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Plasma Enzyme Activities in Coturnix Quail Fed Graded Doses of DDE, Polychlorinated Biphenyl, Malathion and Mercuric Chloride[1]

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Plasma Enzyme Activities in Coturnix Quail Fed Graded Doses of DDE, Polychlorinated Biphenyl, Malathion and Mercuric Chloride. DIETER, M. P. (1974). *Toxicol. Appl. Pharmacol.* 27, 86–98. Male Coturnix quail (*Coturnix coturnix japonica*) were fed diets for 12 weeks containing graded levels of DDE, polychlorinated biphenyl (Aroclor 1254), malathion, and mercuric chloride. Birds were bled prior to exposure and at 2, 4 and 12 weeks, and the plasma used to measure the activities of creatine kinase, aspartate aminotransferase, cholinesterase, fructose-diphosphate aldolase, and lactate dehydrogenase. Abnormal activity of certain plasma enzymes was noted in birds after 2 and 4 weeks, but these changes were not proportional to dose or exposure time. At 12 weeks increases in each of the activities of plasma enzymes of birds fed organochlorines, and decreases in cholinesterase activity of birds fed malathion or mercuric chloride, were proportional to the log dose of the respective agents. In addition, the pattern of enzyme responses in the 4 experimental groups had changed, and was illustrative of the specific type of substance that had been fed. The data suggest that qualitative and quantitative identification of environmental contaminants in birds, and perhaps a variety of wild animals, may be possible by utilization of multiple plasma enzyme assays.

Residue analyses after 12 weeks of feeding showed that DDE accumulated in carcasses and livers at concentrations up to 4-fold higher than those in the diets. In contrast residues of Aroclor 1254 attained in carcasses were identical to, and in livers one-half of, the concentration in the feed. Mercury did not accumulate as much in the tissues; residues attained were one-twentieth or less of those in the feed.

The wide range and diversity of avian species results in their exposure to many types of environmental contaminants. However, even when mortality has occurred and contaminants are suspected as causative agents, it is often difficult to determine whether they were primarily responsible. It is even harder to discern what class of contaminant might have been responsible when reproductive success declines, or when other subtle, physiological alterations ensue in living birds. Usually it is necessary to analyze

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carcasses or organs for residues to confirm that certain kinds of contaminants are involved, which is undesirable when only limited numbers of wild species are available. Multiple plasma enzyme assays may help to identify types of contaminant exposure in living birds.

Alterations in plasma enzyme activities are a manifestation of pathologic changes in several organs or tissues (Hess, 1963). Cellular necrosis and fatty tissue changes, particularly in the liver, kidney, cardiac and skeletal muscles, are associated with leakage of tissue enzymes into the blood (Altland and Highman, 1961; Highman and Altland, 1963; Dieter *et al.*, 1970). A number of pesticides including organochlorines, organophosphates and carbamates, and environmental pollutants in the form of heavy metals, have been shown to alter the activities in the blood of such enzymes as aspartate aminotransferase (Luckens and Phelps, 1969; Grice *et al.*, 1971; Krampl, 1971), lactate dehydrogenase (Luckens and Phelps, 1969; Ringoir, 1970; Grice *et al.*, 1971), fructose-diphosphate aldolase (Krampl, 1971), and cholinesterase (Walker, 1971; Casterline and Williams, 1971). Vos *et al.* (1971), studied the toxicity of hexachlorobenzene in Japanese quail and found that 20 ppm or 120 ppm in the diet for 4 weeks increased the activity of aspartate aminotransferase in the serum 62% and 100%, respectively. A linear response was obtained if enzymatic activity was replotted against the log of toxicant concentration in the feed, suggesting that the amount of toxicant exposure may be positively correlated with the levels of enzyme activity in the blood.

Other studies have shown that organophosphates or carbamates inhibited the activity of serum cholinesterase, but increased the activities of aspartate and alanine aminotransferases (Sasinovich and Voronina, 1971). In addition, the types of isoenzymes of esterase (Walker, 1971) or lactate dehydrogenase (Khaikina *et al.*, 1970; Ringoir, 1970; Grice *et al.*, 1971) released into the blood differed, depending on whether organochlorines, carbamates, or heavy metals were incorporated into the animals' diets. Thus, measurements of enzymes in the blood whose activities show a differential response to separate classes of environmental contaminants, and isoenzyme analysis, may help to distinguish which class of contaminant is involved when these agents are suspected of causing adverse effects in birds.

