Wing and Body Tissue Relationships of DDT and Metabolite Residues in Mallard and Lesser Scaup Ducks

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Introduction and Objectives

Heath and Prouty (1) showed that wings of mallard and black ducks can be used satisfactorily for monitoring pesticides and their metabolites such as dieldrin, DDT, DDD, and DDE. Wings from wild ducks collected and submitted by hunters have been analyzed and results reported by Heath and Stickel (2). Total DDT residues of 1.00 ppm (wet weight) were found in muscle, bone and wing base tissues of four pooled mallards. Complete wing analyses of five other waterfowl species showed a range from 0.15 to 3.90 ppm. Muscle fragments of mallard wings contained a total DDT residue concentration of 0.14 ppm. Wing muscle analyses on ten other wild ducks ranged from 0.03 to 2.74 ppm.

This study investigated the relationships of DDT residues present in wings and in 11 corresponding body tissues in mallard and lesser scaup ducks. Pesticide levels in wings were correlated to concentrations found in breast skin, breast muscle, breast feathers, liver, pancreas, kidney, brain, thyroid, adrenal gland, thyroid gland, and gonads.

Specimens came from our intensive field study involving the accumulation of DDT in the two duck species from a marsh ecosystem.[1]

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Radioactively tagged chlorine-36 technical DDT (ring labeled) was applied at the rate of 0.2 lb/A to a four-acre marsh near Lake Erie, Port Clinton, Ohio in 1964, and 112 ducks were methodically released and collected from the natural marsh area. Ducks were repeatedly exposed for 15, 30, 45, and 90 days over a two-year period. All tissues were analyzed by electron capture gas chromatography (ECGC) for both quantitative and qualitative purposes and by liquid scintillation spectrometry (LSS) for quantitation of total radiotagged residues.

Methods

Sample size. Wing and body tissues from 21 background ducks (13 mallards, 8 lesser scaup) and 104 experimental ducks (59 mallards, 45 lesser scaup) were analyzed by LSS. Tissues from 19 of the experimental birds (11 mallards, 8 lesser scaup) were further analyzed by ECGC to determine the residues of DDT present.

Predetermined uniform sample sizes could not be obtained. Adverse weather, dense marsh cover, eclipse-like secretive behavior, and predation hindered our original plan and unequal samples resulted.

Wing preparation and residue analysis. Since residue and exposure data was available on the tissues of each bird, the wings were analyzed singly rather than pooled as by Heath and Prouty (1).

After the feathers were trimmed and the bones broken, the wing was passed through a food grinder. A gram subsample (wet weight) of pulverized tissue was solubilized with hydroxide of

hyamine 10-X in a glass scintillation vial for radioassay and counted for 100 minutes in a Packard Tri-Carb Scintillation Spectrometer Model 3003. The scintillation solution was 2,5 diphenyloxazole (PPO) and 1,4 bis-2 (4-methyl-5 phenyl-oxazole) benzene (Dimethyl POPOP) in spectrograde toluene as recommended by Hayes (3).

The remaining wing sample (wet weight) was prepared for ECGC and thin-layer chromatography (TLC) by extracting with three washes of glass distilled skelly solve B (hexane) in a Waring blender followed by partitioning in a dimethyl formamide (DMF) system after de Faubert Maunder et al. (4). Pesticide recovery efficiency with this method was 85%. Hexane extracts were injected into a Packard Gas Chromatograph 800 series equipped with a glass column containing 1.5% (by weight) SE-52 liquid phase on a support of Anakrom-ABS (60/80 mesh). Operational temperatures were: flash heater (injector) 230 C, column 200 c, and detector 210 C. DDT and metabolites were verified by TLC.

Correlation coefficients of radioassay data were determined at the Computer Laboratory, The Ohio State University.

Results

Background radiation of wings. The variation in cpm of the background radiation of 21 duck wings was quite low. The 99% confidence interval was calculated from the one-tailed t-distribution since only the maximum activity level of background is important.

Total DDT residue-relationships

Combined species. Radioassay of tissues permitted an evaluation of the total DDT residues present, but no distinction could be made between isomers and metabolites of the pesticide. This highly sensitive analysis method did allow us to follow the route of the originally applied DDT with certainty since labeled experimental residues and previous unlabeled body burden levels of pesticide could be distinguished.

High correlations were found between residues in wings and ten body tissues when the data from all 104 ducks was combined (Table 1). The residue relationship between breast feathers and wings was non-significant, but DDT residue levels in breast skin, breast muscle, uropygial gland, adrenal gland, pancreas, liver, kidney, brain, gonads and thyroid correlated at the .01 significance level. Breast skin, kidney, and breast muscle rank one, two, and three respectively in highest correlations.

Relative residue concentrations also are given as a body-tissue/wing ratio. This ratio based on means shows that breast muscle contained approximately half as much DDT residue as the wing, whereas adrenal tissue contained five times more and the uropygial gland eight times more than the wing.

Single species. Species variations in the residue relationships appear to exist in some tissues when viewing the comparative correlations, rank, and body-tissue/wing ratios of mallards and lesser scaup (Table 2). The significance of the species difference

in correlation coefficients was tested using Z transformations. According to our interpretations, LeClerg et al. (5) imply significant difference at the .15 level while Fisher (6) states significance exists at the .02 level in a test of this type.

