MODULE 4 SPECIAL STUDIES

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Monitoring Pesticide and PCB Exposure in Birds Found Dead or Presenting to Rehabilitators

Report to Maine DEP SWAT Program

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Executive Summary

In Maine, little study of pesticides in birds has been conducted, despite past evidence of organochlorine burdens in marine life and current use of highly toxic pesticides in agricultural settings. Wildlife organizations, rehabilitators and state agencies frequently receive calls reporting dead or injured birds. This provides an opportunity to conveniently and economically sample a diversity of species from a broad geographic range. Further, birds that are injured or otherwise debilitated may be more likely than healthy birds to have underlying contaminant burdens that predisposed them to injury or disease.

In this study, 135 dead birds were collected between July 2004 and July 2005. Fifty-four of these were tested for exposure to organophosphates and carbamates, 28 were tested for organochlorine pesticides, and 8 of these were additionally tested for PCBs. There was no evidence of organophosphate or carbamate exposure in the birds of this study. More focused testing of birds in habitats downstream of agricultural lands will be required to assess the importance of these pesticides to birds.

DDE was the highest OC found in the present study. Overall, while OC levels in the birds of this study are below sublethal ranges, values are elevated in some individuals. Two individuals (double-crested cormorant and hooded merganser) had DDE levels within the range of those associated with egg-shell thinning in double-crested cormorants. In addition, levels are similar to those found to impair immune response in herring gulls and glaucous gulls. Immune effects, even if small, may contribute to the entire suite of stressors that negatively affect populations.

Future study focused on these species could look at biomarkers of immune response relative to OC loads. Such a study could not only provide information on OC effects, but also be used as a screen to identify populations that might be exposed to other immune-modulating contaminants, such as polybrominated diphenyl ether (PBDE), methylmercury, polycyclic aromatic hydrocarbons (PAH), and some pharmaceuticals.

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1. Introduction

Birds play an important role as sentinel species for contaminants in the environment, and are extremely sensitive indicators for certain classes of pesticides, namely the organochlorines (OC) and cholinesterase-inhibiting organophosphates (OPs) and carbamates (CBs). The most notorious of the organochlorine pestic ides is DDT which with its metabolites, is well known for its effects on reproduction and egg-shell thinning. Thresholds for OCs and toxic effects have been established in many species for determination of lethality and reproductive effects, and serial monitoring of OC levels in individual ecosystems can be used to monitor ongoing OC burden and assess population risks. Lethal and reproductive effects are associated with high body burdens of OCs, however recent study has drawn attention to immune effects of lower levels of OCs in birds, with potentially significant implications on health (Fowles 1997).

A second class of pesticides that is highly toxic to birds is the cholinesterase inhibiting organophosphates (OP) and carbamates (CB). Widespread use of OP and CB insecticides has been implicated in the morbidity and mortality of numerous species of birds, which are highly sensitive to toxicity from these pesticides. In Maine, OPs and CBs are used heavily in apple and blueberry agriculture, and sufficient evidence exists to suspect agricultural OP/CB use has measurable impacts on birds in Maine. Recent study by Mineau (*in review*) ranks apple and blueberry agriculture 5th and 24th, respectively, in terms of the lethal risk to birds. This ranking system accounts for relative acreage devoted to each crop on a national scale, thus Maine's risk from blueberries is increased relative to other states. According to Mineau, risk posed by blueberry farming is growing due to increasing intensity of treatment and use of more highly toxic OPs such as phosmet, diazinon and azinphos-methyl. Avian impact from OPs has been detected in birds in habitats downstream from agricultural lands, and sublethal exposure can cause debilitation and disease. In black-crowned night herons, normally beneficial nest beetles feed opportunistically on live tissue of chicks made lethargic by pesticide exposure (Parsons et al. 2000).

2. Survey Purpose

In Maine, little study of pesticides in birds has been conducted, despite past evidence of organochlorine burdens in marine life and current use of highly toxic pesticides in agricultural settings. The project reported upon herein represents a survey of pesticide exposure among injured or found-dead birds brought to rehabilitators. Use of injured and diseased birds allowed for economic sampling of a diversity of species and geographic locales for the purpose of identifying populations potentially at risk. Further, we suspected that birds that are injured or otherwise debilitated might be more likely to have elevated contaminant burdens that could have increased their susceptibility to trauma or disease.

