Insecticides in the Tissues of Four Generations of Rats Fed Different Dietary Fats Containing a Mixture of Chlorinated Hydrocarbon Insecticides

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From weaning, four generations of male and female rats were fed diets containing different fats and a mixture of DDT, DDE, DDD, dieldrin, lindane, BHC, heptachlor epoxide, methoxychlor, and Perthane. DDT, DDD, DDE, and dieldrin were stored in livers and carcasses of weanlings, as well as in livers and body fat of adults. Generation differences were generally small and provided no evidence of a continuing increase with succeeding generations. Differences related to sex were observed in adults but not in weanlings. With DDT, DDE, and DDD, the differences between mated males and females of the F_0 through F_2 generations were the reverse of those in unmated rats of the F_3 generation. No such differences were observed with dieldrin. Distribution of DDT and DDE varied with the tissue investigated. Type of dietary fat exerted little influence on tissue content except for a possible interaction of cottonseed oil and dieldrin.

The persistent occurrence of traces of some of the chlorinated hydrocarbon insecticides (CHI) in our food supply is well recognized. The levels for all except dieldrin remain considerably below currently permissible levels (FAO/WHO, 1968). Many of these insecticides are found regularly in body tissues of humans at levels higher than those in the food supply. There is little information, however, about the possible effect that the continuing long-time ingestion of these insecticides may have on reproductive performance and on the health of succeeding generations. Much of the long-term research with the rat as the experimental animal has dealt with the response to low fat commercial-type animal diets to which have been added one or occasionally a mixture of two of these insecticides.

The need for more information on the response to mixtures of insecticides is apparent from the increasing evidence of their interaction with each other (Street *et al.*, 1969a). The possibility that the kind and level of fat in the diet may influence the accumulation in the tissues of the fat-soluble CHI also needs consideration. The research reported here was undertaken to determine the response of four generations of rats to diets containing a mixture of CHI in proportions found in our food supply. Fat was fed at a level similar to that consumed in the United States. This report deals with the concentrations of insecticides found in the tissues of the weanling and adult rats fed diets containing DDT, DDE, DDD, dieldrin, lindane, BHC, heptachlor epoxide, methoxychlor, and Perthane. The physiological response to these diets will be the subject of a separate report.

EXPERIMENTAL SECTION

From weaning, rats were fed nutritionally adequate, synthetic type diets similar to those described in detail in an earlier publication (Poling *et al.*, 1970). The diets were identical in all respects except for the kind of fat which contributed 20% of the dry weight of the diets or about 38% of the calories. Analyses of the diets gave the following average percentages: H_2O, 3.1; protein, 28.6; fat, 23.2; and ash, 3.8. The fats studied included four commonly used dietary fats: cottonseed salad oil (CS), lard (L), soybean oil (SB), and hydrogenated vegetable oil shortening (S). A composite blend of each fat was prepared by mixing aliquots of three prominent, commercially available brands. The fats used met the arbitrary limit of not more than 0.1 ppm total CHI residue. A fifth fat was prepared by hydrogenating a portion of the cottonseed salad oil just enough to give a negative Halphen test and to contain no detectable cyclopropene fatty acids (CS-CP). Two additional fats were prepared by heating aliquots of CS and L at 182° for 120 hr (HCS and HL) under conditions previously described (Poling *et al.*, 1970). A mixture of CHI was added to each of the five unheated fats and portions of CS and L containing the added insecticides were heated under the same conditions as the control fats.

The "market basket" survey of total diet samples reported by Williams (1964) served as the basis for the kinds and proportions of the insecticides selected. These "market basket" values were multiplied by 200 to provide diets containing levels of the insecticides close to the tolerances permitted at the time the research was initiated. The insecticides were first blended with the test fats in amounts sufficient to produce five times the concentration desired in the experimental diets. Each control fat and each fat containing added insecticides was prepared in sufficient quantities to complete the experiment and was stored until needed in filled sealed glass containers at −18° or below.

Table I summarizes the kinds and amounts of insecticides in each of the seven experimental fats. The insecticides used were, in all cases, reference standards or equivalent. The procedures used for determining these insecticides were carried out in accordance with the FDA Pesticide Manual (1968). An extract of each sample was subjected to an acetonitrile partition cleanup (P.A.M. 211.14a) and further cleanup with a Florisil column (211.14d). The resulting solutions were injected into a gas chromatograph after concentration to a known volume. The analyses were carried out by using a Barber-Colman pesticide analyzer, Model 5360, equipped with a Sr^{90} electron capture detector and a 4 ft × 4 mm glass column packed with 5% DC-200 on 80–100 mesh Crompert XXX. Oven temperature was 200°, injector temperature was 220°, and detector temperature was 240°. Nitrogen was the carrier gas at a rate of flow such that *p,p'*-DDT had a retention time of 6 to 8 min.

