Organochlorine Insecticide Interactions Affecting Residue Storage in Rainbow Trout

by F. L. MAYER, JR., J. C. STREET and J. M. NEUHOLD
Animal Science Department and Ecology Center
Utah State University
Logan, Utah

The biological significance of various levels and combinations of organochlorine insecticides has been investigated only recently. Relatively few studies have been conducted in the area of insecticide interactions, although most organisms are probably exposed to more than one insecticide in their environments. Many types of animals have been found to contain residues of more than one insecticide (1,2,3,4,5,6,7). Varying combinations of insecticides have also been found in single water samples of many U.S. rivers (8).

Evidence that interactions do occur upon combined administration of some of the pesticides is apparent in the literature. Street (9) and Street and Blau (10) found dieldrin storage in rats to be markedly reduced in the presence of DDT. Street and Blau also reported that DDT caused a rapid depletion in preexisting dieldrin residues in the rat and also a reduced storage of dieldrin administered after DDT administration. Further work resulted in finding that excretion of polar metabolites of dieldrin by DDT-treated rats greatly exceeded that by rats given only dieldrin (11). This increase was observed in both feces and urine, with the greatest increase being in urinary products. Street and Blau (10) have postulated that the effect of DDT on cyclodiene storage results from an enhanced activity of metabolizing enzymes in liver.

Whether DDT might induce a reduction of dieldrin storage in fishes was not known since little work has been done with fish in metabolism of xenobiotic compounds. The purpose of this study was to determine insecticide interaction effects in rainbow trout simultaneously treated with up to three organochlorine insecticides. Emphasis was placed on residual insecticide accumulation in the visceral fat body.

---

[1] Present Address: Fish-Pesticide Research Laboratory, U.S.D.I. Columbia, Missouri, 65201

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 4, 1970, published by Springer-Verlag New York, Inc.

Materials and Methods

Three chlorinated hydrocarbon insecticides were used in this study. These were DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane), methoxychlor (2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane), and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,-5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethano-naphthalene). The technical grade chemicals (methoxychlor and dieldrin) were repeatedly recrystallized before use. DDT was purchased as p,p'-DDT and recrystallized. Three levels each of dieldrin (0, 0.04, 0.20 mg), DDT (0, 0.2, 1.0 mg), and methoxychlor (0, 0.6, 3.0 mg) were used in a 3^3 completely randomized factorial design. There were six fish in each of the 27 treatments, with each fish considered as a replicate.

Rainbow trout (Salmo gairdneri) of the Hull-Erickson strain were weighed, marked with single barb plastic dart tags, and placed in sectioned hatchery raceways. The fish weighed 122 g ± 10%. Fish were individually given doses of insecticides per os in corn oil enclosed in gelatin capsules. Doses were given to fish on alternate days, seven doses in all. On the fifteenth day the fish were killed and the visceral fat body analyzed for lipid content and residual insecticides.

Five-tenths g of each fat body was ground together with anhydrous sodium sulfate in a mortar, transferred to 100 ml centrifuge bottles and extracted five times with n-hexane. The five extracts were combined and concentrated to a 50 ml volume. A one percent portion was removed for the estimation of lipid by a dichromate oxidation procedure (12). The remaining extract was chromatographed on deactivated Florisil to separate the insecticides from interfering lipids. The insecticide-containing fraction was analyzed by gas chromatography with electron capture detection. The residual insecticide values are expressed as ppm in extractable lipids of the fat body.

Results

No significant change in weight occurred during the study. All of the fish survived the experimental period, but some were beginning to lose their normal vigor towards the end of the study in the higher dose combinations.

