Distribution of Dieldrin in the Turtle

by

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Introduction

Several investigators have reported on the concentration of dieldrin and the other chlorinated hydrocarbons in the fat depots and some of the other tissues of the body (HUNTER and ROBINSON 1968; EDMUNDSON et al 1969; GARRETTSON and CURLEY 1969; ROBINSON 1969; GUTHRIE and DONALDSON 1970; MORGAN and ROAN 1970). Although these compounds have been monitored in a number of animals in nature (KEANE etal 1969; BRAUND et al 1969; HENDERSON et al 1969) there has been little experimental work done in the laboratory. Other than for the fat depots and brain, information has been sparse on the partition of pesticides in the other tissues of the body (CURLEY et al 1969; SELBY et al 1969)

Cold-blooded animals have certain advantages in experimental work because of their low metabolic rate (COULSON and HERNANDEZ 1964). Changes that ordinarily proceed at a very rapid rate in a small rodent can be "slowed" down by employing a poikilothermic animal. The turtle, Pseudemys scripta elegans, is indigenous to large parts of the Mississippi River Valley and is available in large numbers for experimental work. Pilot studies using the turtle have revealed its value both in distribution studies and as a sentinel animal to detect compounds presenting a potential menace to health.

Methods and Materials

A number of turtles weighing 300 grams to one kilogram were injected with 20 mg/kg of dieldrin in a one-to-one solution of diethyl ether and corn oil (50 mg/kg). Groups were sacrificed at intervals for tissue analysis. These turtles had been kept in concrete tanks with water available at all times and were fed scrap fish once or twice a week previous to injection. The experimental animals were maintained at 28°C after the dieldrin injections.

At timed intervals after the intraperitoneal injection of dieldrin (20mg/kg), the turtles were placed on a turtle board and with a hack-saw the ventral carapace was sawed off. The soft tissues were carefully dissected from the carapace to minimize bleeding. A blood sample was obtained from the heart and was kept chilled until centrifuged to separate the plasma. Sections of liver, kidney, muscle, and brain were

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obtained and quick frozen until analyzed. A sample of brown fat was dissected from the subcutaneous area of the perineal region. The tissue samples were obtained from the same area in all animals to standardize the procedure.

The concentration of dieldrin in the plasma was estimated by gas chromatography. Two milliliters of plasma was extracted three times with 6 ml nanograde hexane using a one minute extraction on a Vortex stirrer. The hexane extracts were combined and the volume for gas chromatographic analysis was adjusted by evaporation of the hexane under a slow stream of air dried through a drierite calcium chloride column.

A modified micro-Mills procedure was used for the extraction of pesticides from muscle, brain, kidney, and liver. The tissue was weighed and then placed in a Duall tissue grinder with 2.5 ml of acetonitrile and homogenized. The homogenate was decanted, acetonitrile again added and the procedure repeated for a total of three extractions. An equal volume of sodium sulfate (7.5 ml) was added to the acetonitrile extract and mixed on a Vortex stirrer. This mixture was extracted three times with 2 ml hexane. The hexane extracts were combined and evaporated to a volume of 0.3-0.5 ml. The evaporated sample was then placed on a micro-Florisil column and eluted with 12 ml hexane followed by 12 ml of hexane-1% MeOH (both were combined to form fraction 1) and a final 12 ml of hexane-1% MeOH (fraction 2). Volumes of the fractions were so large due to high concentrations of dieldrin that the presence of other pesticides could not be detected. Therefore, the two fractions were combined and the volume adjusted for analysis.

For fat analysis, the adipose tissue was dried on filter paper and 0.2 gram was homogenized with 2.5 ml of nanograde petroleum ether in a Duall tissue grinder. The homogenate was decanted and the procedure repeated three times. The petroleum ether homogenates were combined and evaporated to a volume of about 0.3 ml. The samples were passed through micro-Florisil columns similar to the procedures used for the other tissues (see above).

The concentration of dieldrin in the plasma and various tissues was estimated with a Micro-Tek 220 gas chromatograph. The samples were chromatographed on a column of 1.5% OV-17/ 1.95% QF-1 on 100/120 Chromosorb W, H.P. and on a column of 4% SE-30/6% QF-1 on 80/100 Chromosorb W, H.P. The columns were conditioned for 72 hours at 245°C under nitrogen. The operating conditions for analysis were 205°C for the electron capture parallel plate detector (source tritium foil) and 200°C for the columns. Under these conditions dieldrin had a retention time relative to aldrin of 2.40 on the OV-17 and 2.15 on the SE-30 columns.