In the present study Coturnix quail were fed graded levels of DDE, Aroclor 1254, mercuric chloride, and malathion for 12 weeks. Repetitive blood samples were taken to determine the degree of correlation between toxicant ingestion and plasma enzyme activities. Enzyme sensitivity and specificity of response to individual toxicants were determined.

METHODS

Coturnix quail (*Coturnix coturnix japonica*) were raised from brood stock maintained at the Patuxent Wildlife Research Center. At 1 month of age females from the hatch were discarded and males were randomly selected, banded and placed 12 to a section in holding cages in air-conditioned bird rooms with 12 hr:12 hr light:dark cycles. Cage sections were randomly selected for reception of control or contaminated feed. At 2 months of age birds began receiving turkey maintenance mash containing carrier (1% propylene glycol) or mash mixed with 5, 25 and 100 ppm (dry weight)

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DDE[2] or Aroclor 1254,[3] or 2, 4 and 8 ppm mercuric chloride or 8, 35 and 160 ppm malathion.[4] The protocol for mixing contaminants in the feed has been described in detail previously (Heath *et al.*, 1972). The concentrations used were far below published LC50 concentrations (Heath *et al.*, 1972) for DDE, Aroclor 1254, malathion, and mercuric chloride, which are all greater than 1000 ppm. Each of the birds was bled prior to starting experimental feeding and again 2 and 4 weeks later. At 12 weeks they were bled for the last time until dead. All blood samples were collected between 9.00 and 10.00 AM.

Carcasses and livers were weighed, frozen, and shipped to WARF Institute, Inc. for residue analysis. Only the controls were examined for DDE, Aroclor 1254, and mercury residues. The rest of the samples were analyzed specifically for the toxicant which was fed. Livers and carcasses were ground thoroughly in a hand meat grinder and a 10-g subsample of liver or a 20-g subsample of carcass (skinned birds without beaks, wings, feet, and viscera except for heart, testes and kidneys) analyzed. Water and lipid concentrations were also determined in each sample.

DDE. Samples were dried, the fat extracted, eluted on a Florisil column, and the first elutions injected on a gas chromatograph. An aliquot of this solution less than or equal to 5 μg and 20 μg Aroclor 1254 was run through a silicic acid Celite column to separate the polychlorinated biphenyls from DDT[5] and its analogs. The PCB-free samples (10 μl or less) were injected into a Barber-Coleman Model 5000 gas chromatograph and analyzed for DDE, DDD[6] and DDT.

PCB. After drying, fat extraction and elution, 10 μl or less of samples were injected into a Barber–Coleman Pesticide Analyzer, Model 5360 gas chromatograph, which separated Aroclor 1254 into 12 components. To quantitate Aroclor 1254 in a sample 11 peaks occurring at retention times equivalent to 11 in the standard were measured, summed, and the peak heights averaged. The twelfth peak with a retention time equal to DDE was excluded from standard and experimental samples to eliminate a source of error, since in this group of birds no procedure was used to separate PCBs from the chlorinated pesticides.

Mercury. A 5-g subsample of dry material was prepared by acid digestion and mercury determined by cold vapor atomic absorption on a Perkin–Elmer Atomic Absorption spectrophotometer, Model 303, fitted with a cold vapor device.

Malathion. Quail fed this pesticide were not analyzed for residues. Body weights were recorded each time the birds were bled. Samples of blood (2.0 ml) were collected by heart puncture and kept on ice until discarded. An aliquot of whole blood was used for microhematocrit and hemoglobin (Henry, 1964) determinations. Plasma fractions were used for enzyme analyses, which were completed on the same day of collection. Plasma creatine kinase[7] was assayed at pH 9.0 and aspartate aminotransferase[7] at pH 7.4 using

[2] DDE, 1,1-di(*p*-chlorophenyl)-2,2-dichloroethylene.

[3] Polychlorinated biphenyl (Aroclor 1254®), a mixture of chlorinated terphenyls containing 54% chlorine.

[4] Malathion (Cythion®), *O,O*-dimethyl phosphorodithioate of dimethylmercaptosuccinate, kindly donated by American Cyanamide Co., Princeton, New Jersey.

[5] DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane.

[6] DDD, 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane.

[7] Creatine kinase (EC 2.7.3.2; ATP:creatine phosphotransferase); aspartate aminotransferase (EC 2.6.1.1; L-aspartate:2-oxoglutarate aminotransferase); cholinesterase (EC 3.1.1.8; acylcholine acyl-hydrolase); fructose diphosphate aldolase (EC 4.5.1.13; fructose-1,6-diphosphate D-glyceraldehyde-3-phosphate-lyase); lactate dehydrogenase (EC 1.1.1.27; L-lactate:NAD oxidoreductase).