Objectives of this survey were to:

1. Identify avian populations at risk for pesticide impacts by establishing baseline indices for multiple species across a diverse geographic range, and

2. Assess the utility of avian incident monitoring for detecting poisoning events associated with OP/CB pesticides.

3. Methods

Sample collection A total of 135 dead birds were collected between July 2004 and July 2005 from various locations via collaborating entities serving as freezer depot sites. Birds were either found dead by the public and brought to one of these freezer depots pending collection, or else died or were euthanized at a wildlife rehabilitation center and were submitted for the study. Birds were either necropsied before freezing at -30^{0}C or frozen immediately and decapitated and thawed (with heads left in freezer storage) for necropsy. All birds were necropsied and gross changes recorded. Cause of death was established based on a combination of clinical history and post-mortem findings. Location by town, and in sometimes street address, where the bird was found is known for 94 of these.

Cholinesterase assays Heads were moved to -70^{0}C within a month of initial freezing, for storage pending cholinesterase analysis. Heads were shipped on dry ice to the testing lab. Brain cholinesterase was assayed using a modified Ellman technique, with 2-PAM reactivation in approximately 30% of samples (Ellman et al. 1961, Gard and Hooper 1993).

Organochlorine Scan During necropsy, livers were collected in either Teflon sheets or chemical free jars (I-Chem, division of Nalge, New Castle, Delaware 19720) and stored at -20^{0}C pending analysis. Samples were tested in two batches. The first batch was sent to Mississippi State Chemical Laboratory where samples were assayed for organochlorines and total PCB. This scan included HCB, alpha BHC, gamma BHC, beta BHC, delta BHC, Oxychlordane, Heptachlor Epoxide, gamma Chlordane, trans-Nonachlor, Toxaphene, Aroclor – 1242, Aroclor – 1248, Aroclor – 1254, Aroclor – 1260, Total PCBs as Aroclors, o,p'-DDE, alpha Chlordane, p,p'-DDE, Dieldrin, o,p'-DDD, Endrin, cis-Nonachlor, o,p'-DDT, p,p'-DDD, p,p'-DDT, and Mirex. The second batch of samples was sent to the University of Pennsylvania New Bolton Center. There samples were assayed for organochlorine s only. The scan included alpha BHC, Lindane, beta BHC, Heptachlor, Aldrin, Oxychlordane, Heptachlor epoxide, alpha Chlordane, p,p'-DDE, Dieldrin, Endrin, p,p'-DDD, p,p'-DDT, and Methoxychlor. Summary statistics (means and ranges) were determined with non-detect values substituted with one half of the detection limit (Stout and Trust 2002). All results except those for some great blue herons, double-crested cormorants and an osprey had minimum detection limit of 0.020ug/gm (ppm). Samples from six great blue herons, two double-crested cormorants and one osprey were sent to a different lab, where minimum detection limit was 0.010ug/gm.

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4. Results

Primary cause of death or injury (leading to euthanasia) was assigned as unknown trauma (n=47), fishing gear (n=4), trash obstruction (n=2), power line (n=3), collision with vehicle (n=8), shot (n=5), other foreign body (n=2), attack by children (n=1), and other (including undetermined; n=63).

4.1.Cholinesterase

Fifty-four water birds were tested for inhabitation of brain cholinesterase. Significant species differences exist for normal cholinesterase levels and normal reference values have been published for only five species we tested. These are double-crested cormorant, black duck, mallard, herring gull, and ring-billed gull. For other species we established normals by calculating means and looking for values falling greater than 40% below the mean.

4.2. Organic Compounds

Twenty-eight birds were screened for organochlorine pesticide residues (Table 1). These birds were sampled from diverse locations across Maine (Figure 1). DDE was the most commonly occurring OC and was detected in 24 birds. DDE values ranged from undetectable to 3.45ug/gm wet wt., and PCB values ranged from 0.077-2.6ug/gm wet wt. DDE was analyzed by ANOVA for relationship to body condition, sex and age. Birds were scored 1-3 based on pectoral mass, with 1 being emaciated, 2 being moderately thin and 3 being normal. PCB but not DDE was negatively associated with body score, with significantly higher levels in birds with a body score of 1 than 3 ($p=0.04$). There was no difference in PCB or DDE levels between sexes or between first year and older birds. However, first year birds had among the highest OC levels, with the two highest PCB levels found in juvenile great blue herons.

