DDT Residue Persistence in Red-Backed Salamanders in a Natural Environment

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Portions of northern Maine provide an excellent existing field design for studying persistence of DDT in forest ecosystems. DDT was applied from aircraft at the rate of one pound per acre for control of the spruce budworm, Choristoneura fumiferana, in 1967, 64, 63, 61, 60 and 1958. Areas covered in the different programs varied from about 100,000 to 500,000 acres. While there was some overlap in treatments, sizable blocks remain which were treated only once in the years indicated above. Additional areas exist that have been treated two or three times, and there are also surrounding, ecologically similar areas that have no recorded spray history. Within this design, one can obtain within a single season or two samples of organisms or soils that provide data on DDT persistence up to nine years after a single treatment and accumulations of DDT residues following two or three successive treatments.

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Bulletin of Environmental Contamination & Toxicology,
Vol. 3, No. 4, 1968, published by Springer-Verlag New York Inc.

Several species of common animals, occupying a variety of ecological niches, are being studied within the field design described. This report concerns the pattern of DDT persistence in the red-backed salamander, Plethodon cinereus, a common resident of the moist forests of northern Maine.

Methods

Salamanders were collected in 1966 and 1967 by searching among rotten logs, stumps, and in humus. Samples were placed in airtight plastic bags and frozen within six hours of collection, while the salamanders were still alive in all but a few cases. Samples were stored at -18 C until analysis for DDT residues in December, 1967. Each sample represents the total number of salamanders collected in about one man-hour of searching over an area of one to two acres. In most cases, several separate areas were sampled in each type of treatment.

Samples were extracted following the method of Mills, Onley, and Gaither (1). After chromatography on Florisil columns using the procedure of Moats (2), solvent was evaporated and the residues taken up in 5 ml of hexane and 5 microl injected into the gas chromatograph. The gas chromatograph used was an F & M 810 equipped with a Ni 63 electron capture detector and a 4 ft x 1/4 in glass column packed with 6% DC 200 on 50/60 mesh DMCS treated Chromosorb W/AW. The column oven was operated at 200 C and the detector at 240 C, with an argon-methane (95-5%) pressure of 50 psi and flow of 60 ml per minute. The presence and quantities of

DDT isomers and metabolites in samples were determined by comparison of retention times and peak areas with standard samples of these compounds. Identity of the residues was confirmed by thin-layer chromatography. All solvents used for extraction and chromatography were pesticide grade or were redistilled in glass.

Recovery of added, known quantities of DDT has averaged close to 85 percent for several vertebrate and invertebrate species analyzed with the techniques described above. Checks of laboratory contamination have been negative.

Results

The total residues of DDT and metabolites in the salamanders are presented in Table 1. Results of the analyses of 1966 and 1967 collections of salamanders are listed separately since, while they indicate a similar trend, the general residue levels are different in the two years. The suspected reasons for this discrepancy are discussed later.

Among the 1966 collections, those from areas most recently sprayed (1964) are two years removed from treatment. Residue levels are between 0.1 and 0.3 ppm, and similar concentrations apparently persist through six years after treatment (collections from 1961 and 1960-treated areas). Sometime between 6 and 8 years from treatment (1958-treated area), the residue levels drop below 0.1 ppm, and they approach the levels in untreated samples, which presumably also represent pretreatment levels.

TABLE 1

Total residue of DDT and metabolites in red-backed salamanders collected in areas of varying history of DDT-treatment. Number of salamanders in each sample-pool is indicated in parentheses

Year(s) of treatment	1966 samples Residue in ppm	1967 samples Residue in ppm
1967	--	.528 (22)
		.648 (17)
		.168 (6)
		.448 (7)
		2.057 (3)
1964	.237 (7)	
	.104 (2)	
1963		.101 (19)
1961	.128 (6)	.027 (22)
	.110 (7)	.034 (6)
		.058 (8)
		.138 (3)
		.041 (27)
1960	.246 (4)	.055 (25)
	.190 (7)	.036 (29)
		.037 (22)
1958	.044 (16)	.024 (30)
	.079 (5)	.011 (12)
63-60-58	.157 (6)	.074 (7)
64-63-60	.242 (23)	.186 (33)
		.240 (12)
Unsprayed	.042 (6)	.009 (33)
	.035 (7)	.017 (18)
	.011 (8)	.017 (17)
	.064 (7)	.022 (2)

