The total body burden of Dieldrin

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Studies of the chlorinated hydrocarbon insecticide content of human body fat have been reported from a number of countries during the last seven years (Table 1) (1-9). Comparisons between individuals and population groups have been based entirely on the concentration of chlorinated insecticide per gram of fat. In addition, some authors have implied that the total body burden of a chlorinated insecticide was directly related to the concentration of that insecticide per gram of fat (10, 11). The work reported in this paper casts some doubt on the validity of such comparisons. The experimental results suggest that under conditions of equal and constant oral intake, the concentration of a chlorinated insecticide per gram of fat is inversely related to the total body burden of the insecticide and also inversely related to the degree of obesity of the individual sampled.

Bulletin of Environmental Contamination & Toxicology,
Vol. 4, No. 1, 1969, published by Springer-Verlag New York Inc.

TABLE 1.

AVERAGE CONCENTRATION OF CHLORINATED HYDROCARBON PESTICIDES IN BODY FAT OF THE GENERAL POPULATION OF VARIOUS COUNTRIES*

		Storage Level in Body Fat (ppm)					
Country	Year	BHC Isomers	Dieldrin	Endrin	Heptachlor Epoxide	DDT	Reference
United States	1961-1962	0.20	0.15	-	-	2.4	1
United States	1962-1963	0.57	0.11	-	-	-	2
United States	1964	-	0.31	0.02	0.10	-	3
United States	1964	0.60	0.29	0.03	0.24	-	4
England	1961-1962	-	0.21	-	-	-	5
England	1963-1964	0.42	0.26	-	0.1	-	6
England	1964	0.02	0.21	0.02	0.01	-	7
France	1961	1.19	-	-	-	3.5	8
India	1964	1.43	0.04	-	-	4.7	9

*All analyses were carried out by gas-liquid chromatography.

It has been suggested that the concentration of insecticides stored in the body fat of the population of various countries has reached a constant level (5, 7, 12, 13). At this point of storage equilibrium, the amount of insecticide ingested

and absorbed was assumed to be equal to the amount metabolized and excreted. Hunter (14) has fed dieldrin, a chlorinated insecticide, to human volunteers and has achieved a storage equilibrium. At the point of storage equilibrium, three important relationships were observed; (1) the concentration of dieldrin in the blood remained very constant, (2) the concentration of dieldrin in the fat reamined relatively constant, and (3) a direct relationship existed between the concentrations of dieldrin in the blood and fat, in fact, the partition coefficient of dieldrin between these tissues was a constant of 156. Richardson (15) fed dieldrin to dogs and collected data which, when reviewed with the knowledge of Hunter's findings, indicates the existence of similar pharmacodynamic relationships in dogs.

In the experiment reported in this paper, an attempt was made to achieve a storage equilibrium with dieldrin in dogs. The concentration of dieldrin in the blood was measured throughout the experiment. These results were correlated with the concentration of dieldrin in the fat, the total body burden of dieldrin, and the obesity of the animal during the period of storage equilibrium.

Experimental Design

Twelve mongrel dogs, all between twenty-four and twenty-seven months of age, were divided by standard random sampling technique into two groups of six dogs per group. Each group was handled in the following manner:

GROUP	NO. DOGS PER GROUP	SEX	DOSE OF DIELDRIN (Mg/Kg/Day)	DURATION OF ADMINISTRATION (DAYS)
Experimental	6	4M, 2F	1.0	0-5
			0.2	6-59
Control	6	5M, 1F	0	0

In all instances, the recrystallized dieldrin (judged by melting point and thin layer chromatography to be greater than 99% pure 1,2,3,4,10, 10-hexachloro-exo-6,7-epoxy - 1,4,4a,5,6,7,8,8a - octahydro-1,4-endo, exo-5,8 dimethanonaphthalene)* was dissolved in corn oil and the correct volume was measured into a gelatin capsule (size 000). The maximum volume of this solution put into any capsule was 1.2 ml. and only one capsule was fed to a dog each day. This daily oral insecticide dose for each dog was adjusted to the body weight of the dog based on the previous week's weight.

During this experiment, venous blood samples were obtained twice a week from each dog and were routinely analyzed for dieldrin content. A subcutaneous fat sample was obtained surgically from all animals once a month and this was analyzed for its dieldrin concentration. The dog food was analyzed for dieldrin, but none was detected within the limits of sensitivity of the method used (1 PPB). The food consumption, body weight, and hematology data obtained during the experimental and 19-week control periods was similar to that of the control animals and indicated that all of the animals were in good health.

*Dieldrin is the approved name in the United States and Great Britain for a material containing not less than 85% of this compound.

In addition to the above, the total body water space and lean body mass of each animal in the experimental group was determined at the termination of the experiment.

To gain an undistorted view of the processes involved in the metabolism of dieldrin, the animals were, as far as could be determined, isolated from all possible contact with other synthetic organic compounds. Due to the general good health of the animals during the experimental period, no pharmaceutical preparations had to be administered. Also, the insect control procedures normally carried out in the animal quarters were suspended during this experiment.