TABLE 1

RELATIONSHIPS OF TOTAL DDT RESIDUES IN WINGS AND BODY TISSUES OF MALLARD AND LESSER SCAUP DUCKS: COMBINED DATA. (Radioassay analysis)

N = 104	TOTAL DDT RESIDUES ppm mean	STANDARD DEVIATION	STANDARD ERROR from the MEAN	CORRELATION (r) Wing to body tissue	RANK based on r	BODY-TISSUE/WING RATIO based on the mean
Wing	0.51	0.97		1.00	1	
Breast Skin	1.25	3.51	.34	.75	2	2.45
Kidney	0.45	0.75	.07	.73	3	0.88
Breast Muscle	0.28	0.51	.05	.71	4	0.55
Uropygial Gland	4.12	9.23	.91	.67	5	8.08
Adrenal Gland	2.60	6.20	.62	.64	6	5.10
Pancreas	0.54	1.29	.13	.55	7	1.06
Brain	0.44	0.78	.08	.55	7	0.86
Gonads	0.51	1.59	.16	.54	8	1.00
Liver	1.35	2.24	.22	.47	9	2.65
Thyroid	0.44	2.50	.25	.33	10	0.86
Breast Feathers	1.55	3.26	.32	.13 (non-significant)		

.01 significance level of r = .25

TABLE 2

RELATIONSHIPS OF TOTAL DDT RESIDUES IN WINGS AND BODY TISSUES OF MALLARD AND LESSER SCAUP DUCKS: SPECIES VARIATION. (Radio-assay analysis)

	Mallard N = 59				Lesser Scaup N = 45				
	TOTAL DDT RESIDUES ppm Mean	CORRELATION (r) Wing to body tissue	RANK based on r	BODY-TISSUE/WING RATIO based on the mean	TOTAL DDT RESIDUES ppm Mean	CORRELATION (r) Wing to body tissue	RANK based on r	BODY-TISSUE/WING RATIO based on the mean	SIGNIFICANCE LEVEL OF DIFFERENCE BETWEEN SPECIES based on r.
Wing	0.48		1		0.72		1		
Breast Skin	0.80	.73	2	1.67	1.86	.82	2	2.58	.14
Kidney	0.34	.61	6	0.71	0.60	.76	4	0.83	.08
Breast Muscle	0.13	.68	3	0.27	0.51	.78	3	0.71	.15
Uropygial Gland	2.73	.55	7	5.69	6.00	.69	5	8.33	.13
Adrenal Gland	2.12	.63	5	4.42	3.28	.82	2	4.56	.02
Pancreas	0.52	.48	8	1.08	0.59	.69	5	0.82	.06
Brain	0.47	.66	4	0.98	0.50	.66	6	0.69	.50
Gonads	0.37	.27	10	0.77	0.53	.62	7	0.74	.02
Liver	1.25	.18	12	2.60	1.95	.50	8	2.71	.04
Thyroid	0.20	.32	9	0.42	0.74	.37	9	1.03	.39
Breast Feathers	2.10	.31	11	4.38	0.80	.12	11	1.11	.18

.01 significance level r = mallard .32 scaup .37

.05 significance level r = mallard .25 scaup .29

In general, the body-tissue/wing ratios for lesser scaup are slightly higher than for mallards. In each species the breast skin and breast muscle correlations are highest, and the breast feather-wing correlations for mallards are significant at the .05 level. The minimum of the body-tissue/wing ratio range for mallards is breast muscle which contains approximately a fourth as much residue as wings, and the maximum is the uropygial gland with more than five times the mean wing concentration. Breast muscle and brain of lesser scaup show minima of about seven-tenths the wing amount, and the maximum of eight times the wing is present in uropygial glands.

Tissue relationships of DDT and metabolites. Tissues from 19 selected birds were further analyzed for DDT isomers and metabolites present. The majority of these ducks were exposed shortly after the pesticide application which accounts for the relatively higher residue values. Generally, pp'DDT, DDD, DDE, and DIMU were positively identified from these birds. Mean concentrations of these compounds are compared with the total residue levels determined by radioassay (Tables 3 - 5).

DDE is the most abundant metabolite in all cases. Breast feathers, the uropygial gland, breast skin, gonads, and the wing have pp'DDT as the second most abundant compound. The compound DDD is second most abundant in breast muscle, liver, pancreas, kidney, and brain. The intermediate metabolite, DDMU, as described by Peterson and Robison (7) was found only in liver, brain,

and wing tissue.

The existence of pre-experimental body burdens is exemplified by some of the tissues having higher chromatographic concentrations than those found by radioassay.

TABLE 3

COMBINED CONCENTRATIONS (ppm wet weight) OF DDT AND METABOLITES IN TISSUES OF MALLARD AND LESSER SCAUP DUCKS. Lake Erie, Port Clinton, Ohio. 1964-1965.