The results presented represent the mean of at least two separate analyses. The concentrations in the unheated fats were close to the levels sought. Except for DDD and methoxychlor, there was considerable loss of the insecticides added to the heated fats under the conditions of their preparation. No measurable amounts of any of the insecticides added were found in the control fats except

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Table I. Insecticide Content of Dietary Fats

Fat	Insecticide, ppm								
	DDT	DDE	DDD	Dieldrin	BHC	Lindane	Heptachlor epoxide	Methoxychlor	Perthane
	3.50[a]	2.50	2.90	1.90	0.10	0.70	0.30	0.50	2.40
CS	3.42	2.04	1.98	1.50	0.10	0.74	0.31	0.50	2.01
CS-CP	3.54	2.28	2.02	1.46	0.14	0.70	0.33	0.58	2.71
L	3.45	2.29	2.04	1.59	0.14	0.64	0.30	0.39	2.44
SB	3.73	2.12	2.00	1.83	0.10	0.80	0.31	0.58	2.46
S	3.83	2.56	2.30	2.02	0.14	0.72	0.27	0.50	2.67
HCS	2.00	1.15	2.03	0.62	[b]	0.03	0.07	0.40	
HL	2.12	1.07	1.64	0.76		0.07	0.08	0.38	

[a] Values on this line indicate the levels of insecticides sought. [b] No measurable amounts detected.

for small amounts of DDT, DDE, and DDD, all three of which were present except for DDT (which was absent) in the heated fats. The total concentration in any one of the fats did not exceed 0.12 ppm. All rats were supplied water and the appropriate diet *ad libitum* from weaning until death. Animal quarters were artificially lighted and air conditioned with the temperature at 26–27° and relative humidity at 45–50%. Rats were checked daily, body weights were measured weekly, and food disappearances determined weekly during the 12-week postweaning growth phase.

For the parent generation (F_0), 350 weanling rats (Holtzman albinos) were randomized among the 14 diet groups—9 males and 16 females per group. After 12 weeks the animals were mated. Females that had weaned a litter were rested for about 2 weeks before being remated for a second litter. First litters were discarded after the number of each sex weaned, the individual weaning weight, and the apparent health status of each animal had been recorded. Randomly selected weanlings (F_1) of both sexes from second litters were reared for mating and the production of F_2 weanlings. This procedure was continued until there were three generations of second litter weanlings for each diet. Litter origin was not known for the F_0 parents, but F_1 and F_2 males were selected to minimize the mating of siblings to the extent possible.

Adult males and females of each generation, F_0, F_1, and F_2, were killed after weaning of the second litter. The average age of the males was 295 days and of the females was 244 days. The F_3 rats were not mated and were killed 12 weeks from the time of weaning at an average age of 125 days. About three-fourths of the liver and a sample of body fat were removed from randomly selected adult males and females representing each diet group and each generation and were stored frozen for insecticide residue analysis. Also taken for analyses were tissues from rats that became moribund or died before they were scheduled to be killed. The body fat consisted of perirenal fat generally supplemented with abdominal fat taken from the back region to supply adequate samples for the analyses desired. At least two male and two female weanlings from each experimental group were killed when available and stored frozen.

At the time of analysis, the weanling rats were allowed to thaw in the refrigerator for approximately 24 hr. The head was separated from the body, the feet removed at the ankle, the tail removed at the spinal column, and the torso skinned. The brain was removed from the head after the top of the cranium was cut away. After the liver was removed, the remaining carcass was ground. Pooled samples of weanling tissues were prepared for each group. Prior to analysis, all tissues were thoroughly homogenized. A weighed sample was dried, ground with Na_2SO_4, and extracted with suitable solvents. The resulting extracts were carried through the analytical procedures already described.

The "*t*" test of significance was used to evaluate differences when there were sufficient comparable data to warrant statistical analysis. Probability levels of less than 0.05 were considered significant.

RESULTS

DDT, DDE, and DDD. Although there were measurable amounts of DDT, DDE, and DDD in the tissues of rats fed the control fats containing no added insecticides, the levels were low in comparison with those found when the experimental fats were fed. The highest levels were in the adult body fat, averaging 0.37, 0.48, and 0.30 ppm of DDT, DDE, and DDD, respectively. The levels in the other tissues were generally less than 0.10 ppm.

Influence of Generation and Sex. The levels of DDT, DDE, and DDD in the body fat of adult rats and in the carcasses of weanlings fed the fats containing added insecticides are summarized in Table II. For any one experimental condition, the data are limited to the results for two animals. Fortunately, the variation between the values for these pairs of rats was generally small. Data are included only for rats that were apparently healthy and showed no significant weight loss up to the time they were killed. The results for females in the F_0 through F_2 generation included only those rats that were able to rear successfully two normal size litters of young. Although not directly comparable in terms of possible generation differences, data are included for young adult rats of the F_3 generation. These rats were not only younger but differed in several respects from the older rats. The younger rats weighed less (males, 470 g *vs.* 586 g and females, 260 g *vs.* 330 g); their livers were smaller (males, 15.2 g *vs.* 16.1 g and females, 9.0 g *vs.* 13.7 g); and they were not bred.

