with methyl groups in the *ortho*-position can produce similar effects, because pure triesters of 2,3- and 2,4-dimethylphenol are also weakly paralyzing, and technical mixed triaryl phosphates with about 1.5% *o*-cresol exert an effect equal to that of tricresyl phosphates with about 3% *o*-cresol.

The action of triphenyl phosphate in humans is not known directly and cannot be evaluated on the basis of animal experiments at present; if technical preparations contain *o*-cresyl, however, it is still possible in principle for a paralyzing action to be present.

It is highly probable that pyrophosphoric acid esters are not involved in the paralyzing action of technical triaryl phosphates. Tetra-*o*-cresyl pyrophosphate is a parasympathomimetic similar to tetraethyl pyrophosphate (TEPP), but it does not induce paralysis in chickens.

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