Studies on combined effects of Organophosphates or Carbamates and Morsodren in Birds. II. Plasma and Cholinesterase in Quail fed Morsodren and Orally dosed with Parathion or Carbofuran

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We initiated studies on the interaction of mercury and cholinesterase-inhibiting pesticides when it was found that high concentrations of mercury also inhibited cholinesterase activity (DIETER 1974). The first paper of this series reported that mercury would not interfere with the cholinesterase bioassay for organophosphates (DIETER and LUDKE 1975). Nevertheless, we were able to demonstrate an increase in the oral toxicity of parathion in Coturnix quail (Coturnix coturnix japonica) fed 4.0 ppm morsodren (methyl mercury dicyandiamide) for 18 weeks. We attributed a portion of the increase in parathion sensitivity to greater inhibition of blood and tissue cholinesterase caused by the combination of parathion and morsodren. Brain cholinesterase inhibition was almost twice as great in morsodren-fed birds as in clean-fed ones dosed with identical sublethal amounts of parathion. However, the concentrations of morsodren fed in that study resulted in tissue levels of mercury that were higher than levels usually encountered in the environment. The experimental regimen resulted in an accumulation of 21.0 ppm mercury in the liver and 8.4 ppm in the carcass. We were concerned that enhancement of the toxicological effects of parathion might occur at lower mercury concentrations. In the present study, we fed a range of morsodren concentrations that would result in accumulations of tissue mercury similar to levels reported in environmental samples. Because different classes of cholinesterase-inhibiting pesticides interact differently with mercury, we also compared the biochemical interaction between mercury and the carbamate, carbofuran, and mercury and the organophosphate, parathion.

MATERIALS AND METHODS

Male Coturnix quail were raised from our own breeding colony and at 6 weeks of age were randomly selected for treatment. For 18 weeks they were fed either 0.05, 0.50, or 5.0 ppm morsodren (dry weight as methyl mercury) or the carrier (1 percent propylene

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glycol) mixed into turkey breeder mash. Birds randomly chosen from these groups were fasted 30 minutes and orally dosed with a sublethal concentration of parathion or carbofuran (0.5 mg/kg); 60 minutes later their plasma and brain cholinesterase activities were compared.

Blood was obtained from the alar vein in microhematocrit tubes and the plasma separated. Birds were sacrificed by cervical dislocation, and whole brains were immediately removed and homogenized in cold phosphate buffer (0.1 M, pH 8.0). Aliquots of plasma (20 µl) or whole homogenates of brains (200 µl of a 10 mg/ml homogenate) were used to determine cholinesterase activity by the ELLMAN et al. (1961) method. Activity was expressed in milliunits for plasma (nmol substrate transformed/min/ml) or units for brain (nmol substrate transformed/min/mg wet weight).

Livers and carcasses (skinned birds minus beaks, feet, wings, and GI tracts) were analyzed for mercury by cold vapor atomic absorption at WARF, Inc., Madison, Wisconsin.

RESULTS

None of the birds fed morsodren exhibited signs of toxicity. Final body weights and brain weights did not differ between controls and morsodren-fed birds.

There was no consistent effect of morsodren on plasma cholinesterase activity, although a 17 percent reduction did occur in one group of birds fed 5.0 ppm (see undosed birds in Tables I and II). Sixty minutes after 0.5 mg/kg parathion dosage, birds fed clean feed exhibited an 89 percent reduction in plasma cholinesterase activity (Table I). No additional reduction occurred in morsodren-fed birds after dosage with this organophosphate pesticide. However, dosage with the same concentration of carbofuran (0.5 mg/kg) caused a 79 percent inhibition of plasma cholinesterase activity in clean-fed birds (Table II), and an 84 percent reduction in enzyme activity in morsodren-fed birds.

