Relationship of Dietary Intake to Concentration of Dieldrin and Endrin in Dogs

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The relatively high toxicity of endrin when compared to dieldrin and the isomeric relationship between the two provide interesting bases for a study of the biological activity of these chlorinated organics in animals. Both of these insecticides are of interest in long-term exposure studies since they accumulate in animal tissue and generally are metabolized and eliminated slowly.

Investigation has disclosed that fish exposed to a low concentration of endrin in water can accumulate a sufficient quantity to cause death (1). Gas chromatographic analysis of fish blood has shown a significantly higher endrin concentration in the blood of fish that died following exposure to endrin in water than in those surviving similar exposure (1). A correlation has also been reported between dieldrin concentrations in the blood and clinical signs of intoxication in both human beings and dogs (2).

This investigation was designed to determine the following: (a) the feasibility of using the rapid gas chromatographic method

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(3) as a clinical tool in the diagnosis of dieldrin and endrin intoxication, (b) the rate and extent of accumulation of dieldrin and endrin in the blood with continued feeding, (c) the time between ingestion of the insecticide and buildup of detectable concentrations in the blood, and (d) the potential use of blood levels to estimate the body burden of these insecticides.

Experimental Procedure

Eight registered beagle dogs from three litters were obtained from a local breeder and housed in individual cages that provided both comfort and isolation. From each litter one animal was fed a diet containing dieldrin at 0.1 milligram per kilogram (mg/kg) of body weight per day and a second animal was fed a diet containing endrin at the same concentration. The remaining two animals, representing two of the three litters, were maintained as controls. All animals were fed a balanced commercial ration at the rate of 200 grams (g) per day. Insecticide solutions were prepared in ethanol at concentrations of 0.1 milligram per milliliter (mg/ml) and added to the rations at the time of feeding. Insecticide supplementation of the ration was started when the dogs were 9 months old and continued for 128 days.

Collection of blood samples from the jugular vein at weekly intervals was begun 3 weeks before insecticide feeding and continued throughout the feeding period. One week after the final

feeding, animals were anesthetized with Nembutal[1] and sacrificed by exsanguination. Samples of the blood, body fat, and other selected tissues were immediately removed and held in frozen storage until analyzed.

Samples were prepared for analysis by the method previously described for milk (3), modified as follows:

1. Blood samples were delivered directly into the tared beaker portion of the combination reaction-extraction (REX) flask to obviate the need for an anticoagulant.

2. Each sample of tissues containing no more than 1 gram of fat was diced and weighed into the beaker portion of the REX flask.

3. Feed samples were prepared by Soxhlet extraction of 20 g of food with 300 ml of hexane over a 48-hour period. After the hexane was volatilized, the oily residue was prepared for analysis as described previously (3).

Most samples[2] were analyzed on a 6-foot by 1/8-inch aluminum column of 6% QF-1 and 5% SE-30 on 80/100 mesh Gas Chrom Q. Preparation of this column consisted of coating individual portions of the support with each of the liquid phases and packing the columns with a mixture at 1:1 ratio. The packed column was

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(1) Mention of commercial products does not constitute endorsement by the Public Health Service.

(2) Blood samples for the first 15 weeks were analyzed on the column described by Schafer et al. (4)

conditioned by baking at 220° C with a 20-ml/min gas flow for 72 hours. Samples were analyzed at a column temperature of 190° C and carrier gas flow of 30 ml/min.

To reduce the expense and the time involved in determining the validity of the method for animal tissues, the efficiency of recovery was estimated by use of white mice. Concentrations recovered after direct addition of insecticides were compared with those recovered following metabolic distribution of living animals.

In the first experiment eight mice were injected interperitoneally with a known amount of each insecticide. Duplicate animals were sacrificed at 0 hour (assumed to be equivalent to direct addition to tissue) and at 1, 2, and 3 hours to allow in vivo distribution of the insecticides. After sacrifice, the entire animal was homogenized, and the insecticide determined. Analysis of variance of the data indicated no significant difference in the recovery of the insecticides from animal tissue between the time intervals investigated. Since it has previously been shown that metabolites of dieldrin and endrin are not present in blood and tissues (5), such agents are not expected to interfere with these determinations.

The second experiment was concerned with variations in recoveries from different types of tissue. For these trials, 10 mice were sacrificed and dissected into six anatomical groups: pelt, head, kidney, liver, gastrointestional tract, and torso. Duplicate

portions of each anatomical group were contaminated with known amounts of both dieldrin and endrin and analyzed as before. Analyses of these data disclosed that satisfactory recovery was obtained with all groups (100 to 105%) except endrin in liver, which was found to be about 63%.

