TRANSLATION:


At the beginning of 1930 in the USA thousands of persons developed a peculiar form of paralysis. Due to the epidemic-like occurrence of this condition, it was initially believed to be a new infectious disease. However, thorough anamnestic analysis later revealed that the cause was actually ingestion of certain brands of fluid extract of Jamaica ginger [1, 2], which was consumed in large quantities during Prohibition and, owing to tremendous demand, was adulterated with new, oil-like substitutes for the resinous, aromatic ginger extracts. This unintentional mass experiment, which included at least 20,000 cases according to an official estimate [3], went down in medical history as "ginger palsy" [4, 5].

Smith et al. [6-10] found about 2% tricresyl phosphate in bootleg liquors and, during a new wave of poisonings, about 0.5% tricresyl phosphate, whose paralytic effect had remained unknown, despite the already extensive industrial use of this substance.

Shortly thereafter, there were numerous European cases of "polyneuritis" following ingestion of certain apio1 preparations, which were intended as abortifacients; tricresyl phosphate was soon detected as an impurity in these preparations [11-13].

This placed older reports of paralysis after ingestion of phosphoric creosote in a new light. When creosote is treated with phosphorus pentoxide, a portion of the ortho-cresol contained in it esterifies to form toxic com-
pounds. After the first reports of Chaumier [14] and Lorot [15], more than 100 cases of paralysis after ingestion of creosote phosphate had been reported by 1934 [16-26].

The increasing use of tricresyl phosphate in industry was followed by additional reports of poisoning.

Subcutaneous injections of tricresyl phosphate, which was mistaken for paraffin oil, paralyzed several patients [27]. The externally oil-like liquid also got into edible oils by accident or negligence and in some cases intentionally, causing numerous group and mass poisonings [28-56], one of which ended fatally [50]. The shortage of fat during the war and the postwar period led many people who had access to the ester to use it as a frying and baking oil; in this connection, mainly "torpedo oil" components played a role.

Despite appropriate warnings and official protective measures, the wave of illness in Germany did not decline until the fat shortage subsided. However, group illnesses in South Africa have recently been reported [57].

Plastic materials, especially polyvinyl chloride, containing tricresyl phosphate as a softening additive, have been a special source of poisoning since the last war.

Fats and fat solvents are able to dissolve the ester out of such plastic materials relatively easily. Careless handling of articles of daily use resulted in tricresyl phosphate coming into contact with foods, so that it was repeatedly ingested and absorbed through the skin [58-64]. A considerable number of illnesses [56, 65-75] occurred primarily during plastics production and processing and proceeded with somewhat modified symptoms during chronic exposure to the poison [69, 76]. Most cases of poisoning by plastics containing tricresyl phosphate were observed in East Germany.
In a systematic toxicological analysis of the effect of tricresyl phosphates of varied composition, we were able to uncover surprising relationships, which demand a revision of the conventional view of the toxicity of such compounds and at the same time answer some of the questions regarding the etiology and pathogenesis of this poisoning.

I. RELATIONSHIPS OF THE TOXICITY OF TRICRESYL PHOSPHATE TO THE CONTENT OF ortho-CRESOL

Tricresyl phosphate \([C_6H_4(CH_3)O]_3PO\) is formed by reaction of phosphorus oxychloride \((POCl_3)\) with industrial cresol, which is a mixture of the 3 isomers ortho-, meta-, and para-cresol. The first investigators of the toxic effect of tricresyl phosphate \([6-10]\) assumed that the three isomeric triesters of orthophosphoric acid are formed during the synthesis (Formulas I-III).

\[\text{CH}_3\]
\[\text{CH}_3\]
\[\text{CH}_3\]
\[\text{O}\]
\[\text{O}\]
\[\text{O}\]
\[\text{P=O}\]

tri-ortho-cresyl phosphate

\[\text{CH}_3\]
\[\text{CH}_3\]
\[\text{CH}_3\]
\[\text{O}\]
\[\text{O}\]
\[\text{O}\]
\[\text{P=O}\]

tri-meta-cresyl phosphate

3
Since symptomatology similar to that observed in cases of human poisoning could be observed in animal experiments only with the tri-ortho compound (I), researchers formed the opinion that the toxicity of an industrial tricresyl phosphate corresponds to its content of tri-ortho-cresyl phosphate, i.e., the toxicity parallels the proportion of ortho-cresol.

In reality, a mixture of ortho-, meta-, and para-cresol with phosphorus oxychloride yields not only the 3 isomers mentioned above (Formulas I-III), but also mixed esters of orthophosphoric acid with different cresyl radicals (cf. Formulas IV-X).

This possibility had already been recognized by Gross and Grosse [77]. Some of these mixed esters had already been described [78]. However, Gross and Grosse investigated only the pure, easily prepared triesters (I, II, III). In this study, they assumed that the different mixed esters would act similarly to the pure isomers, depending on their content of one component or the other, or would be between them with respect to their effect.

We will show, however, that in the case of tricresyl phosphate, the relationships between toxicity and the content of ortho-cresol are actually quite different.
Methods

The methods generally used to determine the toxicity of substances cannot
be directly applied to the testing of tricresyl phosphates. Different animal
species react very differently to the administration of triaryl phosphates.
Researchers have often failed to consider this fact.

To determine toxic effects, we must try to use those species in which the
symptoms most closely resemble those observed in cases of human poisoning.
Smith, Engel, and Stohlman [10], however, had discovered earlier that in the
present case none of the usual laboratory animals is an ideal experimental
subject from this standpoint.

Tricresyl phosphate produces a totally uncharacteristic clinical picture
in rodents. Rats tolerate even high doses without any signs of illness [10,
80]. Mice regularly develop gastrointestinal symptoms, but they only occa-
sionally develop mild paresis of the legs without the latency period typical
in man [81]; rabbits show similar responses [10, 77, 99]. Therefore, exper-
iments conducted with rabbits [82, 83] and mice [84] on the detection of
ortho-tricresyl phosphate in plastics seem problematic in advance.

A clinical picture quite similar to that of human poisonings can be pro-
duced in cats and, incidentally, in tigers [85]. In particular, we observe
the same sphincter insufficiencies that have been reported in recent clinical
literature, symptoms which have gained considerable importance in the evalua-
tion of our experiments.

Tricresyl phosphate produces similar symptoms of poisoning in chickens;
in particular, in this species there is consistently a dose-dependent asympto-
matic interval between ingestion of the poison and the onset of paralysis.

The nervous system of the chicken has been thoroughly studied in
connection with paralysis due to thiamine deficiency [86, 87]. The changes observed in these studies are almost identical with the damage produced by tri-ortho-cresyl phosphate [88-91]. Therefore, chickens have been regarded as the most suitable experimental animals in recent studies on paralytic effects of phosphoric acid esters [89, 91-93]. We can agree with this only with certain reservations. For example, triphenyl phosphate has no effect on chickens*, but it does paralyze cats, although the symptoms are somewhat different from those produced by tri-ortho-cresyl phosphate [10]; such observations are especially important in regard to our specific interests in this study, as we shall discuss at greater length later in the report. Furthermore, oral administration of tricresyl phosphate to chickens causes hardly any gastrointestinal symptoms, but such symptoms, although they do not occur regularly in man, can be anamnestically demonstrated in varied degrees of severity in the majority of cases.

We used chickens for large series of experiments after we had convinced ourselves, by testing the most important members of this class of substances in a small number of cats, that the dose-effect relationships are the same for both species of animals. In this connection, sufficient numbers of animals were used to allow satisfactory determination of the thresholds of effect.

