In Vivo Effects of Paired Combinations of Five Organic Phosphate Insecticides

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Paired combinations of five organic phosphates were fed to dogs at two levels for 6 weeks. Serum and red blood cell cholinesterase levels were followed by a modified Michel method before and during the feeding period. Many of the combinations produced significant depression of cholinesterase, but these effects were considered only additive. At higher than tolerance levels, the occurrence of potentiation between EPN and Systox was noted. Potentiation was not observed with any pair at tolerance levels, so that this phenomenon would appear to present no public health problem in the present instances.

THE OBSERVATION by Frawley *et al.* (*8*), that malathion {*S*-[-1,2-bis (ethoxycarbonyl)ethyl]*O,O* - dimethyl phosphorodithioate} in combination with EPN (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate) produced severe potentiation of toxicity both acutely and subacutely in the rat and dog, raised the question of the possibility of potentiation between other pairs of organic phosphates. Recently, Frawley and Fuyat (*7*) studied the subacute effect of the simultaneous administration of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) and Systox [a mixture of thiono and thiol isomers of *O,O*-diethyl-(2 ethylmercaptoethyl)thiophosphate] at three levels and over 24 weeks on dog plasma and erythrocyte cholinesterase and found only additive effects. The four phosphate pesticides already mentioned and methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate) appear as legal residues in foods and might, if found to be potentiators, present some health hazard.

Toxicological studies of these individual materials are numerous. EPN has been studied by Hodge *et al.* (*13*) and by Frawley, Hagan, and Fitzhugh (*9*); Systox by Deichmann and Rakoczy (*2*, *3*) and by Barnes and Denz (*1*); malathion by Hazleton and Holland (*12*); methyl parathion by DuBois and Coon (*4*); and parathion by DuBois *et al.* (*5*, *6*), Hagan and Woodard (*10*), and Hazleton and Godfrey (*11*).

The present study evaluates the toxicological hazard, if any, of these five materials when fed in paired combinations subacutely and at low levels as a part of the diet of the dog.

Material and Method

Twenty-two male and 22 female dogs of mixed breeds, unknown ages, and weighing 6 to 10 kg. were used in this study. All animals were housed individually in metabolism cages and had access to food, and water *ad libitum*. The food, prepared each week, consisted of a commercial ground dog chow to which was added the proper concentration of a stock mixture containing a corn oil dilution of the phosphate in question.

All animals were acclimated to the laboratory environment for at least a week. Following this adjustment period, five control cholinesterase determinations per dog were made over the next 4 weeks by the electrometric method of Michel (*14*), as modified by Frawley and Fuyat (*7*).

Except as noted, groups of one male and one female dog were then placed on all possible paired combinations of the five phosphates both at tolerance and at the so-called safe levels—i.e., the highest no-effect levels.

	Levels, P.P.M.	
Phosphates	Tolerance	Safe
EPN	3.0	20.0
Systox	0.75	2.0
Malathion	8	100
Methyl parathion	1.0	5.0
Parathion	1.0	1.0

Blood was drawn from the external jugular vein, and plasma and red cell experimental cholinesterase determinations were made at the end of the 1st, 2nd, 3rd, 4th, and 6th weeks.

Results and Discussion

The results obtained at the end of the 6th week are expressed as final delta pH and per cent pretreatment control in Tables I and II for plasma and red cell cholinesterase, respectively. Pretreatment control levels were determined from five cholinesterase measurements over a period of 4 weeks prior to the beginning of insecticide feeding. Delta pH deviations for each animal in excess of twice the standard deviation of the control were considered significant—i.e., $p < 0.05$. While significant depression was noted with many of the combinations, in only a few cases was potentiation in evidence. With the exception of the combination of EPN (20 p.p.m.) and malathion (100 p.p.m.), potentiation was observed only in the plasma cholinesterase. The criteria for determination of potentiation were based upon the following principle:

Composite male and female dose-response curves for plasma cholinesterase were plotted from previous studies (*7*, *8*) and unpublished data for each of the organic phosphates under study based upon final delta pH levels at the end of 6 weeks of feeding of the individual toxicant. From these curves, the amount of depression expected from the low levels of individual phosphates used in these studies was determined by extrapolation. With the amount of depression induced by compound A, for example, transferred to the dose-response curve of compound B, the amount of further depression expected from the addition of compound B can be plotted. This figure would theoretically represent the additive effect of the two materials. Similarly, the depression expected from compound B can be read from the dose-response curve, this value being transferred to the similar curve for compound A and the additional depression determined which should result from dosing with compound A. These manipulations produce two values. The observed experimental values were compared with these, and potentiation was considered to be present only when the experimental values indicated depression greater than the lower of the two values determined by the method described above.