The results of the analyses of fat bodies for residual insecticides are presented in Tables 1, 2, 3, and 4. Each table is arranged to show the effects on the tissue storage of one insecticide caused by either or both of the other two insecticides. An analysis of variance was computed on the residual insecticide values resulting from each dose level of each insecticide. Summaries of those analyses are listed below

TABLE 1

Residual DDT in trout adipose tissue*

Dieldrin (mg)	Methoxychlor (mg)	DDT (mg) 0.0	0.2	1.0
		Tissue DDT (ppm)		
0.00	0.0	3.17	65.7	173
	0.6	2.20	64.3	147
	3.0	2.00	70.7	287
0.04	0.0	4.12	63.2	334
	0.6	2.70	79.7	529
	3.0	1.63	79.3	361
0.20	0.0	1.95	84.7	444
	0.6	1.30	46.3	318
	3.0	0.82	41.5	255

ANOV

Source	d.f.	M.S.	M.S.	M.S.
Dieldrin	2	10.425[1]	1240.81[3]	197257[1]
Methoxychlor	2	11.714[1]	340.69	4144
Dieldrin x Methoxychlor	4	0.936	1808.05[2]	75766[1]
Error	45	1.926	637.33	16997
Total	53	2.541	737.26	27749

* The fish were given the indicated doses of insecticides per os in corn oil on alternate days, seven doses in all. On the 15th day, they were killed and the fat body analyzed for insecticide residues. Each value is expressed as the mean concentration of DDT in extractable lipids of the fat body from six fish.

1 $P<0.01$

2 $P<0.05$

3 $P<0.25$

TABLE 2

Residual DDE in trout adipose tissue*

Dieldrin (mg)	Methoxychlor (mg)	DDE (mg) 0.0	0.2	1.0
		Tissue DDE (ppm)		
0.00	0.0	0.99	5.47	6.40
	0.6	0.67	3.52	7.78
	3.0	0.51	3.00	12.44
0.04	0.0	0.91	4.35	13.25
	0.6	0.83	4.17	21.00
	3.0	0.80	3.88	18.50
0.20	0.0	1.08	4.20	27.40
	0.6	0.84	2.84	13.77
	3.0	0.49	1.66	15.92

ANOV

Source	d.f.	M.S.	M.S.	M.S.
Dieldrin	2	0.0996	8.196	542.84[2]
Methoxychlor	2	0.7402[1]	15.361[3]	12.96
Dieldrin x Methoxychlor	4	0.0957	2.396	231.75[3]
Error	45	0.1292	9.071	202.08
Total	53	0.1487	8.772	210.04

* The fish were given the indicated doses of insecticides per os in corn oil on alternate days, seven doses in all. On the 15th day, they were killed and the fat body analyzed for insecticide residues. Each value is expressed as the mean concentration of DDE in extractable lipids of the fat body from six fish.

1 $P<0.01$

2 $P<0.10$

3 $P<0.25$

TABLE 3

Residual dieldrin in trout adipose tissue*

DDT (mg)	Methoxychlor (mg)	Dieldrin (mg) 0.00	0.04	0.20
		Tissue Dieldrin (ppm)		
0.0	0.0	0.75	21.8	98.3
	0.6	0.80	8.4	61.0
	3.0	0.48	22.2	89.8
0.2	0.0	0.93	14.0	86.8
	0.6	0.96	19.8	64.7
	3.0	1.05	17.8	60.2
1.0	0.0	0.81	14.5	92.3
	0.6	1.28	14.6	68.8
	3.0	0.86	12.7	73.5

ANOV

Source	d.f.	M.S.	M.S.	M.S.
DDT	2	0.279	70.28[1]	715.18[2]
Methoxychlor	2	0.012	53.18[1]	3548.69[1]
DDT x Methoxychlor	4	1.605[1]	189.27[1]	450.06
Error	45	0.277	9.11	464.91
Total	53	0.367	26.68	589.60

* The fish were given the indicated doses of insecticides per os in corn oil on alternate days, seven doses in all. On the 15th day, they were killed and the fat body analyzed for insecticide residues. Each value is expressed as the mean concentration of dieldrin in extractable lipids of the fat body from six fish.

