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Chlorinated Hydrocarbon Insecticide Residues in Tissues of Rats Before and After Reduction of Body Fat by Dietary Restriction

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Male weanling rats were fed ad libitum until 250 days of age one of three nutritionally adequate diets with or without the addition of 2.80 ppm of a chlorinated hydrocarbon insecticide (CHI) mixture. The mixture comprised DDT, TDE, DDE, lindane, dieldrin, and perthane. Between 251 and 300 days of age, half of the rats in each group were restricted to 50% of their ad libitum food intake. Concentration and total amounts of each insecticide were determined in adipose tissue, liver, and brain. Deposition, and mobilization during weight reduction, of insecticides varied with diet and tissue examined. When food intake was reduced, total DDT amount did not change in the three tissues regardless of diet. However, total amounts of DDT metabolites and dieldrin were altered with reduced intake. Differences associated with diet occurred primarily in adipose and liver tissues. Total DDT amount was not affected by diet.

Although the general use of chlorinated hydrocarbon insecticides (CHI) is prohibited in several countries, the persistence of these compounds in the food chain and their use on a limited scale in agriculture indicate the need for continued research on their effects at low levels. Surveys have shown that all the chlorinated hydrocarbon insecticides are present in humans, but their concentrations vary among tissues (Kutz et al., 1974) and are affected by the physiological status of the tissue (Radomski et al., 1968; Oloffs et al., 1974). The food supply has been the major source of insecticide contamination for the human population as indicated by the report of Duggan and Corneliussen (1972) on typical human diets. Deposition, accumulation, and mobilization of individual chlorinated hydrocarbon insecticides have been examined extensively with the rat as an experimental model. However, information is limited on the effects of feeding two or more insecticides for an extended time (Adams et al., 1974). The use of a mixture of CHI provides a realistic approach for the study of the effects of diet on storage of CHI in the body. For a variety of reasons many individuals are subjected to dietary restriction at intervals during their lives and information is sparse on the fate of a mixture of insecticides in the body during weight reduction. The present report provides data on the influence of diet on the deposition of several chlorinated hydrocarbon insec-

Table I. Composition of Diets[a]

high-fat diet (HF-L)	low-fat diet 1 (LF-PB-BS)	low-fat diet 2 (LF-BS-CO)
20.0% casein	12.8% casein 6.4% lactalbumin	27.0% casein
69.2% lard	6.4% beef suet 20.0% peanut butter	2.0% corn oil 14.0% beef suet
0.0% carbohydrate	40.4% sucrose	50.0% sucrose
1.4% vitamin mix[b]	8.0% yeast	1.0% vitamin mix[b]
0.2% methionine 0.2% additional choline		
7.0% salt mixture[c]	4.0% salt mixture[c]	4.0% salt mixture[c]
2.0% celluflour	2.0% celluflour	2.0% celluflour

[a] In addition, each animal received a weekly dose of percomorph liver oil containing about 3000 units of vitamin A, 400 units of vitamin D, and 36 mg of α-tocopheryl acetate (in 0.01 mL of cottonseed oil). [b] The vitamin mix provides the following amounts per kilogram of final diet: 4.0 mg of menadione, 10.0 mg of thiamin hydrochloride, 10.0 mg of riboflavin, 5.0 mg of pyridoxine hydrochloride, 60.0 mg of niacin, 40.0 mg of calcium pantothenate, 300.0 mg of choline, 0.4 mg of vitamin B_{12}, 0.4 mg of folic acid, 200.0 mg of *p*-aminobenzoic acid, and 400.0 mg of inositol. [c] Jones and Foster (1942).

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ticides in target tissues—brain, liver, and adipose—when a mixture of CHI was fed from weaning to maturity and also data on the subsequent mobilization of CHI when food intake was restricted and weight loss induced.

EXPERIMENTAL SECTION

Experimental Design. Male BHE rats (a mixed strain as reported by Marshall and Hildebrand, 1963) were fed ad libitum one of three nutritionally adequate diets (Table I) with or without (controls) addition of a mixture of CHI (Table II) from weaning until they were 250 days of age. For practical purposes, we used only three experimental diets; however, we made them greatly different in order to study the effect of varied energy sources and kinds of fat on insecticide storage.