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the procedures and kits from Sigma Chemical Co. Cholinesterase[7] and fructose-diphosphate aldolase[7] were assayed at pH 7.4 using the procedures and kits from Boehringer-Mannheim Chemical Corp. Lactate dehydrogenase[7] and its isoenzymes were assayed at pH 8.0 by procedures outlined in Bergmeyer (1965). Enzyme activities were determined at 24–26°C using a recording Beckman ACTA II spectrophotometer and a sample changer at 340 nm by following absorbance changes of NADH, or in the case of cholinesterase by following color production at 405 nm. Reactions were initiated by addition of substrate or plasma; it was shown that reactant concentrations yielded an enzyme velocity that was directly proportional to the amount of plasma used in the assays. The activities of all enzymes were expressed in milliunits (nmol substrate transformed/min/ml plasma). The difference between means was statistically analyzed by Student's *t* test.

RESULTS

There were no visible adverse effects in birds fed toxicants. No mortality occurred and there was no incidence of tremors, convulsions or deviations in normal body weight gains. Repetitive blood sampling did not alter hematocrits or hemoglobin concentrations in control birds. Final body weights, liver weights, hematocrits and hemoglobin concentrations in birds fed toxicants for 12 weeks are summarized in Table 1. Blood hematocrit and hemoglobin concentration of birds fed 100 ppm Aroclor 1254 decreased significantly. There were also significant decreases in hemoglobin concentration of birds fed 5 ppm Aroclor 1254 or 4 ppm mercuric chloride. There was a significant

TABLE 1

CARCASS AND LIVER WEIGHT, HEMATOCRIT AND HEMOGLOBIN CONCENTRATION IN COTURNIX QUAIL FED DIETS FOR 12 WEEKS CONTAINING PESTICIDES OR POLLUTANTS[a,b]

Treatment	Level (ppm)	Carcass (g)	Liver (g/100 g body weight)	Hematocrit	Hemoglobin (g/100 ml)
Control	0	78.1 ± 3.5	2.44 ± 0.10	47.2 ± 0.5	16.2 ± 0.5
DDE	5	79.8 ± 2.5	2.46 ± 0.07	48.1 ± 0.9	16.9 ± 0.5
	25	71.1 ± 1.3	2.78 ± 0.05[c]	45.8 ± 1.2	16.0 ± 0.5
	100	74.8 ± 2.0	2.96 ± 0.11[c]	45.5 ± 0.7	15.2 ± 0.6
Aroclor 1254	5	75.9 ± 2.0	2.27 ± 0.08	48.3 ± 0.8	11.3 ± 0.8[c]
	25	75.7 ± 2.7	2.76 ± 0.12	45.5 ± 1.5	15.0 ± 0.8
	100	74.5 ± 1.4	3.61 ± 0.07[c]	42.0 ± 0.7[c]	13.2 ± 0.5[c]
Mercuric chloride	2	70.0 ± 2.3	2.29 ± 0.07	48.7 ± 0.9	16.1 ± 1.1
	4	75.6 ± 3.5	2.31 ± 0.10	45.7 ± 2.0	14.2 ± 0.4[c]
	8	75.8 ± 2.8	2.38 ± 0.09	47.5 ± 1.2	16.1 ± 0.5
Malathion	8	—	—	49.3 ± 1.0	15.7 ± 1.1
	35	—	—	46.7 ± 0.8	16.7 ± 0.8
	160	—	—	45.1 ± 0.5	15.2 ± 0.3

[a] Carcass weight is weight of skinned bird without beaks, feet, wings, and viscera except for heart, testes and kidneys. Values are means ± SE, $n = 12$.
[b] Carcass and liver weights of birds fed malathion were not measured.
[c] Significantly different from controls, $p < 0.05$, Student's *t* test.

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increase in liver weights of quail fed 25 or 100 ppm DDE or 100 ppm Aroclor 1254 as compared to controls. Increases in organ weights were related to the concentration of toxicants in the feed.

In control quail the carcass residues of DDE averaged 0.03 ppm (Table 2), DDD was not detectable, and those of DDT ranged from 0.008 to 0.031 ppm. DDE residues in the pooled liver samples of control quail were 0.9 ppm, DDD residues were 0.16 ppm and DDT residues were 0.35 ppm. Aroclor 1254 was not detectable in the pooled liver sample of control quail and ranged from not detectable to 0.16 ppm (mean of 10 samples, 0.05 ppm) in the carcasses. Residues of mercury in carcasses and the pooled liver sample of control quail were less than 0.05 ppm.