In order to have homogeneous groups of animals at our disposal, we purchased groups of 60–110 young hens of equal age and weighing 600–800 g (purebred, partridge-colored Italians) from uniform broods at a poultry farm. At the beginning of the tests the animals were 12–14 weeks old. Individual sickly animals were culled. After the appearance of paralytic symptoms, the

*Hierholzer, Noetzel, and Schmidt [94] do not consider this fact; we will come back to the evaluation of triphenyl phosphate elsewhere.
affected animals were isolated. Some nonspecific deaths due to a form of can-
nibalism of stronger animals against weaker and injured animals could not be
prevented.

The liquid ester preparations were mixed with 1 part by volume of olive
oil (with 3 parts by volume of olive oil for very small quantities to ensure
precise dosing). The liquid preparations were administered in 0.5 and 0.75
\( cm^3 \) gelatin capsules. The capsules were administered by insertion in the
craw. High doses (more than 4 capsules) were given on two successive days in
most cases and on three successive days in rare cases. The dosage was varied
in an approximately geometric progression, with a few exceptions designed to
allow more exact determination of the threshold dose. After the threshold of
effect had been approximately determined in preliminary tests, groups of two
or occasionally three chickens were used for testing each dosage level. Sev-
eral animals in each experiment remained untreated and were used as a control
group, since spontaneous epidemic paralysis is occasionally observed in chick-
ens.

Since tricresyl phosphate is unreliably and incompletely absorbed from
the digestive tract of cats \([10]\), these animals received 2 depot injections of
the same mixtures in the thigh musculature.

Each day each chicken was individually observed standing and running; the
observer recorded his observations without knowledge of the previous treatment
of the animals. The degree of paralysis was rated by the scale shown in Fig-
ure 1.

Mild degrees of weakness are not immediately apparent in cats. We
allowed them to run along a long corridor with smooth floor tiles; when the
cats' claws cannot get a firm hold, even slight unsteadiness becomes apparent
Figure 1. Toxicity testing of different tricresyl phosphates in chickens and cats, experimental plan, and results. -- A, B, C, D, E: one closed group. One animal per box, shading according to the severity of the symptoms:
- mild, but definite unsteadiness while walking; severe weakness of the legs; complete paralysis; death due to respiratory paralysis following progressive paralysis; death due to nonspecific cause. KEY: (a) chickens; (b) cats; (c) prep. no.; (d) o-cresol; (e) TOPK - tri-o-cresyl phosphate; and (f) number of animals per dose.

after the animals have been forced to run continuously.

The extensive testing program required several separate groups of tests. Since the sensitivity of the individual groups of animals to tricresyl phosphate varied somewhat (season? early feeding?), within a homogeneous shipment of animals we always ran a series with an ester preparation whose strength of effect, compared to a standard preparation (tri-ortho-cresyl phosphate), was known from an earlier test run. Therefore, in the overall evaluation of the tests, the absolute threshold doses found in the individual series of tests cannot be directly related to one another, but the toxicity of all
preparations can be expressed in relative numbers if the toxicity of the most strongly effective sample is set at unity.

(b) **Strength of Effect of Industrial Tricresyl Phosphates of Old and Recent Production**

To compare the toxicity of industrial tricresyl phosphates with that of pure tri-ortho-cresyl phosphate, we wished to use mainly preparations of the type probably responsible for the cases of poisoning reported in the clinical literature.

In Germany mainly a mixture of aromatic compounds has been used to produce industrial tricresyl phosphate. Such mixtures consisted mainly of varying proportions of the three isomeric cresols commercially available as crude cresol DAB 4 M or tricresol [DAB = German Pharmacopeia]; the content of ortho-cresol usually varies between 25 and 40%, depending on origin and processing. Cresol homologues (phenol, dimethylphenols, ethylphenols, etc.) are also present in small percentages. Tricresyl phosphates from cresol mixtures of this type should have about one-third the toxicity of the tri-ortho ester on the basis of the conventional view mentioned above. According to the same literature view, the toxicity of modern preparations with only 3% o-cresol in the aromatic component should amount to no more than 3% of the toxicity of the tri-ortho compound.

The preparations* that were used and the corresponding analytical data are compiled in Table 1.

---

*We would like to thank Farbwerke Hoechst AG for supplying the samples and performing the analyses.*
TABLE 1. KEY: (a) preparation; (b) contents in the aromatic component*; (c) -cresol; (d) tri-ortho-cresyl phosphate, pure; (e) tricresyl phosphate, industrial product from crude cresol DAB 4 M; (f) the same; and (g) tricresyl phosphate, modern commercial product.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Präparat (a)</th>
<th>Gehalt im Aromatenanteil (b)</th>
<th>α -cresol</th>
<th>m -cresol</th>
<th>p -cresol</th>
<th>Phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Triorthokresylyphosphat rein</td>
<td>99**</td>
<td>0–0,5</td>
<td>0,5</td>
<td>1,7</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Trikresylphosphat, technisches Produkt aus Roh(e) kresol DAB 4 M</td>
<td>30,7</td>
<td>34,9</td>
<td>13,5</td>
<td>1,9</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>desgl. (f)</td>
<td>22,7</td>
<td>37,6</td>
<td>17,3</td>
<td>4,0</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>desgl. (f)</td>
<td>23,3</td>
<td>30,7</td>
<td>13,2</td>
<td>0,5</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Trikresylphosphat, (g) neuer Handelsprodukt</td>
<td>3,3</td>
<td>33,3</td>
<td>29,2</td>
<td>1,0</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>desgl. (f)</td>
<td>2,5</td>
<td>40,4</td>
<td>29,3</td>
<td>1,0</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>desgl. (f)</td>
<td>2,5</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

*After total saponification of the ester preparation, chromatographically determined [95] as & triaryl phosphate, mol. wt. 368 (tricresyl phosphate) and mol. wt. 326 (triphenyl phosphate).

**Calculated by deduction of the proportions of phenol and m- and p-cresol components determined by infrared spectroscopy.

***Not determined.

The results of the tests on 156 chickens and 21 cats are compiled in Figure 1.

In some test series on chickens there is a considerable spread; this was to be expected for a poison with a protracted effect and here remains within limits which do not significantly affect the evaluation of thresholds of effect. The severity of paralysis is approximately parallel to the dosage within a test series; the onset of the first symptoms varied between the 8th and the 15th days after administration of the poison; in chickens the onset of symptoms depended approximately on the amount of poison administered, and in cats there was an exact dependence in this respect.

Surprisingly, tri-ortho-cresyl phosphate (prep. no. I) in experiment A

10  

400884
has only about 10% of the effect of an industrial product (prep. no. II) with 26.7% ortho-cresol. Due to the critical importance of this finding, the comparative determination of the toxicity of these two samples was repeated in experiment B with the same result; this relation was reconfirmed in a later test series (cf. Figure 3). Other industrial ester mixtures with similar contents of ortho-cresol, such as prep. no. II, have approximately the same effect (experiment C); the sample with the lowest content of ortho-cresol (prep. no. III) thus has a somewhat diminished effect; the animals in this test series reacted much less sensitively to this preparation than to the others.

The tricresyl phosphates with ortho-cresol values around 3% (prep. nos. V, VI, VII) have equal but much weaker effects (experiment E); the toxicity of the tri-ortho compound (experiment D) is about 3–5 times higher.

The results in cats are wholly consistent with the results in chickens.

Very surprisingly, industrial tricresyl phosphates from crude cresol DAB 4 H with an ortho-cresol content of approximately 30%, such as were mainly used in industry a few years ago, are thus about 10 times more toxic than pure tri-ortho-cresyl phosphate. However, preparations of this type are very probably responsible for the cases of poisoning observed in Germany during the war and in the postwar period.