Overall evaluation of the results indicated that differences due to diet were small and the data presented in Table II are the averages of all results regardless of diet. In view of the low insecticide levels in the heated fats, the limited results with these fats are separated from those for unheated fats. To show sex and generation differences of possible biological significance, the results have been separated accordingly.

In the fat of adult males and females fed the diets containing unheated fats, the levels of DDT, DDE, and DDD were higher in the F_1 than in the F_0 generation. The decreases in the following generation were small and significant only for DDE. No marked differences in distribution of DDT, DDE, and DDD relating to generation were observed.

The factor or factors responsible for the low level of insecticides in the young male rats of the F_3 generation are not apparent from the data available. The high levels in the F_3 generation females, however, appear to be related to the fact that these rats were not bred. The levels of DDT, DDE, and DDD were significantly higher in the females than in the males of this generation. In contrast, no

Table II. DDT, DDE, and DDD in Adult Body Fat and in Weanling Carcasses of Rats Fed Fats Containing Added Insecticides

Generation	Sex	No.	Insecticide, ppm			Insecticide, % of total		
			DDT	DDE	DDD	DDT	DDE	DDD
			Adult body fat (unheated fat)					
F_0	M	10[a]	7.2 ± 0.5[b]	5.5 ± 0.2	1.4 ± 0.1	51 ± 1	40 ± 1	10 ± 1
F_1	M	12	10.4 ± 0.5	8.1 ± 0.4	2.0 ± 0.1	51 ± 1	39 ± 1	10 ± 1
F_2	M	9	9.7 ± 0.6	6.2 ± 0.4	1.6 ± 0.2	55 ± 1	36 ± 1	9 ± 1
F_3	M	8	6.3 ± 0.4	4.2 ± 0.2	1.7 ± 0.1	51 ± 1	35 ± 1	14 ± 1
F_0	F	10	6.4 ± 0.6	5.1 ± 0.3	2.3 ± 0.1	46 ± 1	37 ± 1	16 ± 1
F_1	F	6	9.1 ± 0.9	7.9 ± 0.4	2.8 ± 0.2	45 ± 1	41 ± 1	14 ± 1
F_2	F	10	7.3 ± 0.7	5.2 ± 0.3	2.0 ± 0.2	50 ± 2	36 ± 1	14 ± 1
F_3	F	7	11.5 ± 1.5	11.4 ± 1.4	4.1 ± 0.4	42 ± 1	42 ± 1	16 ± 1
			Adult body fat (heated fat)[c]					
F_0	M	2[a]	5.6	4.1	1.2	51	38	11
F_1	M	2	7.5	5.6	1.6	51	38	11
F_2	M	2	4.2	3.1	1.2	50	36	14
F_3	M	2	4.5	2.9	2.0	48	30	22
F_0	F	2	4.9	3.7	2.2	45	34	21
F_1	F	2	5.4	5.3	2.4	41	41	18
F_2	F	2	2.8	2.0	0.9	48	36	16
F_3	F	2	6.2	5.7	3.6	40	36	24
			Weanling carcass (unheated fat)					
F_1	M + F	9[d]	0.78 ± 0.10	1.00 ± 0.08	1.13 ± 0.08	27 ± 3	34 ± 1	39 ± 3
F_2	M + F	7	0.05 ± 0.01	0.82 ± 0.08	1.03 ± 0.09	3 ± 1	43 ± 1	54 ± 1
F_3	M + F	9	0.43 ± 0.04	0.77 ± 0.04	1.00 ± 0.08	20 ± 2	35 ± 1	45 ± 2
			Weanling carcass (heated fat)[e]					
F_1	M + F	4[d]	0.28 ± 0.08	0.46 ± 0.04	0.61 ± 0.05	20 ± 5	34 ± 3	46 ± 4
F_2	M + F	4	0.03 ± 0.01	0.50 ± 0.10	0.41 ± 0.10	3 ± 1	53 ± 8	44 ± 8
F_3	M + F	4	0.31 ± 0.03	0.30 ± 0.02	0.47 ± 0.05	29 ± 3	28 ± 1	43 ± 3

[a] Number of tissues analyzed. [b] Standard error of the mean. [c] Heated lard only. [d] Number of composites analyzed; two rats per composite. [e] Heated CS and L.

significant differences were observed between males and females in any one of the three generations of rats that were bred. Further evidence suggesting that the lower values of the mated rats may be due to a loss of these insecticides to the milk produced during lactation is seen in data obtained from some females of the F_1 generation. The results for five rats in the F_1 generation are excluded from Table II because of a problem encountered in their reproductive performance. The rats were fertile and produced normal sized litters. No young from the first litters survived, although second litters of 7–8 pups were successfully reared. This problem, which was encountered more often with this generation, occurred with control as well as with the experimental fats and appeared to be unrelated to the insecticide level in the diet. The body fat of these five rats contained the following, in ppm: DDT, 14.3 ± 1.2; DDE, 11.7 ± 1.4; and DDD, 0.06 ± 0.2. Levels of DDT and DDE were significantly higher than those seen in Table II for the six rats that were able to rear successfully two litters of young. Both groups produced similar numbers of pups (18 and 20) and the pups that failed to survive were generally dead within 3 days after birth.