TABLE 1

Concentration of dieldrin (average and range in ppm) in tissues of the turtle after injection

	Controls	1 hr	3 hr	6 hr	12 hr	1 day	2 days	3 days	25 days	45 days	70 days
No. animals	3	3	3	3	3	3	2	2	3	2	2
Plasma	0.003 0.002- 0.007	0.021 0.002- 0.039	1.51 0.033- 3.11	0.345 0.045- 0.781	0.122 0.057- 0.166	1.19 0.518- 1.87	0.409 0.171- 0.883	1.18 0.076- 2.31	1.01 0.780- 1.47	2.33 2.24- 2.41	5.38 3.58- 7.18
Liver	0.037 0.030- 0.050	0.158 0.104- 0.234	1.39 1.05- 1.89	3.19 1.08- 6.32	4.27 4.03- 4.49	5.02 0.746- 7.34	13.99 3.64- 26.78	13.55 7.36- 24.37	50.08 26.60- 52.08	67.82 62.82- 72.69	81.43 46.07- 116.80
Muscle	0.005 0.001- 0.008	3.52 1.86- 4.75	6.27 0.133- 18.51	0.316 0.226- 0.473	5.31 0.216- 10.19	7.02 3.20- 10.85	2.45 0.820- 5.32	3.37 0.376- 5.30	6.02 5.84- 6.20	1.62 0.071- 3.02	5.51 3.69- 7.32
Kidney	0.036 0.001- 0.096	2.09 0.99- 4.33	5.27 1.11- 10.06	9.18 2.91- 14.99	2.09 0.969- 2.91	7.40 6.40- 9.91	24.19 1.65- 43.12	11.01 8.08- 12.82	9.67 8.30- 11.94	14.50 8.51- 20.49	36.19 13.45- 58.92
Brain	0.005 0.002- 0.009	0.464 0.306- 0.567	0.544 0.287- 0.518	0.292 0.247- 0.345	1.63 1.10- 2.12	1.97 0.932- 3.44	6.42 2.16- 14.44	11.60 0.820- 24.73	11.93 8.15- 16.63	14.74 9.66- 19.82	17.25 7.62- 26.87
Fat	0.142 0.100- 0.217	6.01 3.10- 8.82	2.85 1.68- 4.97	4.20 0.090- 7.15	10.27 0.101- 15.50	3.60 0.128- 8.71	12.95 2.54- 23.38	25.07 16.66- 40.89	618.00 505.59- 722.50	320.95 297.56- 344.33	887.21 445.21- 1329.21

Results

The rate of absorption of dieldrin in the turtle after intraperitoneal injection was slow and unpredictable. The results at any single time were variable due to the erratic absorption from the intraperitoneal site. In time (Table 1) the fat depots gradually increased until the concentration of dieldrin approached 1000 ppm or 0.1% of the weight of the fat. For some unexplained reason, the muscle tissue had the tendency to concentrate dieldrin early after injection. Following early increases in the muscle/plasma ratios, the muscle tissue slowly came into equilibrium with the extracellular fluids (Table 1). Next to the fat, the liver had the greatest propensity to concentrate dieldrin. For many compounds, the kidney has the greatest concentrating power of all the tissues of the body, but in these experiments, the kidney was only slightly superior to the brain and muscle. The inability of the brain to concentrate dieldrin was somewhat of a surprise (Table 1).