The toxicity of modern commercial preparations with a greatly reduced ortho-cresol content (about 3%) is, contrary to expectations, still about one third the toxicity of tri-ortho-cresyl phosphate. On the other hand, compared to the highly toxic products with about 30% ortho-cresol, its effect is only about 1/30 as great.

As we have already mentioned, our experimental setup allowed us to
express the toxicity of all preparations as a percent of the effect of the most strongly effective sample. The relationships, which we have already briefly reported [96], are summarized in Figure 2.

Contrary to accepted belief, there is no correlation between the content of ortho-cresol and the strength of the effect. Instead, starting from tri-ortho-cresyl phosphate, the toxicity increases with decreasing content of

\[
100% \rightarrow 50% \quad \text{[TOCP]} \propto \frac{1}{\text{tox}}
\]

\[
\frac{1}{2} \rightarrow 0\% \quad \text{[TOCP]} \propto \text{tox}.
\]
ortho-cresol, reaches a maximum at intermediate values, and falls below the toxicity of the pure tri-ortho compound only at very low concentrations; tricresyl phosphates free of ortho-cresol are most likely nontoxic.

(c) Strength of Effect of Isomeric Mixed Tricresyl Phosphates

To clarify the relationships between toxicity and configuration of tricresyl phosphate mixed esters, we tested (together with Bayer [97]) all isomeric tricresyl phosphoric acids (cf. Formulas I–III and IV–X) in chickens by the same experimental setup.

All of compounds IV–X are oily liquids. They were systematically
synthesized via the monocresyl dichlorides and dicresyl monochlorides*. The analytical data and boiling points are given in Table 2.

All preparations were administered in a closed test series to a uniform group of animals; for comparison, a highly toxic ester preparation with an ortho-cresol content of about 30% was also tested (prep. no. II, cf. Table 1).

Figure 3 shows the results in 82 chickens; only mixed esters with ortho-cresyl radicals have a paralytic effect. The o-m-m-, o-p-p-, and o-m-p-...
phosphates (hereafter called mono-ortho esters) are the most strongly active compounds. There are small differences in effect in the following order o-m-p > o-m-m > o-p-p-cresyl phosphate. The average toxicity of all 3 esters is equivalent to that of an industrial ester mixture with an ortho-cresol content of 26.7%. The isomers with two ortho-cresyl groups (di-ortho esters) have a significantly lower toxicity. The o-o-p compound is more active than o-o-m-TCP; on the average, both are about half as strong as the mono-ortho esters. The toxicity of the tri-ortho compound is calculated to be only 1/10 the toxicity of the strongest mono-ortho esters. A comparison of tri-ortho-cresyl phosphate with the industrial ester preparation (ortho-cresol content 26.7%) confirms the 1:10 effect ratio determined in the first series of tests (see above). The mixed esters without ortho-cresyl radicals (m-m-p- and m-p-p-cresyl phosphate) are tolerated without symptoms in doses of 2.5 cm³/kg.

The o-o-o-, o-o-p-, and o-p-p-esters were recently studied by Hine et al. [92] for their paralytic effect without exactly determining the thresholds of effect; they did not seem to differ significantly. This apparent contradiction of our results may possibly be explained by the fact that the authors did not use uniform animal material. In addition, the two tested mixed esters o-o-p-TCP and o-p-p-TCP may not have been prepared by systematic synthesis, as was the case in our study, but rather from corresponding cresol mixtures; unfortunately, the report contains no information on this question and no analytical data.

Smith et al. [10] were able to show that the specific paralytic effect is due neither to the orthophosphoric acid (or phosphorous acid) nor to the
ortho-cresol alone; therefore, only the molecular group \( p-o-C_6H_4 \cdot CH_3 \) could be the active principle.

To a certain extent, our findings conflict with this theory, according to which the group \( p-o-C_6H_4 \cdot CH_3 \) would be the only determining factor; for in this case the strength of the effect should increase in the following order:

\[
\text{mono-ortho ester} < \text{di-ortho ester} < \text{tri-ortho ester}
\]

with increasing occurrence of the group.

However, knowledge of the strengths of effect of all isomeric tricresyl phosphates offers the possibility of clarifying the causes of the surprising differences in toxicity among industrial ester preparations with different contents of ortho-cresol.

(d) ortho-Cresol Component and Toxicity

In the esterification of orthophosphoric acid with mixtures of the three isomeric cresols, ten isomeric tricresyl phosphates can form (Formulas I–X). Since only the esters with ortho-cresyl groups cause paralysis, and the degree of their paralytic effect depends on the number of o-cresyl groups in the molecule, we are interested in knowing the given proportions of these toxic isomers in an ester mixture with a known content of bound ortho-cresol. Calculation of these proportions is possible if the isomeric cresols are equivalent with respect to those of their chemical properties which are of interest here (ester equilibria), i.e., they do not affect one another during esterification.

If two substances: \( X \) (orthophosphoric acid) and \( Y \) (cresol) react with one another in such a way that one unit of \( X \) accepts three units of \( Y \), and a
portion of $Y$ ($Y' = \text{ortho-cresol}$) is especially distinguished, compounds of the following types can form:

$$XY', XY', XY'Y, XY'Y,'$$

If $n$ - the number of occupied sites of $X$ and $k$ - the number of sites occupied by $Y'$, the following general formula applies to the compounds that are formed:

$$XY_{n+k}Y'$$

(1)

Furthermore, if $q$ is the proportion of $Y'$ (o-cresol) in $Y$ ($q = Y'/Y$), and if a fully statistical distribution is assumed, the probability of the formation of such compounds is given by the following general formula:

$$w_t = \left[\frac{n!}{k!}\right] \cdot q^k (1-q)^{n-k}$$

(2)

For mono-ortho esters ($XY_2Y'$):

$$w_1 = 3q - 6q^2 + 3q^3$$

(3)

For di-ortho esters ($XY_2Y'$):

$$w_2 = 3q^2 - 3q^3$$

(4)

For the tri-ortho ester ($XY_3'$):

$$w_3 = q^3$$

(5)

Distribution curves for variable o-cresol contents were calculated from the probability functions (3), (4), and (5) and are plotted in Figure 4. Mono-ortho compounds predominate at low ortho-cresol contents, di-ortho compounds predominate in medium and higher ranges, and finally the tri-ortho ester, which, by definition, is present to the extent of 100% in the starting mixture ($q - 1$) in the case of pure o-cresol, predominates in the highest range.

Since $w = f(q)$, the maxima of the distribution curves, i.e., those percentages of o-cresol at which maximum proportions of the given compounds are
present, are determined by

\[
\frac{d\mu}{d\lambda} = \mu = \eta.
\]

![Statistical distribution of the o-cresyl-containing esters and hypothetical toxicity of the total preparation.](image)

**Figure 4.** Statistical distribution of the o-cresyl-containing esters and hypothetical toxicity of the total preparation. -o-o- mono-ortho esters; -oo-oo- di-ortho esters; -ooo-ooo- tri-ortho ester; -A-A- hypothetical total toxicity. KEY: (a) % o-cresol.

Accordingly, the proportion of di-ortho esters is highest at 66 2/3% o-cresol in the total cresol, and the proportion of mono-ortho esters is highest at 33 1/3% o-cresol in the total cresol; however, this is the range in which the ortho contents of the highly toxic industrial products fall, which are the basis of most of the cases of human poisoning reported in the German literature during the war years and the postwar period. We will demonstrate this significant fact later.