Although comparable data for the heated fats containing added insecticides are limited, the results for the levels of these insecticides in the body fat of adults fed heated lard are included to provide further evidence of the consistent sex and generation differences even with the lower levels of these insecticides in the dietary fat. With both heated and unheated fats, no marked differences in distribution of DDT, DDE, and DDD relating to generation were observed and sex differences were due chiefly to the higher percentages of DDD in the females.

In the carcasses of the weanling rats, DDT, DDE, and DDD were consistently present but there were no significant differences between males and females. Generation differences were apparent with both unheated and heated fats. Although the differences were small, the levels in the F_1 weanlings, as in the F_1 adults, tended to exceed those of succeeding generations, with the total of these three products significantly higher than that for either the F_2 or F_3 generation. There is no apparent explanation for the extremely low level of DDT in the F_2 generation. The distribution of DDT, DDE, and DDD differed from that in adult body fat, with the percent DDT significantly lower and DDD significantly higher in the weanling carcass.

Table III summarizes the data for the concentration of DDT and related products in livers of adult and weanling rats fed the diets containing added insecticides. The levels of DDT in the livers of both adults and weanlings were consistently low and no greater than those obtained in comparable tissues from control rats. In adult males fed unheated fats, the level of DDE was significantly lower in the F_2 generation than in the preceding generations. The highest concentration of DDD was in the liver of F_1 rats, but generation differences were not significant. The levels of DDE and DDD in the livers of adult males were significantly higher than in the livers of females of the corresponding generations except in the F_3 generation, where sex differences were not significant. The levels of DDE and DDD in adult females were significantly higher in unmated rats of the F_3 generation than in mated females of the preceding generations. Although significant differences related to reproductive performance were not observed in the livers of F_1 females, the results showed a trend similar to those observed in body fat even with the relatively low levels of these insecticides in the livers. Total DDE and DDD in the livers of rats rearing only one litter averaged 0.51 ppm in contrast to an average of 0.37 ppm in the rats successfully rearing two litters. The relative proportion of DDE was higher and of DDD lower in the F_0 generation of both males and females than in succeeding generations. In the livers of adult rats fed heated fats, the concentrations of these insecticides were low but in general sex differences were similar to those found in the livers of rats fed the unheated fats.

In the livers of weanlings fed unheated or heated fats,

Table III. DDT, DDE, and DDD in Livers of Adults and Weanlings Fed Fats Containing Added Insecticides

Generation	Sex	No.	Insecticide, ppm			Insecticide, % of total		
			DDT	DDE	DDD	DDT	DDE	DDD
			Adults (unheated fats)					
F_0	M	10[a]	0.05 ± 0.01[b]	0.31 ± 0.02	0.50 ± 0.03	6 ± 1	36 ± 2	58 ± 2
F_1	M	12	0.09 ± 0.02	0.31 ± 0.02	0.65 ± 0.06	9 ± 2	30 ± 1	62 ± 2
F_2	M	9	0.03 ± 0.01	0.20 ± 0.02	0.55 ± 0.02	4 ± 1	25 ± 1	71 ± 2
F_3	M	8	0.06 ± 0.02	0.16 ± 0.01	0.49 ± 0.06	8 ± 2	24 ± 2	68 ± 1
F_0	F	10	0.05 ± 0.01	0.12 ± 0.02	0.16 ± 0.02	14 ± 2	37 ± 2	49 ± 2
F_1	F	6	0.06 ± 0.01	0.10 ± 0.02	0.27 ± 0.06	14 ± 3	24 ± 2	62 ± 3
F_2	F	10	0.06 ± 0.01	0.09 ± 0.02	0.23 ± 0.03	10 ± 3	24 ± 1	66 ± 4
F_3	F	7	0.14 ± 0.06	0.22 ± 0.02	0.51 ± 0.04	14 ± 4	26 ± 2	61 ± 3
			Adults (heated fats)					
$F_0 + F_1 + F_2$	M	9[a]	0.05 ± 0.01	0.18 ± 0.02	0.35 ± 0.02	7 ± 2	32 ± 2	61 ± 2
$F_0 + F_1 + F_2$	F	11	0.03 ± 0.01	0.07 ± 0.01	0.17 ± 0.02	10 ± 2	25 ± 3	65 ± 4
F_3	M	4	0.05 ± 0.03	0.08 ± 0.01	0.26 ± 0.02	11 ± 6	21 ± 3	69 ± 5
F_3	F	4	0.07 ± 0.04	0.10 ± 0.02	0.29 ± 0.05	12 ± 4	22 ± 3	66 ± 3
			Weanlings (unheated fats)					
F_1, F_2, F_3	M	14[c]	0.11 ± 0.03	0.36 ± 0.02	0.99 ± 0.04	7 ± 2	25 ± 1	69 ± 2
F_1, F_2, F_3	F	11	0.11 ± 0.03	0.36 ± 0.03	0.92 ± 0.05	7 ± 2	26 ± 1	67 ± 1
			Weanlings (heated fats)					
F_1, F_2, F_3	M	6[c]	0.08 ± 0.02	0.20 ± 0.03	0.63 ± 0.07	10 ± 3	21 ± 2	69 ± 2
F_1, F_2, F_3	F	6	0.12 ± 0.06	0.20 ± 0.03	0.56 ± 0.11	14 ± 5	24 ± 2	63 ± 5

[a] Number of tissues analyzed. [b] Standard error of the mean. [c] Number of composites analyzed; two rats per composite.