Our modification of a high-fat lard (HF-L) diet (Barboriak et al., 1958), in which fat is the only nonprotein energy source, produced extremely obese rats. The other two diets were high in sucrose but low in fat; LF-PB-BS provided a greater proportion of plant fats and LF-BS-CO, of animal fats. The compositions of the diets are presented in Table I.

A mixture of six insecticides (Table II) was first dissolved in an aliquot of the dietary fat and then blended with the remaining dietary ingredients. The relative amounts of each insecticide were based on those reported for market basket surveys made prior to 1965 (Williams, 1964). However, we used 200 times the reported amounts in order to detect any possible dietary effects. At the time this investigation was initiated in 1966, these higher levels were considered safe by the Food and Drug Administration and the World Health Organization.

The animals were individually housed, and the air was maintained at 26–27 °C and a relative humidity of 45–50%. Their body weights and food intakes were recorded weekly. Body fat and water were estimated in vivo at 250 and 300 days of age by ^{40}K counting and tritiated water uptake, respectively. At 250 days seven or eight animals from each diet group were killed. Half of the remaining animals (12–15 in each group) continued to receive the diet ad libitum; the other half was given only 50% of their previous intake. At 300 days, all remaining animals were killed. A gross pathological examination was performed on all animals and livers were scored for fattiness (0 = no fat and 4 = extremely fatty). Brain, liver, epididymal, and perirenal fat pads were excised, weighed, and stored frozen for analyses. Carcass slurries were prepared for analyses according to the method of Womack et al. (1964).

Analytical Methods. *CHI.* Frozen adipose tissue, liver, and brain were thawed (epididymal pads and perirenal pads combined) and homogenized. An aliquot of the homogenate was weighed, dried, and ground with sodium sulfate. The resulting mixture was extracted for gas-chromatographic analysis of CHI and analyzed by Crobaugh Laboratories as described by Adams et al. (1974). Perthane was not resolved by this method.

Body Composition. We estimated body fat in vivo by the whole-body counting method of ^{40}K (Pommer and Lakshmanan, 1975). We determined total body water in vivo by measuring the uptake of tritiated water from a physiological saline solution containing 1.6 μCi ^{3}H/mL that was injected intraperitoneally. After 1 h, tail blood was collected in a heparinized capillary tube and centrifuged. Plasma, 50 λ, was placed in 6 mL of the following scintillation mixture: 800 mL of dioxane, 200 mL of absolute alcohol, 100 g of naphthalene, 7 g of 2,5-diphenyloxazole (PPO), and 50 mg of *p*-bis(*o*-methylstyryl)benzene (bis-MSB). After the scintillation sample was shaken for 20 min and stored in the liquid scintillation freezer for 2 h, its radioactivity was counted.

Total fat and water were determined directly on aliquots of the individual carcass slurries. Fat was analyzed by the method of Conway and Adams (1975). For determination of carcass water content, an aliquot of carcass slurry was dried under an infrared lamp for 2 h, cooled in a desiccator for 15 min, and then weighed. The sample was repeatedly dried at 30-min intervals until its weight remained constant.

Statistical Methods. A split-plot analysis of variance was made on the data. When F values were significant ($P < 0.05$), treatment means were compared according to the Duncan multiple range test (1955). Differences among treatments were considered significant at $P < 0.05$.

RESULTS

Small amounts of all insecticides studied were detected in the control diets (without added insecticides), but totaled less than 0.10 ppm. CHI levels found in the tissues of rats fed control diets were low in comparison with levels in rats fed experimental diets (with added insecticides). Food intake, body weight, weight loss, organ weights, or carcass fat and water were not significantly different in rats given added insecticide than in rats fed the same diet without added insecticide. Therefore, only data for the insecticide-fed rats are presented. Pathological lesions could not be attributed to ingestion of the insecticide mixture.

Food Intake, Body Weight, and Carcass Fat and Water. Table III presents data on food intake and body weight, fat, and water. Cumulative food intakes for restricted rats were calculated only from weaning until 250 days of age, the period of ad libitum feeding. Carcass water data are reported for only those rats whose body water was estimated at 250 days by ^{3}H uptake. Food intakes and body weights at 300 days were similar among animals fed the low fat diets. Although rats fed the high-fat diet ate less by this age, they weighed more than rats fed either low-fat diet because of the higher caloric content of the high-fat diet. Between 250 and 300 days, no further weight gain was observed in rats fed ad libitum, regardless of diet.