1 $P<0.01$

2 $P<0.25$

TABLE 4

Residual methoxychlor in trout adipose tissue*

Dieldrin (mg)	DDT (mg)	Methoxychlor (mg) 0.0	0.6	3.0
		Tissue Methoxychlor (ppm)		
0.00	0.0	0.0	100	774
	0.2	0.0	110	591
	1.0	0.0	71	759
0.04	0.0	0.0	69	1154
	0.2	0.0	129	916
	1.0	0.0	162	890
0.20	0.0	0.0	86	996
	0.2	0.0	83	618
	1.0	0.0	156	672

ANOV

Source	d.f.	M.S.	M.S.	M.S.
Dieldrin	2	0.0	3063.00	392592[1]
DDT	2	0.0	9067.50[2]	347206[1]
Dieldrin x DDT	4	0.0	8545.25[2]	46330
Error	45	0.0	2348.00	49581
Total	53	0.0	3096.26	73511

* The fish were given the indicated doses of insecticides per os in corn oil on alternate days, seven doses in all. On the 15th day, they were killed and the fat body analyzed for insecticide residues. Each value is expressed as the mean concentration of methoxychlor in extractable lipids of the fat body from six fish.

1 $P<0.01$

2 $P<0.05$

each data column in the tables. That form of statistical analysis allowed testing only the two-way interactions. Significant interactions were also found for three-way interactions, but the data are presented in two-way form for convenient visualization of dose effects.

When DDT and dieldrin were fed in combinations to fish, an accompanying increase in tissue storage of both DDT and DDE occurred (Tables 1 and 2). These increases were most apparent with the higher DDT dose. The reality of the interaction was more apparent in DDT storage than in DDE storage. The effect of methoxychlor was a reduction in DDT storage. This was highly significant when fish were dosed with methoxychlor alone, but only in the case of the background levels of tissue DDT. However, feeding of methoxychlor resulted in a reduction of DDE storage from the 0.2 mg DDT treatment, but was of low significance. A dieldrin and methoxychlor interaction occurred in storage of both DDT and DDE. The results were of varying degrees of significance, but the overall trend was a reduction in both DDT and DDE in the fat body.

Dieldrin storage was reduced by the presence of either DDT or methoxychlor (Table 3). The alteration of tissue dieldrin was more apparent when the fish were fed the low dose of DDT rather than the high dose. The effect of methoxychlor on reduction of dieldrin storage was more pronounced than that of DDT. A DDT and methoxychlor interaction effect occurred on dieldrin storage, but the trend was not definable since dieldrin storage fluctuated.

The influence of dieldrin on increasing methoxychlor storage was highly significant when fish were fed the high dose of methoxychlor (Table 4). Conversely, the presence of DDT resulted in a decrease in methoxychlor storage. The dieldrin and DDT interaction resulted in a decreased storage of residual methoxychlor and was significant only in the presence of the low dose of methoxychlor.

Discussion

DDT, and possibly methoxychlor, could bring about a selective induction of drug metabolizing enzymes in the liver of trout. This hypothesis has merit since Creaven, Davies, and Williams (13) and Buhler (14), demonstrated drug metabolizing enzyme activity in trout liver. The data in this study reinforced this hypothesis; feeding of DDT or methoxychlor with concomitant dieldrin administration resulted in decreased dieldrin storage. That such interaction would be less in fish than in higher vertebrates could also be hypothesized, as inferred from studies by Creaven *et al.* (13).

A comparison of the results of the present study and those of Street and Blau (10) gives additional reinforcement to these hypotheses. A 6 to 36 percent reduction in residual dieldrin in the trout fat body resulted from DDT administration in this study. The results of Street and Blau with rats indicated a 25 to 93 percent reduction of residual dieldrin in tissue. A dieldrin storage reduction in trout of 49 percent was achieved in a later study, but the dose of DDT was so great that 78 percent of the fish died before termination of the study. In that study, trout were treated with 0.2 mg dieldrin or 5 mg DDT and 0.2 mg dieldrin every other day for seven days, four doses in all. The respective dieldrin storage levels were 45.5 ppm and 23.2 ppm. Methoxychlor appeared to have a greater effect on dieldrin reduction in trout as compared to rats.