N = 19 n = none detectable

Analysis Method	ECGC					LSS
	DDMU	DDE	DDD	DDT	TOTAL DDT RESIDUES	TOTAL DDT RESIDUES
Wing	0.14	0.56	0.11	0.20	1.01	1.99
Breast Feathers	n	1.01	0.02	0.63	1.66	3.04
Uropygial Gland	n	9.25	0.21	2.42	11.88	15.95
Breast Skin	n	3.98	0.34	2.61	6.93	4.64
Breast Muscle	n	0.50	0.07	0.04	0.61	0.88
Liver	0.02	1.35	0.33	0.11	1.81	4.62
Pancreas	n	1.81	0.47	0.28	2.56	2.38
Gonads	n	0.76	0.11	0.17	1.04	2.24
Thyroid	no tissue available					1.90
Adrenal	no tissue available					9.59
Kidney	n	1.17	0.30	0.06	1.53	1.59
Brain	0.13	1.28	0.40	0.22	2.03	1.30

TABLE 4

COMPARATIVE CONCENTRATIONS (ppm wet weight) of DDT AND METABOLITES IN MALLARD DUCK TISSUES. Lake Erie, Port Clinton, Ohio. 1964-65.

N = 11 n = none detectable

Analysis Method:

	ECGC					LSS
	DDMU	DDE	DDD	DDT	TOTAL DDT RESIDUES	TOTAL DDT RESIDUES
Wing	0.01	0.24	0.05	0.09	0.38	1.39
Breast Feathers	n	n	n	n	n	4.52
Uropygial Gland	n	5.66	0.17	3.23	9.06	10.77
Breast Skin	n	2.80	0.43	2.25	5.48	3.80
Breast Muscle	n	0.26	0.07	0.03	0.36	0.39
Liver	0.02	0.68	0.34	0.01	1.05	4.13
Pancreas	n	1.13	0.40	0.28	1.81	2.42
Gonads	n	0.76	0.11	0.17	1.04	1.70
Thyroid	no tissue available					0.71
Adrenal	no tissue available					8.02
Kidney	n	0.47	0.15	0.08	0.70	1.21
Brain	0.17	0.55	0.28	0.17	1.17	1.12

TABLE 5

COMPARATIVE CONCENTRATIONS (ppm wet weight) OF DDT AND METABOLITES IN LESSER SCAUP DUCK TISSUES. Lake Erie, Port Clinton, Ohio. 1964-1965.

N = 8 n - none detectable

Analysis Method	ECGC					LSS
	DDMU	DDE	DDD	DDT	TOTAL DDT RESIDUES	TOTAL DDT RESIDUES
Wing	0.32	1.01	0.23	0.35	1.91	2.30
Breast Feathers	n	1.35	0.02	0.84	2.21	1.19
Uropygial Gland	n	20.06	0.35	n	20.41	23.09
Breast Skin	n	8.68	n	4.03	12.71	5.95
Breast Muscle	n	0.89	0.07	0.06	1.02	1.00
Liver	0.03	2.56	0.30	0.30	3.19	5.26
Pancreas	n	3.00	0.58	0.27	3.85	2.33
Gonads	no tissue available					2.99
Thyroid	no tissue available					3.39
Adrenal	no tissue available					11.55
Kidney	n	2.91	0.56	n	3.47	2.06
Brain	n	3.21	0.72	0.32	4.25	1.53

Discussion and Summary

A highly significant positive relationship (.01 level of significance) exists between total DDT residues in wings and in each of 10 body tissues of mallard and lesser scaup ducks taken collectively. These significant body tissues include breast skin, breast muscle, uropygial gland, liver, pancreas, gonads, thyroid, adrenal, kidney and brain. Residues in breast feathers did not correlate with wing levels in the combined sample.

When each species was analyzed separately, all but one mallard tissue correlated with the wing. Residue levels in mallard liver were not significant; breast feathers were significant. Both of these were based on the .05 significance level. Also related to species variation, some significant correlation coefficient differences do exist.

Examining tissues individually, it is reasonable that breast skin and breast muscle correlations are high since these two types of tissues are major components of wings. Breast feather relationships do not follow such a consistent pattern. Perhaps the inconsistent levels on breast feathers are caused by residue adhesion to feathers from sporadic physical contact with the pesticide in the marsh environment and in preening, whereas DDT residues in skin and muscle are present due to physiological processes and therefore more consistent.

In addition to pp'DDT, DDD, and DDE reported by Heath and Stickel (2) and Heath and Prouty (1), we found and verified by TLC

the presence of DDMU in wing tissue.

Finally, the ranges of the total DDT residues (means) in wings are as follows:

combined species	0.51 to 1.99 ppm
mallard	0.48 to 1.39 ppm
lesser scaup	0.72 to 2.80 ppm

Ranges of total DDT residues (means) in breast muscle tissue are:

combined species	0.28 to 0.88 ppm
mallard	0.13 to 0.39 ppm
lesser scaup	0.51 to 1.00 ppm

These ranges incorporate the values from both radioassay and gas chromatography and correspond to the values reported earlier by Heath and Stickel (2).

The positive correlations and other relationships presented here support the use of duck wings for pesticide monitoring of DDT and its metabolites.

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