Environmental Health Perspectives
Vol. 13, pp. 77–81, 1976

Physicochemical Properties of Some Organophosphates in Relation to Their Chronic Toxicity

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Organophosphates in increasing amounts and wider varieties of molecular structure are being used to replace the organochlorine pesticides. It has been assumed that the organophosphates as a class would have a minimal chronic toxicity due to rapid hydrolysis and an unfavorable partitioning as regards to lipids. The physicochemical properties of a number of representative organophosphates were determined, including the octanol/water partition coeffecient, hydrolysis, and binding to proteins. Those having a halogenated aryl substitution were found to have partition coefficients not largely different from the organochlorines, suggesting the possibility of fat deposition. Certain of these compounds are sufficiently stable toward hydrolysis to allow bioaccumulation to occur. These preliminary findings appear to indicate a correlation between readily determined physical properties and the potential for chronic effects in this class of compounds.

Introduction

As use of the organochlorine pesticides has been phased out, greater quantities of the organophosphates have come to be employed. The shorter persistence and presumed lack of cumulative effects on mammals have been features of the organophosphates that have led to their substitution for the organochlorine compounds, at least in some instances (*1*). In other situations, the organophosphates have provided the means of control of insects that have developed genetic resistance to other substances (*1, 2*).

One of the earliest used organosphosphates was TEPP (tetraethyl pyrophosphate) (*1*). While highly effective, TEPP had two serious drawbacks: an acute mammalian toxicity and a very short residual effect. These shortcomings were shared by a number of the earlier introduced organophosphates; in order to overcome this, a large number of different organophosphates have been developed (*1,3*). Among these were derivatives of phosphonic acid, phosphorothioates, phosphorodithioates, and phosphoroamidates. The objectives in development of this variety of phosphorus-containing pesticide were to increase the selectivity and effectiveness as insecticides, reduce mammalian toxicity, and increase the length of residual effect.

Many of the organophosphates are highly labile under environmental and physiological conditions (*4*). A common method of breakdown is the hydrolysis of the organophosphorus ester yielding products of substantially lower toxicity. However, the substitution of larger groups, e.g., substituted phenyl groups, on the phosphorus-containing compounds have yielded substances more refractory to hydrolysis. As will be shown, some of these compounds are quite stable under hydrolytic conditions that would normally result in rapid dismutation of other organophosphates. Because of the rapid

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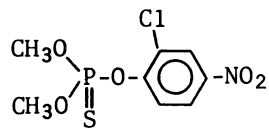
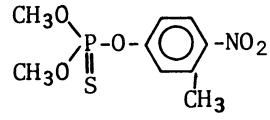
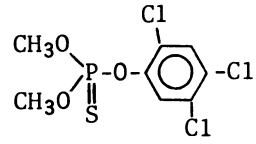
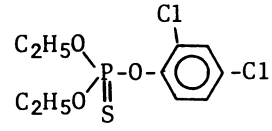
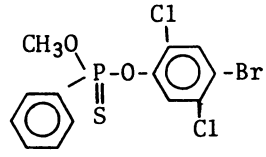
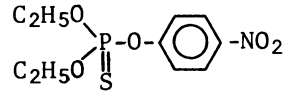
detoxication of phosphates, it has been widely assumed that little in the way of cumulative effects would be expected from a single or limited number of exposures (*3*). Such cumulative effect as has been studied has largely been depression of cholinesterase activity from repeated exposures. However, there are indications of accumulation of certain of the organophosphates in mammalian tissues with subsequent chronic effect (*2,3*). This appears to be particularly true of the substituted aryl derivatives.

A number of studies have been conducted to attempt to relate the structure and/or properties of the organophosphates to their biological activity. Since one of the primary biological effects of the organophosphates is inhibition of cholinesterase, the active site of which has a dimension of between 4.2 and 5.4 A°(*1*), it has been possible to establish this as a critical dimension between the anionic and cationic portions of the active compound. However, these dimensions do not determine exclusively either the intrinsic activity of the organophosphate or its spectrum of selectivity. Consequently, investigators have examined the enzyme inhibition constant in relation to structure, hydrolysis constant, sigma values, π values, and molecular orbital parameters in relation to activity (*5–8*).

The study reported here was concerned with the structure–property relationships of the

Table 1. Chemical structure and LD_{50} of organophosphates.

