Trial Monitoring of Pesticides in Wings of Mallards and Black Ducks

by ROBERT G. HEATH and RICHARD M. PROUTY
Bureau of Sport Fisheries and Wildlife
U. S. Department of Interior
Patuxent Wildlife Research Center
Laurel, Maryland

To be suitable for use in a nation-wide program of pesticide monitoring, a species of wildlife should be relatively abundant throughout most of the nation, be readily collectable, and have feeding habits that sample some target cross-section of the environment. Very few of the many species found within the United States meet these requirements.

Guided by these criteria, the Bureau of Sport Fisheries and Wildlife selected the wild mallard, or where necessary the closely related black duck, to be used as part of its phase of the Federal pesticide monitoring program. The combined range of the species includes virtually all of the continental United States, the mallard being relatively abundant in all but the Atlantic coast States where the black duck predominates. Both species are omniverous, feeding on a variety of vegetation and aquatic animals. In some regions mallards feed heavily on grain crops. Thus, their varied diets and migratory habits cause the species to sample a broader segment of the habitat than most forms of wildlife.

The feasibility of monitoring these species depended upon the suitability of the duck wing as a medium for pesticide analysis and as an indicator of body residue levels. An established nation-wide survey to measure annual waterfowl productivity each fall provides the Bureau with tens of thousands of duck wings mailed by cooperating hunters. The wings, identified by species, age, sex, and county of kill, were routinely discarded once the above data were recorded. Thus a

Bulletin of Environmental Contamination & Toxicology,
Vol. 2, No. 2, 1967, published by Springer-Verlag New York Inc.

monitoring sample was virtually readymade if wings proved to be a suitable medium for analysis. It was otherwise inconceivable that these prized species of waterfowl could be adequately sampled for monitoring on a national scale.

To learn if pesticides were detectable, analyses were made of 20 samples of wings from 14 species of ducks collected in the fall of 1963. These determinations, made at the Bureau's Patuxent Wildlife Research Center at Laurel, Maryland, and the Wisconsin Alumni Research Foundation, revealed the presence of pesticides in all but one sample.

To test whether wing residue levels correlate with those in other parts of the body, the Patuxent Center contracted the services of Dr. T. J. Peterle and D. Dindal at Ohio State University who were using DDT ring-labeled with chlorine-36 to trace DDT dispersion in a marsh ecosystem. Their preliminary findings based on radio-assays of 104 mallards and scaup ducks revealed highly significant positive correlations (.01 level of significance) between wing residue levels and those in 10 of the 11 body parts examined. Wing levels correlated with those in the skin, muscle, brain, liver, kidney, pancreas, adrenal, gonad, thyroid, and uropygial gland; they did not correlate with levels in feathers. (Correlation coefficients are still subject to verification and are not reported here.)

A pilot collection of black duck and mallard wings was scheduled for the 1964 duck-wing survey to develop and test our proposed monitoring protocol prior to a full-scale operation. Sampling was restricted

to wings from the States of New York and Pennsylvania where both species of ducks were adequately represented in the kill. The wings were sampled according to species, State of kill, and age, separating birds of the year from adults. The age separation was made on the premise that adult birds, through longer exposure, could have higher residue levels than immatures. Both species were examined because black ducks feed more heavily than mallards on aquatic animals known to concentrate certain pesticides and conceivably could develop higher residues.

The most precise and important estimate to be derived in monitoring these species is not the pesticide levels in the wings themselves but the quantification of any trends in levels that might develop within their continental populations. In addition, findings should be relatively applicable to other species of waterfowl with similar ranges and feeding habits, and intuitively should reflect pesticidal trends within the overall environment.

Since the primary aim in monitoring is to measure trends in pesticide levels, it was decided for greater efficiency to "pool" a fixed number of wings in each chemical analysis. While pooling fails to measure extremes in levels among individual wings, it provides a more precise estimate of average levels for a given number of analyses. Clearly, trends are most readily determined by detecting significant changes between <u>average</u> residue levels over some period of time. A pool size of 25 wings was thought to be adequate.