Dieldrin treatment resulted in an increase in DDT and DDE in both trout and rats. The largest increase of DDT and DDE in rats was 45 and 54 percent, respectively (10) while administration of dieldrin resulted in a maximum of 157 percent increase in DDT storage and 328 percent increase in DDE storage in trout.

Street and Blau (10) found no significant effects of dieldrin or DDT on methoxychlor storage in rats. Feeding of dieldrin with methoxychlor to trout resulted in an increase of residual methoxychlor, much the same as in the effect of dieldrin on DDT and DDE storage. This is not surprising since DDT and methoxychlor are similar in structure and might possibly possess similar storage and enzyme inducement properties in a species having a very low capacity to metabolize either compound.

Since the rat and trout studies were not identical in design, one might infer that many of the interaction differences between these two organisms were due to duration of dose and dose level. Recently Macek (15) reported that DDT caused a reduction of dieldrin storage in pyloric caeca of rainbow trout. That experiment was 140 days in duration and dieldrin storage reduction was still much less than that found in rats.

Adamson, Dixon, and Francis (16) found fish and amphibians lacked oxidative microsomal drug enzymes. La Du *et al.* (17), in *in vivo* studies, concluded that aminopyrine, methylaniline, and hexobarbital were not affected by fish liver microsomes. They also reported that these compounds were excreted unchanged by fish. Brodie and Maickel (18) have reported that liver microsomal drug metabolizing enzymes were not found in fishes. They concluded that fish probably dispose of foreign lipid-soluble compounds by diffusion through the gills and skin into the surrounding water rather than by metabolism and hepatorenal excretion.

Other workers have reported that drug metabolizing enzymes do occur in fishes. Creaven, Parke, and Williams (19) have shown that trout liver preparations can hydroxylate biphenyl. Adamson et al. (16) have reported the presence of azo- and nitro-reductase activity in certain fishes. Hepatic hydroxylation of aniline, reduction of nitrobenzoic acid, and N-dealkylation of aminopyrine have been found in several fishes (14). Buhler also reported that pretreatment of rainbow trout with DDT or phenylbutazone brought about a selective induction of drug metabolizing enzymes. Increased enzyme activity, stimulated by the presence of heptachlor, heptachlor epoxide, gamma chlordane, and nanochlor, has been considered as a factor in declining residue levels in bluegill sunfish (20).

However, in vitro studies of rainbow trout liver microsomes led to the conclusion that the low epoxidative activity was the result of a deficiency in the epoxidative enzymes (Chan, et al., 21). This observation is also reflected in the data of Boyle, Burttschell, and Rosen (22). They found large quantities of dieldrin in both viscera and muscle tissues of fish exposed to aldrin for 22 days. However, little dieldrin was found in fish that had died after five days of a massive dose of aldrin.

Discrepancies in detecting drug metabolizing enzyme activity in fish are probably due to the techniques used. Creaven et al. (13) and Adamson et al. (16) have noted that enzymatic activities of fishes were greater at temperatures lower than 37 C. The optimum temperature for enzymatic activity was from 20 to 26 C. However, these activities still occurred at a slower rate than in mammals. Creaven et al. (13) hypothesized that drug metabolizing enzymes occur in fish but at lower activity than in mammals.

This study presents evidence that insecticide interactions do in fact occur involving their residual storage in enzyme induction in trout. It is also evident that a large difference in capacity for organochlorine pesticide metabolism exists between trout and rats. However, ultimate effects of these interactions are yet to be recognized in the natural environment.

Summary

Three dose levels each of dieldrin (0 mg, 0.04 mg, 0.2 mg), DDT (0 mg, 0.2 mg, 1.0 mg), and methoxychlor (0 mg, 0.6 mg, 3.0 mg) were used in a 3^3 completely randomized factorial design. All 27 possible compound and dose combinations were used in the study. Six fish were used per treatment. The fish were individually given doses of insecticides per os in corn oil enclosed in gelatin capsules. The fish

were dosed on alternate days, seven doses in all. On the fifteenth day, the fish were killed and the fat body analyzed for lipid content and residual insecticides.