Morsodren alone, even at the highest concentration fed, had no effect on brain cholinesterase activity. Values in a group of eight birds fed 5.0 ppm morsodren for 18 weeks had a mean and standard error of 10.55 ± 0.43 units; this mean was essentially identical with those for control birds in Tables I and II, and was similar to that reported in our previous study (10.03 ± 0.29 brain cholinesterase units) from birds fed 4.0 ppm morsodren (DIETER and LUDKE 1975).

Parathion dosage caused a 29 percent inhibition of brain cholinesterase activity in clean-fed birds, and a progressively greater degree of enzyme inhibition in birds fed morsodren (Table I and Figure 1). Generally, the differences between the morsodren-fed and clean-fed groups were not significant, except

TABLE I

Plasma and Brain Cholinesterase Activity in Coturnix Quail Fed Graded Levels of Morsodren or Clean Food, and Dosed with Parathion. Means ± S.E., N=8.

Dosage	CLEAN FOOD	MORSODREN IN FOOD (ppm) 0.05	0.50	5.00
		PLASMA		
None	1892±147	2040±173	2208±273	2182±220
0.05 mg/kg Parathion	214± 13[a]	225± 30[a]	277± 41[a]	247± 38[a]
		BRAIN		
None	10.92±0.31	--	--	--
0.5 mg/kg Parathion	7.76±0.42[a]	7.47±0.42[a]	7.02±0.44[a]	6.45±0.50[a,b]

[a]Significantly different from birds not dosed, P<0.01.
[b]Significantly different from dosed birds on clean feed, P<0.01.

TABLE II

Plasma and Brain Cholinesterase Activity in Coturnix Quail Fed Graded Levels of Morsodren or Clean Food, and Dosed with Carbofuran. Means ± S.E., N=8.

Dosage	CLEAN FOOD	MORSODREN IN FOOD (ppm) 0.05	0.50	5.00
		PLASMA		
None	2100±92	1900±137	2000±115	1750±87[a]
0.5 mg/kg Carbofuran	440±42[b]	323± 31[b,c]	326± 23[b,c]	285±16[b,c]
		BRAIN		
None	10.37±0.30	--	--	--
0.5 mg/kg Carbofuran	7.39±0.40[b]	5.99±0.29[b,c]	5.14±0.26[b,c]	5.33±0.32[b,c]

[a]Significantly different from birds on clean feed, P<0.01.
[b]Significantly different from birds not dosed, P<0.01.
[c]Significantly different from dosed birds on clean feed, P<0.01.

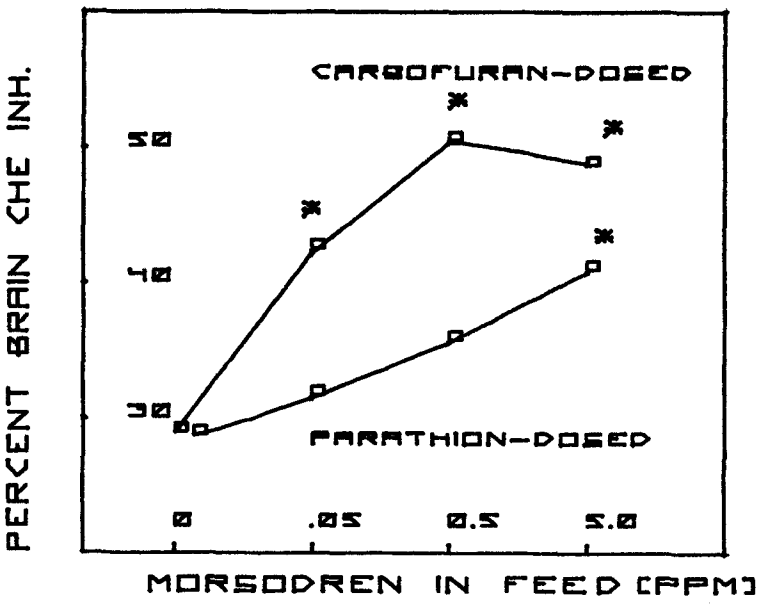

Figure 1. Brain cholinesterase inhibition compared with undosed controls. Asterisk indicates inhibition significantly greater than in dosed birds on clean feed (Student's t test, $P<0.01$).

in one instance. Parathion caused a 41 percent inhibition of brain cholinesterase activity in those fed 5.0 ppm morsodren.