Chemical	Structure	Molecular weight	LD_{50}, mg/kg Oral	LD_{50}, mg/kg Dermal
Decapthon		297.6	475	> 1000
Fenitrothion		277.2	250	> 3000
Ronnel		321.5	1250	2000
Dichlofenthion (VC-13)		315.2	270	6000
Leptophos		344.4	90	> 800
Parathion		291.3	13	21

organophosphates in relation to possible accumulation and chronic effects in mammals. A number of physical properties or organophosphates were determined more accurately than heretofore reported, or are reported for the first time. An attempt is made to relate some of these properties as well as the structures to toxic effect.

Experimental and Results

Materials

The organophosphates (Table 1) used were all analytical standards ranging in purity from 95 to 100%. Other chemicals used were: *n*-octanol (J. T. Baker), doubly distilled; hexane (Mallinckrodt), nanograde; fatty acid-free bovine serum albumin (Sigma Chemical). Water utilized was distilled and run through a 2 × 10 cm XAD-2 resin column. This was done in order to remove dissolved organics. Other chemicals used were analytical grade or the best grade available.

Technique

A gas–liquid chromatographic technique was used to analyze the samples. A Tracor 550 GLC utilizing a ^{63}Ni electron capture detector was used. The glass column used was 10 ft × 1/8 in. packed with 1.5% OV-17, 1.95% OV-210 on 60/80 Q. Oven temperature varied from 200 to 260°C, depending on the compound.

Methods

Partition Coefficient Determination. The octanol was doubly distilled and equilibrated with water prior to the experiment. Previous studies had indicated that the reagent grade *n*-octanol was unsuitable for partitioning, possibly due to trace impurities which served to stablize the emulsion formed at the octanol-water interface. Further investigation (hydrogen flame GLC) confirmed the presence of several impurities which were subsequently removed by distillation.

A stock solution of ~ 1000 μg/ml organophosphate in octanol was prepared, and 2 ml of stock solution was added to 35 ml of water in a glass-stoppered 50-ml centrifuge tube. The tubes were shaken gently in a horizontal position for 24 hr at 20°C. A 1-ml portion of octanol was removed for analysis and the remainder withdrawn and discarded along with the top few milliliters of the aqueous phase. The remaining aqueous phase was centrifuged to 3 hr. (Servall centrifuge at 5000 rpm) at 20°C. Several milliliters were again discarded to remove octanol separated by centrifuging and a 10 ml sample withdrawn for analysis. The transfer pipet was rinsed with ca. 3 ml hexane, the hexane then being used for the first extraction of the aqueous phase. All samples were extracted three times with hexane and then made up or blown down to convenient concentrations for analysis via GLC. A hexane dilution of the octanol phase was made followed by direct GLC analysis.

The partition coefficient K of an organophosphate is defined eq. (*1*) as the ratio of its concentration in octanol and water. The K values and the water solubility of organophosphates are given in Table 2.

$$K = [C]_{octanol}/[C]_{water} \quad (1)$$

Table 2. Partition coefficient and water solubility of organophosphates at room temperature.

Compound	Purity, %	K	Solubility, ppm
Fenitrothion	98.5	2,381 ± 3%	30[a]
Dicapthon	99.8	3,785 ± 2%	7.8[b]
Parathion	98.5	6,431 ± 4%	20[c], 24[d]
Ronnel	95 +	75,266 ± 11%	1.7[b], 44[c], 100[e]
Dichlofenthion	97.0	137,220 ± 4%	0.245[d]
Leptophos	100	2,024,000 ± 13%	0.009[b], 0.03[f], 2.4[f]

[a]Data of Melnikov (*9*).
[b]Values determined in this laboratory.
[c]Data from Perrine Standard Catalog (*10*).
[d]Data of Spencer (*11*).
[e]Data of Zweig (*12*).
[f]Manufacturers' data (*13*).

Binding with Bovine Serum Albumin. The binding of organophosphates with the bovine serum albumin (BSA) protein was studied by equilibrium dialysis technique. Dialysis tubing was prepared by soaking in hot EDTA and $NaHCO_3$ solution for 4 hr and rinsing repeatedly with stock buffer solution. Buffer solution was 0.025*M* K_2HPO_4 and 0.025*M* NaH_2PO_4 with a pH of 6.83.

