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Uptake of Methoxychlor from Food and Water by the American Toad (*Bufo americanus*)

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Various studies (MULLA et al. 1963; FERGUSON and GILBERT 1967; COOKE 1973) have examined the effects of pesticides on anuran amphibians, but the routes of contaminant uptake by transformed amphibians have not been evaluated. The need of frogs and toads to imbibe water from natural surface water would seem to make them vulnerable to uptake from this source. Fish are known to accumulate pesticides from both food and water (JARVINEN et al. 1977). Toads are more terrestrial and tend to lose water less readily than most other amphibians (THORSON and SVIHLA 1943); hence they should be a conservative estimator of the potential for uptake of pesticides from water.

Methoxychlor is an analogue of DDT and has been widely used to replace it. It is a good model of many pesticides in current use in its low persistence in animal tissues (GARDNER and BAILEY 1975), and its long half life in water (WOLFE et al. 1977). It is also suitable for study because of its relatively low toxicity to amphibians (KAPLAN and OVERPECK 1964) and its easily detected residues. In the present study we measured the accumulation of methoxychlor from food and water in a preliminary evaluation of the two routes of exposure.

MATERIALS AND METHODS

Toads (Bufo americanus) were captured at the Patuxent Wildlife Research Center and were maintained on a diet of mealworm larvae. Levels and durations of exposure were meant to resemble field conditions. In one experiment two tanks of toads grouped as four adult males per tank were fed mealworms containing 0.024 ppm methoxychlor for 1 and 6 days. A group of five subadult toads was fed mealworms with higher methoxychlor residues (0.32 ppm) for one day. The lower dosage mealworms had been fed 1 ppm (A.I., technical methoxychlor, Dupont Co.) pesticide and the the higher dosage group had been fed 10 ppm in their bran diets. In a second experiment, 3 groups of 4 male toads each were exposed to 0.069 ± 0.035 ppm methoxychlor in water for periods of 1, 6, and 36 days. Pesticide was added to unchlorinated well water in an acetone carrier to produce a nominal concentration of 0.1 ppm in a flow through apparatus that delivered about 40 l/day to each tank. Tanks were tilted so that toads could enter or leave the water at will. Groups of controls were maintained for each treatment group. Animals were fed throughout the experiments.

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Animals were killed by freezing immediately after cessation of dosage and skins and gastrointestinal tracts were removed. Methoxychlor levels in water and tissues were measured by electron-capture gas chromatography. Water samples were extracted with hexane: methylene chloride 85: 15 and dried with anhydrous sodium sulfate, extracted for 7 hours with hexane on a Soxhlet apparatus and cleaned by passage through a Florisil column (PROUTY et al. 1977). The gas chromatograph column was packed with 1.5% SP-2250/ 1.95% SP-2401 on 100/120 mesh Supelcoport. The lower limit of detection was 0.01 ppm. Average recovery was 94 percent for water samples and 79 percent for tissue samples. Tissue levels are expressed on a whole body wet weight basis.

RESULTS AND DISCUSSION

Results of residue analyses are summarized in Table 1. Toads fed 0.024 ppm methoxychlor in the diet accumulated residues averaging 0.010 ppm. Animals on the dietary dosage for one and six days showed no significant differences in accumulation. All toads fed 0.32 ppm prey had detectable residues; the mean was 0.033 ppm. Tissue levels averaged about 0.4 times the dietary level in the lower dosage groups and 0.11 times dietary concentrations in the higher dosage group. No residues were detected in the controls. Toads exposed to methoxychlor in water had mean residue levels of 0.192 ppm or approximately 2.8 times the concentration measured in the water. Residue levels are not correlated with duration of exposure.

No gross signs of pathology were seen during autopsies, nor were there changes in organ weights, feeding, behavior, or survival.

Indications that increased time of exposure do not increase the residue levels suggest a rapid attainment of equilibrium between uptake and elimination of methoxychlor. The high partition coefficient of the pesticide (GARDNER and BAILEY 1975) would favor uptake from water, and only rapid elimination would explain the low residues found. Toads resemble other animals in this ability to eliminate methoxychlor (GARDNER and BAILEY 1975).

Of the possible modes of exposure, the aquatic one is clearly more significant. Neither mealworms nor toads showed much tendency to store the pesticide taken up with food. Mealworms accumulated residues to levels only 2 percent of those in the diet and the levels accumulated by toads were only about 30 percent of their dietary intake. Uptake from water is proportionately several times greater, although less than the reports of concentration of more persistent pesticides by fish.

Most pesticides in current use resemble methoxychlor in its rapid elimination from animals and its relatively great persistence in the abiotic environment, but some of them may be much more toxic to amphibians. Amphibians which inhabit sprayed forest land or the vicinity of cultivated fields may be significantly exposed to pesticides in small or temporary pools of water even when precautions are taken to protect more permanent aquatic habitats.

TABLE 1
Mean Residues of Methoxychlor Accumulated From Food and Water

Dosage Group	Days of Exposure	N	Residues of Methoxychlor (ppm Wet Weight) Geometric Means and 95% Confidence Limits
0.024 ppm in food	1	4	0.013 (0.003-0.043)
	6	4	0.008 (0.002-0.024)
0.325 ppm in food	1	5	0.033 (0.012-0.088)
0.069 ± 0.036 ppm in water	1	4	0.145 (0.065-0.323)
	6	4	0.244 (0.124-0.482)
	36	4	0.124 (0.048-0.327)
controls	-	12	not detected

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