In contrast to the results with parathion, carbofuran dosage of any of the groups fed morsodren resulted in significantly greater brain cholinesterase inhibition compared with dosed birds fed clean food (Table II and Figure 1). Enzyme inhibition was 42 percent below controls in birds fed 0.05 ppm, and 50 percent below controls in birds fed 0.5 or 5.0 ppm morsodren.

Mercury in carcasses and livers of the morsodren-fed birds was directly proportional to the concentrations fed for 18 weeks. Table III lists the concentrations of mercury attained and Figure 2 illustrates the linear correlation between liver and carcass mercury concentrations. The eight values for mercury concentrations in the carcasses and livers of birds previously fed 4.0 ppm morsodren (DIETER and LUDKE 1975) fell on the same regression line, and are included in Figure 2 for comparison.

TABLE III

Carcass and Liver Residues of Mercury in Coturnix Quail Fed Graded Levels of Morsodren. Means ± S.E.

Conc. in Feed	N	Mercury (ppm) Carcass	Liver
0	4	<0.05	<0.05
0.05	8	0.31 ± 0.03	1.10 ± 0.12
0.50	8	1.76 ± 0.11	4.13 ± 0.22
5.00	8	14.40 ± 0.89	35.80 ± 1.8

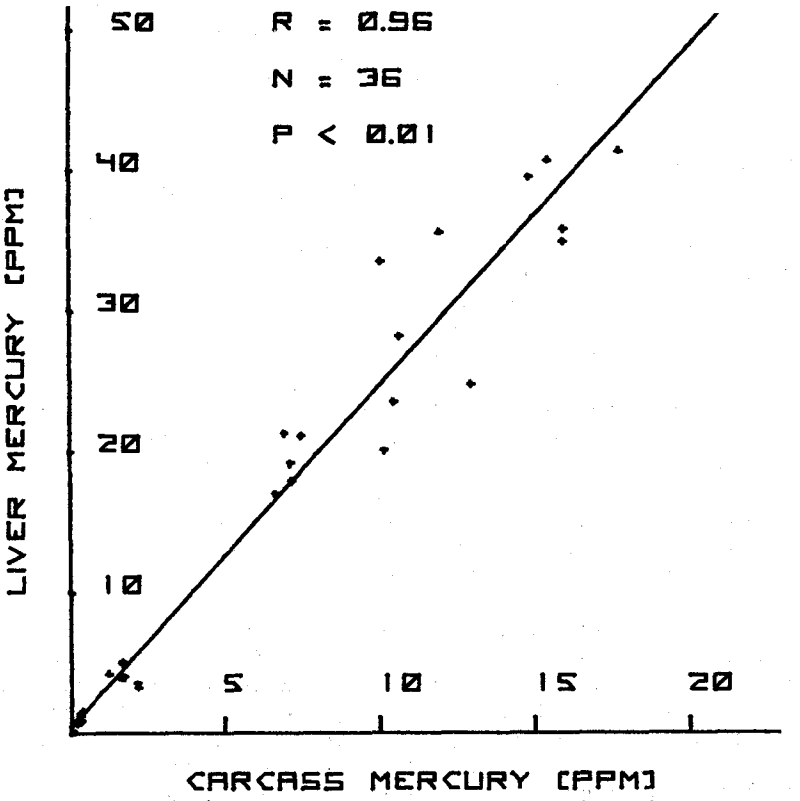

Figure 2. Correlation between mercury concentrations in carcass and liver. The line of best fit is described by the linear regression: liver mercury = 0.45 + 2.4 carcass mercury.