Solutions of organophosphates were prepared by saturating the buffer solution with the desired organophosphate at 4°C. The saturated solution was then diluted with buffer solution to obtain the desired concentrations.

The dialysis experiments were carried out by placing 1 ml of BSA solution (20 mg/ml) in a dialysis bag and dialyzing the bag against 40 ml of

organophosphate solutions of varied concentration. The dialysis was carried out for 24 hr at 4°C. After this period of time a change in the concentration of organophosphate was determined. The analysis of organophosphate was carried out by extraction of the solution with hexane and subsequent gas-liquid chromatography extract.

The decrease in concentration of organophosphate from the control solution was an indication of binding. A series of runs were also done to determine the binding of organophosphates to the bags in absence of protein. This was taken into account when expressing the binding of organophosphates to protein.

The binding constant K for the BSA-organophosphate interaction was determined by the well known Scatchard equation (*14*):

$$\nu/A = K(n-\nu) \qquad (2)$$

where ν is the average number of moles of organophosphate bound per mole or protein, A the equilibrium concentration of organophosphate, and n the binding site.

The binding constants and the number of binding sites are given in Table 3. Leptophos and diclofenthion showed extremely low binding to BSA and thus the K values were not determined.

Table 3. Binding characteristics of organophosphates to bovine serum albumin.

Organophosphate	Binding	Binding constant	Number of binding site(s)
Parathion	Weak	~ 800	
Dicapthon	Moderate	5.2×10^3	~ 1
Leptophos	Weak		
Fenitrothion	Strong	5.6×10^4	~ 1
Dichlofenthion	Weak		
Ronnel	Strong	6.4×10^4	~ 1

Hydrolysis Studies. The hydrolysis rate of organophosphates was measured by the procedure described by Ruzicka et al. (*15*). An initial concentration 6 μg/ml of the chemical in 20:80 ethanol water mixture at pH 6.0 buffer was kept at 70°C in a water bath, and the concentration of organophosphate was determined as a function of time. The halflife was determined from the first-order rate plot. In view of the extremely slow degradation of organophosphate at this pH value a temperature of 70°C was used. This enabled us to complete the experiment in a shorter period of time. Although these conditions are rather extreme, good indication about the comparative hydrolysis rates may be obtained. The halflife or organophosphates is given in Table 4.

Table 4. Halflife of organophosphates.

Chemical	Halflife, hr
Parathion	43.0[a]
Dicapthon	6.4
Dichlofenthion	19
Leptophos	48
Ronnel	10.5
Fenitrothion	11.2[a]

[a]Data of Ruzicka et al. (*15*).

Discussion

One of the more striking measurements was that of the partition coefficient. It will be noted that at least two of the organophosphates (dichlofenthion and leptophos) show partition coefficients into the organic phase within the order of those found with organochlorine compounds. These high partition coefficients would indicate the possibility of accumulation of these materials in fatty depots. Indeed, the case has been described for dichlorfenthion (J. E. Davies, personal communication) where an individual ingesting a single oral dose of this material, showed toxic levels of the compound for at least 30 days following the exposure. For many of the less fat-soluble and more labile organophosphates, the hydrolysis and excretion will be essentially complete in 3–10 days.

The solubility of all of the organophosphates examined in this study is higher in lipid type solvent than in water. From this information alone, it may be predicted that these compounds would tend to accumulate in fat depots, at least to a limited extent. However, studies of the dynamics of a number of these compounds in the mammalian body indicate that this is not the case. Either the compound is found in another compartment, or it is so labile that accumulation does not occur. In a few instances, protein binding may allow for some accumulation, but for the most part the rapid metabolism of the compounds probably accounts for the lack of accumulation. In examination of the hydrolytic halflife, many of these compounds tend to substantiate this conclusion. Of the two compounds (dichlofenthion and leptophos) known to both accumulate and have a tendency to chronic

effect, one finds both have a high partition coefficient into the lipid layer, and a slow rate of hydrolysis. It is interesting to note that these two chemicals show extremely weak binding to the protein bovine serum albumin. These factors then strongly suggest the possibility of chronic effects ensuing from fat depots accumulation and slow metabolism. Thus we have what appears to be a quite good correlation between the physical properties of certain of the organophosphates, and their tendency for accumulation and excretion of a chronic toxic effect.

Acknowledgement

This work was supported in part by the grant ES-00210 from NIEHS.

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