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Residues of 2,4,5-T in the American Coot (*Fulcia americana*)

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Since 1970 there has been a renewed interest in research dealing with (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), following reports indicating that 2,4,5-T was teratogenic to mice (COURTNEY et al. 1970, WILSON 1972). Fears of environmental contamination and the potential hazards that these residues might present to humans arose as a result of a report by MACLEAD (1973). His report indicated that the incidence of birth defects in the Vietnamese population followed massive use of agent orange[1] as a defoliant by the military forces. COURTNEY and MOORE (1971) confirmed that the teratogenic effects shown by mice used in laboratory experiments was largely due to TCDD which occurs as a by-product in the manufacture of 2,4,5-T. SHAPLEY (1973) reported TCDD in Vietnamese fish and 3 reports (YOUNG 1974, BARTLESON et al. 1974; YOUNG et al. 1976) showed residues of TCDD at parts per trillion (ppt) levels in rodents, birds, fish and insects collected on military test sites treated with thousands of kg of agent orange over a period of 8 years.

Although there is a great amount of published data on 2,4,5-T and TCDD there is relatively little information on levels of these residues in wild animals. This kind of information is of interest because 2,4,5-T is used extensively in the United States on forests and rangelands to control undesirable vegetation. Rates of application seldom exceed 2.24 kg/ha.

This paper reports the levels of 2,4,5-T and the absence of TCDD in 6 tissue types from the American Coot (*Fulcia americana*) collected in a reservoir that received runoff from a watershed on which 1093 ha were treated with 2,4,5-T in June 1976 to control honey mesquite (*Prosopis glandulosa*). The rate of application was 0.56 kg a.e. 2,4,5-T/ha. However, a number of sites in the watershed have been treated during past years including some areas immediately adjacent to the lake.

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[1] A combination of the n-butyl esters of 2,4-D and 2,4,5-T in approximately equal amounts.

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Because TCDD may persist for 12 years or more (YOUNG et al. 1976) and 2,4,5-T for periods greater than a year (PLUMB et al. 1977) it is possible that these residues may enter aquatic systems in runoff and be accumulated in aquatic organisms and biomagnified through successive levels of food chains. This study is one part of an investigation conducted to test this hypothesis.

MATERIALS AND METHODS

Twenty nine coots were shot at White River Lake, Texas, August 1976 through mid-December 1976. Birds were wrapped in aluminum foil, placed in plastic bags and returned to the laboratory and kept frozen until analysis. Sample extracts were prepared in duplicate where possible from individual birds using 10 g of tissue (fresh weight) of each tissue type. When individual tissue weights were insufficient, the tissues of several birds were pooled. Tissues included: breast muscle, fat, gizzard, liver, brain and heart tissue. Pooled brain tissues yielded 5 preparations and pooled hearts yielded 10.

Procedures were adapted from WOOLSON and ENSOR (1973). Final extracts were reduced to 1.0 mL; and 1.0 to 5.0 µL volumes were subjected to analysis for 2,4,5-T and TCDD using a Perkin Elmer 3920B gas chromatograph equipped with a Ni^{63} electron capture detector. Glass columns were 1.8 M by 2.0 mm i.d., packed with 5% OV-17 on chromosorb G-HP, 80-100 mesh. Operating temperatures were: column and injector ports 190 C, interface 240 C, and detector 275 C. Prepurified nitrogen was used as the carrier at a flow rate of 60 mL/min. Standing current was at 0.5 µV. Relative retention time $\pm$ 3% was 403 sec. for 2,4,5-T and 743 sec. for TCDD.

Residue concentrations were electronically determined by a Perkin Elmer model 1 programable integrating calculator calibrated and programed for the external standards method 3B (SPECTRA PHYSICS INC. 1974, PERKIN ELMER CORP. 1975). One and 2 µL volumes of a mixture of 2,4,5-T and TCDD containing 0.05 ng/µL were used in calibration.

Recovery of 2,4,5-T and TCDD from samples fortified with levels of 0.02, 0.05, and 0.1 ng/µL of each residue was 75 to 90% for 2,4,5-T and 65 to 80% for TCDD.

Data were analyzed by analysis of variance and significant means were separated using Duncan's multiple range test.

