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Effect of Skin Removal on Estimated Levels of Organochlorine Pesticide Contamination in Passerine Birds

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In recent years there has been increasing concern about the potential deleterious effects of organochlorine (OC) and other pesticides on passerine bird populations (Robbins et al. 1989; Terborgh 1989; Askins et al. 1990; Colborn et al. 1993; Gard et al. 1993; Gard et al. 1995; Block et al. 1995). However, there is a paucity of published data on OC levels in Neotropical migrant passerines (i.e., birds that winter in the West Indies, Mexico, Central and South America, but breed in the North Temperate zone of North America). Furthermore, differences in the methods of data collection in the few studies that do exist may complicate efforts to draw conclusions across studies. For example, in some of the studies published on this topic (e.g. DeWeese et al. 1986; Fyfe et al. 1991) the plucked skins of the birds were ground up with the carcasses and were included in the pesticide analyses. The rationale for including the skin is that it often has a high fat content and therefore may contain high levels of lipophilic OC compounds. However, in other studies (e.g. White and Krynitsky 1986; Harper et al. 1996) the skins were excluded from the analyses. If not destroyed in the pesticide analysis, these skins represent an important resource because they can be preserved as museum study skins (Harper et al. 1996). Although some data on plumage and morphology can be taken from the carcass prior to destroying the skin, preserving the skin in a permanent collection allows new data to be taken from it at a later date. Such data can enhance the understanding of pesticide contamination patterns. If the skin is included in the chemical analysis, such potentially useful information is lost. These skins can be used to identify the individual to the level of subspecies by comparison with study specimens housed at museums. Different subspecies may winter or breed in different geographic areas and may therefore receive different levels of exposure to OC compounds.

No studies have been published on the effects of skin removal on estimating OC levels in passerines. Because a preserved study skin is a valuable resource for pesticide, systematic, and ecological studies of passerines, it may be desirable not to include skin in the pesticide analysis if this does not affect estimates of OC levels. The objective of this study was to determine if removing the skin prior to

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quantifying OC pesticide levels in passerine carcasses resulted in a significant underestimation of levels of OC contaminants.

METHODS AND MATERIALS

Thirty ovenbirds (*Seiurus aurocapillus*), a Neotropical migrant passerine, were salvaged at a television tower kill in Livingston County, Illinois (8.2 km ESE of Pontiac, Illinois), USA, on 16 May 1996. The birds were placed on ice upon collection and then transferred to a -80°C freezer. The carcasses were randomly placed into one of three treatment groups. Birds of the first group (skinned birds) were thawed and the skins were removed and prepared as museum study specimens. Care was taken to remove all visible subcutaneous fat from the skin, and this fat was included in the pesticide analysis. All skins were deposited in the Illinois State University Department of Biological Sciences bird collection. The carcasses, including torso, proximal limbs, brain, eyes and subcutaneous fat, were refrozen until chemical analysis could be performed. Birds of the second group (plucked birds) were thawed, the feathers were removed, and the carcass was refrozen to await chemical analysis. In an attempt to account for the variability of pesticide levels expected from each sample, birds of the third group (bisected birds) were thawed and cut in half. One half was randomly assigned to be skinned while the other half was assigned to be plucked. Special care was taken to divide asymmetrical organs, such as the heart, lower digestive tract and gonads, equally. Both halves were refrozen until chemical analyses were performed. A scale modified from McCabe (1943) was used to estimate the amount of subcutaneous fat (none = 0, light = 1, moderate = 2, heavy = 3, extremely heavy = 4) present in whole carcasses and carcass halves from all treatment groups.

Just prior to analysis each carcass was thawed and the digestive tract was rinsed with water to remove the contents. Sodium sulfate (not exceeding 50% of the weight of the carcass) was added to the carcass, which was then ground into a paste with a tissue homogenizer. The mixture was then transferred to a soxhlet thimble and extracted with hexane (approximately 150 ml, pesticide-grade, Fisher) for 15-24 h. The extract was concentrated to under 5 ml and then transferred to a chromatography column containing Florisil® (20g, 60-100 mesh, activated at 130°C for 16 h) and sodium sulfate (1-2 cm). The column had been washed with hexane (~40 ml). The column was eluted with 200 ml portions of 6% diethyl ether in hexane (fraction 1), 15% diethyl ether in hexane (fraction 2), and 50% diethyl ether in hexane (fraction 3). These elutions were collected, concentrated to about 5 ml using a rotary evaporator and rediluted to 10.0 ml in a volumetric flask.

Each fraction was analyzed with a Hewlett Packard (HP) 6890 series gas chromatograph equipped with two Ni^{63} electron capture detectors operated at 300°C. One microliter injections were made with an autosampler (HP 18596-C) into a split/splitless injector operated at 230°C. The analyte was separated on two different fused silica capillary gas chromatograph columns using helium as the