The three highest DDE leve ls were found in a juvenile double-crested cormorant in South Portland (5/27/05), an adult hooded merganser in Nobleboro (6/16/05), and a hatchling osprey in Nobleboro (6/21/05). The three highest PCB levels were two juvenile great blue herons found in Boothbay Harbor and Greene in 9/29/04 and 10/11/04, respectively, and a juvenile double-crested cormorant found in Portland in November 2004.

There was no relationship between DDE or PCB levels in relation to body score. There was no evidence that species feeding lower on the trophic scale (e.g. mallard and wood duck) had lower contaminant burdens, though the numbers were very small.

5. Discussion

DDE was the highest OC found in the present study. Of the DDT metabolites, this is one of the most toxic and extensively studied (Blus, 1996). Overall, while OC levels in the birds of this study are below sublethal ranges, values are elevated in some individuals. Several individuals had DDE levels within the range of those associated with egg-shell thinning in double-crested cormorants (Custer et al. 2000). In addition, levels are within the range of those found to impair immune response. DDE levels in herring gull eggs between 0.6 to 7.4 ug/gm are positively associated with heterophil and lymphocyte counts (Grasman et al. 1996, 2000), as are blood DDE levels in glaucous gulls averaging 0.062 ug/gm (Bustnes et al. 2004). PCBs are also known to influence a variety of immune responses (Fowles et al. 1997). In the present study, two individuals had liver PCB levels (2.1ug/gm, 2.6ug/gm) that approach the level (3.5 ug/gm) shown to be associated with elevated heterophils and lymphocytes in glaucous gulls (Bustnes et al. 2004).

Species-specific comparisons with previously published studies of herring gulls and double-crested cormorants show overlapping levels of PCBs and DDE levels. Herring gulls collected in 1966 from Lincoln, Sagadahoc, and Washington counties by Wiemeyer et al. (1978) had DDE and PCBs concentrations ranging from 1.7-5.9ug/gm and 2.8-17ug/gm, respectively. Herring gulls collected from the Bay of Fundy in the Gulf of Maine area had mean liver concentrations of DDE of 2.08ug/gm and liver PCB concentrations of 6.50ug/gm (Zitko and Choi 1972; Zitko and Hutzinger 1972).

Double-crested cormorants collected from the Bay of Fundy in the Gulf of Maine area had mean liver DDE of 4.16ug/gm and liver PCB of 2.13ug/gm (Zitko and Choi 1972; Zitko and Hutzinger 1972). Double-crested cormorants collected from Muscongus Bay in 1966, contained tissue concentrations of DDE ranging from 1.5ug/gm in the brain to 6.5ug/gm in gonads (Kury 1969). In 1967 Kury (1969) found mean DDE concentrations of 0.34ug/gm in brain. In that study, researchers concluded exposure to the pesticides occurred in some other area, possibly its wintering grounds in Florida. In the present study, juvenile birds are among those with the highest contaminant levels, suggesting exposure is significant in Maine.

There was no evidence of OP/CB exposure in the birds of this study. Behavioral effects from these compounds can be seen at sublethal doses, and typically these are associated with 40% or greater reduction of enzymatic activity and 2-PAM reactivation (Walker 2003). These levels were not seen in the present study, however our findings should not be taken to assume no exposure in Maine. At the same time, it can be concluded that avian incident monitoring is not a sensitive tool for detecting effects from this class of pesticides. More focused testing of birds in habitats downstream of agricultural lands will be required to assess the importance of these pesticides.

OC levels in this study were potentially high enough in several individuals to cause immune effects, which, even if small, may contribute to the entire suite of stressors that negatively affect populations. Future study focused on these species in which values were elevated could look at biomarkers of immune response relative to OC loads. Such a study could not only provide information on OC effects, but also be used to identify populations that might be exposed to other immune-modulating contaminants, such as polybrominated diphenyl ether (PBDE), methylmercury, polycyclic aromatic hydrocarbons (PAH), and some pharmaceuticals.