The combinations of parathion and methyl parathion at 1.0 p.p.m. each induced a mean depression to 54% of the pretreatment control. A comparison of the expected depression as a result of addition alone, based upon the analysis described above, indicates an expected spread of from 55 to 71%. Potentiation if present is minimal. A similar condition existed when Systox and methyl parathion were fed at 0.75- and 1-p.p.m. (levels). The expected spread

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Table I. Dog Plasma Cholinesterase Levels after 6 Weeks of Feeding Phosphate Mixtures

		Final ΔpH	% [b]	Methyl Parathion, 5.0 P.P.M. Final ΔpH	% [b]	Parathion, 0.5 P.P.M. [a] Final ΔpH	% [b]	EPN, 20.0 P.P.M. Final ΔpH	% [b]	Malathion, 100.0 P.P.M. Final ΔpH	% [b]	
	♂			0.54 [c]	73	0.78 [c]	76	0.36 [c]	42	0.38 [c]	66	Systox, 2.0 P.P.M.
	♀			0.55 [c]	92	0.77 [c]	83	0.42 [c]	47	0.84 [c]	63	
Methyl Parathion, 1.0 P.P.M.	♂	0.46 [c]	84			0.54 [c]	76	0.51 [c]	65	0.78	89	Methyl Parathion, 5.0 P.P.M.
	♀	0.35 [c]	61			0.54 [c]	59	0.68	83	0.84	83	
Parathion, 1.0 P.P.M.	♂	0.46 [c]	50	0.57 [c]	53			0.43 [c]	67	0.70 [c]	74	Parathion, 0.5 P.P.M. [a]
	♀	0.51 [c]	59	0.42 [c]	55			0.81	89	0.59 [c]	72	
EPN, 20.0 P.P.M.	♂	0.95 [c]	86	0.71 [c]	89	0.47 [c]	71			0.88	99	EPN, 20.0 P.P.M.
	♀	0.81 [c]	135	0.76 [c]	83	0.56 [c]	60			0.62	81	
Malathion, 8.0 P.P.M.	♂	0.70	80	1.06	78	0.53 [c]	61	0.67	89			Safe Levels
	♀	1.02 [c]	80	0.92	88	0.52 [c]	54	0.92 [c]	76			Tolerance Levels
		Systox, 0.75 P.P.M.		Methyl Parathion, 1.0 P.P.M.		Parathion, 1.0 P.P.M.		EPN, 3.0 P.P.M.				

[a] Parathion levels are 1/2 safe levels. [b] Per cent of pretreatment control. [c] Significance at 5% level.

Table II. Dog Red Cell Cholinesterase Levels after 6 Weeks of Feeding Phosphate Mixtures

		Final ΔpH	% [b]	Methyl Parathion, 5.0 P.P.M. Final ΔpH	% [b]	Parathion, 0.5 P.P.M. [a] Final ΔpH	% [b]	EPN, 20.0 P.P.M. Final ΔpH	% [b]	Malathian, 100.0 P.P.M. Final ΔpH	% [b]	
	♂			0.82 [c]	89	0.68	103	0.63 [c]	85	0.61 [c]	83	Systox, 2.0 P.P.M.
	♀			0.83	115	0.89 [c]	85	1.09 [c]	89	0.97	104	
Methyl Parathion, 1.0 P.P.M.	♂	1.03	93			0.76	97	0.79 [c]	81	0.60 [c]	81	Methyl Parathion, 5.0 P.P.M.
	♀	0.97	99			0.51 [c]	90	0.73	90	0.99	90	
Parathion, 1.0 P.P.M.	♂	0.73 [c]	93	1.12	93			0.87	97	0.69 [c]	72	Parathian, 0.5 P.P.M. [a]
	♀	0.69	97	0.97	114			0.49 [c]	94	0.52 [c]	71	
EPN, 3.0 P.P.M.	♂	0.80	105	0.95	97	1.02	100			0.19	35	EPN, 20.0 P.P.M.
	♀	0.72	95	1.12	100	1.14	99			0.21	33	
Malathion, 8.0 P.P.M.	♂	1.07	100	0.88	95	0.73	90	0.81	88			Safe Levels
	♀	1.117 [c]	110	0.97	101	0.92 [c]	86	1.02	98			Tolerance Levels
		Systox, 0.75 P.P.M.		Methyl Parathion, 1.0 P.P.M.		Parathion, 1.0 P.P.M.		EPN, 3.0 P.P.M.				

[a] Parathion levels are 1/2 safe levels. [b] Per cent of pretreatment control. [c] Significance at 5% level.

based upon dosage response curves was from 74 to 85% of pretreatment control; the actual study indicated a mean depression to 72.5%. The 1.5% difference indicates questionable potentiation.

The administration of safe levels of Systox and EPN at 2.0 and 20.0 p.p.m., respectively, produced a mean depression in plasma cholinesterase to 44% of pretreatment control (Table I). This value is 12% below the estimated low of 56 (spread 56 to 78%). Some potentiation is present in this combination at these levels. No other combinations induced enough plasma cholinesterase depressions potentiation.

