Chlorinated Hydrocarbon Insecticide Residues in Adipose, Liver, and Brain Samples from Iowa Mink

by

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Biomagnification of chlorinated hydrocarbon insecticides has been well documented in aquatic systems (KEITH, 1966; MEEKS, 1968), and high concentrations have been found in predatory birds at top trophic levels (AMES, 1966; HICKEY and ROELLE, 1969). However, less work has been done concerning predatory mammals associated with aquatic systems. Our study was designed to measure chlorinated hydrocarbon insecticide residues in the mink. Objectives were to quantify residues in adipose, liver, and brain tissues, and to examine the relationship between age and insecticide level.

METHODS

During the 1970-71 fur season, 35 wild mink carcasses were collected from a central Iowa watershed located in parts of Story, Boone, and Hamilton counties. Liver, brain, and adipose tissues were removed from each animal and weighed, then frozen until preparation for residue analysis. Mandibles were retained for age determination.

The mink were aged by counting annual rings in the periosteal zone of mandible sections. KLEVEZAL and KLEINENBERG (1969) have reviewed this method in nine mammalian orders and found that the age of mink in years equals the number of annuli. Mandibles were decalcified, sectioned, and stained according to methods modified from LINHART and KNOWLTON (1967).

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Methods for extraction and cleanup of samples for residue analysis were modified from MILLS et al. (1963) as described by THOMPSON (1971). The gas chromatograph used was a Packard, Model 7631, with dual-column oven (Packard Instrument Co., Inc., Downers Grove, Ill.), equipped with an electron-capture detector (300 mc tritium). Temperatures for injector, column, detector, and outlet were 220°, 190°, 220°, and 235°C, respectively. Samples were analyzed quantitatively by using a 4-mm i.d. x 132-cm glass column packed with 1.5% OV-17/1.95% QF-1 on a solid support of Chromosorb WHP, 100/120 mesh. The carrier-gas flow rate was 120 ml/min (pre-purified nitrogen, Matheson Gas Products, Joliet, Ill.). A qualitative check was run simultaneously by using a similar column packed with 5% OV-210 on Chromosorb WHP, 80/100 mesh, with a nitrogen flow rate of 75 ml/min. Column packing materials were obtained from Supelco, Inc., Bellefonte, Pa. Average retention times (in minutes) for standards on the 5% OV-210 and 1.5% OV-17/1.95% QF-1 columns, respectively, were: lindane, 1.5 and 1.9; β-BHC, 1.8 and 2.4; aldrin, 1.9 and 3.0; heptachlor epoxide, 3.8 and 5.0; p,p'-DDE, 4.1 and 8.0; dieldrin, 6.0 and 8.2; o,p'-DDT, 5.4 and 11.5; p,p'-DDD, 7.5 and 13.2; p,p'-DDT, 8.2 and 15.9.

Peak height ratios were used to quantify sharp, early eluting peaks (lindane, β-BHC, heptachlor epoxide, p,p'-DDE and dieldrin). For broad, later eluting peaks (o,p'-DDT, p,p'-DDD and p,p'-DDT) 0.5 (peak height x width) was used as an approximation of peak area, and ratios were calculated with respect to standards. Recovery of known amounts of standard insecticides from blank samples was 85-95%. The data are presented as obtained from samples; they are not corrected for percentage recoveries. Limits of detectability of standards were: o,p'-DDT, p,p'-DDT, and p,p'-DDD, 0.01 ppm; p,p'-DDE, aldrin, dieldrin, and heptachlor epoxide, 0.005 ppm; lindane and β-BHC, 0.001 ppm. Analytical precision was evaluated by measuring the error of duplicate observations. Two adipose samples from each of six different mink carcasses were subjected to the extraction and analytical processes, and the standard deviation (expressed as a percentage of the mean of duplicate values; coefficient of variation) was calculated.

Thin-layer chromatography was used to confirm identifications made by gas chromatography. Methods were modified from KOVACS (1963) as described by the U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE (1969). To reach the limits of detectability (0.002 µg), five 8-ml samples, each containing insecticide residues from a mink adipose sample,

were combined and concentrated to 50 µliters. Then 25 µliters were spotted at each of two points along the baseline on the glass plates. Insecticide standards were spotted with samples on the same plate.

RESULTS

Results of adipose tissue analysis and aging are listed in Table 1. Total residues of DDT and related compounds ranged from 0.27 to 9.51 ppm. Of the total, p,p'-DDE made up 23 to 80%, p,p'-DDT 4 to 50%, p,p'-DDD 6 to 41%, and o,p'-DDT 2 to 34%. In six samples, however, one or more of the following was not detected: p,p'-DDD, p,p'-DDT, and o,p'-DDT. Dieldrin residues ranged from 0.02 to 4.90 ppm. Low levels of heptachlor epoxide and β-BHC (up to 0.20 and 0.11 ppm, respectively) were found in some samples. No lindane was detected in adipose tissue. Footnote a of Table 1 also gives the analytical error for individual compounds as expressed by the coefficient of variation. Gas-chromatographic identifications of o,p'-DDT, p,p'-DDT, p,p'-DDE, and dieldrin were confirmed by thin-layer chromatography.