The distribution curves determined for phosphates containing ortho-cresyl radicals can be used to make purely computational determinations of the toxicity to be expected for tricresyl phosphates with varying ortho-cresol contents.
Assuming that all of the compounds present in the ester mixture act additively, the relationship between total toxicity and ortho-cresol content is obtained by superposition of the 3 probability functions. In this connection, the toxicity of the mono-ortho esters is regarded as 100\%, that of the di-ortho esters as 50\%, and that of the tri-ortho ester as 10\%. With the aid of Equations (3), (4), and (5), we thus obtain the total toxicity:

\[
T = 3\gamma - 2\gamma^2 + 3\gamma^3 - 3\gamma^4 - \frac{1}{16}\gamma^5
\]

\[
= \frac{16}{16}\gamma^5 - \frac{9}{2}\gamma^4 + 3\gamma
\]  

(6)

The toxicity curve calculated from Equation (6) is drawn in Figure 5 over the distribution curves. Its maximum is calculated \((dT/d\gamma = 0)\) to be 43.4\% o-cresol in the total cresol. This greatest achievable toxicity would thus be (cf. Figure 4) about 6 times greater than that of the tri-ortho ester. In fact, we found industrial ester mixtures with about 30\% o-cresol 10 times stronger than the tri-ortho compound. This is probably explained primarily by the fact that in the esterification of cresol with phosphorus oxychloride, the ortho isomer is less lightly bound than meta-cresol and para-cresol due to steric hindrance; suitable analyses of the starting cresols and phosphates confirm this. The formation of tri-ortho and di-ortho esters is thus suppressed in favor of the mono-ortho esters. Furthermore, there is the possibility that cresol homologues (phenol, dimethylphenols, ethylphenols, etc.), which occur in small proportions in industrial cresols, sometimes form mixed esters, which are even more toxic than the mono-o-di-(m,p)-cresyl esters. In both cases the result is an increase in toxicity mainly in the intermediate and low ortho-cresol ranges and a shift of the toxicity peak towards lower values (near 30\% o-cresol), so that the curve shown in Figure 5 (broken curve)
Figure 5. Toxicity of tricresyl phosphate mixtures with varied proportions of ortho-cresol. - actual toxicity curve; -A-A- hypothetical curve for a purely statistical distribution of the mixed esters containing o-cresyl radicals (cf. Figure 4); --- hypothetical toxicity according to the accepted view (proportional to the content of ortho-cresol). KEY: (a) % o-cresol.

can be estimated; in this connection, the values over 100, 25-30, and over 3% o-cresol are confirmed.

At these low ortho contents, the actual toxicity falls below the hypothetical values. **Detoxification mechanisms probably play a greater role in relatively less toxic mixtures of this type,** which must be administered in high doses in the determination of the thresholds of effect.

The results of our animal experiments make it necessary to revise the previous evaluation of clinical cases of poisoning. Previous calculations of the toxic human dose were based on the amount of ortho-cresol contained in a preparation and related this amount to tri-ortho-cresyl phosphate, in the belief that the bound proportions of meta-cresol and para-cresol have no effect on the toxicity of the total preparation. **However, since the meta and para isomers that are present cause the formation of mono-ortho and di-ortho**
esters, and since, as we were able to show, the toxicity of these mixed esters is much greater than that of tri-ortho-cresyl phosphate, the old method of calculation, which only recently was again used to obtain an incorrect value for the toxic limiting dose [94], is invalid. In an investigation of a mass poisoning, Staehelin [30] calculated a minimal toxic dose of 0.12–0.15 g of tri-ortho-cresyl phosphate in adults. However, as he explicitly states (cf. also Iselin [100]) the substance actually tested was 0.5 g of industrial tricresyl phosphate with about 30–40% o-cresol in the total cresol; accordingly, 0.5 g of such a preparation must be regarded as the toxic limiting dose.

A comparison with the thresholds of effect determined in our animal experiments (cf. Figure 1) shows that in chickens and cats about 12 mg/kg is the smallest dose of an industrial tricresyl phosphate with about 30% ortho-cresol in the aromatic component that was still able to produce paralysis or weakness of the rear extremities. The threshold doses for man and animals are thus very similar. We will return to this important coincidence in the evaluation of modern tricresyl phosphates [154].

Nevertheless, in possible future poisonings by tricresyl phosphate, chemical analysis of the underlying ester mixtures should definitely be performed. However, it is no longer permissible to relate an analyzed proportion of ortho-cresol to tri-ortho-cresyl phosphate. In evaluating the amount of poison absorbed by an individual, it is necessary, rather, to determine the dose of total tricresyl phosphate and, at the same time, its content of ortho-cresol. When the relationships between toxicity and ortho-cresol content determined in our animal experiments are taken into consideration, it is then possible to draw conclusions about dose-effect relationships in man.
(e) Symptomatic and Histological Differences in Poisoning by Different Tricresyl Phosphates

In the course of our experiments it was observed that pure tri-orthocresyl phosphate produces only flaccid paralysis in chickens and cats. On the other hand, industrial mixed preparations, whose cresol component contains only 30% ortho cresol, and isomers of the mixed ester type produce chiefly spastic paresis; in addition, cats exhibited marked sphincter paralysis. It was to be assumed that the two types of tricresyl phosphate affect the nervous system in different ways. Therefore, we removed the brains, spinal cords, and portions of peripheral nerves from some animals to investigate the question of different types of effect by histological techniques.

Chickens which died of respiratory paralysis were prepared as quickly as possible after death; otherwise, the animals were anesthetized with Pernocton, and then the brain and spinal cord were removed dorsally. The organs were fixed in 5% Formalin solution. The preparations were embedded in paraffin (section thickness 10 μm). The nerve cells were stained with gallocyanine dye after Einarson; the axis cylinders were stained by the silver impregnation method in the modification of Glees and Marsland [101]. Myelin sheaths with fatty degeneration were made visible by Marchi's osmic acid method.

The histological nervous system damage caused in chickens by tri-orthocresyl phosphate was first studied by Smith and Lillie [9] and then by Brouwer [102]. Recent systematic studies were published by Barnes and Denz [89] and by Cavanagh [90]. Myelin sheaths and corresponding axis cylinders of the peripheral nerves are the most severely affected; the damage is greatest in the distal segments and becomes progressively less intense in the proximal direction. Axis cylinders and myelin sheaths simultaneously undergo changes in the
sense of wallerian degeneration [90, 91]. After high doses, three nerve pathways are affected: (1) the analogue of Goll's fiber in mammals, (2) a laterally situated tract corresponding to the spinocerebellar tract, and (3) the ventral tract; it very probably corresponds to the pyramidal tract in man, but it does not arise in the cortex but rather probably in a control site in the midbrain [90]. Primarily the long fibers with a thick myelin sheath are affected; the lesions are concentrated in the cervical and lumbar intumescences. The degenerated fibers of the spinocerebellar tract can be followed all the way into the cerebellum; isolated damaged fibers can also be encountered in the midbrain and in the vestibulocochlear system. The motor cells in the spinal cord are not regularly changed, but when they are, the changes are mild. Moderate chromatolysis becomes apparent only after several weeks and does not differ from the chromatolysis observed after neurotomy. Fat granules in the cytoplasm are occasionally found [9, 90].

With respect to the type and localization of the nervous system lesions, our observations agree with the findings of the earlier researchers cited above. In most of the severely affected animals we also found more or less strongly pronounced signs of damage in many palisade cells in the cerebellum with shrinkage of central apparatus and Golgi apparatus, darkening [?; German Verdämmung -- Tr. Ed.] of the cell body, and neuronophagia; a finding which has never been described before but which is not surprising, since, of course, the fibers of the spinocerebellar tract are damaged, and since, especially in birds, the cerebellum is exceedingly important to motor coordination.