A foreseeable limitation to the use of the mallard and black duck as indicator species is that, should an increase in residue levels be detected within some segment of their population, the mobility of the species might permit only a general location of the source of contamination. However, monitoring of localized material (soil, crops, etc.) by other agencies should help identify such concentrations.

Methodology

Random selections of wings within each of the eight wing-categories (2 States x 2 species x 2 age groups) provided five 25-wing pools from all categories except that of Pennsylvania adult black ducks where wings for only three pools were available. (Two of the five New York adult mallard samples were lost in a laboratory accident.) Feathers were removed from each wing by trimming and singeing. The defeathered wings of each pool were then ground into an homogenous 25-wing mass with a Hobart Food Cutter (Model 8418-D), and a 20-gram tissue sample was taken from each pool-homogenate for analysis. Sample preparation and chemical analyses were performed at the Patuxent Wildlife Research Center by Richard M. Prouty and William L. Reichel with assistance from James D. Frye; James W. Spann segregated the wings and randomized them into 25-wing pools.

The tissue samples were mixed with sufficient sodium sulphate to bind any moisture present, then extracted for 7 hours with petroleum ether (30° - 60° C. b.p.) in a Soxhlet extractor. After evaporation

of the petroleum ether, the lipids were partitioned with acetonitrile and hexane and the remaining fats removed by passage through a florisil column.

All analyses were made by electron capture gas chromatography, and verified with thin layer chromatography. The gas chromatography utilized a 5 percent SE-30 column on Anakrom ABS. Thin layer chromatographic plates coated with aluminum oxide were developed with hexane and visualized by spraying with silver nitrate and exposing to UV light.

Tissue pools were analyzed in a randomly selected order so that, should a progressive analytical error ever develop, it would be randomly distributed among all wing categories. Otherwise error confined to certain categories could be falsely interpreted as residue differences.

Results

All 36 wing pools contained measurable amounts of DDT and its metabolites, DDD and DDE; dieldrin was detected in 32 of the pools. Only the above chemicals were identified by gas and thin layer chromatography.

Tables 1, 2, and 3 show the average residue levels of pools in ppm wet weight of DDT and DDD combined, of DDE, and of dieldrin. DDD has been included with DDT since it is a known early breakdown product of the latter and also is toxic.

All data shown and the accompanying statistical analyses are based on the gas chromatographic readings. Statistical analysis of the thin layer readings was not attempted, since some pool readings were too low

to be quantified. Agreement between the two methods was considered acceptable in all cases, differences seldom exceeding 0.5 ppm.

Analysis of variance of the DDT plus DDD data failed to establish differences (.05 level of significance) in residue levels among species, age, State of kill, or their interactions. The difference between the mean residue level (0.83 ppm) in black ducks and that in mallards (0.53 ppm) closely approached significance and warrants further study.

Mean levels of DDT plus DDD in adult wings were virtually identical to those in immatures (0.68 and 0.67 ppm, respectively); however, levels of DDE were significantly higher in adults (1.39 and 0.52 ppm, respectively). The fact that average levels of DDE in adults were more than twice those in immatures and double those of DDT plus DDD in both age groups presumably reflects a longer period of exposure to this relatively stable, readily stored compound, whether acquired directly from the environment or metabolized from DDT.

The difference in DDE levels between species was not significant, nor were there significant differences between the two States either in levels of DDT plus DDD or in DDE. The dieldrin data were not subjected to a complete statistical analysis because of obvious similarity between most means. The apparent difference between State means was not statistically significant (.05 level).

Duplicate analyses made on 5 of the 36 pools yielded "within-pool" coefficients of variation of 8 percent for DDT plus DDD and 25 percent for DDE. The larger coefficients of variation for the total analyses

(66 and 106 percent, respectively, Tables 1 and 2) indicate that most of the variation between pools in any one wing-class is not an artifact of chemical methodology. Evidently, then, there is considerable variation in residue levels among individual wings, although its magnitude has not yet been studied.