Rats fed the HF-L diet deposited about twice as much body fat as rats fed either low-fat diet. Between 250 and 300 days, total body fat did not increase in unrestricted, ad lib fed rats but was reduced approximately 50% in restricted rats, regardless of diet. The ratio of percentage of fat loss to percentage of weight loss was 95 for rats fed the HF-L diet, 62 for rats fed LF-PB-BS, and 75 for rats fed LF-BS-CO.

Total body water did not change during weight loss, regardless of diet. At 250 days, total body water did not differ significantly among rats fed the three diets, but at 300 days rats fed HF-L had significantly more total body water than rats fed the low-fat diets.

Gross pathological examination of the animals killed at 250 or 300 days of age revealed that livers of rats fed the HF-L diet tended to be fatty. They scored an average of 2.1, whereas rats fed the low-fat diets scored less than 0.5. With food restriction, liver fat of rats fed HF-L was utilized or mobilized as suggested by the mean score of 0.9 in the restricted rats.

Adipose Tissue Weight and Insecticide Content. The weight of the epididymal and perirenal fat pads, i.e., adipose tissue, and the concentration and content (total amount) of DDT, DDE, TDE, and dieldrin in the fat pads of rats fed the three experimental diets are summarized in Table IV. Fat pads from rats fed HF-L weighed approximately twice as much as those from rats fed either

Table II. Insecticide Mixture

insecticide	concn, ppm
DDT	0.72
TDE	0.58
DDE	0.50
lindane	0.14
dieldrin	0.38
perthane	0.48

low-fat diet. Fat pads weighed about half as much in restricted as in unrestricted rats.

Between 250 and 300 days, concentration (ppm) of CHI in the fat pads did not change significantly regardless of diet. However, total amounts (microgram) of DDE increased in adipose tissue with the low-fat diets, and of TDE and DDT with LF-BS-CO. With reduced food intake there was no appreciable loss of DDT or DDE in spite of the loss of body fat regardless of diet. In contrast, dieldrin and TDE contents (microgram) decreased along with body fat when either low-fat diet was fed.

At 250 days, diet did not influence the amount of insecticide in adipose tissue except for the elevation of DDE and dieldrin with the LF-BS-CO diet. The amounts were significantly greater with this low-fat diet than with either of the other diets. At 300 days, rats fed LF-BS-CO also had in their fat pads significantly more DDT and TDE as well as DDE and dieldrin than LF-PB-BS or HF-L. In addition, the increased amount of DDE between 250 and 300 days in rats fed LF-PB-BS resulted in higher amounts of DDE in this group than in the HF-L group.

Although lindane was present in adipose tissue, concentrations were low (0.2 ppm or less). Differences due to diet and age were not significant. However, the total amount of lindane was reduced significantly with dietary restriction from 8 to 4 μg in rats fed HF-L; 5 to 2 μg in rats fed LF-PB-BS; 6 to 3 μg in rats fed LF-BS-CO.

Liver Weight and Insecticide Content. Neither concentration (data not presented) nor amounts of CHI

Table III. Food Intake, Body Weight, Fat and Water Contents of Rats Fed Diets with Chlorinated Hydrocarbon Insecticides[a]

	treatment			250 days			300 days		
diet	diet regime	age killed, days	cumulative food intake, g	weight, g	fat by ^{40}K, g	water by ^{3}H, g	weight, g	fat by extraction, g	water by drying, g
HF-L (7)	unres[b]	250	2294 c	728 a	352 a				
HF-L (12)	unres	300	2774 d	713 a	344 a		752 (11) a	395 a	
HF-L (12)	res	300	2311 c	703 a	337 a	256 (10) a	512 b	151 b	295 (10) a
LF-PB-BS (7)	unres	250	3195 bc	501 b	136 b				
LF-PB-BS (14)	unres	300	3839 a	518 b	155 b		542 b	164 b	
LF-PB-BS (15)	res	300	3152 c	501 b	146 b	226 (12) a	394 c	78 c	249 (12) b
LF-BS-CO (8)	unres	250	3303 bc	508 b	152 b				
LF-BS-CO (15)	unres	300	3999 a	513 b	154 b		540 (14) b	171 b	
LF-BS-CO (13)	res	300	3376 b	523 b	164 b	240 (9) a	409 (12) c	69 c	237 (9) b
HF-L (31)			2486 c	713 a	343 a		627 (23) a	273 (24) a	
LF-PB-BS (36)			3427 b	508 b	148 b		466 (29) b	119 (29) b	
LF-BS-CO (36)			3619 a	516 b	157 b		479 (26) b	124 (28) b	
	unres (22)	250	2948 b	569 a	209 a				
	unres (41)	300	3586 a	573 a	210 a		601 (39) a	234 (41) a	
	res (40)	300	2972 b	576 a	210 a	239 (31)	423 (39) b	97 (40) b	260 (31)