RESULTS

No TCDD was detected in any of the samples and no 2,4,5-T was detected in brain and heart samples from pooled birds. Residues of 2,4,5-T were detected in breast muscle of 14 birds, in fat of 12 birds and in the liver and gizzards of 5 birds. Of the 14 birds containing residue in breast muscle only 2 contained

levels greater than 1,000.0 ppb, 1 contained 474.0 ppb while 3 contained levels between 300.0 and 60.0 ppb. Residue levels in the other 8 birds ranged from 8.0 to 144.0 ppb. Fat residue levels in 5 birds were between 30.0 and 60.0 ppb, and from 5.0 to 20.0 ppb in the other 7 birds. Residues in liver were between 68.0 to 118.0 ppb in 2 birds but were only 2.0 to 30.0 ppb in the other 3. The gizzards of 2 birds contained levels of 36.0 to 41.0 ppb while the other 3 birds contained levels from 6.0 to 21.0 ppb.

Residue levels for a given tissue type compared by month (TABLE 1) were significantly ($P<.05$) higher in liver in August while levels in breast muscle were significantly higher in October and November. Residue levels in gizzard were significantly higher in November and residue levels in fat did not differ over the 4 months.

TABLE 1

Mean concentrations of 2,4,5-T (ppb) in 4 tissue types from the American coot,[1]

Month	Number of birds collected, tissue type, and number of birds with residue.								
	N[2]	breast muscle	WR[3]	fat	WR	liver	WR	gizzard	WR
		(ppb)		(ppb)		(ppb)		(ppb)	
Aug.	6	45.0c	2	18.0a	1	70.0a	3	0.0	0
Oct.	11	603.0a	4	19.0a	3	0.0	0	6.0b	1
Nov.	5	139.0b	5	25.0a	3	0.0	0	39.0a	2
Dec.	7	10.0c	3	21.0a	5	8.0b	2	18.0b	2
Mean		199.0		21.0		39.0		18.0	
Total	29		14		12		5		5
Range		(8.0-1,338)		(5.0-30.0)		(2.0-118.0)		(6.0-41.0)	

[1]Means within a column followed by the same letter are not significantly ($P<.05$) different.

[2]N = number of birds collected.

[3]WR = number of birds with residue.

Residue levels compared between tissues for a given month showed that residues in liver for August were significantly ($P<.05$) higher than in the other tissues and that levels in breast muscle were significantly higher than levels in other tissues in October and November. Residue levels were not different between tissues in December.

The grand means for these tissues compared to one another showed the order of concentrations to be: breast muscle>liver> fat>gizzard and only the levels in the breast muscle were

significantly higher than in the other tissues.

DISCUSSION

The absence of TCDD and only ppb levels of 2,4,5-T in coot tissues is not completely surprising in view of the low levels of the herbicide used within the watershed, the time interval between treatment and collection of birds, and the migratory nature of this bird species. In addition, results from a companion study (authors unpublished data) revealed the absence of TCDD in water, mud, and organic matter from White River Lake and levels of 2,4,5-T were seldom greater than 2.0 ppb over the same time period during which the coots were collected. Furthermore analysis of fish revealed no residue of either TCDD or 2,4,5-T while skeletal muscle from only a few herbivorous turtles contained levels of 2,4,5-T less than 20.0 ppb.

Most surprising were the ppm levels of 2,4,5-T detected in the breast muscle of 2 birds. These residue levels suggest relatively recent exposure. The phenoxy herbicides in general are rapidly eliminated, at least by mammalian species (ST. JOHN et al. 1964; GEHRING et al. 1973; CLARK et al. 1975; KOSCHIER and BRANDT 1976). The residues detected in these birds are therefore not believed to have been contributed by in-lake sources. Many of the birds had only recently arrived at the lake and possibly acquired the residue somewhere along their migratory route.

The absence of TCDD is less surprising if the estimates of the CAST report (1978) are correct. The amount of TCDD in 2,4,5-T and silvex distributed in the United States is estimated at no more than 28g/year.

Based on the information gathered in this study along with other data and published reports it is only logical to arrive at a few relevant conclusions. Namely the levels of 2,4,5-T detected in coots is of no toxicological significance as residues are well below the reported LD_{50} and EC_{50} levels for birds (KENAGA 1975). Residues of TCDD are not present at detectable levels, and 2,4,5-T is apparently not biomagnified through food chains. This further suggests that 2,4,5-T used at recommended rates would not present any serious hazards to wildlife species such as coots.

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