Table IV. The Influence of Dietary Fat on Dieldrin Content of Tissues of Adult and Weanling Rats Fed Diets Containing Added Insecticides

Generation and sex	Dieldrin, ppm[a]							
	CS	CS–CP	L	SB	S	Mean, all unheated fats	HCS	HL
					Adult body fat			
F_0 M	0.50	0.54	0.26	0.32	0.52	0.43 ± 0.05 (10)	0.13	0.20
F_1 M	0.27	0.15	0.34	0.22 (3)	<0.05 (3)	0.18 ± 0.05 (12)	<0.05 (1)	0.07
F_2 M	0.16	0.06 (1)	0.06	0.18	0.10	0.12 ± 0.03 (9)	no data	<0.05
F_3 M	No data	0.34	0.13	0.30	0.19	0.24 ± 0.03 (8)	<0.05	0.12
F_0 F	0.78	1.72	0.98	1.36	1.32	1.23 ± 0.15 (10)	0.44	0.64
F_1 F	3.16	1.52	0.56	0.18	0.38 (3)	1.09 ± 0.34 (11)	0.38	0.32
F_2 F	0.42	0.78	1.10	0.36	0.19	0.57 ± 0.13 (10)	0.31 (1)	0.14
F_2 F	No data	1.90	2.00	0.13 (1)	3.39	2.10 ± 0.44 (7)	1.04	0.36
					Adult livers			
F_0, F_1, F_2, F_3 − M	0.04 (6)	0.03 (7)	0.03 (8)	0.03 (9)	0.03 (9)	0.03 ± 0.01 (39)	<0.02 (5)	<0.02 (8)
F_0, F_1, F_3 − F	0.08 (6)	0.04 (6)	0.03 (6)	0.06 (6)	0.03 (7)	0.05 ± 0.02 (31)	0.03 (5)	0.02 (6)
F_3 − F	No data	0.12	0.12	0.14 (1)	0.09	0.11 ± 0.01 (7)	0.04	0.06
					Weanling carcass			
F_1 − M + F	0.22 (1)	0.29	0.34	0.27	0.28	0.29 ± 0.02 (9)	0.05	0.14
F_2 − M + F	No data	0.12	0.14	0.02 (1)	0.10	0.09 ± 0.02 (7)	<0.01	0.04
F_3 − M + F	0.09 (1)	0.21	0.21	0.12	0.15	0.16 ± 0.02 (9)	0.04	0.04
					Weanling livers			
F_1, F_2, F_3 − M + F	0.08	0.11 (6)	0.12 (6)	0.14 (5)	0.12 (6)	0.12 ± 0.01 (25)	0.02	0.05
					Moribund and dying rats, body fat			
F_1 − M	0.10 (1)	0.47	0.15 (1)	0.24 (1)	0.05 (1)		0.21 (3)	0.07 (1)
F_1 − F	4.59				0.09 (1)		0.18	0.62

[a] Data represent the mean of two analyses unless otherwise indicated by numbers in parentheses.

no generation differences were observed and the significant sex effect noted with adults was not apparent. DDE and DDD were present in proportions similar to those found in adults of the F_2 and F_3 generations.

Although DDT, DDE, and DDD were consistently present in the brains of weanling rats, the levels were low and no differences due to generation, sex, or kind of dietary fat were evident. The concentrations of these insecticides were often as low in rats fed the experimental diets as in controls, although the overall average was somewhat higher with 0.06, 0.06, and 0.04 ppm of DDT, DDE, and DDD, respectively, in the controls and 0.09, 0.08, and 0.05 ppm in the rats fed the fats containing added insecticides.

Influence of Dietary Fat. Although generation and sex differences were significant, the similar patterns observed with each fat make possible a comparison of the overall response to the individual dietary fats. No consistent differences related to dietary fat were observed in the levels of DDT, DDE, or DDD in the body fat of adults or in the carcasses of weanling rats fed the unheated fats. In terms of total DDT, DDE, and DDD, the averages for the individual fats ranged from 16.2 to 18.6 ppm in adult body fat and from 2.2 to 2.6 ppm in weanling carcasses. The corresponding ranges for the adult livers were 0.5–0.8 and for weanling livers 1.3–1.6 ppm. Although the differences were small, the levels of DDE and DDD in the livers of

both adult and weanlings showed a consistent tendency to be lower in rats fed L or S than in the livers from rats fed the other unheated fats.