Great blue herons and double-crested cormorants were among those with elevated contaminant levels, which is not unsurprising given their location high on the food web. Previous study has shown higher OC exposure among great blue herons and double-crested cormorants in estuarine vs. non-estuarine habitats, presumably due to greater bioaccumulation rates due to particulate deposition from watershed and higher biomagnification rates due to species differences at lower trophic levels, as well as past agricultural use (Harris et al. 2003). For this reason, estuarine populations should be targeted to maximize detection of bioaccumulative compounds.

There are several limitations to the present study. First, comparisons based on location, species and sex are difficult due to small sample size, and pooling data among species prevents statistical determination of inter- or intraspecific variability of contamination levels. Additionally, some of the study birds were thin, causing artifact of at least elevated PCB levels. PCB and DDE concentrations are influenced by body condition, with higher concentrations found in birds with low fat scores (Wienburg and Shore 2004). It is likely DDE levels in thin birds were also elevated as artifact this study, though this was not

statistically significant. Bustnes (2003) found in glaucous gulls changes in body condition and amount of blood lipids were of lesser importance than trophic level. Finally, birds were collected in different seasons, so that while season is unlikely to significantly affect contaminant levels, increased movement of birds in late summer and early fall prevents locating source of exposure.

6. Summary

Overall, while OC levels in the birds of this study are below lethal ranges, values are elevated in individuals of some species (double crested cormorant, great blue heron, hooded merganser, and osprey). Further study of those species is warranted to detect immune effects of documented organochlorines, as well as to screen for other immunomodulating contaminants of concern in Maine. To measure impact of agricultural OP/CB use, more focused study of birds inhabiting waters adjacent to agricultural lands is recommended.

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Figure 1. Origin of birds screened for organochlorines.