TABLE 2

RESIDUES IN CARCASSES AND LIVERS OF COTURNIX QUAIL FED GRADED LEVELS OF DDE, AROCLOR 1254, OR MERCURIC CHLORIDE IN THE DIETS FOR 12 WEEKS[a]

Treatment	Level (ppm)	Residue (ppm) in Carcass	Residue (ppm) in Liver
DDE	0	0.03 ± 0.0 (10)	0.9
	5	25.3 ± 2.3 (10)	11.4
	25	125.2 ± 8.6 (11)	34.2
	100	415.8 ± 37.1 (11)	207.0
Aroclor 1254	0	0.05 ± 0.0 (10)	ND[b]
	5	5.1 ± 0.8 (8)	1.9
	25	20.4 ± 2.3 (6)	12.5
	100	87.9 ± 11.7 (11)	71.9
Mercuric chloride	0	<0.05 (10)	<0.05
	2	<0.05 (8)	0.09
	4	0.05 ± 0.00 (11)	0.25
	8	0.11 ± 0.02 (9)	0.64

[a] Quail fed malathion were not analyzed for this pesticide. Liver samples were pooled for each treatment group. Means ± SE for carcass residues, sample numbers in parentheses.
[b] ND, not detectable.

Table 2 also lists residues attained in carcasses and livers of experimental birds after 12 weeks feeding of DDE, Aroclor 1254, or mercuric chloride. Concentrations of DDE attained were 4-fold higher in carcasses, and 2-fold higher in livers, than the concentration in the diets. In contrast residues of Aroclor 1254 attained in carcasses were identical to those fed, and in livers one-half that fed in the diets. Mercury residues attained in carcasses were at or below limits of detection in birds fed 2 and 4 ppm and in those fed 8 ppm only 0.1 ppm residue was found. Residues attained in livers of quail fed mercuric chloride were about one-twentieth of those in the diets.

Percent lipid in the carcasses of quail fed DDE or Aroclor 1254 was no different than in controls. This parameter was not analyzed in quail fed mercuric chloride. Percent

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lipid in pooled liver samples was 4.38% in the control and 5.60, 5.01 and 7.46% in quail fed 5, 25 and 100 ppm DDE, and 4.29, 8.12 and 6.88% in quail fed 5, 25 and 100 ppm Aroclor 1254.

The activities for the 5 enzymes in control birds are presented in Table 3. It is obvious that repetitive blood sampling, per se, did little to alter the activities of the plasma enzymes. The oscillations in enzyme activity were not significant, except for increases in fructose-diphosphate aldolase.

TABLE 3

PLASMA ENZYME ACTIVITY IN CONTROL COTURNIX QUAIL FED DIETS CONTAINING CARRIER (PROPYLENE GLYCOL)

Enzyme[a]	Intervals bled (weeks)			
	0	2	4	12
Creatine kinase	48.4 ± 6.7	48.1 ± 3.4	46.2 ± 4.2	57.8 ± 4.2
Aspartate aminotransferase	103.5 ± 12.0	110.1 ± 2.9	106.3 ± 4.5	121.1 ± 9.4
Cholinesterase	2172 ± 56	2304 ± 82	2085 ± 91	1895 ± 99
Fructose-diphosphate aldolase	5.9 ± 1.5	10.8 ± 1.6	7.3 ± 0.9	11.5 ± 1.8
Lactate dehydrogenase	29.3 ± 3.6	26.4 ± 4.9	28.3 ± 4.9	44.6 ± 11.2

[a] Enzyme activity in International milliunits (nmol substrate transformed/min/ml plasma). Means ± SE, $n = 12$.

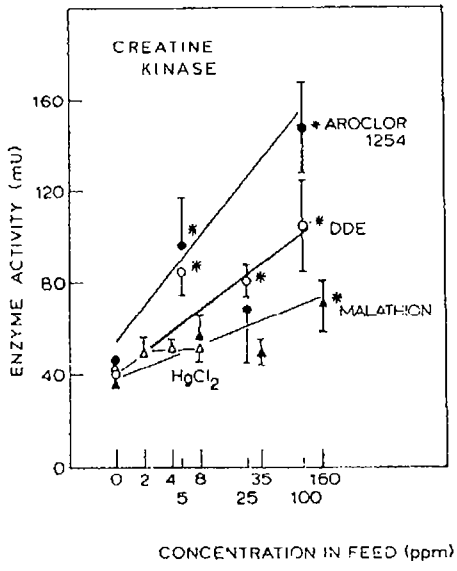


FIG. 1. Plasma creatine kinase activity in Coturnix quail fed diets for 12 weeks containing 5, 25 and 100 ppm DDE or Aroclor 1254, or 2, 4 and 8 ppm mercuric chloride, or 8, 35 and 160 ppm malathion. Each point represents mean enzyme activity for respective treatment groups either prior to toxicant exposure (0 ppm, 36 samples) or after 12 weeks' exposure (3 dose levels, 12 samples each). Vertical lines indicate SE. The best straight lines through the points of this and the following figures were drawn by visual inspection. * Significantly different from respective preexposure values, $p < 0.05$, Student's t test.