Significant differences (7.3 ppm for HCS and 12.4 ppm for HL) were observed in the levels of these insecticides in the body fat of adults fed the heated fats, although the concentrations of these insecticides in these dietary fats were similar. In the other tissues, levels were low but showed a similar trend. With HCS the averages were 0.99 ppm in weanling carcasses, 0.32 ppm in adult livers, and 0.87 ppm in weanling livers; with HL the corresponding values were 1.26, 0.51, and 0.92 ppm.

Levels in Dying Rats. For comparison with the results for healthy animals, limited data were collected on the insecticide content of the livers and body fat from F_1 rats that were moribund or died before they were scheduled to be killed. Data were obtained for 16 controls as well as for 17 rats fed the fats containing added insecticides. Average age of death was 250 days; the range was from 100 to 350 days. The levels of insecticides varied considerably among animals but no relationship between cause of death and tissue concentration could be established. DDT, DDE, and DDD were present consistently in the tissues of controls and of experimental rats. Differences due to sex were not apparent.

The levels in the livers were low and only reflected intake when dietary levels differed considerably. Thus, the average concentration of total DDT, DDE, and DDD in the rats fed the control fats was 0.13 ppm. The average for rats fed the fats containing added insecticides was 0.64 ppm and no differences related to the levels of insecticides in the heated and unheated fats were observed. The higher levels in body fat reflected dietary intake more closely and differed little from the values found for comparable healthy rats of this generation. The average total concentration for controls was 2.3 ppm, for rats fed heated fats containing added insecticides 15 ppm, and for those fed the unheated fats containing added insecticides 31 ppm. In general, the results indicate that the distribution of these three insecticides follows patterns similar to those observed in the tissues of healthy rats.

Dieldrin. Although the pairs of analyses obtained for each experimental condition were in good agreement in most instances, differences in dieldrin concentration in the tissues and especially in body fat of rats fed diets containing added insecticides varied considerably with diet. Sex and generation differences were also apparent. The results in Table IV, therefore, are summarized by diet for each generation, with the data for males and females separated when sex differences were significant. Included also are overall averages of the levels in the tissues of rats fed unheated fats. The tissues of control rats contained less than 0.02 to 0.05 ppm, concentrations too low to obtain an accurate measure of the levels in the tissues examined.

The levels of dieldrin in the body fat of male rats varied with diet and the data were too limited to establish significant diet differences. There was a definite trend with generation and the overall averages of the levels in the F_1 and F_2 generations were significantly lower than for the F_0 generation. Although the concentration of this insecticide in the young adult rats of the F_3 generation was consistently higher than that found in comparable older rats of the preceding generation, the differences were generally small and in the reverse direction from those observed for DDT and metabolites in the young F_3 males.

In the fat of females, levels of dieldrin were consistently higher than those found in males under comparable conditions. The concentrations varied markedly with diet, especially in the F_1 generation. The highest concentration in the F_1 generation was found in the rats fed CS. Except for L, the concentrations were less in the F_2 generation than in the F_0 generation. The concentrations in the fat of unmated F_3 females were consistently high, except for the concentration in the one rat fed SB, and much higher than for males of the same generation.

The dieldrin concentration in adult livers was generally low. In males, no diet or generation differences were found. In the livers of F_0, F_1, and F_2 females, generation and diet differences were small except possibly for the somewhat elevated level of 0.16 and 0.12 ppm in the F_1 generation rats fed CS and SB, respectively. In the F_3 generation of unmated females, the concentrations of dieldrin with all diets were higher than in the preceding generations of mated females.

The presence of dieldrin in the weanling carcass was not influenced to any great extent by diet, no significant sex differences were observed, and generation differences were small, following somewhat the same pattern as that observed in the fat of adult males of the same generation.

Weanling livers contained similar levels of dieldrin regardless of diet, and no generation or sex differences were apparent. The levels of this insecticide in livers of weanlings were consistently higher than found in those of adult males and were similar to levels found in livers of adult females of the F_3 generation.

Data for moribund or dying rats were limited to those for the F_1 generation and varied considerably. Here, as in the F_1 generation of healthy females, the levels of dieldrin in the adipose tissues of the dying females fed CS were very high. The other concentrations observed were low and not necessarily related to the levels in comparable healthy rats of the same generation.

Other Chlorinated Hydrocarbons. Although the levels of the other insecticides added to the experimental fats were generally low, there were measurable amounts of lindane, BHC, and heptachlor epoxide (HE) in some of the tissues. Methoxychlor and perthane were not detected. None of these insecticides were found in the F_0 and F_2 generations. The analyses for these two generations were carried out prior to those for the F_1 and F_3 generations. The ability to detect low levels of these insecticides in the later analyses may have been due to some improvement in sensitivity of the columns used for these measurements.