[a] For comparable means for each variable, means followed by the same letter or letters are not significantly different at the 5% level according to Duncan's multiple range comparisons (Duncan, 1955). [b] Unrestricted (unres) rats were fed ad libitum throughout the experiment, restricted (res) rats were fed ad libitum until they were 250 days of age and at 50% of ad libitum levels from 251 to 300 days of age. Numbers in parentheses at left designate numbers of rats included in mean of each row; those at right designate exceptions to the row.

Table IV. Weight and Chlorinated Hydrocarbon Insecticide Content of Adipose Tissue[a]

	treatment			concentration, ppm				total amount, μg			
diet	diet regime	age killed, days	wt of tissue, g	DDE	TDE	DDT	dieldrin	DDE	TDE	DDT	dieldrin
HF-L (7)	unres[b]	250	65.5 a	2.2 e	0.9 e	0.4 ef	0.3 e	144 d	61 bc	24 bc	23 bc
HF-L (12)	unres	300	71.7 a	2.5 e	1.0 e	0.3 f	0.4 e	177 d	73 b	21 c	30 b
HF-L (12)	res	300	39.6 b	4.8 de	1.4 de	0.6 def	0.4 e	176 d	58 bc	22 bc	15 bc
LF-PB-BS (7)	unres	250	23.6 cd	7.3 cd	1.9 cd	1.0 cde	1.2 bc	164 d	46 bc	23 bc	29 bc
LF-PB-BS (14)	unres	300	33.9 bc	7.3 cd	2.1 c	0.7 def	1.0 cd	242 c	73 b	25 bc	32 b
LF-PB-BS (15)	res	300	17.5 d	12.8 b	2.3 bc	1.8 b	0.7 de	209 cd	39 c	26 bc	13 c
LF-BS-CO (8)	unres	250	30.3 bc	9.0 c	2.3 bc	1.2 cd	1.9 a	258 bc	67 b	31 b	51 a
LF-BS-CO (15)	unres	300	36.3 b	9.6 c	2.7 b	1.2 c	1.6 ab	333 a	96 a	39 a	59 a
LF-BS-CO (13)	res	300	17.1 d	21.6 a	3.9 a	2.9 a	1.9 a	316 ab	67 b	43 a	30 b
HF-L (31)			57.9 a	3.3 c	1.2 c	0.5 c	0.4 c	169 b	64 ab	22 b	22 b
LF-PB-BS (36)			25.1 b	9.6 b	2.1 b	1.2 b	0.9 b	213 b	54 b	25 b	23 b
LF-BS-CO (36)			28.0 b	13.8 a	3.0 a	1.8 a	1.7 a	310 a	79 a	39 a	47 a
	unres (22)	250	39.4 b	6.3 b	1.7 b	0.9 b	1.2 a	192 b	59 b	26 a	35 a
	unres (41)	300	45.9 a	6.7 b	2.0 b	0.8 b	1.0 a	256 a	82 a	29 a	41 a
	res (41)	300	24.0 c	13.3 a	2.6 a	1.8 a	1.0 a	234 a	54 b	30 a	19 b

[a] For comparable means for each variable, means followed by the same letter or letters are not significantly different at the 5% level according to Duncan's multiple range comparisons (Duncan, 1955). [b] Unrestricted (unres) rats were fed ad libitum throughout the experiment, restricted (res) rats were fed ad libitum until they were 250 days of age and at 50% of ad libitum levels from 251 to 300 days of age. Numbers in parentheses at left designate numbers of rats included in mean of each row; those at right designate exceptions to the row.

in liver (Table V) changed between 250 and 300 days in ad libitum fed rats. Dietary restriction did not significantly influence the concentrations (ppm) of DDE, TDE, or DDT in the liver (data not presented). Absolute liver size was reduced, however, with all three diets. DDE content (μg) significantly decreased in rats fed HF-L; amounts of TDE and dieldrin were also reduced in the livers of rats fed HF-L or LF-BS-CO. The amount of DDT in the liver was not significantly changed after weight reduction, regardless of diet.