Some 1967 collections provide data for salamander samples taken in a year of treatment. These average nearly 0.8 ppm of DDT and metabolites but with variation from 2.0 to 0.16 ppm among samples. The remaining 1967 collections show the same trend as the 1966 samples, but at lower residue levels throughout. Residues have dropped to a lower level (1963-treated sample) which persists with little significant change through seven years from spraying (1960-treated samples) but by nine years from spraying (1958-treated samples) residue levels are similar to those in areas never treated. Combining the trends seen in both series of collections, one sees a relatively high level of DDT and metabolites in salamanders in the season of spraying; a lower level of residue has been reached by two years after spraying, however, this level apparently persists with little change through six or seven years from treatment. Residues approaching pretreatment levels are apparently reached after eight or nine years.

Residues presented in Table 1 are totals of DDT and metabolites. DDE comprised 40 to 60 percent of the total residue in nearly all samples with no changes in populations more remote in time from spraying. Only DDE was detected in four samples. These were two untreated samples and one each from 1958 and 1960 treated plots, all with low residue levels. DDD comprised 5 to 10 percent of total residue in seven samples, those with the highest total residues. It may have been present in similar proportions in the remaining samples but below the level of detectability.

Single salamanders were too small (0.5-1.0 gms) for individual analysis, making it difficult to assess within-sample variance and predict minimum adequate sample size. Among larger animals of other species collected and analyzed from the same research area, variability among individuals is such that 4 to 8 crayfish, for example, will estimate residues with a 95 percent probability of being within 10 percent of the mean where residue levels are 0.2 and 0.1 ppm respectively (3). Among young-of-the-year robins, the same accuracy is attained with 3 to 4 birds at residue levels around 0.5 ppm (4). With no reason to suspect greater variability among salamanders, it appears that size of many of the samples listed in Table 1 is adequate, particularly where there are a dozen or more individuals. Using the same method of calculation (5) of variance between sample-pools and using variance computed from the five 1963-treated areas sampled in 1967, it is found that eight area-samples will give an estimate within 10 percent of the mean (95 percent probability) and four samples for accuracy within 20 percent of the mean. While most of the sample groups for different treatments achieved at best the criterion for the lower accuracy level, the agreement in trend between the 1966 and 1967 collections, and the agreement between, for example, the 1960 and 61-treated samples among the 1967-collected samples lend strength to the data.

Discussion

One of the reasons for selecting red-backed salamanders for study, in addition to ease of collection, is their close association with forest litter. Several studies (4, 6) have indicated prolonged persistence of DDT in forest soils, and it seemed reasonable to expect chronic persistence in soil- or litter-associated organisms. Woodwell and Martin (6) have shown, that forest soil residues of DDT persist above pretreatment levels beyond ten years. This has been confirmed (4) in the areas sampled for salamanders in this study. Belyea (4) suggested, in addition, that the DDT is passed from soil to earthworms to robins throughout the period of its persistence, whereas the present data indicate that the contamination reaching salamanders is reduced to pretreatment levels after 8 or 9 years. The difference between the salamanders and the robins in duration after treatment of contact with persistent soil residues may stem from differing food habits. Robins depend heavily on earthworms, which penetrate deep into the soil, whereas salamanders, while taking worms when available, exploit as food chiefly arthropods that are associated with the upper forest litter (7). Belyea (4) showed that, while DDT is persistent in forest soils, there is a tendency with time for a downward movement of the residue as contaminated litter below decomposes and is replaced by relatively uncontaminated material on the surface. Thus, with passage of time there would be more of a tendency for less contact between the surface-feeding salamanders and the persistent soil residues than in the case of robins.

Table 1 showed that while 1966 and 1967-collected samples followed similar trends in DDT persistence, the 1966 samples contained residue levels several times higher than samples collected in 1967, in both untreated and treated samples. This difference is probably related to the fact that 1966 samples were collected in June whereas collections were made in late July and August in 1967. A seasonal variation in residue level is suggested with higher levels in the spring. Possible explanations for such a variation might include differing age structures in the population, the fact that females have deposited eggs by late summer, perhaps eliminating considerable DDT, or seasonal differences in food habits. There is presently no evidence to suggest the correct answer.

While the highly persistent residue levels in salamanders are low and perhaps present no direct hazard to these forms themselves, they cannot be considered harmless because of the potential for food chain magnification threatening populations of higher carnivores. The fact that residues persist for many years following a single, minimal DDT treatment suggests that additional searching for ecological damage is justified.

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