Recovery studies for dieldrin and endrin in blood were conducted by the direct addition of insecticides to the blood of control dogs at monthly intervals. Samples analyzed as previously described yielded average recoveries of 95.6 and 98.9% for dieldrin and endrin, respectively.

Results and Discussion

Blood samples drawn before initiation of feeding were found to contain about 0.002 ppm of DDT + DDE, but did not contain measurable amounts of dieldrin or endrin (<0.001 ppm). Table 1 shows the concentrations of dieldrin and endrin in the blood with continued feeding of these insecticides. Analysis of these data shows that dieldrin concentrations approach a maximum after about 114 to 121 days. Toxicity symptoms were not evident in the dogs at these maximum concentrations. DDT + DDE concentrations remained at essentially the original levels in all animals throughout the experiment.

A linear relationship between the concentration of $\log_{10}$ of dieldrin in ppb and $\log_{10}$ of time (days 2 through 128) is shown in Figures 1-3, where it will be noted that the slopes of the three lines are nearly equal, with the lines differing only in actual

TABLE 1

Dieldrin and Endrin Concentrations in Blood with Continued Feeding of these Insecticides (PPB)

Days	Endrin			Dieldrin		
	Dog No. 1	Dog No. 3	Dog No. 5	Dog No. 2	Dog No. 4	Dog No. 6
2	3.0	3.0	2.8	4.0	4.7	0.9
9	3.3	3.0	2.2	19.2	17.4	10.0
16	8.2	7.4	7.0	34.0	31.2	20.0
23	5.5	3.8	3.6	35.0	48.0	21.0
30	2.8	2.2	2.0	40.0	44.0	23.0
37	6.4	3.4	3.4	82.0	58.0	38.0
44	6.6	3.0	3.0	84.0	66.0	44.0
51	2.6	2.0	1.4	82.0	64.0	40.0
58	7.0	3.0	3.3	104.0	82.0	64.0
65	8.4	5.4	5.4	90.0	70.0	36.0
72	4.8	3.4	3.2	110.0	88.0	60.0
79	8.0	6.5	5.6	170.0	120.0	75.0
86	2.0	4.0	2.0	120.0	63.0	50.0
93	5.4	6.4	3.2	154.0	110.0	50.0
100	6.3	5.2	5.8	184.0	110.0	50.0
107	4.4	3.8	4.3	150.0	110.0	60.0
114	4.5	1.7	1.9	180.0	120.0	97.0
121	2.1	2.2	2.7	190.0	124.0	76.0
128	4.0	3.7	3.1	174.0	106.0	78.0

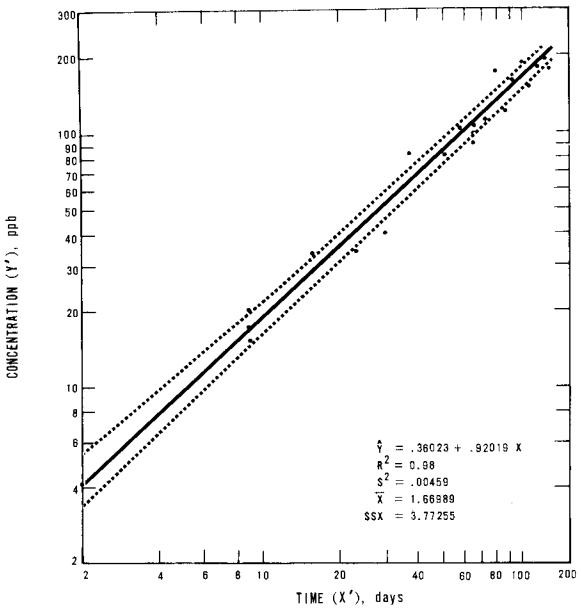

Figure 1. Linear regression and 95% confidence interval relating blood levels of dieldrin and time for Dog No. 2.