At 250 days the livers of rats fed LF-BS-CO contained significantly higher amounts of DDE and dieldrin than the livers of rats fed LF-PB-BS. At 300 days the amounts of DDE and dieldrin were also greater in rats fed LF-BS-CO than in rats fed LF-PB-BS, but the differences were significant only for dieldrin. At 300 days the amounts of DDE as well as TDE were also significantly higher in the livers of rats fed HF-L than in the livers of rats fed LF-PB-BS. DDT content in liver was not affected by diet.

Since lindane was rarely detected in the liver, and when present was at a concentration less than 0.05 ppm, data are not shown in Table V.

Brain Weight and Insecticide Content. The concentrations (data not given) and amounts of insecticides in the brain (Table V) remained virtually unchanged between 250 and 300 days with all three diets fed the unrestricted group. With dietary restriction the amount of DDE in the brain increased significantly with both low-fat diets. TDE also increased in the brains of rats fed LF-BS-CO after 50 days of limited food intake. In contrast, the amount of dieldrin in the brains of rats fed LF-BS-CO was reduced. DDT remained unchanged during this period.

The amount of DDE was consistently lower in the brains of rats fed HF-L than of rats fed the low-fat diets. The differences were significant except between rats fed HF-L and LF-PB-BS at 250 days. Highest amounts of dieldrin were observed in the brain of rats fed LF-BS-CO. Amounts of dieldrin were significantly greater with this diet than either HF-L or LF-PB-BS, particularly in unrestricted rats. Diet had no effect on DDT in brain and only limited influence on TDE. In the restricted group the amount of TDE appeared significantly greater in rats fed LF-BS-CO than in rats fed HF-L.

Lindane was normally found in the brain at a concentration and amount (data not presented) equivalent to DDT. As with DDT, neither diet nor treatment had a significant effect on its content in brain.

DISCUSSION

The effect of metabolic stress caused by weight reduction or starvation on the mobilization of DDT and its derivatives (Dale et al., 1962; Brown, 1970; and Shtenberg et al., 1975) and of dieldrin (Zabik and Schemmel, 1973) has been studied in the rat. We estimated fat and water losses in an attempt to determine their individual contributions to the weight loss caused by dietary restriction. After 50 days on a restricted diet, fat rather than water was lost in both obese and normal-weight rats. Chlorinated hydrocarbon insecticides are stored in adipose tissue and fat of organs and damage nerve tissue when released in toxic amounts from fat (Dale et al., 1962). We determined the CHI content of only the perirenal–epididymal fat pads. With all diets, prolonged weight reduction caused mobilization of about 50% of the dieldrin from adipose tissue and loss of about 50% in the weight of the fat pads (Table IV). Fat pad weight losses also approximated 50% for all three diets. These data agree with those of Zabik and

Table V. Weight and Chlorinated Hydrocarbon Insecticide Content of Liver and Brain[a]