Tissue storage of DDT and DDE increased when DDT and dieldrin were fed in combination. The same response had been noted in rats but was of much greater magnitude in fish. Dieldrin storage was reduced by the presence of either DDT or methoxychlor. The effect of methoxychlor was more pronounced than that of DDT. The effect of DDT on tissue dieldrin reduction is much less in fish than in rats. Feeding of DDT with methoxychlor resulted in decreased methoxychlor storage. The influence of dieldrin on increasing tissue methoxychlor was highly significant ($P < .01$) when fish were given doses of 3.0 mg methoxychlor. Insecticide interactions occur in fish, but large differences in these responses exist between fish and rats.

Acknowledgements

This research was sponsored by the United States Public Health Service, National Institutes of Health Toxicology Training Grant (GM-1179), Environmental Health Sciences Research Grant (ES-00255), and the United States Department of the Interior, Federal Water Pollution Control Administration Fellowship (F1-WP-31).

References

1. R. J. Greenwood, Y. A. Greichus, and E. J. Hugghins, J. Wildl. Mgmt. 31, 288 (1967).

2. G. L. Moore, Y. A. Greichus, and E. J. Hugghins, Bull. Environ. Contam. and Toxicol. 3, 269 (1968)

3. W. L. Reichel and C. E. Addy, Bull. Environ, Contam. and Toxicol. 3, 174 (1968)

4. M. K. Causey, F. L. Bonner, and J. B. Graves, Bull. Environ. Contam. and Toxicol. 3, 274 (1968).

5. E. A. Epps, F. L. Bonner, L. D. Newsom, R. Carlton, and R. O. Smitherman, Bull. Environ. Contam. and Toxicol. 2, 333 (1967).

6. H. Cole, D. Barry, and D. E. H. Frear, Bull. Environ. Contam. and Toxicol. 2, 127 (1967).

7. L. C. Dugal, J. Fish. Res. Bd. Can. 25, 169 (1968).

8. L. Weaver, C. G. Gunnerson, A. W. Breidenbach, and J. J. Lichtenberg, Public Health Rept. 80, 481 (1965).

9. J. C. Street, Science, 146, 1580 (1964).

10. J. C. Street, and A. D. Blau, Toxicol. Appl. Pharmacol. 8, 497 (1966).

11. J. H. Bragdon, J. Biol. Chem. 190, 513 (1951).

12. J. C. Street and R. W. Chadwick, Toxicol. Appl. Pharmacol. 11, 68 (1967).

13. P. J. Creaven, W. H. Davies, and R. T. Williams, Life Sci. 6, 105 (1967).

14. D. R. Buhler, Federation Proc. 25, 343 (1966).

15. K. J. Macek, Proc. Symp. on Biol. Impact of Pesticides in the Envir., Oregon State Univ., Corvallis (1970).

16. R. H. Adamson, R. L. Dixon, and F. L. Francis, Proc. Nat. Acad. Sci. 54, 1386 (1965).

17. B. N. LaDu, L. Gaudette, N. Trousof, and B. B. Brodie, J. Biol. Chem. 214, 741 (1955).

18. B. B. Brodie and R. P. Maickel, Proc. 1st Internat. Pharmacol. Meeting 6, 299 (1962).

19. P. J. Creaven, D. V. Parke, and R. T. Williams, Biochem. J. 96, 879 (1965).

20. A. K. Andrews, C. C. VanValin, and B. E. Stebbings, Trans. Amer. Fish. Soc. 95, 297 (1966).

21. T. M. Chan, J. W. Gillett, and L. C. Terriere, Comp. Biochem. Physiol. 20, 731 (1967).

22. H. W. Boyle, R. H. Burttschell, and A. A. Rosen, Advances in Chem. 60, 207 (1966).