carrier gas. A 30-m DB-35 (0.32-mm inside diameter) served as the primary column for pesticide quantification, while a 30-m DB-1701 (0.32-mm inside diameter) was used for pesticide confirmation. The oven temperature was raised from an initial temperature of 150°C to 200°C at a rate of 8°C/min, then from 200°C to 290°C at 4°C/min, and maintained at 290°C for 7 min. Data were collected and analyzed with HP environmental analysis software. Peak areas from eight calibration standards were used to calculate response factors. Curve fit was performed by linear regression, and linearity of each calibration curve was verified by determining the coefficient of determination of the line formed by the eight response factors for each pesticide. The coefficient of determination was always greater the 95%. The average response factor from the calibration curve was used to quantify pesticide levels. In all cases the retention times of the peaks closely matched the standards. The chemicals assayed for were aldrin, 2,2-Bis(4-chlorophenyl)-1,1-dichloroethane (p,p'-DDD), 2,2-Bis(4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE), 2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethane (p,p'-DDT), dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, alpha-hexachlorocyclohexane, beta-hexachlorocyclohexane, delta-hexachlorocyclohexane, lindane, and methoxychlor. Detection limits were 0.01 µg for all pesticides except the following: heptachlor (0.02 µg), aldrin (0.03 µg), endosulfan I (0.03 µg), and endosulfan sulfate (0.10 µg). Positive identification of pesticides was made when sample retention times were within ±0.05 min of the average retention time of the calibration standards on both columns. Levels of OC pesticides in duplicate samples were within five percent of each other.

Only p,p'-DDE was found in a sufficient number of carcasses for statistical analyses (see below). In order to increase confidence in the data, only carcasses that contained p,p'-DDE at twice the minimum detection level (0.02 µg) were included in statistical analyses. This was also the lowest concentration used when generating the calibration curve. After log transforming the data to meet the assumption of normality, a t-test was used to analyze differences in p,p'-DDE between skinned and plucked birds (Sokal and Rohlf 1995). Data reported for skinned and plucked birds are untransformed means and 95% confidence limits (Sokal and Rohlf 1995). Differences in fat scores between skinned and plucked carcasses were analyzed with a Mann-Whitney U test (Sokal and Rohlf 1995). A t-test for paired comparisons (Sokal and Rohlf 1995) was used to analyze differences in p,p'-DDE levels in skinned and plucked halves of bisected birds.

RESULTS AND DISCUSSION

DDT, p,p'-DDD, dieldrin, and heptachlor epoxide were detected in ovenbird carcasses (Table 1). There was no significant difference in levels of p,p'-DDE in skinned ($\bar{x}$ = 19.50 ng/g; 95% confidence limits = 14.63 - 24.37 ng/g) versus plucked carcasses ($\bar{x}$ = 51.60 ng/g;; 95% confidence limits = -0.22 - 103.42 ng/g) (t = -1.45, df = 8.11, p = 0.18). This t value was corrected for unequal variances

Table 1. Frequency of detection and lowest and highest detected levels of OC compounds (ng/g)in ovenbird carcasses.

Compound	Frequency of Detection	Lowest Level	Highest Level
DDT	3/30 carcasses	15.0	32.0
p,p'-DDD	7/30 carcasses	4.7	52.0
p,p'-DDE	28/30 carcasses	8.5	404.0
Dieldrin	16/30 carcasses	6.6	243.0
Heptachlor epoxide	10/30 carcasses	6.2	64.0

Table 2. Levels of p,p'-DDE (ng/g) in skinned and plucked halves of bisected ovenbird carcasses.

	Treatment Group	
Sample Number	Skinned	Plucked
1	154.0	404.0
2	46.0	63.0
3	29.0	28.0
4	213.0	335.0
5	26.0	23.0
6	97.0	171.0

between the samples as determined by Levene's test for equality of variances (Norusis /SPSS Inc. 1993). Likewise there was no significant difference in levels of p,p'-DDE in skinned and plucked halves from bisected carcasses ($t = -2.17$, $df = 5$, $p = 0.08$; Table 2). Fat scores did not differ significantly between skinned and plucked carcasses ($U = 26.5$, $p = 0.52$), and were identical for halves of bisected carcasses.

The lack of significant differences in levels of p,p'-DDE both in skinned and plucked carcasses and in the skinned and plucked halves of bisected birds suggest that skin removal does not affect estimates of OC levels in passerine carcasses. However, in three bisected carcasses that contained relatively high levels of p,p'-DDE (greater than 100 ng/g), levels were substantially higher (albeit statistically nonsignificant) in the plucked halves than in the skinned halves. This suggests that skinning may lead to an underestimation of OC pesticide levels when OC's are present at higher levels. Controlled studies with larger sample sizes in which noncontaminated passerines are fed known concentrations of OC pesticides and then subjected to similar treatments would provide more definitive answers to this question.

Although no studies on this topic have been conducted using passerines, these results differ from Porter and Wiemeyer (1972), who analyzed the carcasses and skins of captive American kestrels (*Falco sparverius*) that were treated with

p,p'-DDE as part of their diet. They found substantially higher levels in the skins than in the carcasses, but it is uncertain whether the subcutaneous fat was included with the skins or the carcasses in their analysis.

Because of the lipophilic nature of OC contaminants, it is important to include the subcutaneous fat in chemical analyses, even if the skins are saved as museum study specimens. However, it can be difficult to remove all subcutaneous fat from the skin in migrant passerines with heavy to extremely heavy fat scores (Capparella, personal experience), which may lead to underestimation of OC levels. This was likely not a problem in this study because the ovenbirds had moderate to heavy fat scores. It should also be noted that the removal of subcutaneous fat in large birds, especially ducks and herons, is more difficult than in passerines (Capparella, personal experience).

In studies that involve passerines with described subspecies, museum study skin specimens may be important to a complete understanding of pesticide contamination patterns. Museum study skin specimens can also be used in systematic and other ecological and evolutionary studies of passerine birds. Passerine skins should be preserved as museum study skin specimens if excluding skins does not alter estimates of pesticide levels. However, in the absence of a controlled study as suggested above, including skins in the pesticide analysis should be left to the discretion of individual researchers.

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