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Each group of 12 birds fed a particular level of toxicant was bled prior to exposure, and at 2, 4 and 12 weeks. After 2 or 4 weeks of toxicant feeding, the changes in plasma enzyme activities were neither dose-related nor time-related. Figures 1–6 illustrate the relationship between activities of plasma enzymes in quail fed toxicants for 12 weeks and the concentration of the toxicant in the feed. Control values for each group of birds fed a particular toxicant are represented as an average of the enzyme activities obtained in these birds prior to toxicant exposure.

Exposure to graded levels of organochlorines (DDE, Aroclor 1254) resulted in significant increases in activities of all 5 enzymes measured. The responses were almost linear when enzyme activity was plotted against the log of the toxicant concentration in the feed. Maximum increases above control levels in activities of cholinesterase

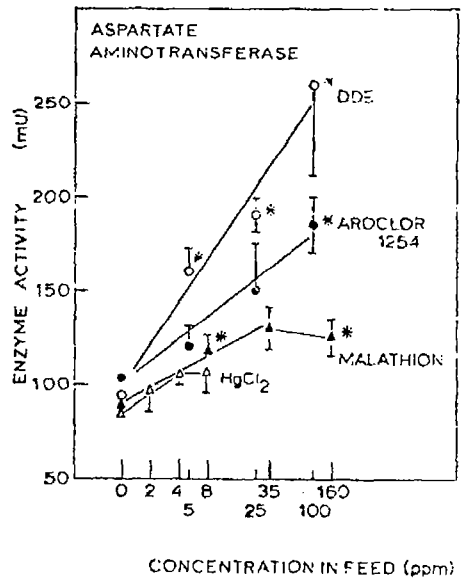


FIG. 2. Plasma aspartate aminotransferase activity in Coturnix quail fed diets for 12 weeks containing 5, 25 and 100 ppm DDE or Aroclor 1254, or 2, 4 and 8 ppm mercuric chloride, or 8, 35 and 160 ppm malathion. Each point represents mean enzyme activity for respective treatment groups either prior to toxicant exposure (0 ppm, 36 samples) or after 12 weeks' exposure (3 dose levels, 12 samples each). Vertical lines indicate SE. * Significantly different from respective preexposure values, $p < 0.05$, Student's t test.

(80%), fructosediphosphate aldolase (5- to 6-fold), and lactate dehydrogenase (9-fold) were similar in birds fed either DDE or Aroclor 1254 (Figs. 3–6). Creatine kinase activity rose higher in Aroclor 1254 (4-fold maximum increase) than in DDE-treated birds (2.5-fold maximum increase), but the maximum increases in aspartate aminotransferase were greater after DDE (2.5-fold) than after Aroclor 1254 (75%) exposure (Figs. 1 and 2).

In contrast, in malathion-treated birds the increases in creatine kinase, aspartate aminotransferase and lactate dehydrogenase activities were much less than in birds fed organochlorines and were not always linear (Figs. 1, 2 and 6). Fructose-diphosphate

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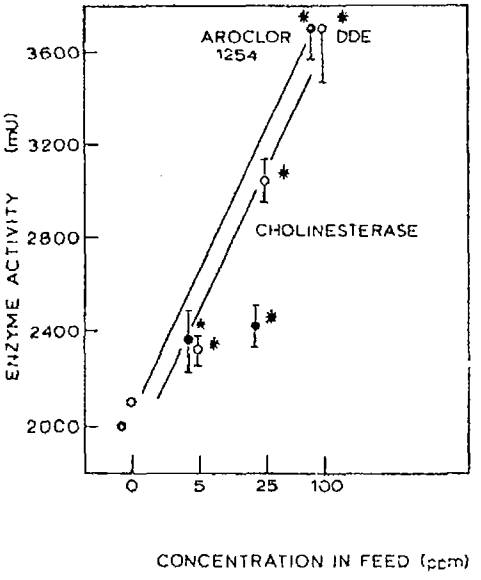


FIG. 3. Plasma cholinesterase activity in Coturnix quail fed diets for 12 weeks containing 5, 25 and 100 ppm DDE or Aroclor 1254. Each point represents mean enzyme activity for respective treatment groups either prior to toxicant exposure (0 ppm, 36 samples) or after 12 weeks' exposure (3 dose levels, 12 samples each). Vertical lines indicate SE. * Significantly different from respective preexposure values, $p < 0.05$, Student's t test.

