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Persistent Organochlorine Pesticide Contamination of Birds Collected in Connecticut During the Year 2000

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In the summer and fall of 2000 The Connecticut Agricultural Experiment Station (CAES) screened in excess of 200 bird carcasses from across the state for the West Nile virus (WNV), the Eastern Equine Encephalitis (EEE) virus and various other viruses. The brain tissues of twenty birds in the present study were found to contain WNV and two of the birds tested positive for EEE (denoted in Table 2 by a single or double asterisk, respectively). In parts of Connecticut where WNV or EEE was found in mosquitoes, ground spraying of the insecticide Scourge® (a formulation composed of resmethrin, piperonyl butoxide and petroleum distillates) was conducted by the Connecticut Department of Environmental Protection (DEP) with the goal of controlling the transmission of these viruses to humans. To ensure that bird mortality was not a direct cause of the use of these chemicals, tissues from 129 of the birds tested for the viruses were screened for resmethrin and other pesticide residues using a modification of a multi-residue pesticide screen developed in our laboratories (Pylypiw, 1993).

All of the birds in this study were wild and collected within 24 hours after death. Fifty-two crows were sacrificed intentionally on November 4, 2000 in an effort to track the progress of viruses within the state. Unexpectedly, 43% of the 129 birds analyzed were found to contain residues of one or more Persistent Organochlorine Pesticides (POPs). The use of POPs on food crops was banned in the U.S. in 1978 (Pylypiw, 1997). Residues of these pesticides continue to persist in the environment and their uptake and accumulation by crops such as squash, cucumbers and carrots have been well documented (Mattina, et al., 2000, Pylypiw, et al., 1991, Pylypiw, et al., 1997). These POPs are known to accumulate in insects and insect larvae where they may enter the food chain (Stansley and Roscoe 1999a). Less well documented has been the continuing persistence of POPs in birds and other forms of wildlife. Okoniewski and Novesky (1993) reported 122 cases of avian mortality caused by poisoning with cyclodienes used on turf in New York State, and Stansley and Roscoe (1999b) reported on chlordane poisoning of birds in New Jersey from 1996-1997. More recently Lötter and Bouwman (2001) have reported the presence of POPs in hunted guineafowl in South Africa where the use of POPs is less restricted. We report herein the findings from our pesticide residue study that further document the continued widespread occurrence of these POPs in avian tissues.

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MATERIALS AND METHODS

Bird carcasses, predominantly from Fairfield and New Haven Counties, arrived at CAES for testing throughout the summer and fall months of 2000 from various sources including the DEP. A total of 129 birds were necropsied to provide 260 samples of brain (54), gizzard (125), and liver (81) tissue for pesticide residue testing. Tissue samples were weighed into 40 mL glass vials or 250 mL glass jars and the wet weight recorded. Enough methanol was added to cover the sample (1 to 222 mL) (JT Baker, Ultra Resi-Analyzed®) and the sample allowed to soak for a minimum of 24 hours. An aliquot of the methanolic solution was withdrawn and filtered through a two-micron external filter tip (Labsciences, Inc., Reno, NV) into glass autosampler vials.

Samples were analyzed using an Agilent 6890 Gas Chromatograph (GC) equipped with a 7683 injector and autosampler and a model 5973 Mass Selective Detector (MSD) operating in full scan mode over the range m/z 70 to m/z 430 (Agilent Technologies, Palo Alto, CA). The instrument was fitted with a Supelco MDN-12 fused silica capillary column, 30m x 0.25 mm ID x 0.25 μm (Supleco, St. Louis, MO, part no. 24388). Operating conditions were as follows: initial oven temperature 75 °C; no initial hold time; ramp to 200 °C at 25 °C/min; ramp to 230 °C at 2 °C/min; ramp to 250 °C at 20 °C/min; ramp to 280 °C at 30 °C/min; hold at 280 °C for 16 min. The MSD transfer line was set at 280 °C. Operating the injector in pulsed splitless mode, 2μL of each sample was injected; pulse pressure 30 psi until 1 min; purge flow to split vent 50 mL/min at 1 min; gas saver on 20 mL/min at 10 min. The Agilent split/splitless inlet was set to 250 °C, with a flow of 9.2 psi. A Merlin Microseal™ duckbill septum was employed. The carrier gas was helium, and an Agilent mass spectrometer gas purifier was used. The data were processed using Hewlett Packard (HP) Chemstation *Plus* (Rev.B.02.00) software on a HP Kayak XA computer.