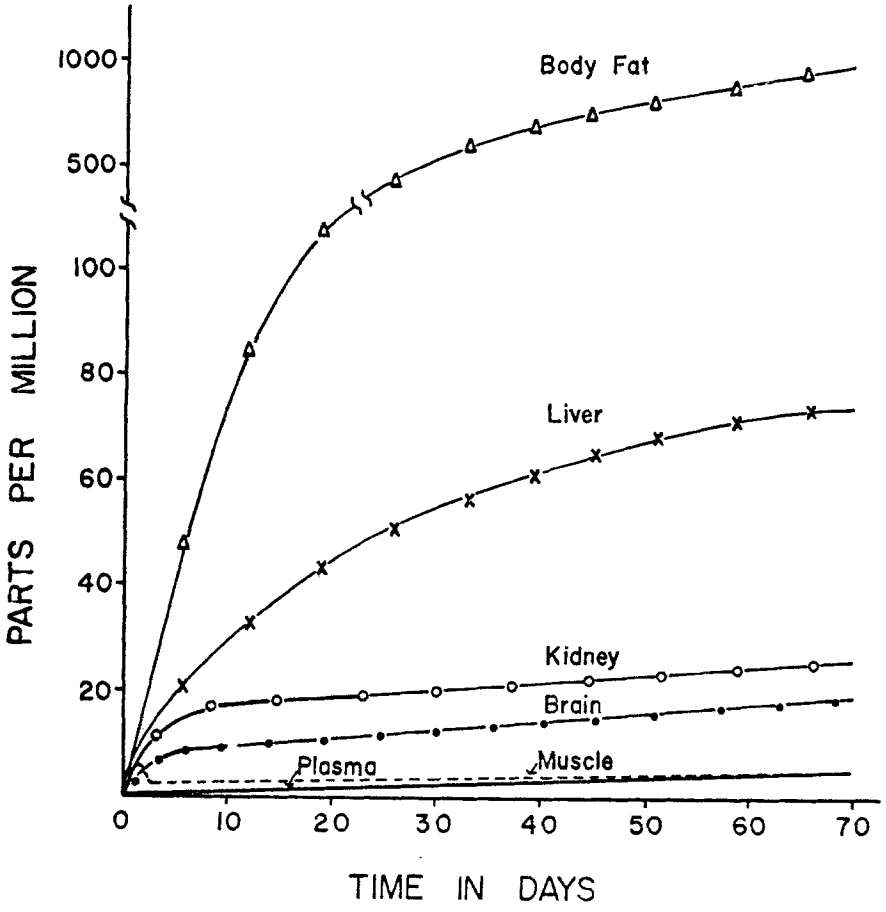

FIGURE 1 Rate of absorption of dieldrin in the turtle following intraperitoneal injection of 20 mg/kg.

An average plot of the data in Table 1 shows the relationship between the different tissues and the plasma in dieldrin concentration after the intraperitoneal injection of 20mg/kg of dieldrin (Figure 1). The fat depots are capable of storing large amounts of chlorinated hydrocarbons. The dieldrin concentrations in the fat approached 1000 ppm after a period of 70 days, indicating the rather slow absorption from the injection site. The fact that the concentration of dieldrin in the other tissues was increasing with time indicates that the great increase in the fat was not at their expense. The experimental turtles were fasted during this time and it is reasonable to assume that the fat stores were greatly depleted after 70 days (even in turtles with low metabolic rates). This may explain the high concentration of dieldrin observed in the diminished fat reserves (about 0.1%).

References

BRAUND, D.G., L.D. BROWN, J.T. HUBER, N.C. LEELING, and M.J. ZABIK: J. Dairy Science 52, 1988 (1969).

COULSON, R.A. and Y. HERNANDEZ: Biochemistry of the Alligator, Baton Rouge, La., Louisiana State University Press (1964).

CURLEY, AUGUST, M. FRANK COPELAND, and RENATE D. KIMBROUGH: Arch. Environ. Health 19, 628 (1969).

EDMUNDSON, WALTER F., JOHN E. DAVIES, MORRIS CRANMER, and GIGI A. NACHMAN: Industr. Med. Surg. 38, 55 (1969).

GARRETTSON, LORNE K. and AUGUST CURLEY: Arch. Environ. Health 19, 814 (1969).

GUTHRIE, F.E. and W.E. DONALDSON: Toxic. Appl. Pharmacol. 16, 475 (1970).

HENDERSON, CROSWELL, WENDELL L. JOHNSON, and ANTHONY INGLIS: Pesticides Monit. J. 3, 145 (1969).

HUNTER, C.G. and J. ROBINSON: Fd. Cosmet. Toxicol. 6, 253 (1968).

KEANE, WILLIAM T., MITCHELL R. ZAVON, and SAMUEL H. WITHERUP: Brit. J. Ind. Med. 26, 338 (1969).

MORGAN, DONALD P. and CLIFFORD C. ROAN: Arch. Environ. Health: 20, 452 (1970).

ROBINSON, J.: Canad. Med. Assn. J. 100, 180 (1969).

SELBY, LLOYD A., KENNETH W. NEWELL, GEORGE A. HAUSER, and GLADYS JUNKER: Arch. Environ. Res. 2, 247 (1969).