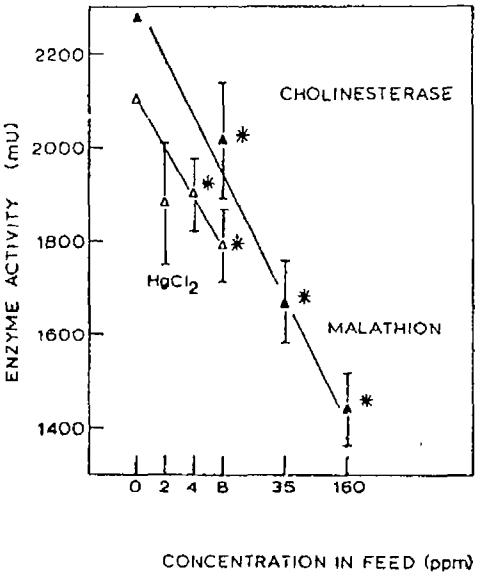


FIG. 4. Plasma cholinesterase activity in Coturnix quail fed diets for 12 weeks containing 2, 4 and 8 ppm mercuric chloride or 8, 35 and 160 ppm malathion. Each point represents mean enzyme activity for respective treatment groups either prior to toxicant exposure (0 ppm, 36 samples) or after 12 weeks' exposure (3 dose levels, 12 samples each). Vertical lines indicate SE. * Significantly different from respective preexposure values, $p < 0.05$, Student's t test.

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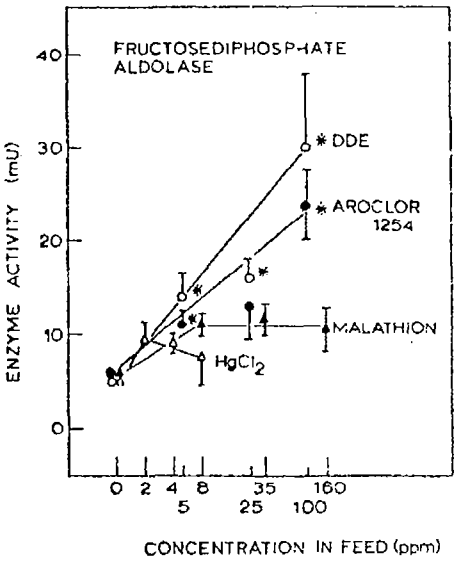


FIG. 5. Plasma fructose-diphosphate aldolase activity in Coturnix quail fed diets for 12 weeks containing 5, 25 and 100 ppm DDE or Aroclor 1254, or 2, 4 and 8 ppm mercuric chloride, or 8, 35 and 160 ppm malathion. Each point represents mean enzyme activity for respective treatment groups either prior to toxicant exposure (0 ppm, 36 samples) or after 12 weeks' exposure (3 dose levels, 12 samples each). Vertical lines indicate SE. * Significantly different from respective preexposure values, $p < 0.05$, Student's t test.

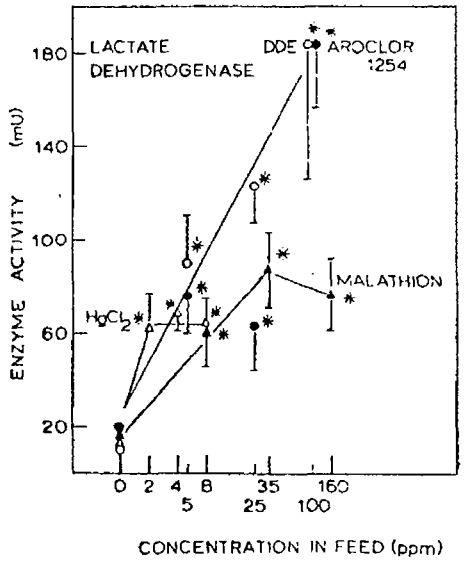


FIG. 6. Plasma lactate dehydrogenase activity in Coturnix quail fed diets for 12 weeks containing 5, 25 and 100 ppm DDE or Aroclor 1254, or 2, 4 and 8 ppm mercuric chloride, or 8, 35 and 160 ppm malathion. Each point represents mean enzyme activity for respective treatment groups either prior to toxicant exposure (0 ppm, 36 samples) or after 12 weeks' exposure (3 dose levels, 12 samples each). Vertical lines indicate SE. * Significantly different from respective preexposure values, $p < 0.05$, Student's t test.