Under these conditions the POPs found in this study eluted at the following retention times: oxychlordane 12.6 min (OXY), heptachlor epoxide 12.7 min (HEPX), *trans*-nonachlor 14.6 min (TN), p,p'-DDE 15.6 min (DDE), and *cis*-nonachlor 18.1 min (CN). The retention times of each POP matched the retention time of the corresponding authentic standard. Comparison of the mass spectra exhibited by each of these POPs to the mass spectra of laboratory standards and to the NIST 98 library confirmed their identities. An additional octachlorinated compound was found that eluted between *trans*-chlordane (TC), 10.7 min, and *cis*-chlordane (CC), 11.2 min. This compound had a retention time of 10.9 min, and exhibited a mass spectrum identical to that of the two chlordane diastereomers. It was found to have the same retention time as a component in technical chlordane, and to exhibit the same mass spectrum as this component. Based upon its relative retention time the compound was tentatively designated 'MC5,' a component of technical chlordane.

Calibration was performed using external standard calibration curves generated from mixed standards of the five compounds at 25, 50, 100, 250, and 500 ng/mL in methanol (JT Baker, Ultra Resi-Analyzed®). The mixed standards, except for MC5, were prepared by dilution from stock solutions that were prepared from neat analytical standards. Quantitation of MC5 was based on the response factor for TN. A set of the five standards bracketed each run and one of the standards was injected after every tenth sample. The limit of detection was established to be 10 ng/mL injected concentration at approximately 3:1 signal to noise. Randomly selected samples of brain, liver, and gizzard tissues (n=2 each) were spiked with mixed standards such that final expected concentration would be 250 ng/mL. The tissues were then extracted with methanol as above. For the recovery analysis each sample was injected in duplicate and each standard in triplicate. The average recoveries across all the tissue types for each of the POPs were found to be: OXY, 81%; HEPX, 123%; TC, 102%; TN, 127%; DDE, 91%, CN, 135%. No recovery data are available for the 'MC5' component of technical chlordane because an authentic standard was not available.

RESULTS AND DISCUSSION

Table 1 is an overview of the findings of this study. Table 2 provides a detailed summary of the 260 tissue samples collected from 129 birds studied. Blanks in the table indicate birds from which no tissue sample was available for testing, and ND represent samples which were tested but in which no pesticide was detected. All values are in μg/g of wet tissue. The recoveries of POPs from spiked tissue were as described in the Materials and Methods section. None of the values contained in Table 2 is corrected for the recovery of the individual pesticides studied. None of the tissue samples examined was found to contain resmethrin. Two of the samples were found to contain diazinon, an insecticide known to be acutely toxic to avian species, (Extension Toxicology Network, 2001, US Public Health Service, 1995) at concentrations of 2.97 (crow) and 333 (cardinal) μg/g based on the wet weight of the gizzard. A triple asterisk in Table 2 denotes these samples. These were the only non-POPs found in this study. A total of 55 birds (43%) were found to contain residues of POPs, namely residues of technical chlordane-related compounds; DDE (a metabolite of DDT); or HEPX (a metabolite of heptachlor {HEP}). The POPs detected which originate from technical chlordane were CN, TN, MC5, and OXY (a metabolite). Although HEP was a component of technical chlordane, it was also used as a stand-alone pesticide. Therefore, HEPX is not summed with the other components of technical chlordane. Mattina *et al.* (2000) and Dearth and Hites (1991) provide detailed discussions of technical chlordane and its components. Of the three tissue types examined in this study, POPs were found most often in gizzard tissue and less often in brain and liver tissue (Table 1). This may in part account for the higher percentage of POP findings in the current report (43%) relative to earlier reports that examined only brain tissue. In previous studies of chlordane residues in avian species, Stansley and Roscoe (1999b) report that 10% of brain tissue contained chlordane while Okoniewski (1993) reports POPs in 17% of brain