diet	treatment: diet regime	age killed, days	liver: wt of tissue, g	liver: total amount DDE, μg	liver: total amount TDE, μg	liver: total amount DDT, μg	liver: dieldrin, μg	brain: wt of tissue, g	brain: total amount DDE, μg	brain: total amount TDE, μg	brain: total amount DDT, μg	brain: dieldrin, μg
HF-L (7)	unres[b]	250	17.7 b	6.3 abc	2.8 ab	1.4 a	1.3 ab	2.13 a	0.08 e	0.05 ab	0.04 a	0.06 cd
HF-L (12)	unres	300	22.0 a	7.3 a	3.9 a	1.3 (11) a	1.3 ab	2.05 a	0.10 e	0.03 b	0.03 a	0.04 d
HF-L (12)	res	300	11.9 de	4.0 bcd	1.6 bc	0.72 a	0.35 c	2.07 a	0.14 de	0.04 b	0.03 a	0.04 d
LF-PB-BS (7)	unres	250	13.8 cd	2.8 cd	1.3 bc	0.54 a	0.75 bc	2.20 a	0.15 cde	0.04 b	0.02 a	0.06 cd
LF-PB-BS (14)	unres	300	15.7 bc	4.3 bcd	1.9 bc	0.62 a	0.75 bc	2.13 a	0.21 cd	0.04 b	0.03 a	0.08 (13) cd
LF-PB-BS (15)	res	300	10.4 ef	2.5 d	0.9 c	0.83 a	0.25 c	2.23 a	0.29 b	0.05 ab	0.02 a	0.06 cd
LF-BS-CO (8)	unres	250	13.8 cd	6.5 ab	2.6 b	0.89 a	1.8 a	2.08 a	0.20 cd	0.05 b	0.02 a	0.13 ab
LF-BS-CO (15)	unres	300	15.0 c	5.8 abc	2.4 b	0.81 a	1.9 a	2.21 a	0.23 bc	0.04 b	0.02 a	0.14 a
LF-BS-CO (13)	res	300	9.7 f	4.7 abcd	1.2 c	0.82 a	0.64 bc	2.15 a	0.46 a	0.06 a	0.04 a	0.09 bc
HF-L (31)			17.1 a	5.8 a	2.8 a	1.1 (30) a	0.93 b	2.07 a	0.11 c	0.04 a	0.03 a	0.05 b
LF-PB-BS (36)			13.1 b	3.3 b	1.4 c	0.69 b	0.54 b	2.19 a	0.23 b	0.05 a	0.03 a	0.07 (35) b
LF-BS-CO (31)			12.8 b	5.6 a	2.0 b	0.83 ab	1.4 a	2.16 a	0.31 a	0.05 a	0.03 a	0.12 a
	unres (22)	250	15.0 b	5.3 a	2.2 a	0.94 a	1.3 a	2.13 a	0.15 b	0.044 ab	0.03 a	0.09 ab
	unres (41)	300	17.3 a	5.7 a	2.7 a	0.88 (40) a	1.3 a	2.13 a	0.19 b	0.040 b	0.03 a	0.09 (40) a
	res (40)	300	10.6 c	3.7 b	1.2 b	0.80 a	0.41 b	2.16 a	0.30 a	0.051 a	0.03 a	0.07 b

[a] For comparable means for each variable, means followed by the same letter or letters are not significantly different at the 5% level according to Duncan's multiple range comparisons (Duncan, 1955). [b] Unrestricted (unres) rats were fed ad libitum throughout the experiment, restricted (res) rats were fed ad libitum until they were 250 days of age and at 50% of ad libitum levels from 251 to 300 days of age. Numbers in parentheses at left designate numbers of rats included in mean of each row; those at right designate exceptions to the row. Mean for 11 rats; one abnormally high value, 17 μg excluded. Mean for 13 rats; one abnormally high value, 57 μg, excluded.

Schemmel (1973), who investigated the storage of dieldrin in obese, normal, and semistarved rats and found that the perirenal–retroperitoneal fat pad lost weight and dieldrin at approximately the same rate. They also found that in rats of normal weight, dieldrin losses were higher than tissue weight losses, on a percentage basis. We found no such differences between obese and normal-weight rats.

DDT and its metabolite DDE were retained in adipose tissue during weight reduction regardless of diet. Brown (1970) also reported the same response of DDT, DDE, as well as TDE in the fat of rats subjected to exercise and starvation. Brown also found that concentrations of DDT metabolites increased in liver and brain and in several other tissues when animals were starved for 1 week. When Shtenberg et al. (1975) starved rats for 20 days, DDT concentrations increased in the liver but not in the brain or fat tissues of male Wistar rats. When we restricted food intake of rats, amounts of DDE, TDE, and dieldrin in liver decreased and liver weight also decreased; thus concentrations of the metabolites in liver were generally unaffected. However, the amount and concentration of DDE in the brain consistently increased, and these differences were significant in rats fed low-fat diets. Our data demonstrate in rats the effect of previous diet on the chlorinated hydrocarbon insecticides during weight reduction caused by a 50-day restriction of food intake.