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aldolase activity was not altered in birds fed malathion. There was, however, a significant and progressive decline in plasma cholinesterase activity (approximately 50% below controls) that was linearly related to the log of malathion concentration in the feed (Fig. 4). In quail fed mercuric chloride, only cholinesterase and lactate dehydrogenase activities were altered (Figs. 4 and 6). The maximum decrease in cholinesterase activity amounted to 25% below that in controls, and was almost linear in relation to the log dose of contaminant in the feed. Irrespective of the quantity of mercuric chloride fed, lactate dehydrogenase activity increased 3-fold above control values.

The isoenzymes of plasma lactate dehydrogenase were separated and the activities assayed in quail fed toxicants for 12 weeks. The results were expressed as a percentage of the total lactate dehydrogenase activity. While each of the toxicants induced significant increases in total lactate dehydrogenase activities (Fig. 6), Table 4 shows that isoenzymes originating from the heart and kidney accounted for a significantly greater percentage of these elevations than isoenzymes from the liver and muscle.

TABLE 4

PLASMA LACTATE DEHYDROGENASE ISOENZYMES (PERCENT OF TOTAL ACTIVITY) IN COTURNIX QUAIL FED DIETS FOR 12 WEEKS CONTAINING PESTICIDES OR POLLUTANTS[a]

Treatment	Level (ppm)	Isoenzyme	
		Heart + kidney	Liver + muscle
Control	0	45.2 ± 2.6	54.8 ± 2.6
DDE	5	50.8 ± 5.1	49.2 ± 5.1
	25	64.4 ± 2.5[b]	35.6 ± 2.5[b]
	100	65.3 ± 3.1[b]	34.7 ± 3.1[b]
Aroclor 1254	5	54.2 ± 3.6	45.8 ± 3.6
	25	53.1 ± 3.0	46.9 ± 2.0
	100	54.6 ± 2.8[b]	45.4 ± 2.8[b]
Mercuric chloride	2	53.3 ± 9.6	46.7 ± 9.6
	4	53.9 ± 1.8[b]	46.1 ± 1.8[b]
	8	56.2 ± 2.7[b]	43.8 ± 2.7[b]
Malathion	8	42.3 ± 2.7	57.7 ± 2.7
	35	57.9 ± 2.0[b]	42.1 ± 2.0[b]
	160	56.8 ± 3.0[b]	43.2 ± 3.0[b]

[a] Enzyme activity in International milliunits (nmol substrate transformed/min/ml plasma). Means ± SE, $n = 12$.
[b] Significantly different from controls, $p < 0.05$, Student's t test.

DISCUSSION

The incidence and levels of DDE and polychlorinated biphenyls in wildlife species have been the subject of several recent reviews (Stickel, 1968; Dustman and Stickel, 1969; Peakall and Lincer, 1970; Dustman *et al.*, 1971). Peakall and Lovett (1972) have also summarized the information concerning the occurrence of mercury in the environment. The concentrations of these residues in tissues of wild birds were well within the range of the lower levels (2 ppm to 25 ppm) fed in this study. However, it was found

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that DDE residues in quail fed 25 or 100 ppm, or Aroclor 1254 residues in quail fed 100 ppm, attained greater than 25 ppm in the carcasses. The higher levels of the toxicants fed and attained in the carcasses were less than one-tenth of the published LC50 for Coturnix quail (Heath *et al.*, 1972).

Despite the use of sublethal levels of toxicants, marked increases in plasma enzyme activities occurred. Apparently the complex equilibria between toxicant ingestion, absorption, accumulation, metabolism, and that of enzyme release into and removal from the blood were not attained until after 12 weeks, when increases in plasma enzyme activities became proportional to the log dose of the contaminants fed.