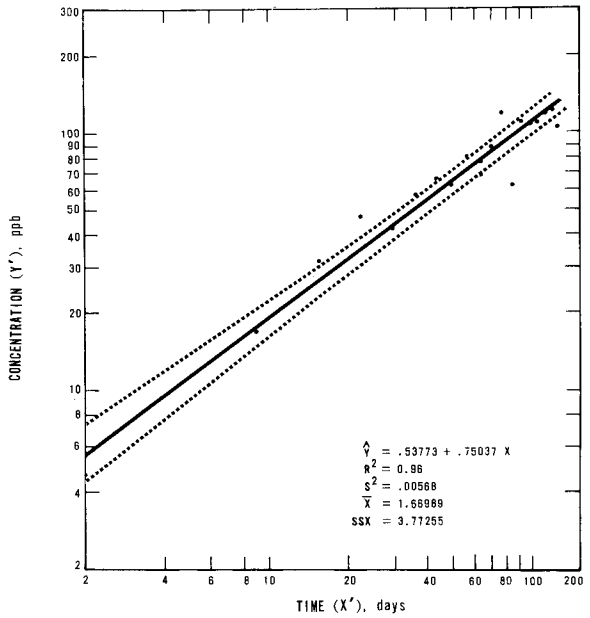

Figure 2. Linear regression and 95% confidence interval relating blood levels of dieldrin and time for Dog No. 4.

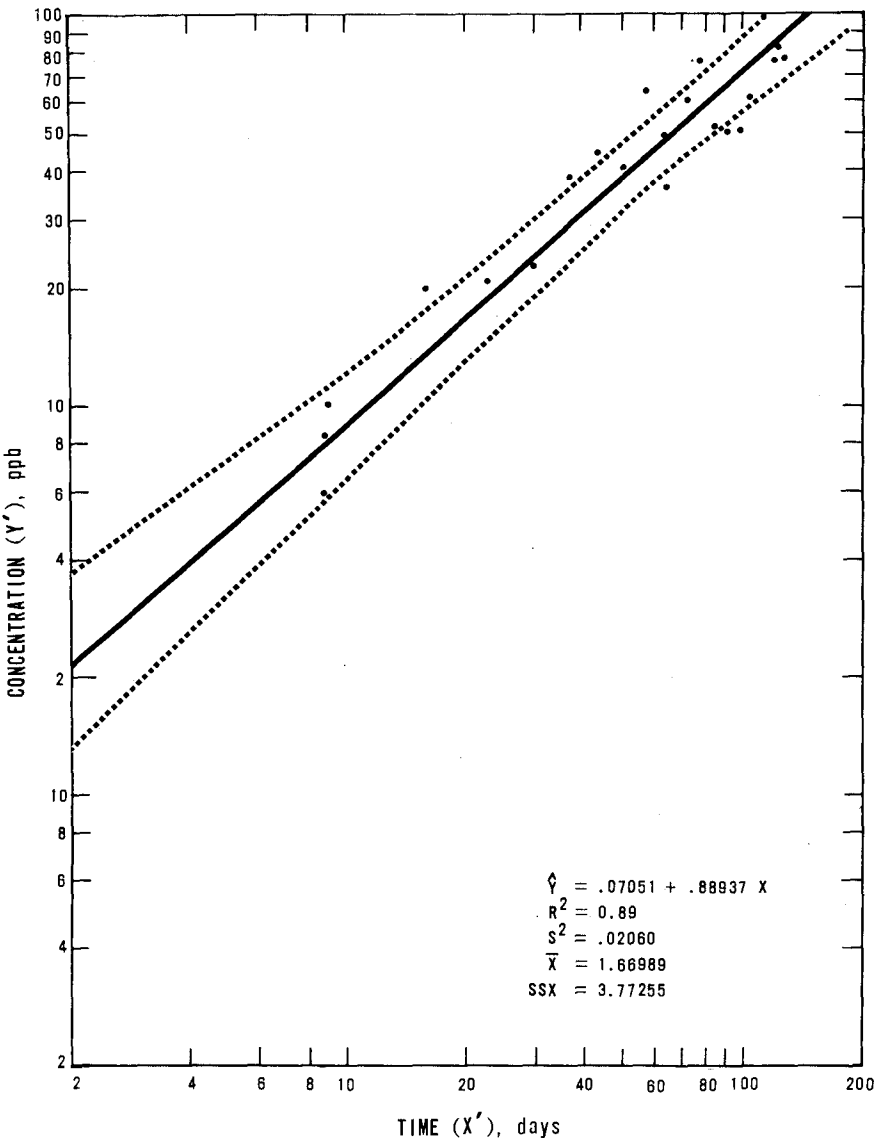

Figure 3. Linear regression and 95% confidence interval relating blood levels of dieldrin and time for Dog No. 6.

position. For these calculations, the time was assumed to be measured without error, and $\log_{10}$ of concentration was assumed to be normally distributed with common error variance for all time observations (X). Ostle (6) describes the method of least-squares fit with confidence intervals. The intervals shown on Figures 1-3 are 95% intervals, meaning that the true regression line lies between the upper and lower boundaries 95% of the time. Each figure has the equation correlation index (R^2), error variance (S^2), mean $\log_{10}$ of days ($\bar{X}$), corrected sum of squares of X (SSX), and the equation for predicting concentrations ($\hat{Y}$) listed in the lower right hand corner. The S^2, $\bar{X}$, and SSX are given so that the variance for a predicted Y can be calculated.