Paired mixtures of the same phosphates had appreciably less effect on the red cell cholinesterase (Table II) than on the plasma. No indication of potentiation was seen in red cell cholinesterase except with EPN and malathion, where the extent of the potentiation has been described in detail by Frawley (*8*) and has been confirmed in this study (Table I). The mean depression noted by Frawley (*8*) in the red cell cholinesterase was to 46% pretreatment control level, while the mean depression in this study was to 40%—a slightly greater de-

gree of potentiation than previously reported. Using the criteria described earlier, the spread is 90 to 90%; thus the differential is 50% for this pair, indicating definite potentiation.

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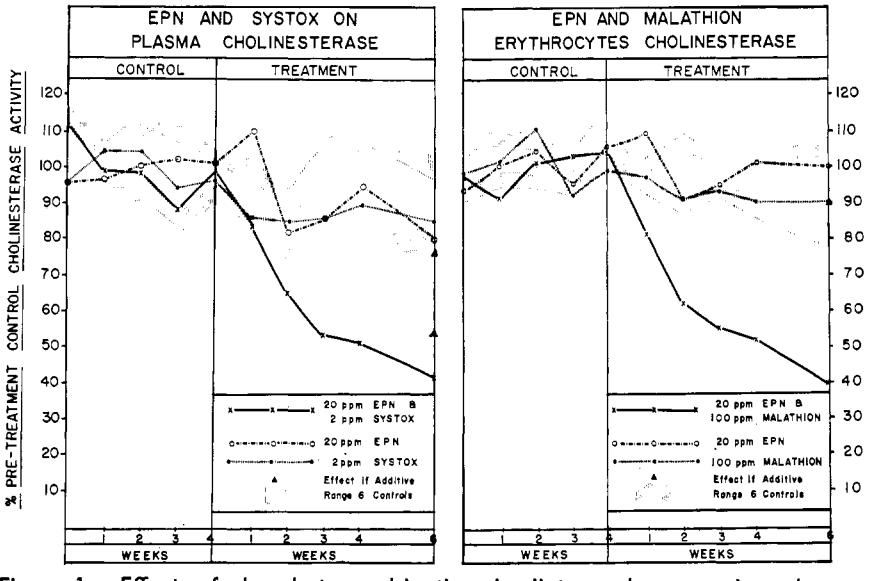

Figure 1. Effects of phosphate combinations in diet on plasma and erythrocyte cholinesterase

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INSECTICIDE RESIDUES

Endrin Content of Body Tissues of Steers, Lambs, and Hogs Receiving Endrin in Their Daily Diet

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Steers, lambs, and hogs fed endrin at dietary levels of 0.1 p.p.m. for 12 weeks showed little tendency to deposit endrin in body tissues. After 12 weeks of endrin feeding at 0.25 p.p.m., the endrin content of the fat of these animals was not higher than 0.2 p.p.m. Other tissues contained no detectable endrin at this level of intake. The analyses were performed with a method specific for endrin and sensitive to 0.1 p.p.m.

THE USE OF RESIDUAL INSECTICIDES against pests of forage crops is a practice of great potential value to agriculture. However, the problem of residues remaining when the crop is consumed by livestock and the extent of subsequent contamination of meat products must be investigated.

Claborn (*3*) measured the residues present in the fat of steers and sheep after these animals ingested feed contaminated with several chlorinated hydrocarbons. Several authors have reported the presence of DDT in animal tissues. It has been found in the tissues of sheep (*6, 13*), hogs (*1, 7*), steers (*5*), and calves (*12*) after the ingestion of DDT-treated feed. Toxaphene accumulates in the fat of steers and sheep (*2, 4*), and high levels of lindane in the diet result in the deposition of this insecticide in fat (*9*).

The present report describes analytical results obtained when the chlorinated hydrocarbon insecticide, endrin (1,2,3,4,10,10 - hexachloro - 6,7 - epoxy-1,4,4*a*,5,6,7,8,8*a* - octahydro - 1,4 *endo-endo* - 5,8 - dimethanonaphthalene) was fed to sheep, steers, and hogs, and various tissues from these animals were analyzed for endrin residues. A more complete report listing all relevant data is available in mimeograph form (*11*).

Experimental

The steers and lambs were purchased from local sources and the hogs were from the Oregon State College swine herd. The steers were stanchioned throughout the experiment. Hogs and lambs were housed in individual pens. All animals were kept under shelter.

The ration for steers and lambs was composed of barley, oats, and grass hay. The hog ration consisted of barley, oats, alfalfa meal, tankage, steamed bone meal, oyster shell flour, and iodized salt. Salt and water were available *ad libitum*. All animals were fed twice daily in individual feeders. Analyses of the rations prior to the feeding experiments indicated that they were free from endrin contamination.

The rations were fortified by distributing endrin in acetone solution over the entire ration at each feeding. A separate glass syringe was used for each level of toxicant. The endrin solutions were prepared so that the desired level of fortification could be attained by