However, clear differences were observed in the distribution of the damage for pure tri-ortho-cresyl phosphate, on the one hand, and for the highly toxic industrial tricresyl phosphates with about 30% ortho content, on the
TABLE 3. DISTRIBUTION OF THE DAMAGE IN THE NERVOUS SYSTEM OF CHICKENS AFTER POISONING WITH DIFFERENT TRICRESYL PHOSPHATES. KEY: (a) animal no.; (b) preparation; (c) dose, cm³/kg; (d) interval, days; (e) duration of paralysis, days; (f) cervical cord; (g) lumbar cord; (h) TOCP; (i) industrial TCP, 26.7% o-cresol; (j) the same; and (k) o-m-p-TCP.

<table>
<thead>
<tr>
<th>No.</th>
<th>Preparation (b)</th>
<th>Dose (c), cm³/kg</th>
<th>Interval (d), days</th>
<th>Duration of paralysis (e), days</th>
<th>cervical cord (f)</th>
<th>lumbar cord (g)</th>
<th>Nervous tract marked</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TOKP (h)</td>
<td>0.4</td>
<td>11</td>
<td>17</td>
<td>(−)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>TOKP</td>
<td>0.6</td>
<td>10</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>TOKP</td>
<td>0.3</td>
<td>10</td>
<td>−</td>
<td>(−)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>tech. TCP</td>
<td>0.2</td>
<td>13</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>26.7% o-KP</td>
<td>0.05</td>
<td>28</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>o-m-p-TKP</td>
<td>0.03</td>
<td>27</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>(−)</td>
</tr>
<tr>
<td>7</td>
<td>o-m-p-TKP</td>
<td>0.03</td>
<td>27</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>o-m-p-TKP</td>
<td>0.1</td>
<td>21</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>(−)</td>
</tr>
<tr>
<td>9</td>
<td>− n-TKP</td>
<td>0.4</td>
<td>27</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

On the other hand, the quantitative evaluation (Table 3) showed mainly involvement of the peripheral nerves and relatively minor damage to the central tracts in animals paralyzed by tri-ortho-cresyl phosphate. On the other hand, chickens that had been poisoned by industrial tricresyl phosphates with an o-cresol content of about 30% only occasionally showed damaged fibers in the peripheral nerves but had severe degeneration of the spinal cord. This degeneration also shows clear qualitative differences from the degeneration observed in the case of tri-ortho-cresyl phosphate. Whereas in poisoning by the tri-ortho ester the degenerated fibers are limited to the three tracts mentioned above and to some extent can be recognized as belonging to definite systems, in animals poisoned by industrial tricresyl phosphates the affected fibers appear to be diffusely dispersed through the entire white matter, and assignment to specific tracts is not possible. The type and distribution of the damage caused by mixed esters (animal nos. 6-9, Table 3) correspond to the type and
distribution of the damage produced by industrial tricresyl phosphate mix-
tures.

We presently know as little about the causes of the different effects of
the different tricresyl phosphates as about the fine mechanism of action
itself. The question of the substrate of the tricresyl phosphate effect
seemed at least partially answered when Bloch [103] discovered the inhibition
of serum cholinesterase by tri-ortho-cresyl phosphate, and Earl and Thompson
[103a] found that the activity of pseudocholinesterase in the nerve sheaths is
practically eliminated after poisoning with this substance. The hypothesis
based on these findings was quickly shaken by Mendel and Myers [80], who
reported that rats are not paralyzed by tri-ortho-cresyl phosphate, despite
complete inhibition of the pseudocholinesterase of the brain; the supposed
relationship between pseudocholinesterase and paralytic effect was completely
refuted by Barnes and Denz [89], who were able to show that of a large number
of phosphoric acid esters that act on cholinesterases, only three produce the
typical protracted paralysis (tri-ortho-cresyl phosphate, diisopropyl fluoro-
phosphate, and bis(isopropylamino)fluorophosphine oxide). Our studies on
relationships between structure and paralytic effect tend to show (especially
in regard to the qualitative differences in the pattern of effects) that there
are still great difficulties involved with solving the problem of the mecha-
nism of paralysis by this class of substances.

II. EPICRITICAL CONSIDERATION OF TRICRESYL PHOSPHATE POISONING
ON THE BASIS OF NEW TOXICOLOGICAL KNOWLEDGE

Our understanding of the nature of tricresyl phosphate poisoning has
changed during the last 10 years or so. Whereas purely peripheral paralysis
was reported in connection with the American "ginger" poisonings and the "apiol" poisonings, recent German papers emphasize chiefly involvement of the central neuron. We have critically examined the clinical literature, because our new knowledge about the toxicity of tricresyl phosphates (see Section I) promised clarification of various disputed questions.

There is considerable agreement about the symptoms in the first stage of the poisoning; in this connection, we can refer to earlier review papers [44, 58, 71, 135]. However, the descriptions from the different poisoning epidemics differ greatly with respect to several very important symptoms and especially with respect to the subsequent course of the disease.

(a) Differences in the Clinical Picture

The signs of reduced perfusion in the affected extremities, i.e., cyanosis, lowered skin temperature, chills, cold sweats, and paresthesia, are specially emphasized in recent German papers on "torpedo oil" poisoning and "Igelit" poisoning [30, 33, 38, 44, 58, 71].

Parnitzke [58] speaks of "massive, dripping-wet sweating of the feet". According to Scheid [44], trophic disturbances are among the "most constant and . . . intractable symptoms . . ."; these symptoms were regularly present in the cases reported by Staehelin [30]. On the other hand, vasomotor symptoms of this type are rarely mentioned by American authors, despite the much larger number of observed cases [104]; mild trophic disturbances are mentioned in review works [4, 5]. In the reports on "apiol" poisoning, we found only one mention of such symptoms, namely, by Geithner [105], who noted a feeling of coldness in the legs in one case; Kastan [106] found the appearance and condition of the skin to be completely normal.
In recent years bladder disturbances have been observed with remarkable frequency in poisonings with tricresyl phosphates of the "torpedo oil" type. Scheid [44] describes bladder problems in one-third of his approximately 100 cases, and Parnitzke [58] describes them in 10% of 125 cases; in severe cases these problems can persist for years [44]. 24 of the 80 patients described by Staehelin [30] suffered from incontinence. The cases described by Mertens [71] included one case of cystoproctoparalysis which persisted for 4 weeks. On the other hand, most authors do not mention these kinds of symptoms in connection with "ginger paralysis". In a critical review Weber [3] speaks of "little or no involvement" of the sphincters. Kiely and Rich [4] found disturbances in only 16 of 201 cases. Kidd and Langworthy [5] point out in their review paper that they observed no cases of urinary or fecal incontinence. The apiol literature contains no mention of disturbances of the bladder or rectum.

The conspicuous differences between these various types of poisoning led to a search for changes in the cerebrospinal fluid.

No abnormalities were found in the "apiol" cases published up to 1934 (that is, in those cases in which the cerebrospinal fluid was tested) [135]; all 11 cases of Ter Braak and Carillo [108] had normal cerebrospinal fluid findings. Large-scale studies were conducted in the American poisoning cases: Of the 120 lumbar punctures that were performed, only two showed a positive globulin reaction [4, 109]; some reports mentioned slightly elevated globulin and cell increases (up to 11 per mm³), but in most cases the fluid was normal [5]. The recently reported findings of German authors are quite different: Mertens [71] found nonspecific elevation of protein in six of eight tested cases; Scheid [44] obtained a slightly positive Pandy reaction in one-third of
his more than 100 patients, who all had normal cell values; Parnitzke [58] found mild, uncharacteristic protein elevation in 42% of 125 patients. The changes persist for a long period of time and in some cases are still observed after 2 years [71].

It is especially important for us to consider the further course of the illness.

The flaccid paralysis subsides in a few months in mild cases. In severe cases it largely subsides in 1–2 years, although it may persist even longer in rare cases. Remission of symptoms occurs in the exact opposite order of development.