Several investigations with rats have explored the effect of dietary ingredients on storage and toxicity of CHI compounds. Experiments dealing with level and kind of protein or fat and with vitamin deficiencies have primarily focused on individual chlorinated hydrocarbon insecticides (Sauberlich and Baumann, 1947; Lee et al., 1964; Tinsley, 1966; Weatherholtz, et al., 1969; Stoewsand et al., 1970; Oshiba and Kawakita, 1972; Zabik and Schemmel, 1973; and Miranda and Webb, 1974).

Adams et al. (1974) studied the response of rats to a mixture of nine chlorinated hydrocarbon insecticides when source and treatment of dietary fat were the variables. They reported that the type of dietary fat in general exerted little influence on tissue content of DDT, DDE, and dieldrin. In the present study, however, we found differences in the amounts of CHI in tissues that were associated with diet. Many of the significant differences in CHI deposition in the tissues were between the two low-fat diets, LF-PB-BS, which provided a high proportion of plant fats, and LF-BS-CO, which provided mainly animal fats. Rats consumed similar amounts (Table III) of these two diets, but deposited significantly more CHI, especially in fat, with the LF-BS-CO diet than with LF-PB-BS. The high-fat diet contained solely animal products, and intake was lowest because of its high caloric content. As a result, rats fed HF-L consumed less CHI than rats fed the low-fat diets. However, amounts of CHI in adipose tissue of rats fed HF-L (Table IV) and generally in liver and brain (Table V) were comparable to the amount in rats fed LF-PB-BS.

The influence of diet on the deposition of insecticides in the liver differed from that observed in adipose tissue, especially with respect to the HF-L diet. The amount of CHI in the liver of rats fed HF-L (Table V) was either comparable to or higher than the amount in liver of rats fed the low-fat diets. Possibly the high level of fat in the liver of rats fed the high-fat diet influenced the storage and/or catabolism of CHI and was responsible for the elevation of CHI in livers of these rats. Radomski et al. (1968) and Oloffs et al. (1974) showed that pathological alteration of human livers significantly affected CHI deposition: *p,p'*-DDE, *p,p'*-DDT, and dieldrin levels were elevated in persons with portal cirrhosis but not in those with fatty infiltration in the absence of necrosis or cirrhosis. They also noted depressed levels of *p,p'*-TDE in patients with portal cirrhosis, which suggested that conversion of DDT to TDE was impaired. In the present study, the level of TDE in livers of rats fed HF-L were not lower but higher than or comparable to those in rats fed the low-fat diets, indicating that conversion of DDT to TDE was normal. Some differences in liver CHI amount were also observed between the rats fed the two low-fat diets. Fat deposition, however, was negligible with these diets.

In contrast to its effect in adipose tissue, dietary manipulation had no effect on DDT and only a limited effect on TDE storage (Table V), in either liver or brain. The overall effect of diet, regardless of treatment, indicated that LF-BS-CO resulted in the highest amounts of dieldrin in all three tissues. The response of DDE to diet, however, varied with each tissue. In adipose tissue, DDE levels were highest in rats fed LF-BS-CO, regardless of treatment, and were generally similar in rats fed LF-PB-BS or HF-L. In liver, nevertheless, DDE differences among diets are not as clear-cut as those in fat. In some ad libitum fed rats, the statistical significance of DDE levels was lowest with LF-PB-BS when treatments are combined. In brain, DDE amounts again were highest with LF-BS-CO but lowest with HF-L when treatments were combined. This effect was consistent in 300-day-old animals. The amount of fat in the diet as well as that deposited in the body may be a contributing factor to the low storage of DDE in brain with the high-fat diet, but neither level nor source of fat account for the many other differences observed in the three tissues.

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Decamethrin Metabolites from Oxidative, Hydrolytic, and Conjugative Reactions in Mice

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Mouse metabolism of orally administered decamethrin differs from previous findings with rats as follows: the feces contain less decamethrin and more monohydroxy and dihydroxyester metabolites; more acid moiety metabolites are hydroxymethyl derivatives and less alcohol moiety metabolites are phenolic compounds: metabolites in mouse but not rat excreta include 3-(2,2-dibromovinyl)-2-*trans*-hydroxymethyl-2-methylcyclopropanecarboxylic acid sulfate, 3-phenoxybenzaldehyde, 3-phenoxybenzyl alcohol and its glucuronide, glucuronides of 4′-hydroxy-3-phenoxybenzyl alcohol and 5-hydroxy-3-phenoxybenzoic acid, and 3-phenoxybenzoyltaurine. Intraperitoneal (ip) administration yields the same metabolites but in different ratios. Decamethrin is hydrolyzed in vitro by esterases in blood, brain, kidney, liver, and stomach preparations. Mice pretreated with piperonyl butoxide (PB) or *S,S,S*-tributyl phosphorotrithioate (DEF) metabolize decamethrin less readily than normal mice by oxidative or hydrolytic pathways, respectively. Equitoxic doses of decamethrin (twice the LD_{50}, 6–191 mg/kg) administered orally or ip with different vehicles or ip to PB- or DEF-treated animals yield similar levels (~0.5 ppm) of decamethrin in the brain. Severe poisoning symptoms result on introducing this level of decamethrin into the brain by direct injection.