Liver and brain samples from five mink were analyzed (Table 2). The values for DDT and related compounds were much lower in these tissues, but the highest levels correlated with high adipose levels, and low levels correlated with low adipose levels. Dieldrin residues ranged up to 0.04 ppm in brain and 0.07 ppm in liver. Lindane and β-BHC were detected in brain at levels up to 0.04 and 0.08 ppm, respectively. No heptachlor epoxide was detected in brain or liver.

Table 3 lists the mean residues of DDT and related compounds and of dieldrin for different year classes of mink. Analysis of variance showed that the distribution of total DDT and dieldrin residues among year classes of male mink was not significant ($F = 0.56$, 3 and 10 df, $P > 0.1$; $F = 0.38$, 3 and 10 df, $P > 0.1$). Statistical results were similar for total DDT and dieldrin residue distributions among female year classes ($F = 2.11$, 1 and 14 df, $P > 0.1$; $F = 1.14$, 1 and 14 df, $P > 0.1$). Combining all age classes, the overall dieldrin-residue mean for males was not significantly different from the female dieldrin-residue mean ($F = 1.75$, 1 and 33 df, $P > 0.1$). Similarly, total DDT-residue means for males and females were not significantly different ($F = 0.32$, 1 and 33 df, $P > 0.1$).

TABLE 1.
Chlorinated hydrocarbon insecticide residues (ppm) in mink adipose tissue.

Specimen No.	Sex	Year Class	DDT and Related Compounds[a]					Dieldrin	Heptachlor Epoxide	β-BHC
			p,p'-DDE	p,p'-DDD	p,p'-DDT	o,p'-DDT	Total			
1	F	1	1.33	0.53	0.24	0.14	2.24	0.30	[b]	0.03
2	M	1	0.37	0.27	0.27	0.06	0.97	0.09	0.07	[b]
3	M	1	3.18	1.28	1.85	0.81	7.12	0.25	[b]	[b]
4	M	2	0.49	0.61	0.54	0.49	2.13	0.08	0.05	[b]
5	M	1	1.80	0.37	0.16	0.09	2.42	0.14	[b]	[b]
6	F	[c]	1.86	0.18	0.30	0.32	2.66	0.04	[b]	[b]
7	M	3	0.30	0.04	0.11	0.02	0.47	0.44	0.06	[b]
8	M	2	1.33	0.22	0.63	0.30	2.48	0.21	[b]	[b]
9	F	1	0.74	0.38	0.18	0.10	1.40	0.14	[b]	[b]
10	F	1	5.40	1.54	1.35	1.22	9.51	1.01	[b]	0.11
11	F	1	0.43	0.20	0.40	0.19	1.22	0.08	0.03	[b]
12	F	2	0.11	0.11	[b]	0.11	0.33	0.02	0.02	0.01
13	F	2	0.72	0.09	0.05	0.04	0.90	0.15	0.03	[b]
14	M	1	0.49	0.07	0.12	0.03	0.71	0.16	0.11	[b]
15	F	1	6.91	0.67	0.31	0.18	8.07	2.27	0.20	0.05
16	M	2	1.66	0.20	0.22	0.18	2.26	0.11	[b]	[b]
17	F	2	0.49	[b]	0.23	0.14	0.86	0.22	0.05	[b]
18	F	[c]	0.39	0.10	[b]	[b]	0.49	0.11	[b]	[b]
19	M	3	1.37	0.16	0.31	0.33	2.17	0.16	0.04	0.03
20	F	1	0.32	0.05	[b]	0.03	0.40	0.64	0.03	[b]
21	M	1	0.90	0.48	0.27	0.18	1.83	1.09	0.10	[b]
22	M	[c]	0.92	0.25	0.35	0.10	1.62	0.28	0.04	[b]
23	F	1	0.49	0.23	0.18	0.02	0.92	0.07	[b]	[b]
24	M	1	1.00	0.09	0.31	0.04	1.44	0.13	0.20	0.02
25	F	1	0.29	0.04	0.08	0.03	0.44	0.48	0.03	[b]
26	M	4	0.48	0.18	0.15	0.12	0.93	0.11	0.04	[b]
27	M	[c]	0.19	0.04	[b]	0.04	0.27	0.13	0.03	[b]
28	M	3	0.23	0.04	0.04	0.02	0.33	0.05	0.02	[b]
29	F	2	0.77	0.46	0.19	0.12	1.54	0.35	0.17	[b]
30	F	[c]	1.52	0.74	0.78	0.20	3.24	4.90	[b]	[b]
31	F	1	3.91	0.39	1.44	0.35	6.09	0.56	[b]	0.03
32	F	1	0.42	0.10	0.06	0.09	0.67	0.09	[b]	[b]
33	F	2	0.19	0.13	[b]	[b]	0.32	0.23	[b]	[b]
34	M	2	1.06	0.36	0.17	0.05	1.64	0.12	0.02	[b]
35	F	1	0.64	0.20	0.90	0.08	1.82	0.04	[b]	[b]

[a]Analytical error expressed as the coefficient of variation: p,p'-DDE, 23%; p,p'-DDD, 25%; p,p'-DDT, 17%; o,p'-DDT, 14%; total, 18%; dieldrin, 11%.