The biological samples were chemically prepared by a procedure originally reported by Schafer (16).

All dogs were fed their respective capsule of recrystallized dieldrin in corn oil, five days a week, Monday through Friday, between 8:30 and 9:30 A.M. Blood samples to be analyzed for dieldrin were collected on two days each week (Monday and Thursday) between 9:00 and 10:00 A.M. On these days the dogs were fed their capsule containing dieldrin immediately after the blood was collected.

Materials and Methods

The concentrations of dieldrin in the biological samples were determined with a dual column gas-liquid chromatograph equipped with two electron capture detectors.

The total body water space in each animal was determined by isotope dilution technique in which tritiated water was measured

with a liquid scintillation counter (17). Results of the total body water determination combined with the value obtained for the lean body mass made possible a calculation of the intracellular and extracellular water for each dog. An edematous state in any of the animals, which would have made the lean body mass measurements useless, could have been detected by these procedures. None of the animals were edematous according to the results of these determinations.

An estimate of the lean body mass (LBM) was obtained by using a whole body counter which quantitated the ^{40}K in each animal. The majority of all potassium in the mammalian body is stored intracellularly, and since 0.012 per cent of all naturally occurring potassium (^{39}K) is radioactive potassium (^{40}K), the above measurement permitted the calculation of the total amount of potassium stored intracellularly within the body. This information can be converted, with the aid of a conversion factor (62.25 meq ^{39}K/Kg. LBM), into the lean body mass expressed in kilograms for each dog. Subtraction of the LBM from the total body weight yields an accurate estimate of the total amount of adipose tissue present in the body of the animal. This is a well-established and accepted, though not routine, clinical procedure.

This procedure is based on the assumption that the lean body mass has a constant potassium content, and that neutral fat does not bind electrolytes (18). The conversion factor employed was derived from a paper by Moore (19) and agrees with the factor

usually used for calculating human lean body mass (68.1 meq K/Kg. LBM).

Discussion of Results

The concentration of dieldrin in the blood of all the animals remained very constant from day 7 to day 59; therefore, a storage equilibrium of dieldrin was believed to have existed during this period (Table 2).

TABLE 2.

MICROGRAMS OF DIELDRIN PER 100 GRAMS OF WHOLE BLOOD

Day	7	10	14	17	21	24	28	31
1966	5-23	5-26	5-30	6-2	6-6	6-9	6-13	6-16
Experimental Group	Oral-Dose (0.2 mg/Kg/day)							
1	10.0	8.2	9.9	9.8	12.3	9.3	15.0	11.4
4	9.9	7.2	8.5	8.8	9.8	7.9	8.9	8.9
8	11.7	15.1	12.2	12.5	17.6	15.8	13.5	12.4
9	11.1	11.4	11.8	13.4	14.8	13.1	13.3	13.1
13	10.7	11.2	10.1	11.5	12.8	13.6	13.6	12.3
18	12.7	7.9	9.4	9.8	12.0	12.9	13.3	10.4
Mean	11.0	10.3	10.4	10.9	13.2	12.1	12.9	11.4

TABLE 2. (CONTINUED)

MICROGRAMS OF DIELDRIN PER 100 GRAMS OF WHOLE BLOOD

Day	35	38	42	45	49	52	56	59
	6-20	Oral-Dose (0.2 mg/Kg/day)						
1	10.8	14.0	13.9	15.8	13.8	11.3	12.2	11.7
4	8.5	10.4	8.9	11.3	10.4	10.4	8.8	10.3
8	12.0	15.5	13.1	16.5	17.1	13.2	17.2	16.3
9	11.8	14.0	14.5	19.2	16.6	13.8	15.9	15.5
13	10.5	8.6	12.9	15.4	14.0	13.5	13.7	12.6
18	11.8	13.9	11.2	14.0	15.7	13.7	13.7	15.1
Mean	10.8	12.8	12.4	15.4	14.6	12.7	13.5	15.2

Two surgical fat samples, on days 16 and 50, were obtained during the experiment. In order to more definitely establish the existence of a storage equilibirum during the period in question (days 7 through 59), the fat/blood ratios (partition coefficients) were calculated (Table 3).

TABLE 3.

THE RATIO OF THE CONCENTRATION OF DIELDRIN IN THE FAT TO THE CONCENTRATION OF DIELDRIN IN THE BLOOD DURING THE STEADY STATE PERIOD

DATE: 6-1-66 (16 days since the beginning of the experiment)

Dog No.	Micrograms of Dieldrin Per Gram of Fat (PPM) / Micrograms of Dieldrin Per Gram of Whole Blood (PPM)
4	173
1	249
18	242
9	233
13	233
8	165
MEAN	216

DATE: 7-5-66 (50 days since the beginning of the experiment)

4	192
1	183
18	170
9	184
13	152
8	181
MEAN	177

The fat/blood ratios remained relatively constant for each dog during the experiment, but the smallest range for the entire group of six dogs was observed on day 50 when the mean fat/blood ratio

was 177. These results, when reviewed in the light of Hunter's (14) findings, support the contention that a true storage equilibrium of dieldrin existed during this interval.