Decamethrin [(S)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,-3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] (Figure 1), a highly potent synthetic pyrethroid insecticide (Elliott, 1977; Elliott et al., 1974), is metabolized by rats (Ruzo et al., 1978) and mouse liver microsomal enzymes (Shono and Casida, 1978; Shono et al., 1979) by pathways that include hydroxylation of the methyl group trans to the carboxyl group, hydroxylation at the 2′, 4′, and 5 positions of the alcohol moiety, and hydrolysis of the parent compound or its hydroxy derivatives. In rats, the phenolic metabolites are excreted as glucuronides and sulfates and the carboxylic acid metabolites as glucuronides and glycine conjugates (Ruzo et al., 1978). The cyano fragment is converted via HCN to SCN^-, which is temporarily localized in the stomach and skin prior to excretion, and small amounts of 2-iminothiazolidine-4-carboxylic acid which is excreted more rapidly (Ruzo et al., 1978). Analogous reactions (Casida et al., 1979) are established in rats for compounds with the unresolved alcohol moiety (i.e., α*RS*) esterified with α-(4-chlorophenyl)isovaleric acid (i.e., fenvalerate) (Ohkawa et al., 1979) or 2,2,3,3-tetramethylcyclopropanecarboxylic acid (Crawford and Hutson, 1977).

It is of interest to compare the metabolic fate of decamethrin in mice and rats since both species are important for acute and chronic toxicity studies and for safety evaluations. Mice are generally more susceptible than rats to pyrethroid poisoning (Miyamoto, 1976; Ruzo and Casida, 1977). Treatment of mice with the oxidase inhibitor piperonyl butoxide (PB) or the esterase inhibitor *S,S,S*-tributyl phosphorotrithioate (DEF) further increases their susceptibility to decamethrin (Soderlund and Casida, 1977).

This study compares decamethrin metabolism in mice with our earlier report (Ruzo et al., 1978) in rats. It also examines the significance of oxidative, hydrolytic, and conjugative pathways and the importance of brain decamethrin levels in the susceptibility of mice to decamethrin poisoning.

MATERIALS AND METHODS

Chromatography and Radiocarbon Analyses. Thin-layer chromatography (TLC) utilized silica gel 60 F-254 20 × 20 cm chromatoplates with 0.25-mm layer thickness (EM Laboratories, Inc., Elmsford, NY) and the following solvent systems: BAW, 1-butanol–acetic acid–water (6:1:1); BE, benzene–ethyl acetate (6:1); BFE, benzene (saturated with formic acid)–ether (10:3), two developments; CE, carbon tetrachloride–ether (3:1); EFW, ethyl acetate–formic acid–water (35:2:2); EH, ether–hexane (1:1), three developments; EMW, ethyl acetate–methanol–water (2:1:1); HE, hexane–ether (4:1), three developments. R_f values for decamethrin derivatives are given in Table I. In referring to solvent systems for two-dimensional development, (BFE × CE) indicates development in the first direction with BFE and in the second direction with CE. Unlabeled standard compounds were detected first with UV light (254 nm) and then by spraying with either $PdCl_2$ (0.5% w/v in 12 N HCl) or phosphomolybdic acid (20% w/v in ethanol) and heating at 110 °C for up to 30 min. Procedures for radioautography, ^{14}C quantitation, and cochromatography of ^{14}C metabolites or their derivatives with unlabeled standards are given by Ueda et al. (1975).

Chemicals. Metabolite Designations and Standards. Metabolites are designated as shown in Figure 1, e.g., 4′-HO-dec and 4′-HO-PBacid are decamethrin and

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