Conclusions

The results of the trial monitoring indicate that mallard and black duck wings are satisfactory monitoring media, that pesticidal residues were present in all pools, and that due to the variability in levels, the pool size should not be reduced from 25 wings unless there is a wing shortage. Differences in DDE residues between adult and immature wings of both species indicate that the age classification should be maintained in sampling. For analytical reasons, State identification will be necessary in nation-wide monitoring even though significant residue differences between New York and Pennsylvania wings were not demonstrated.

Table 1. Levels of DDT plus DDD (ppm wet weight) in pools of 25 wings of mallards and of black ducks shot in New York and Pennsylvania during the fall of 1964 (Measurements by gas chromatography)

		Mallards			Black ducks			Means, combined species		
		Adults	Immatures	Ages combined	Adults	Immatures	Ages combined	Adults	Immatures	Ages combined
N. Y.	Number of pools	3	5		5	5				
	Pool means	0.48	0.74	0.61	1.05	0.94	1.00	0.77	0.84	0.80
	Range	(0.3-0.8)	(0.5-1.2)		(0.4-1.8)	(0.2-2.2)				
Pa.	Number of pools	5	5		3	5				
	Pool means	0.64	0.27	0.45	0.53	0.72	0.63	0.59	0.50	0.54
	Range	(0.4-1.3)	(0.2-0.4)		(0.4-0.6)	(0.4-1.1)				
Means, combined States		0.56	0.50	0.53	0.79	0.83	0.81	0.68	0.67	0.67

Error variance = 0.194

Coefficient of variation = 66 percent

Note: No differences are statistically significant (.05 level of significance)

Table 2. Levels of DDE (ppm wet weight) in pools of 25 wings of mallards and of black ducks shot in New York and Pennsylvania during the fall of 1964 (Measurements by gas chromatography)

		Mallards			Black ducks			Means, combined species		
		Adults	Immatures	Ages combined	Adults	Immatures	Ages combined	Adults	Immatures	Ages combined
N. Y.	Number of pools	3	5		5	5				
	Pool means	0.87	0.52	0.69	1.08	0.50	0.79	0.97	0.51	0.74
	Range	(0.5-1.4)	(0.2-0.8)		(0.2-1.7)	(0.2-0.9)				
Pa.	Number of pools	5	5		3	5				
	Pool means	2.48	0.28	1.38	1.13	0.78	0.96	1.81	0.53	0.69
	Range	(0.6-5.8)	(0.1-0.6)		(0.5-2.0)	(0.1-1.5)				
Means, combined States		1.67	0.40	1.04	1.11	0.64	0.87	1.39*	0.52*	0.96

Error variance = 1.04

Coefficient of variation = 106 percent

*Difference between age classes statistically significant (.05 level of significance)

Table 3. Levels of dieldrin (ppm wet weight) in pools of 25 wings of mallards and of black ducks shot in New York and Pennsylvania during the fall of 1964 (Measurements by gas chromatography)

	Mallards			Black ducks			Means, combined species		
	Adults	Immatures	Ages combined	Adults	Immatures	Ages combined	Adults	Immatures	Ages combined
Number of pools	3	5		5	5				
Pool means	0.03	0.11	0.07	0.06	0.06	0.06	0.05	0.09	0.07
Range	(0 -0.05)	(0.04-0.20)		(0-0.20)	(0.02-0.10)				
Number of pools	5	5		3	5				
Pool means	0.11	0.12	0.12	0.10	0.10	0.10	0.11	0.11	0.11
Range	(0-0.30)	(0.02-0.40)		(0.04-0.20)	(0.02-0.20)				
com- States	0.07	0.12	0.10	0.08	0.08	0.08	0.08	0.10	0.09

Differences not of sufficient magnitude to warrant statistical analysis.