Lindane was found in all of the tissues of adult rats in the F_1 and F_3 generations although the levels were sometimes too low to permit a quantitative measure of its concentration. In the body fat of controls, the levels were generally less than 0.05 ppm, the limit of sensitivity in this tissue. The livers of controls and of experimental rats contained less than 0.02 ppm. In the fat of adult rats (Table V) no consistent differences due to diet were apparent except for the lower levels generally found in the tissues of rats fed the heated fats. The levels of this insecticide tended to be lower in the young males of the F_3 generation than in the older males of the F_1 generation. The concentration of lindane in the fat of female rats fed the unheated fats was considerably higher than that in males. In contrast to the results with DDT and metabolites, no differences were observed between the mated females of the F_1 generation and the unmated females of the F_3 generation. Neither weanling carcasses nor weanling livers contained detectable amounts of this insecticide.

The levels of BHC in the diets were extremely low. The concentrations were also low in the tissues of adult rats, generally less than 0.05 ppm in body fat and less than 0.02 ppm in the liver except in the body fat of some females in the F_3 generation. In this generation small but measurable levels were observed when the diets contained added insecticides and the fats were CS-CP, L, and SB (Table V). As with lindane, this insecticide was not detected in the tissues of weanlings.

HE was not present in detectable amounts in the fat of male or female adults in the F_0, F_1, and F_2 generations or

Table V. Concentration of Lindane, BHC, and Heptachlor Epoxide in Body Fat of Rats Fed Fats with Added Insecticides

Fat	Lindane, ppm[a]				BHC, ppm[a]		Heptachlor epoxide, ppm[a]	
	M, F_1	M, F_3	F, F_1	F, F_3	M, F_3	F, F_3	M, F_3	F, F_3
CS	0.18	No data	0.32	No data	No data	No data	No data	No data
CS–CP	0.14	0.08	0.22	0.24	<0.05	0.10	0.25	1.34
L	0.16	0.09	0.31	0.32	<0.05	0.12	0.24	0.74
SB	0.11	0.10	0.28	0.56	<0.05	0.06 (1)	0.24	1.16 (1)
S	0.14	0.09	0.18	0.32	<0.05	<0.05	*b*	
HCS	0.07	<0.05	0.06	<0.05	<0.05	<0.05		0.10
HL	0.08	0.08	<0.05	<0.05	<0.05	<0.05	0.06	0.25

[a] Two animals per value unless otherwise indicated by number in parentheses. [b] Analyses made but HE not detectable.

in the livers of males or females of any generation. Significant amounts were found in the fat of F_3 rats with surprisingly high levels in females fed CS–CP, L, or SB. This insecticide, however, was still not found in male or female rats fed the S-containing diet nor in males fed HCS. Small amounts were also found in the weanling carcasses of this generation. In the carcasses of rats fed the unheated fats containing added insecticides, the average concentration was 0.07 ppm with no apparent diet, sex, or generation differences.

The results for moribund or dying rats of the F_1 generation fed the fats containing added insecticides were similar to those for healthy animals of the same generation. The average level for lindane was 0.16 ppm in the adipose tissues of males fed unheated fats and 0.08 ppm for those fed heated fats. The corresponding values for the more limited data on female rats were 0.32 and 0.08 ppm. The low levels of BHC in liver and adipose tissues and the lack of HE in these tissues were in general agreement with the results obtained for comparable healthy rats.

DISCUSSION

Many factors are recognized as influencing the storage of insecticides in body tissues such as level of intake, duration of exposure, age, sex, and species. This report provides evidence of the levels of insecticides found in the tissues of male and female rats fed a mixture of the chlorinated hydrocarbon insecticides through several generations and of the storage of these insecticides in the tissues of weanlings. With the products fed, except for methoxychlor and perthane, it was apparent that all could be stored in the body fat of rats.

According to Kunze *et al.* (1950) storage of methoxychlor occurs to a very limited extent, and its lack in body fat was not surprising considering the very low level of this insecticide in the diet. Perthane, however, was present in the diet in concentrations comparable to DDT and dieldrin but still was not detected in any of the tissues investigated. Although chemically related to DDT, perthane has a lower storage potential and is much less toxic to mammals than is DDT (Negherbon, 1959). As a biodegradable product which is still toxic to many species of insects, this insecticide has been suggested as a possible alternative for DDT (Halladay *et al.*, 1972).

With DDT and its metabolites, tissue storage was found even with the low intake of the control rats. There was no evidence of any continuing increase in the levels of these insecticides with succeeding generations. The results provide no answer as to the cause of the significant increases in the concentrations of DDT and DDE in the body fat of both males and females in the F_1 generation. Environmental conditions were carefully controlled and no differences related to body weight or food intake were apparent. In spite of the low levels of these insecticides in the control fats and in the body fat of the control rats, a similar increase was observed in the F_1 controls. Both the control and the experimental diets contained a high level of fat, much higher than that found in the diets of stock rats. The results obtained with the F_1 rats may be the results of a gradual adaptation of these rats to the high fat diets. The amounts of the insecticides fed were low compared with those used in the relatively short time studies which have shown DDT capable of inducing enzymes for its own degradation or which have demonstrated an interaction of DDT and metabolites with other chlorinated hydrocarbons (Street, 1969b). That such reactions could have contributed to the slow leveling off observed in the levels of these insecticides is still a possibility considering the lengthy duration of exposure to these insecticides.