[b]Not detected.

[c]Mandibles were not available for aging.

TABLE 2.
Chlorinated hydrocarbon insecticide residues (ppm) in brain and liver tissue from five selected individuals.

Specimen No.	Year Class	DDT and Related Compounds: p,p'-DDE	p,p'-DDD,	p,p'-DDT	o,p'-DDT	Total	Dieldrin	Lindane	β-BHC
Brain									
10	1	0.08	0.05	a	a	0.13	0.02	0.04	0.08
15	1	0.05	0.04	a	a	0.09	0.02	0.01	0.07
4	2	0.02	a	a	a	0.02	0.04	0.02	a
23	1	a	a	a	a	a	a	0.01	0.02
26	4	a	a	a	a	a	a	0.01	0.01
Liver									
10	1	0.11	0.03	0.01	0.01	0.16	0.07	a	a
15	1	0.05	a	0.01	a	0.06	0.01	a	a
4	2	0.03	a	a	a	0.03	0.01	a	a
23	1	0.01	a	a	a	0.01	0.01	a	a
26	4	0.01	a	a	a	0.01	0.01	a	a

[a]Not detected.

TABLE 3.
Mean residues (ppm) of total DDT and related compounds and dieldrin distributed among year classes of mink.

Sex	Year Class	Number of mink	DDT and Related Compounds: p,p'-DDE	p,p'-DDD	p,p'-DDT	o,p'-DDT	Total	Dieldrin
Male	1	6	1.29	0.43	0.50	0.20	2.42	0.31
	2	4	1.13	0.35	0.40	0.25	2.13	0.13
	3	3	0.63	0.08	0.16	0.12	0.99	0.22
	4	1	0.48	0.18	0.15	0.12	0.93	0.11
Female	1	11	1.90	0.39	0.47	0.22	2.98	0.52
	2	5	0.46	0.16	0.09	0.08	0.79	0.19

DISCUSSION

The chlorinated hydrocarbon insecticide residues found in adipose, liver, and brain tissues were well below lethal levels suggested for ranch mink (AULERICH et al., 1969; AULERICH and RINGER, 1970). These authors fed ranch mink a commercial ration containing one of three insecticide treatments; 100 ppm DDD, 5 ppm dieldrin, or 100 ppm DDT plus 50 ppm DDD. Maximum total accumulations of p,p'-DDT, o,p'-DDT, p,p'-DDD and p,p'-DDE ranged from 771 ppm in fat to 6.10 ppm in brain. The 5-ppm dieldrin ration reached a toxic level during stress, but DDD and DDT plus DDD were tolerated during the entire life cycle of the mink, although growth was slower than in controls. Kits from females on all rations containing insecticides exhibited an unusually high mortality up to 4 weeks after birth, but there seemed to be no detrimental effects on the number of kits whelped, the number of stillborn kits, or the birth weights of kits.

According to the preceding data, the residues of individual insecticides found in our study would probably have little effect on reproductive performance. However, the effect of all chlorinated hydrocarbon insecticides considered together is unknown because little study has been done relating synergistic effects to effects caused by individual insecticides.

The relative ratios of mean residues of DDT and related compounds were rather constant between age classes (Table 3). This indicates that these compounds may reach their particular relative proportions in a short time and that each age class metabolizes them in a manner that keeps the ratios relatively constant. As a result, no differential buildup or reduction of any single compound would occur, even though the total residue level may decline with increasing age. AULERICH et al. (1971) studied the relative proportions of DDT and related compounds in fat up to 12 weeks after feeding a ration contaminated with 100 ppm technical grade DDT and 50 ppm DDE. They found that shifts in percentages of compounds did occur, but it may be that, after a matter of months, a steady state is reached.

Several questions are raised by this study. (1) What is the synergistic effect on mink reproduction of p,p'-DDE, p,p'-DDD, p,p'-DDT, o,p'-DDT, dieldrin, and heptachlor epoxide at the levels that occur in the natural environment? (2) What are the residue levels in the natural diet that produce the given residues in mink tissues? (3) What is the relationship between insecticide residue levels and age? Future study in these areas is necessary to understand the full impact of insecticide residues on wild mink.

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