Mild poisonings by "torpedo oil", "Igelit", machine gun oil, and other industrial tricresyl phosphates produced during the war and in the postwar period in Germany usually ended with complete return of functional normalcy. In the vast majority of severe cases of this group, disappearance of the flaccid paralysis was accompanied or immediately followed by the development of spastic paresis, first in the proximal sections of the extremities and later in the calves and forearms. The knee reflexes, which were often diminished and in some cases completely absent at the height of the paralytic stage, showed an increase at this stage and in pronounced cases caused adductor spasms; ankle clonus could also be provoked in many cases. Spastic scissor gait was observed. An increased patellar tendon reflex and often an increased achilles tendon reflex gave the impression of being permanent symptoms after many years, even in mild cases [44].

Spastic paresis, which is prognostically less favorable than flaccid paralysis, indicates that the central neuron is also involved.
The actual pyramidal signs may be absent. The spinal damage must already occur in the early stage parallel with the involvement of the peripheral motor fibers, since no progression of the spasticity was observed over a period of several years, which would be expected in secondary degenerative processes in the central organ. In fact, fascicular twitching and fibrillary contractions were observed very early in such cases, i.e., after a few weeks, even in non-paralyzed muscle groups (Scheid [44]). Walthard [110] also reports on, in his opinion, "pseudospastic" symptoms during the first weeks after poisoning. The damage of the central organ is supposedly masked by the motor deficiencies in the peripheral nerves; only after subsidence of the flaccid paralyses, which show a relatively good remission tendency, would spastic signs then become manifest (Scheid [44], Mertens [71], Parnitzke [58]).

This "symptom transformation" (Parnitzke) has already been observed in American follow-up tests of some cases of "ginger paralysis" [3, 107, 109]. Of course, it is overlooked that only the most severe cases, which remained in nursing homes, could be determined here.

The follow-up investigators themselves concede that there could be the impression that only a minority of the victims from 1930/1931 may have been afflicted with such severe residual symptoms; unfortunately, however, there are no exact numerical data, which are undoubtedly difficult to obtain during follow-up studies on lower socioeconomic groups. However, aside from the differences already mentioned above, there is sufficient evidence to suggest the probability of an altered course of the "ginger paralysis" compared to the "torpedo oil" poisonings. The early reports of the American authors extend to an observation period of up to 1½ years in some cases. Nevertheless, signs of involvement of the central neuron were seen only in very isolated cases [111].
Aring [107] gives a detailed report of a case of poisoning with signs of central involvement, which did not occur until 7 years after the great poisoning epidemic by ginger brandy; to be sure, the patient had ingested a very large quantity. If we use the information given by Smith et al. [7, 8], according to whom the ester content of the brandies was 2%, then the 8 U.S. fluid ounces consumed by the man contained 6 g of tricresyl phosphate.

Increased knee reflexes were only occasionally observed in the American cases [107]. In their review paper, Kidd and Langworthy reported no spastic symptoms after observing the patients over a period of 18 months. In his comprehensive study, Weber [3], who was able to perform follow-up examinations of 35 severely ill patients 6 years after the epidemic of poisonings, characterizes the symptoms of the first years as "predominantly [those] of a flaccid paralysis"; examination of his case histories, all of which involve very severe cases, shows that the change from flaccid paralysis to spastic symptoms in these cases cannot have been completed until after several years; some of these patients were bedridden for up to 2 years.

Parnitzke [58], on the other hand, reports that in poisonings by tricresyl phosphates of the "torpedo oil" type, the flaccid paralysis generally disappeared after about 9-10 months. Scheid [44] reports tension and tightening in the thigh muscles and spontaneous clonus only a few weeks or months after the poisoning.

Finally, the reports on "apiol" paralysis [105, 106, 108, 112-134] give hardly any indication of spastic symptoms. One exception is a case reported by Wuite [125], in which ankle clonus interpreted as medullary damage was present after 2 years; some of his contemporaries [135] disputed an etiological connection. The flaccid paralysis was completely cured after 1-1½ years in.
many cases; even in severe cases the motor functions were later well restored without spastic residual symptoms being superimposed, as a pertinent case reported by Jagdhold [135] shows especially clearly. Follow-up studies after 3 years [136] confirmed previously expressed suppositions regarding a favorable prognosis for a complete cure. There are no reports on the subsequent fate of the patients, supposedly because at that time, after the supposed clarification of the etiology of the illness, there was "apparently very little interest in the further course" (Scheid [44]). On the other hand, as in the later "torpedo oil" poisonings, one probably would have taken the opportunity to make such remarkable findings known if they had actually been present.

The paralysis occurring after creosote phosphate, ginger brandy, and "apiol" was formerly interpreted as "toxic polyneuritis". On the other hand, on the basis of the definite involvement of central tracts in paralysis caused by "torpedo oil", recent authors point out that, as a general rule, the concept of a "toxic polyneuritis" can no longer be accepted for the syndrome of tricresyl phosphate poisoning (Scheid [44], Mertens [71], Parnitzke [58]). However, in a recently published review, Scheller [137] points to the still unexplained discrepancy between apiol paralysis, on the one hand, and torpedo oil and ginger brandy paralysis, on the other hand.

To follow up on the above-described differences in the clinical symptoms, we would like to distinguish three large groups of tricresyl phosphate poisonings: (1) paralysis by certain apiol preparations with almost exclusive involvement of the peripheral pathways; (2) paralysis by tricresyl phosphates of the "torpedo oil" type, which also include "Igelit" poisonings and the Swiss group poisoning, with definite signs of involvement of the central
neuron; and (3) ginger brandy paralysis would fall between the first two types of paralysis; in ginger paralysis there is central involvement only in especially severe cases, so that this type of poisoning is less severe on the whole.

(b) Etiological and Pathogenetic Characteristics of Different Tricresyl Phosphate Poisoning Epidemics

As we were able to show above, there are significant differences with respect to both the poisoning symptoms and the histological findings in chickens. Pure tri-ortho-cresyl phosphate affects mainly the peripheral motor neuron and produces flaccid paralysis, whereas industrial tricresyl phosphates containing mainly mono-ortho-cresyl esters cause only slight damage to the peripheral nerves and instead affect predominantly the long tracts of the spinal cord and produce spastic paresis. Similar differences were most likely present in the different poisoning epidemics, although the differences were perhaps not as sharp as in our animal experiments.

Naturally, the various types of damage found in the animals can be related to the illness produced in man only with reservations. A comparative analysis of the nervous system damage in animals and in man is difficult because only a few reports are available on usually pathoanatomically incompletely investigated cases of tricresyl phosphate poisoning.

In addition to the expected damage to musculature and peripheral nerves, Smith and Lillie [9] and various other authors [138-141] found fatty degeneration of the white substance of the spinal cord in occasional patients who died of other causes during the "ginger epidemic", but they failed to provide detailed information regarding localization. Aring [107] was able to conduct
systematic studies on 36 cases after several years and found greater changes in the lateral pyramidal tract and Goll's tract and thickened leptomeninges and considerable damage to the cells of the anterior and lateral horns of the spinal cord; in most cases there were signs of thromboangiitis obliterans. Since the disease had existed for several years in almost all of the cases that he investigated, no definite answer can be given to the question of a relationship between changes in the vessels and nervous elements. In one case there were also changes in the cells in the nucleus dentatus of the cerebellum [142], and in another case there was softening of the nucleus lentis and spongiosis of the cerebral cortex 8 years after "edible oil poisoning" [143]; with respect to the clinical symptoms in tricresyl phosphate poisoning, a causal relationship seems doubtful here.