The variation which does exist in the fat/blood ratios calculated for day 16 may be due to the fact that, during the first five days of the experiment, the animals were fed 1.0 mg. of dieldrin/Kg/day in order to reach the equilibrium state very rapidly. Subsequent to the fifth day and through the remainder of the experiment, the animals were fed 0.2 mg/Kg/day.

The experimental results obtained on day 16, though variable, will be included in all subsequent investigations into the relationships that existed between the concentration of dieldrin in the fat, the obesity of the animal, and the total body burden of dieldrin during the storage equilibrium. There were two reasons for this approach; (1) the concentration of dieldrin in all body compartments were still fluctuating at this time, but were believed to be relatively close to a constant level, and (2) any relationships found to exist in the absence of an ideal storage equilibrium condition would be more acceptable when applied to a population study.

The first relationship which was investigated was that which existed between the concentration of dieldrin in the fat and the total kilograms of adipose tissue in the animal's body. Visual inspection of the data associated with these two parameters (Table 4) suggested that they were inversely related.

TABLE 4.

THE CONCENTRATION OF DIELDRIN IN THE FAT AND IN THE BLOOD, THE TOTAL BODY BURDEN OF DIELDRIN, AND THE TOTAL MASS OF ADIPOSE TISSUE FOR EACH DOG

6-1-66 (16 days since the beginning of the experiment)

Dog No.	Total Kilograms Of Fat In Body	Micrograms Of Dieldrin / Grams Of Fat	Total Body Burden Of Dieldrin In Milligrams	Micrograms Of Dieldrin / 100 Grams Of Whole Blood
4	5.83	14.8	86.2	8.5
1	2.10	24.7	51.9	9.9
18	1.83	22.8	41.7	9.4
9	1.28	27.5	35.2	11.8
13	0.87	23.6	20.5	10.1
8	0.60	20.2	12.1	12.2

7-5-66 (50 days since the beginning of the experiment)

4	5.86	20.0	117.2	10.4
1	2.02	25.4	51.3	13.8
18	1.35	26.6	35.9	15.7
9	0.63	31.0	19.5	16.6
13	0.63	30.5	19.2	14.0
8	0.39	21.2	8.3	17.1

This inverse relationship would not be expected to occur if the partitioning of dieldrin between the blood and fat compartments was a simple chemical equilibrium. A simple chemical equilibrium was not substantiated by the experimental results. In fact, concentrations of dieldrin in the blood and fat were not

the same in all dogs as would be expected. Therefore, this simple approach and the assumptions associated with it need modification. No explanation of the mechanism involved is available, but it is possible to speculate that one or more of the following processes may be involved:

1. A decreased rate of absorption of dieldrin from the alimentary tract of the more obese animals.
2. A more rapid rate of metabolism and/or excretion of dieldrin by the liver of the obese animals.
3. The metabolism or conversion of dieldrin to another compound within the adipose tissue of the more obese animals.
4. The larger fat depot and blood volume in the more obese animals.

The next relationship to be investigated was that which existed between the concentration of dieldrin in the fat and the total body burden of dieldrin. The total body burden of dieldrin in each animal was obtained by multiplying the concentration of dieldrin in the fat of each animal by the total body fat in kilograms. The data in Table 4 suggests that the greater the total body fat, the greater will be the total body burden of dieldrin. Review of this table also suggests that the concentration of dieldrin in the fat was <u>inversely</u> proportional to the total body burden of dieldrin.

Review of the data in Table 4 also suggests that a direct relationship exists between the concentrations of dieldrin in the fat and blood. These results substantiate the findings of other researchers (14, 15).

Summary

Numerous authors of articles concerned with insecticides had suggested that both blood and fat analyses could be used as indices of the total body burden. It has been implied that the concentrations of dieldrin in the blood and fat were directly related to the total body burden of dieldrin. The results of this experiment indicate an indirect relationship rather than a direct relationship.

These relationships may have important epidemiological implications. It appears that the results of fat or blood analyses alone are insufficient on which to base any predictions concerning the total body burden of dieldrin of any one individual. It is necessary to have some knowledge of the degree of obesity of the individual. Also, the average concentration of dieldrin in the fat of small groups of individuals in different countries are being compared to yield some information concerning the degree of storage of this compound in the fat of the populations in these same countries. But more importantly, any differences observed in fat storage are being extrapolated to represent differences in the total body burdens of the populations of these countries. The average stature or degree of obesity of these two populations are not usually considered.

This experiment appears to have been the first attempt to quantitate the total body burden of an insecticide, and since these results differ from those which have been assumed to occur, this appears to be an area which warrants additional investigation. This new concept is additionally significant since the supporting data does not disagree with the results of extensive experimentation in human volunteers reported by Hunter (14). In fact, the relationship exhibited between the concentrations of dieldrin in the blood and fat supports the contention of Hunter that the concentration of a chlorinated insecticide in the blood can be used as an index of the concentration of that insecticide in the fat.

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