DISCUSSION

Quail fed 5.0 ppm morsodren and dosed with parathion exhibited 41 percent inhibition of brain cholinesterase activity. This was the same magnitude of inhibition obtained in a previous study with parathion-dosed quail fed 4.0 ppm morsodren (DIETER and LUDKE 1975). Our results confirm that the interaction between parathion and mercury is dose-dependent, and significant only when high tissue concentrations of mercury are attained. The threshold for the response in the two studies was reached when birds had accumulated 21 ppm liver mercury (DIETER and LUDKE 1975) or more (36 ppm liver mercury, Table III). At these mercury concentrations brain cholinesterase inhibition after parathion dosage was significantly greater than in clean-fed birds.

By contrast this level of brain cholinesterase inhibition occurred in carbofuran-dosed birds fed only 0.05 ppm morsodren. Realistically this means that birds with liver concentrations of about 1 ppm mercury are more susceptible to what should normally be sublethal dosages of carbofuran. Bird populations with tissue residues of 1 ppm mercury or more are not uncommon (see HEINZ 1976 for review). This illustrates the potential hazard that may occur from indiscriminate usage of cholinesterase inhibitors.

We can only speculate on the mode of interaction between mercury and these two different pesticides. Parathion and carbofuran were equally effective cholinesterase inhibitors in birds given clean feed, but prior mercury exposure rendered carbofuran a much more potent brain cholinesterase inhibitor. Organophosphates like parathion are usually regarded as irreversible inhibitors whereas carbamates are reversible (MATSUMURA 1975); mercury might interfere with the reversibility of carbofuran, increase the duration of the enzyme-inhibitor complex, and make it more toxic than normal. WILKINSON (1976) has hypothesized that in addition to the esteratic and anionic site of the cholinesterase enzyme, several others (hydrophobic, charge transfer, indophenyl) are present that may react with cholinesterase inhibitors and account for their observed different potencies. Perhaps mercury has occupied some critical enzyme sites and resulted in greater brain cholinesterase inhibition with carbofuran than with parathion.

Another explanation for the observed difference between the mercury and parathion or the mercury and carbofuran interaction is based on the fact that mercury inhibits mixed function oxidases in the liver (LUCIER et al. 1972), thereby possibly reducing the formation of the toxic organophosphate metabolite, paraoxon, but prolonging the existence of the parent carbamate inhibitor, carbofuran. They also reported that pretreatment with methyl mercury hydroxide resulted in reduced oral LD_{50} values for carbaryl in rats.

We have tested the possible interaction between other heavy metals and parathion. In a similar experimental regime neither lead nor cadmium resulted in significant biochemical interactions when fed at 100 ppm (lead nitrate or cadmium chloride) for 18 weeks. They also had no effect on plasma or brain cholinesterase activities by themselves.

Thus far we have tested only parathion and carbofuran for biochemical interactions with mercury. These kinds of non-persistent pesticides have largely replaced persistent organochlorines and now enjoy widespread usage. Studies of possible interactions between non-persistent pesticides and metals should continue. For instance cholinesterase-inhibiting pesticides like carbofuran, that are found to significantly interact with mercury, could be substituted by non-reactive ones like parathion.

SUMMARY

The degree of interaction between mercury and cholinesterase inhibiting pesticides was determined by comparing enzyme responses to sublethal dosages of parathion or carbofuran in quail fed 0.05, 0.5, or 5.0 ppm morsodren for 18 weeks. A statistically significant interaction was defined as greater brain cholinesterae inhibition in morsodren-fed than in clean-fed birds following pesticide dosage. The tissue residues of mercury that accumulated before significant mercury-parathion interactions occurred were higher than levels that might be expected in natural populations, but significant mercury-carbofuran interactions occurred in birds that had only accumulated 1.0 ppm liver mercury. The results indicate that indiscriminate usage of cholinesterase inhibiting pesticides are dangerous, since natural populations of fish-eating birds oftentimes contain this magnitude of mercury.

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