Two of the 5 enzymes measured, aspartate aminotransferase and lactate dehydrogenase, were the most sensitive to the agents. In previous studies on multiple stress, lactate dehydrogenase was also the most sensitive of 5 enzymes measured (Dieter *et al.*, 1969, 1970, 1971). Lactate dehydrogenase was also the enzyme that showed the greatest increase in activity after toxicant exposure. For instance, in birds fed organochlorines, the activity of plasma lactate dehydrogenase was as much as 9-fold greater than in controls; in those fed mercuric chloride or malathion, the activity was as much as 3- to 4-fold greater. More importantly, even in birds fed the lowest levels of these compounds, the activity of this enzyme increased 3-fold above controls. Although the lower residue levels found in this study (<0.05 ppm mercury, 5.1 ppm Aroclor 1254, and 25.3 ppm DDE) are nonlethal and comparable to those found in samples of wild populations of birds, rises in plasma enzyme activities are known to precede and are indicative of serious, impending histopathologic defects (Altland and Highman, 1961; Dieter *et al.*, 1969, 1970). As organochlorines and mercury show cumulative effects and are persistent in the environment (Stickel, 1968; Peakall and Lincer, 1970; Peakall and Lovett, 1972), measurement of plasma enzyme activities would provide a sensitive "early warning" system to monitor the presence of contaminants in wild animals. It is known that irreversible liver and kidney damage, accompanied by further increases in plasma enzyme activity, ultimately occurs when higher concentrations of these contaminants accumulate in the tissues (Vos *et al.*, 1971; Grice *et al.*, 1971).

In some instances it was possible to quantify the amount of toxicant fed in the diets by measuring plasma enzyme responses. There was an almost exact relationship between the log of the concentration of DDE or Aroclor 1254 in the diet, and the responses of aspartate aminotransferase, cholinesterase, and lactate dehydrogenase. Vos *et al.* (1971) obtained almost identical results when they measured serum aspartate aminotransferase in Coturnix quail fed hexachlorobenzene for 4 weeks. Thus three different types of chlorinated hydrocarbons (DDE, Aroclor 1254 and hexachlorobenzene) caused a dose-related release of enzymes from organs into the blood, suggesting that this particular class of environmental contaminant may be quantitatively identified by plasma enzyme assays. There was also a quantitative relationship between the concentration of malathion fed to Coturnix and the decline in their plasma cholinesterase activity. This method is commonly used to identify the presence of organophosphates or carbamates, because these compounds undergo rapid biological degradation (Fukuto and Metcalf, 1969). However, present results indicate that mercurial contaminants might interfere with this bioassay, since log-dose related decreases in plasma cholinesterase activity were also obtained in birds fed very low levels of mercuric chloride.

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A qualitative identification of these environmental contaminants was also afforded by plasma enzyme measurements, as the patterns of enzyme activities were different in birds exposed to the separate classes of toxicants. The organophosphate (malathion) and the organochlorines (DDE and Aroclor 1254) were readily distinguished by comparing plasma cholinesterase responses. The pattern of enzyme responses was obviously different in birds fed mercuric chloride as opposed to the other three toxicants. It was more difficult to separate the effects of DDE from those of Aroclor 1254, although the former appeared to cause greater increases in activities of plasma aspartate aminotransferase and fructose-diphosphate aldolase, and the latter greater increases in creatine kinase. The explanation for the differential response of the enzymes to toxicants is undoubtedly related to the extent that particular organs are affected by the different toxicants, and to the distribution of the enzymes in various tissues of the birds. For example, creatine kinase, which is located almost exclusively in the skeletal muscle (Bergmeyer, 1965), appeared in larger quantities in the blood of birds fed Aroclor 1254 than in those fed DDE, and, in turn, responses were greater in the latter birds than in those fed malathion. Apparently cell membrane permeability in the skeletal muscle of these birds was altered in various degrees by these toxicants, allowing different proportions of this enzyme to leak into the blood. Isoenzyme analysis also helped to identify the source of enzymes released into the blood of birds fed toxicants. In addition to a release of lactate dehydrogenase from skeletal muscle, there was a release from cardiac muscle, liver and kidney. A greater percentage of the lactate dehydrogenase released from these latter organs originated from the heart or kidney. Single ip injections of mercurials elevated total lactate dehydrogenase activities in rat sera, and isoenzyme analysis together with histologic examination indicated that the kidney was the primary source of this enzyme (Ringoir, 1970; Grice *et al.*, 1971). In the present study, it was found that lactate dehydrogenase was the only enzyme that increased significantly in the plasma of quail fed mercuric chloride, suggesting that this toxicant also had its primary effect on the kidney.

The practicality of utilizing multiple plasma enzyme assays to identify qualitatively and quantitatively the presence of environmental contaminants is borne out in this study. Despite the range of enzyme activities found in control birds, the lowest level of toxicants fed induced from 50% to 200% changes in activities of the various enzymes, when compared to the upper limit of control values. Similar enzyme procedures are being successfully utilized in toxicologic studies in rats (Luckens and Phelps, 1969; Sasinovich and Voronina, 1971) and humans, and should prove to be equally useful in wildlife research.

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