This small amount of information does not provide any definite evidence of the correctness of our hypothesis that the different poisoning epidemics were based on chemically different tricresyl phosphates. There are absolutely no pathoanatomical findings for the cases of "apio1" paralysis and "torpedo oil" paralysis. We should also keep in mind that the cases investigated by Aring [107] involved especially severe poisoning with ginger brandy and therefore cannot be regarded as a representative cross section.

We felt that the critical finding was that there were any differences at all in the effect of different tricresyl phosphates. We then decided to try to clarify the relationships between the types of paralysis and the tricresyl phosphates causing the paralysis on the basis of experimentally gained knowledge, available published information, and our own follow-up studies.
1. Paralysis by Creosote Phosphate

"Polyneuritides" after therapeutic administration of phosphoric creosote occurred predominantly as group poisonings.

In connection with the illness of 7 pulmonary patients in Haarlem, Huet [20] reports that chemical analysis of the medication that was used revealed only 12.5% phosphate (as $\text{P}_2\text{O}_5$) instead of the required 20-25%. In addition, it was difficult to saponify, and the boiling range was well above the prescribed range of 190-203°C.

We investigated the question of which toxic component may have been present in the creosote phosphate by preparing such a preparation by an old method [144] and testing it and the residual fraction chemically and in animal experiments.

125 g of officinal creosote was gradually treated with 35 g of phosphorus pentoxide and 5 g of sodium, as the mixture was continuously stirred. The reaction mixture was stirred for another 24 hours and was then fractionally distilled. The major portion passed over between 190 and 202°C as a clear liquid that was insoluble in water; starting at 212°C, a second, pale-yellow fraction distilled, which was immiscible with the first fraction and was more oily in quality. On further distillation, the boiling point rose continuously to 250°C; further reactions probably occurred in the distillation vessel. The two fractions that were obtained were separately dissolved in ethanol and precipitated with water.

In the first fraction, which distilled between 190 and 202°C and supposedly represented the drug in the specified procedure, no phosphate was detectable by the very sensitive molybdate reaction after baking with
magnesia. We also obtained pharmaceutical confirmation of this important finding*. The paralytic effect was tested in 2 chickens by administering a total dose of 5 g/kg, divided into 10 single doses given on 10 consecutive days; except for transient signs of local irritation of the mucous membranes in the crop and esophagus, the animals remained free of symptoms for 4 weeks. The second, higher-boiling fraction contained 1.44% phosphate (calculated as $P_2O_5$; determination by Wurzschmitt [145]). We fed 2 chickens with 1 or 3 g/kg of this sample, whereupon they promptly became affected with spastic paresis 12 days and 9 days, respectively, after administration of the last dose.

These observations place the etiology of the paralysis by creosote phosphate in a totally different light. Presumably, this preparation has been produced not only by large companies, but also occasionally in laboratories (cf. Huet). Here and there, the distillation may have been performed incorrectly, so that a portion of the toxic, high-boiling fraction got into the preparation.

A similar assumption was made by Martinius [146], who believed that the extremely nonvolatile tri-ortho-cresyl phosphate cannot get into the medication if the specifications for preparing phosphoric creosote are followed exactly. According to the above discussions, however, the tri-ortho ester cannot arise in such reactions or at most can form in only extremely small quantities; instead, mixed esters form, which can contain not only other phenol derivatives, but also ortho-cresyl radicals. The statement by Tiffeneau

*We would like to thank Dr. List of the Pharmaceutical Department of the University of Würzburg for preparing an analogous preparation and analyzing it for phosphate.
[147] that up to 15% tri-ortho-cresyl phosphate could be present in creosote phosphates (this statement is cited in many later papers) has not been analytically demonstrated and thus must be regarded as purely speculative. The amount of ortho-cresyl-substituted phosphoric acid esters contained in such preparations can hardly have been high. In our second fraction we found only 1.44% $P_2O_5$; if we convert this to mono-ortho-cresyl phosphates, their content (with respect to moles of tricresyl phosphate) would be a maximum of about 7.5% in the total preparation. We believe that this is confirmed by the data on the amounts actually ingested in such cases of poisoning; in the cases reported by Huet, amounts of up to 120 g of creosote phosphate had been consumed in short periods of time.

2. "Ginger Paralysis"

On the basis of the available clinical reports, we classified the type of paralysis observed in the American ginger brandy cases as hypothetically between "apiol" paralysis and "torpedo oil" poisoning. Involvement of the central motor tracts was demonstrated only in the severe cases of this poisoning epidemic. A comparison with our animal experimental findings suggests that these poisonings were based on a tricresyl phosphate intermediate between the preparations that were thoroughly tested by us (pure tri-ortho-cresyl phosphate and industrial products with 25-30% o-cresol), i.e., a tricresyl phosphate that must have had a high content of ortho-cresol. In fact, according to reports in the technical literature in the USA, an industrial tri-ortho-cresyl phosphate was used as a plasticizer of celluloid and lacquers.

Long before the toxic effects of the tri-ortho ester became known, tri-o-tolyl phosphate was being discussed as a camphor substitute in pertinent
published reports and patent documents [148]. In all probability, pure ortho-
cresol was not being produced for such industrial products because its prepa-
ration is very expensive; the starting material that was used probably still
contained a certain amount of the isomers and homologues of ortho-cresol.

Our search of the literature uncovered certain information pointing in
this direction that is important to our problem. Smith et al. [6–10] used an
"industrial tri-ortho-cresyl phosphate" for their first animal experimental
studies on the etiology of ginger paralysis. In a review report on the ginger
brandy cases, Kidd and Langworthy [5] state that a commercial tricresyl phos-
phate was sold under the name Lindol or Lyndol, which consisted mainly of tri-
ortho-cresyl phosphate with only small percentages of meta and para compounds.
After the paralytic effect of the tri-ortho compound became known, the non-
toxic meta and para esters were used to produce Lindol. According to Sampson
[32], a "Lindol" was the cause of the illnesses in 1937/38 in Durban. Accord-
ing to "careful analysis", a contaminated soybean oil, which was responsible
for the mass poisoning, contained "0.28% tricresyl phosphates", which con-
sisted of the 3 isomers in the following proportions: tri-ortho- 58.2%, tri-
para- 23.7%, and tri-meta- 18.1%.

According to these reports, a tricresyl phosphate with a high content of
ortho-cresol and therefore a high content of tri-ortho ester was very probably
present in the toxic ginger brandies. According to our present discussion,
such preparations produce predominantly peripheral paralysis. Consistent with
this, spastic symptoms are not reported in the victims of the poisoning epi-
demic in Durban, even after an observation period of more than 3 years [32].
3. "Apioi" Paralysis

Samples of apiol, which had been demonstrated to produce paralysis, were reported to contain 28–50% tri-ortho-cresyl phosphate; it is explicitly stated in these reports that chemical analysis showed the presence of tri-ortho-cresyl phosphate [11-13]. This seems to confirm our hypothesis that a pure or almost pure tri-ortho-cresyl phosphate was present in the toxic varieties of apiol on the basis of the clinical picture and the effect in the animal experiment.

However, critical review of those analytical reports shows that the phosphoric acid content was probably quantitatively determined, but cresol was determined only by qualitative methods. In particular, the presence of ortho-cresol was determined only on the basis of a positive Melzer sample [11, 12]; a quantitative ortho-cresol determination was not performed, and apparently there was also no testing for the presence of the meta- and para-isomers.

Since the only pure triester that is active is the tri-ortho compound, the analytical results were automatically related to tri-ortho-cresyl phosphate under the influence of the old conception, according to which only the three specified compounds exist in industrial tricresyl phosphates. There was thus no firm foundation for the statements that were then repeatedly cited, to the effect that the paralyzing apiol samples contained 28–50% tri-ortho-cresyl phosphate.