tissues. Both studies confirm that POPs pass through the blood brain barrier. In contrast to the reports from Okoniewski (1993) and Stansley (1999b) who detected dieldrin frequently, in the present study dieldrin was found in only one bird, specifically in a robin, which is denoted by a quadruple asterisk in Table 2. DDE and dieldrin are well known to co-elute on many types of GC columns, and on OV-17 and OV-101 columns they are separated by only two or three seconds (McMahon, 1994). Without MS confirmation it is possible that these compounds may be misidentified.

Table 1. Summary of Tissues Containing POPs

	NUMBER OF SAMPLES	POSITIVE FOR POPs	PERCENTAGE POSITIVE
Liver	81	15	18.5
Gizzard	125	53	42.4
Brain	54	18	33.3
Total Tissue Samples	260	86	33.1

There are reports in the literature that the effect of cyclodiene poisoning is additive (Stansley and Roscoe 1999a). This is an area that may warrant further review. The majority of the birds testing positive for POPs in this study, 74.6% of the 55 birds, were found to contain chlordane and at least one additional POP. While substantial amounts of OXY are found, no TC and CC was observed. This is consistent with previous studies of avian tissues (Okoniewski, 1993; Stansley and Roscoe 1999b). Since OXY is a metabolite of TC and CC, this observation suggests that metabolism in birds may be very rapid. However, it has been reported that in Japanese beetles, a common avian food source, the relative amounts of TN, OXY, and HEPX increase while the relative amounts of TC and CC decrease in comparison to these ratios in technical chlordane (Stansley and Roscoe 1999a). Thus, the major alteration in the component profiles noted in avian tissues has already begun to occur in a major food source, chlordane-resistant beetles. Causes of bird mortality are difficult to predict from this study. A number of the birds in this study were sacrificed in an attempt to follow the spread of viruses within the state. The majority of the birds in this study that tested positive for WNV or EEE were also found to contain POPs (73%). Whereas previous reports of avian mortality have focused on brain tissue (Okoniewski, 1993; Stansley and Roscoe 1999b), this study has shown that POPs are more prevalent in gizzard than in either the brain or liver tissue. POPs have not been used in this country since 1988 when they were banned because of health concerns. Nevertheless, POPs continue to pervade our environment. The fact that a majority of the samples testing positive for POPs were those of gizzard tissue indicate that POPs are still widely present in our environment and actively being ingested by avian species. Their presence in liver and brain indicate that they are still being accumulated in birds where they are likely being introduced into higher levels of the food chain.

Table 2. Concentrations of Positive POPs Detected in Birds Expressed as μg/g Wet Tissue Weight.