There is considerable evidence that rats store more DDT and DDE in the body fat of females than in males (Hayes, 1965), information based on studies comparing only unmated males and females. The sex differences observed under the conditions of the present research with young F_3 rats are in general agreement with these previous reports and are in contrast to the results obtained with mated males and females. No comparable reports dealing with sex differences in the livers of rats were located.

Hormonal differences (Durham *et al.*, 1956) may be a factor in some of the sex differences observed. However, the contrasting results with the mated and not mated rats fed DDT, DDE, and DDD suggest that the low levels in the livers of mated females and the failure to store high levels of these insecticides in their body fat are due to the passage of these insecticides to the young. The limited data with rats of the F_1 generation suggest that this reduction in the storage of these rats occurs during lactation. The data for the mated females were obtained shortly after weaning of their last litter, and provide no information as to the possible levels that these rats would have attained later in life with continued exposure to the insecticides.

Information on the distribution of DDT and metabolites in the tissues of rats is limited and generally based on feeding DDT alone rather than a mixture such as may occur in present-day diets. From the results of the study reported here it is not possible to differentiate between those products stored as fed and those resulting from the metabolism of DDT. It is apparent, however, that under the conditions investigated, the relative proportions of DDT, DDE, and DDD differed not only from those in the diet but also from tissue to tissue. In terms of total DDT and metabolites, the diets contained approximately 45% DDT, 29% DDE, and 26% DDD. DDD was the major product in the liver, accounting for about 66% of the total, whereas the proportion of DDT was low in both adults and weanlings, generally less than 10%. In body fat, DDT accounted for 50% of these insecticides stored; DDD accounted for 10 to 16%. Similar differences in the distribution of DDD and DDT in liver and perirenal fat were reported by Ottoboni and Ferguson (1969) when rats were fed a diet containing 20 ppm of technical DDT. In the carcasses of weanlings the relative proportions of these

products were intermediate between those in body fat and livers. DDE generally accounted for a slightly higher percentage of the total in the tissues than in the diet and varied less from tissue to tissue than did DDT or DDD.

The consistently lower levels of DDD and DDE in the livers of adults and weanlings with the highly saturated fats, L and S, may represent a real difference although, at the level fed, diet differences were small and would seem to be of little physiological significance. There were no apparent reasons for the significant differences in the storage of these insecticides in the body fat and carcasses of rats fed the heated fats.

Interpretation of the results with dieldrin is complicated by the great variation in tissue levels with diet as well as with generation and sex. This variation may be related to factors influencing the absorption of dieldrin from the gastrointestinal tract. Absorption occurs chiefly *via* the portal vein (Heath and Vandekar, 1964) in contrast to absorption of DDT which occurs mainly through the thoracic lymph duct (Hayes, 1965).

Dieldrin was stored in the tissues of weanlings and of adults. When compared with the storage of DDT or DDE, the storage of dieldrin in the body fat of males was low in relation to intake. Generation differences were observed in the body fat of males and in weanling carcasses but did not follow the same pattern as that seen with DDT and its metabolites. Although the overall averages for the levels in the body fat of females showed a trend similar to that found with males, the variation among diets was too great to warrant any conclusions concerning generation differences with females. As with DDT, no differences related to sex were observed in the levels of dieldrin in the tissues of weanlings. In contrast, sex differences were apparent with adult rats following a pattern which differed consistently from that observed with DDT. The concentration of dieldrin was higher in the fat of females, mated or not mated, than in the fat of comparable males, but in the livers no sex or generation differences were seen except for the significantly higher level in unmated females of the F_3 generation.

Because of the many factors influencing the response to dieldrin, it was not possible to establish significant diet differences. However, the results with CS in the F_1 generation suggest the possibility of real differences with this fat. The levels in the body fat of females of the F_1 generation fed CS were consistently high in both healthy and moribund rats, with the values for four rats ranging from 2.8 to 5.5 ppm. Except with CS–CP (1.48 and 1.57 ppm) the levels with the other fats for this generation were well below 1 ppm. Problems were encountered in this generation in obtaining sufficient healthy weanlings fed either CS or CS containing added insecticides to carry on the reproduction study, but the results were too limited to establish the possibility that the reproductive problems with CS were aggravated by the presence of the insecticides added to this fat. No data have been reported on reproductive performance with diets comparable to those fed in this study. There has been, however, some evidence that dieldrin, when fed in a low fat diet at a concentration of 2.5 ppm for three generations, may interfere with reproductive performance and that this effect tends to disappear in succeeding generations (Treon and Cleveland, 1955).

The dietary levels of lindane, BHC, and HE were too low to establish possible generation differences in the tissue concentrations of these insecticides. However, the presence of measurable amounts of HE in the tissues of unmated rats of the F_3 generation is noteworthy considering that this insecticide was not detected in the tissues of mated adults. Differences in the tissue levels of males and females were seen with all of these insecticides. With lindane, as with dieldrin, the levels in the fat of both mated and unmated females exceeded those in males. With BHC and HE, sex differences were found in the F_3 unmated rats.

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