Despite comprehensive follow-up studies, including studies by official testing institutes and by manufacturers of tricresyl phosphate, we were unable to procure reliable documentation about the composition of the ester preparation that had been added to some apiols. In addition to the agreement between clinical symptomatology and animal experiments with tri-ortho-cresyl...
phosphate, there is further evidence that the "apiol" paralysis very probably involved the tri-ortho ester. At the beginning of the 1930s, there was a strong increase in demand for the abortifacient apiol as a result of the economic depression, especially since just prior to this the specific effect of this preparation had been clinically confirmed [149–151]. In isolated cases the increased demand may have induced unscrupulous individuals to replace parsley extracts by a substance with similar physical properties; it is possible that someone with this intention thought to use the likewise bluishly opalescent, oily substance tricresyl phosphate. The intensity of the opalescence depends partly on the content of ortho-cresol; therefore, it seems understandable that one would use tri-ortho-cresyl phosphate to adulterate apiol.

4. Paralysis by Tricresyl Phosphates of the "Torpedo Oil" Type

Reliable information is available on those tricresyl phosphates that caused a large number of poisonings in Europe and especially in Germany during the war and the postwar years. Commercial crude cresol or tricresol, whose o-cresol content can vary between 25 and 40%, was used as the starting material for producing tricresyl phosphates.

The group poisoning described by Staehelin was based on an industrial phosphate whose ortho content is given as 40% [30, 100]. The tricresyl phosphate produced at that time in Bitterfeld and used in East German soft Igelit products contained about 30% o-cresol in the aromatic component, as is apparent from official comments [152] and analyses [82]. The aryl phosphates that were added to the torpedo oil were produced from crude cresol of the exact same type.
By analyzing the clinical symptoms in man and the poisoning symptoms and pathoanatomical changes in experimental animals and by examining the chemical literature, we have been able to show that three different types of poisoning based on chemically different tricresyl phosphates can be distinguished in the observed tricresyl phosphate poisonings in man. Depending on the content of ortho-cresol in an ester mixture, damage to the peripheral motor fibers is accompanied by more or less strong involvement of the central nervous pathways in the degeneration process. This is most markedly the case in paralysis caused by tricresyl phosphates of the "torpedo oil" type, which, with an ortho-cresol content of about 30%, contain predominantly mono-ortho-substituted mixed esters and were identified in the toxicity test as the relatively strongest preparations. Central involvement is less prominent in the case of tricresyl phosphates with high ortho values, as was the case with the ginger brandies; according to available records, the o-cresol contents were at least twice as high as in the phosphates of German production during the war and postwar years, and the content of mixed esters was thus significantly lower. Finally, pure or almost pure tri-ortho-cresyl phosphate produces almost exclusively peripheral paralysis. Although final conclusive proof has not yet been produced due to a lack of suitable chemical analytical data, this type of paralysis appears to have been present in the "apiol" poisonings.

This would be an additional argument in favor of Schaltenbrand [116] (cf. also [58]), who at that time correctly characterized "apiol" paralysis as "elective polyneuritis".

The clinical picture is described as tri-ortho-cresyl phosphate poisoning in almost all relevant papers, textbooks, and reference books. The concept on which this term is based is now outdated. As was shown above, the observed
illnesses were not usually produced by the tri-ortho-cresyl ester of phosphoric acid, but rather by a relatively high content of ortho-cresyl radicals in the preparations and especially by highly toxic mono-ortho-di-(meta, para)-cresyl esters. However, since industrial mixtures of triphosphates of isomeric cresols are always involved, we should generally speak of tricresyl phosphate poisoning.

SUMMARY

According to the conventional view, industrial tricresyl phosphate is a mixture of the 3 isomers tri-ortho-, tri-meta-, and tri-para-cresyl phosphate, and its toxicity corresponds to the content of tri-ortho compound or ortho-cresol.

In comparative toxicological animal experiments, older industrial tricresyl phosphates with about 30% ortho-cresol are, surprisingly, 10 times more toxic than pure tri-ortho-cresyl phosphate, whereas modern preparations containing about 3% o-cresol are about 3 times weaker than the tri-ortho ester and about 30 times weaker than those highly toxic ester mixtures. Accordingly, there is no correlation between the toxicity and the ortho-cresol content.

Contrary to the prevailing published opinion, industrial tricresyl phosphates contain not only the three uniform triesters specified above, but also seven other isomeric mixed esters. Only tricresyl phosphates with ortho-cresyl radicals were found to have toxic paralytic effects. Their toxicity decreases in the order mono-, di-, and tri-ortho-cresyl esters in the proportions 10:5:1.

A mathematical analysis of the theoretically possible proportions of
these mixed esters in tricresyl phosphates, which could arise from creosol mixtures with variable contents of ortho-cresol with a purely statistical distribution, yields the basis for understanding the differences in toxicity in industrial tricresyl phosphates. The content of the strongest mono-ortho esters is highest at 33 1/3% o-cresol, i.e., the range of ortho-cresol contents of the highly toxic industrial phosphate mixtures that produced most of the poisonings of the war years and postwar period.

The newly gained knowledge requires a revision of the previous toxicological evaluation of tricresyl phosphate poisoning; in particular, the calculation of minimal toxic doses may no longer be related to tri-ortho-cresyl phosphate, but rather must be related to the total preparation.

Comparative consideration of the clinical pictures of tricresyl phosphate poisoning shows that 3 types of poisoning can be distinguished, which were observed during the major poisoning epidemics: certain "apiol" preparations produced almost exclusively peripheral paralysis without demonstrated involvement of central pathways; in the American "ginger epidemic" involvement of the central neuron was reported only in very severe cases; cases of paralysis that occurred during the war and in the postwar period ("torpedo oil poisoning", "Igelit poisoning") were accompanied by spinal damage in the majority of moderately severe and severe cases.

The conclusion that the different types of poisoning were caused by chemically different tricresyl phosphates is supported by our own animal experiments. Industrial tricresyl phosphates with an ortho-cresol content of about 30% and mixed esters containing ortho-cresyl radicals produce predominantly spastic symptoms, whereas pure tri-ortho-cresyl phosphate produces only flacid paralysis. Histological studies confirm that the two types of tricresyl...
phosphates trigger different pathogenetic processes. Pure tri-ortho-cresyl phosphate produces lesions mainly in the peripheral nerves with only slight involvement of certain nervous pathways of the spinal cord; with respect to the highly toxic tricresyl phosphates, the damage is concentrated in the spinal cord, and there is only slight involvement of the peripheral nerves.

This knowledge leads to a new interpretation of the etiology and pathogenesis of the various poisoning epidemics that have occurred. The "ginger paralysis" in America was very probably caused by industrial tricresyl phosphate with a high ortho-cresol content; on the other hand, the poisonings which occurred in Europe during the war and in the postwar period were probably caused by tricresyl phosphates that contained about 30% ortho-cresol and consisted chiefly of mixed esters. The cases of paralysis observed after ingestion of apiol preparations were very probably caused by an almost pure tri-ortho-cresyl phosphate; in this variant of the poisoning, which at the time was justifiably characterized as "motor polyneuritis", it seems that no spastic symptoms were ever observed.

The therapeutic use of creosote phosphate has resulted in numerous cases of paralysis since the turn of the century. Our own experiments lead to the conclusion that the medication is occasionally contaminated by high-boiling phosphoric acid esters containing ortho-cresyl radicals with paralytic properties due to improper preparative technique.

The results that have been presented here show that the term "tri-ortho-cresyl phosphate poisoning" should no longer be used; instead, we should use only the more general and more accurate term "tricresyl phosphate poisoning".
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