Bird Type	Family	CLDN												HEPX			DDE		
		Gizzard				Liver				Brain				Gizzard	Liver	Brain	Gizzard	Liver	Brain
		Oxy	MC5	TN	CN	Oxy	MC5	TN	CN	Oxy	MC5	TN	CN						
Hawk* n = 3																			
Hawk	Accipitridae					0.16	0.06	0.19	0.03	0.16	0.04	0.14	0.02		0.25	0.36		0.66	0.48
Hawk	Accipitridae	0.86	0.68	2.30	0.49					0.76	0.49	1.39	0.26	0.60		0.61	3.08		1.35
Blue Jay n = 5																			
Blue Jay*	Corvidae	ND	ND	ND	ND	0.23	0.23	0.14	0.01	0.02	0.02	0.03	ND	ND	0.07	ND	0.08	0.66	0.35
Blue Jay*	Corvidae	0.08	0.03	0.03	ND	0.09	0.06	0.04	ND	0.22	0.10	0.15	ND	0.06	0.12	0.21	0.10	0.10	0.70
Blue Jay*	Corvidae	0.74	0.64	0.94	0.04	1.66	1.58	2.04	0.10	0.46	0.30	0.42	0.00	0.84	2.10	0.67	1.01	1.88	0.51
Blue Jay*	Corvidae	0.96	0.88	1.56	0.23									1.05			3.60		
Crow*(x 3) n = 43																			
Crow*	Corvidae	ND	ND	ND	ND	0.04	0.04	0.07	ND	ND	ND	ND	ND	ND	0.02	ND	0.06	0.66	0.02
Crow*	Corvidae	0.04	0.02	0.02	ND					ND	ND	ND	ND	0.10		ND	0.05		ND
Crow*	Corvidae	ND	0.002	0.002	ND	ND	ND	ND	ND	0.03	0.01	0.04	ND	0.02	ND	0.03	0.01	ND	0.06
Crow*	Corvidae	0.03	0.01	0.03	ND	ND	ND	ND	ND	0.02	ND	0.02	ND	0.03	ND	0.04	0.10	ND	0.07
Crow*	Corvidae	0.01	0.01	0.03						0.01	0.02	0.03	ND	0.06		0.09	ND		ND
Crow*	Corvidae	ND	ND	ND	ND									ND			0.04		
Crow	Corvidae	ND	ND	ND	ND									ND			0.03		
Crow***	Corvidae	ND	0.03	0.03	ND									0.03			0.02		
Crow*	Corvidae	0.02	0.02	0.05	ND									0.03			0.02		
Crow*	Corvidae	0.03	0.03	0.05	ND									0.07			0.05		
Crow*	Corvidae	0.08	0.07	0.11	ND									0.11			0.06		
Crow	Corvidae	0.05	0.06	0.15	ND									0.08			ND		
Crow*	Corvidae	0.17	0.14	0.33	0.01									0.35			0.86		
Crow	Corvidae	0.22	0.17	0.41	0.10									0.19			0.71		
Cardinal n = 3																			
Cardinal	Fringillidae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.12	0.42	0.32
Cardinal	Fringillidae	ND	ND	ND	ND									ND			0.05		
Cardinal***	Fringillidae	0.22	0.04	0.01	ND									0.20			0.51		
Finch n = 2																			
House Finch**	Fringillidae	ND	ND	ND	ND	ND	0.18	0.16	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gull n = 3																			
Herring Gull	Laridae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.24	ND	0.34
Ring-Billed gull	Laridae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.06	0.04	0.48

Catbird n = 10	Mimidae	ND	ND	ND	ND									ND			0.12		
Catbird	Mimidae	ND	ND	ND	ND									ND			0.09		
Catbird	Mimidae	0.21	0.10	0.30	ND									0.18			0.16		
Catbird	Mimidae	0.69	0.39	0.69	ND									0.28			0.91		
Catbird	Mimidae	0.41	0.15	0.24	0.01									1.54			2.09		
Catbird	Mimidae	4.60	1.15	3.70	0.08									3.71			64.30		
Mockingbird n = 2																			
Mockingbird	Mimidae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.22	0.99	0.85
Mockingbird	Mimidae	3.16	0.71	2.41	0.29									2.60			1.84		
Titmouse n = 1																			
Titmouse	Paridae	ND	ND	ND	ND									ND			0.23		
Sparrow n = 13																			
House Sparrow	Ploceidae	ND	ND	0.09	ND									ND			0.41		
House Sparrow	Ploceidae	2.23	0.45	1.12	0.40									1.79			3.57		
House Sparrow	Ploceidae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.65	7.12	1.59
House Sparrow	Ploceidae	0.13	0.037	0.025	ND	0.19	0.06	0.05	ND	0.24	0.10	ND	ND	0.10	0.05	0.12	1.04	0.72	2.32
House Sparrow	Ploceidae	0.10	0.07	0.16	ND	0.30	0.13	0.34	ND	0.14	ND	0.24	ND	0.28	0.47	0.53	0.57	1.60	1.03
House Sparrow	Ploceidae	ND	0.06	ND	ND									0.07			0.12		
House Sparrow	Ploceidae	ND	0.05	0.06	ND									0.08			1.38		
House Sparrow	Ploceidae	0.16	0.03	0.003	ND									0.11			1.10		
House Sparrow	Ploceidae	0.96	0.64	1.82	0.07									0.62			0.24		
House Sparrow	Ploceidae	1.26	0.39	1.29	0.21									0.55			3.84		
House Sparrow	Ploceidae	3.58	2.23	4.84	1.22									2.18			11.12		
Owl n = 2																			
Horned Owl	Strigidae	0.02	ND	ND	ND	0.05	0.01	ND	ND	0.04	0.002	ND	ND	0.02	0.05	0.03	0.003	0.02	0.12
Horned Owl	Strigidae	0.19	0.02	ND	ND	0.04	ND	ND	ND					0.05	0.03		0.84	0.30	
Starling n = 3																			
Starling	Sturnidae	0.06	ND	0.01	ND									0.05			0.16		
Starling	Sturnidae	0.29	0.01	0.07	ND									0.17			0.57		
Starling	Sturnidae	1.36	0.22	2.80	0.14									0.91			2.99		
Robin n = 4																			
Robin	Turbidae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05	0.38	0.45
Robin	Turbidae	ND	ND	ND	ND									ND			0.33		
Robin*	Turbidae	0.24	0.06	0.35	0.02									0.06			0.53		
Robin****	Turbidae	2.74	0.39	2.65	0.37									1.52			3.14		