All data were transformed to $\log_{10}$ and the equations of the line yielded estimates of $\hat{Y}$ from values of $\log_{10}$ of days (X'). The estimates used to plot the lines and confidence intervals were transformed back to antilogs so that they could be plotted on graphs. R^2 is the proportion of sum of squares explained by linear regression, and $1-R^2$ represents that proportion of the sum of squares due to experimental error and lack of fit of the model chosen.

Endrin data were also subjected to the log transformation; however, the correlation was not significant from zero in any animal. The largest R^2 (0.007) justified the conclusion that there is no relationship between the concentration of endrin in the blood and the duration of feeding. Analysis of these data shows

that endrin does not accumulate in the blood, and this finding is in agreement with that of a previous investigation (1). The data further indicate that the metabolism of the stereoisomers, dieldrin and endrin, is different in the dog.

Dieldrin and endrin levels were recorded, Tables 2 and 3, for blood and tissues at the time of sacrifice. Note that dieldrin was found in both sets of animals. Although the presence of dieldrin in endrin-supplemented animals is not apparently explainable, a feeding error was most likely involved because the dieldrin content of the untreated ration was insignificant (about 0.0009 ppm) and the control animals did not contain detectable dieldrin residues.

TABLE 2

Dieldrin in Blood and Tissues of Dogs (PPB)

Animal	Blood	Heart	Liver	Kidney center	Pancreas	Spleen	Lung	Fat	Muscle
2	190	2,030	6,610	3,320	3,050	1,450	1,560	44,000	880
4	170	850	4,820	2,150	30,360	320	1,640	20,000	500
6	90	380	1,830	1,520	8,680	360	470	12,000	320
1	8	32	187	76	133	24	36	430	190
3	4	11	92	25	59	8	19	260	110
5	3	11	48	45	61	7	20	260	170

These observations on the six dogs containing dieldrin were used to calculate linear correlations between the concentration of

TABLE 3

Endrin in Blood and Tissues of Dogs (PPB)

Animal	Blood	Heart	Liver	Kidney center	Pancreas	Spleen	Lung	Fat	Muscle
1	8	170	84	71	280	2,620	33	760	170
3	5	125	77	38	87	7	17	520	120
5	1	170	77	82	110	120	27	250	310

dieldrin in blood and in tissues. Each correlation was tested for significance from zero at $\alpha = 0.01$. Since these multiple tests are not independent, the probability of at least one test being significant is approximately 0.08. Table 4 shows the correlation index

TABLE 4

Correlation Index (R^2)
Dieldrin in Blood and Tissues of Dogs

Relationship	R^2
Blood - Heart	0.81[a]
Blood - Liver	0.96[a]
Blood - Kidney	0.96[a]
Blood - Pancreas	0.43
Blood - Spleen	0.68
Blood - Lung	0.96[a]
Blood - Fat	0.86[a]
Blood - Muscle	0.70

[a]Significant at the overall 0.08 probability level.

for blood and various tissues. Since it has been shown that proteins in blood are able to retain dieldrin in the absence of fat (7), the high correlation between blood and other low-fat tissues is not surprising. Although no explanation can be offered for the absence of correlation between blood and spleen and blood and pancreas, these observations suggest possible roles for the spleen and pancreas in metabolism or detoxification of dieldrin.

No definite relationship was found between the levels of endrin in blood and those in the body tissues with the exception of fat (See Table 3).

Neither dieldrin nor endrin were found in the blood or body tissues of the control animals.

Summary

The method utilized in this investigation was simple, rapid, and quantitative for the measurement of dieldrin and endrin in the blood and tissues of dogs and mice and should be useful in the diagnosis of unknown toxicity syndromes in human beings. Levels of dieldrin in the blood were indicative of the concentrations of these agents in tissues and vital organs; however, there was no correlation between levels of endrin in blood and tissues other than fat.

Feeding equal quantities of dieldrin and endrin resulted in approximately equal concentrations in blood after 48 hours. Thereafter, dieldrin accumulated rapidly as feeding was continued,

whereas endrin did not. These data indicate different modes in the metabolism of these stereoisomers in dogs.

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