No residues found in: Chickadee n=1; Morning Dove* n=1; Goose n=5; Ovenbird n=1; Parula n=1; Pigeon n=5; Thrush n=6; Turkey n=2; Vireo n=1; Warbler n=4; Woodpecker n=3. Notations in Table: * = WNV; ** = EEE; *** = Diazinon; **** = Dieldrin. ND = Not Detected, Blank = No Tissue Available.**

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REFERENCES

Dearth MA, Hites RA (1991) Complete analysis of technical chlordane using negative ionization mass spectrometry. Environ Sci Technol 25:245-254

Extension Toxicology Network, Pesticide Information Profiles; Oregon State University. http://ace.orst.edu/cgi-bin/mfs/01/pips/diazinon.htm?37#mfs (accessed February 2002)

Lötter L, Bouwman H (2001) Organochlorine pesticide residues in helmeted guineafowl (*Numida meleagris*), South Africa. Bull Environ Contam Toxicol 66:162-170

Mattina MJI, Iannucci-Berger W, Dykas L (2000) Chlordane uptake and its translocation in food crops. J Ag Food Chem 48:1909-1915

McMahon BM, Wagner RF (1994) Pesticide analytical manual volume I, 3rd Edition, U.S. Department of Health and Human Services, Public Health Service Food and Drug Administration, Washington D.C.

Okoniewski JC, Novesky E (1993) Bird poisonings with cyclodienes in suburbia: links to historic use on turf. J Wildl Manage 57(3):630-639

Pylypiw HM, Naughton E, Hankin L (1991) DDT persists in soil: uptake by squash plants. J Dairy Food and Environ Sanit 11:200-201

Pylypiw HM (1993) Rapid gas chromatographic method for the multiresidue screening of fruits and vegetables for organochlorine and organophosphate pesticides. J AOAC Int Ed 76:1369-1373.

Pylypiw HM, Misenti T, Mattina MJI (1997) Pesticide residues in produce sold in Connecticut 1996. Bulletin 940. The Connecticut Agricultural Experiment Station, New Haven, CT

Stansley W, Roscoe DE (1999a) Food chain aspects of chlordane poisoning in birds and bats. Soc Environ Toxicol Chem 20th Annual Meeting, Philadelphia, PA, USA, 14-18 Nov 1999, Abstract Book SETAC, Pensacola, FL, p 206

Stansley W, Roscoe DE (1999b) Chlordane poisoning of birds in New Jersey, USA. Environ Tox and Chem 18(9):2095-2099

U.S. Public Health Service Hazardous Substance Data Bank (1995) Washington, DC, p 5-9