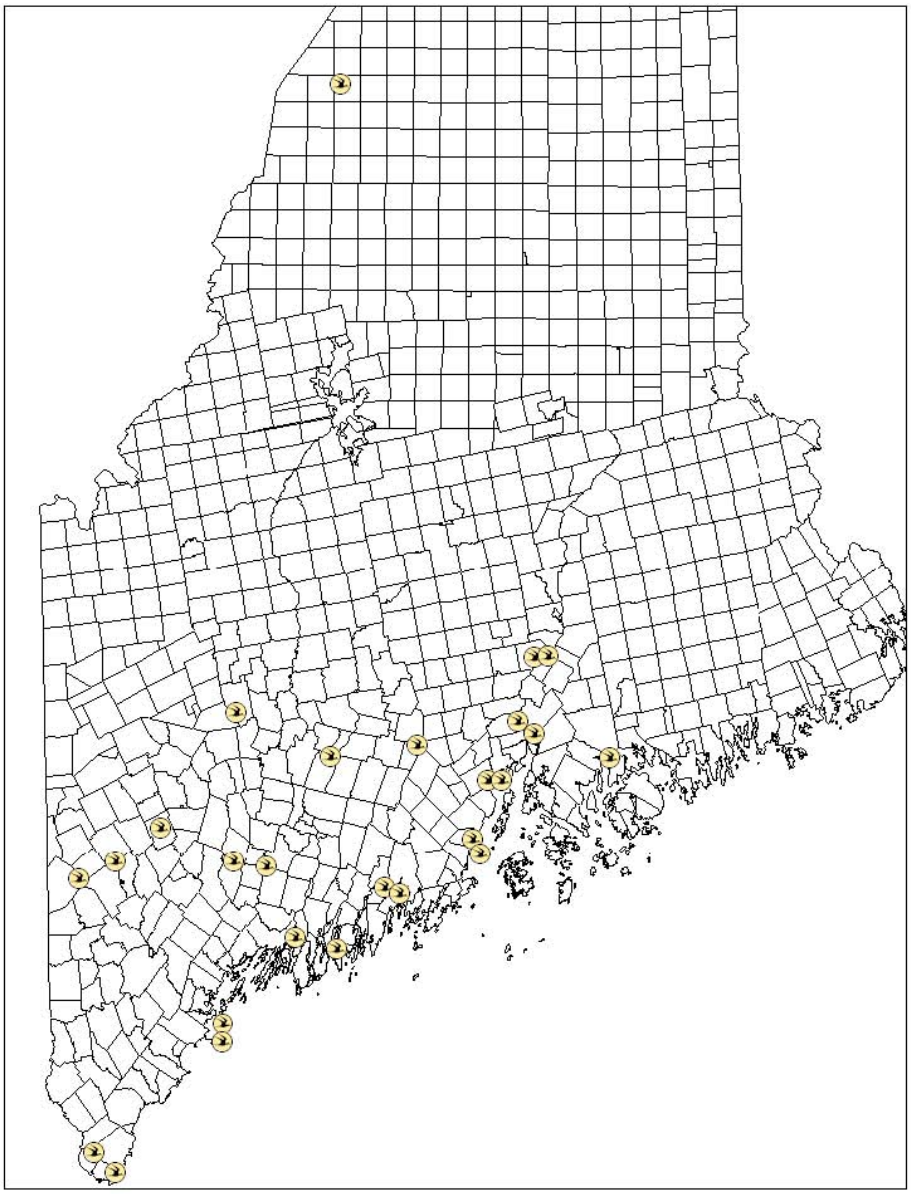

Table 1. Hepatic OC concentrations (mean (range), ug/gm wet wt). N/A=not assayed . Values preceded by “<” are below the method detection limit.

	uck		**led**	**n**	**n**	**ser**
Bs						
ne						
ordane		0.039)				
		.435)			.633)	
hlor epoxide	=1	=2	=1	=1	=3	=1

		lue heron	**crested cormoran**	**billed murre**	**gull**
Bs	1	.077-2.6) n=6	63-1.4) n=2		
	<0.010-0.024) n=3	0.010-0.010) n=6	=4	=1	=4
hlordane	N=3	=6	=4	=1	=4
ordane	N=3	0.010-0.027) n=6	0.010-0.014) n=4	=1	=4
	0.023-1.450) n=3	.012-0.690) n=6	.110-2.161) n=4	1	.052-0.776) n=4
	N=3	=6	=4	=1	=4
hlor epoxide	N=3	0.010-0.012) n=6	=4	=1	=4

*includes nestling

4.2 PCB LABORATORY COMPARISONS

The Maine Center for Disease Control (MCDC, formerly Maine Bureau of Health) issued several fish consumption advisories for various rivers in Maine and for striped bass and bluefish in general based wholly or partially on high levels of PCBs found from the SWAT program. Over the years samples have been analyzed by several different labs, often with different results, although each had a quality assurance (QA) plan and met the requirements. To determine if the current lab used by DEP, Pace Analytical Services (PAS) was providing credible PCB data, 5 samples were split and sent to PAS and the Geochemical and Environmental Research Group lab at Texas A&M University (TAMU), a lab approved for use for these analyses by the US Fish and Wildlife Service. The samples were to be analyzed by high resolution gas chromatography mass spectrometry (HR GC/MS).

Equipment issues at TAMU delayed HR analyses, and in the interim, preliminary low resolution (LR GC/MS) results were delivered to DEP. The results were biased low compared to the PAS results, but within the 30% relative percent difference (RPD) allowed by the QA plan (Table 4.2.1). HR results were biased high but much closer to the PAS results than were the LR results and well within the RPD required. These results indicate that the results from PAS are likely reasonably accurate.

Table 4.2.1. 2004 PCBs in striped bass and bluefish from Maine rivers

STATION	SPECIES	PAS PCB ppt	TAMU PCB LR ppt	RPD %	PAS PCB ppt	TAMU PCB HR	RPD %
Androscoggin R at Brunswick	STB	201			201		
	ARB-STB-03	226	183	**21.0**	226	**243**	**-7.2**
Kennebec R at Augusta	STB	170			170		
	KAG-STB-02	208	151	**31.8**	208	226	**-8.3**
Penobscot R at Orrington	STB	211			211		
	PBO-STB-04	201	140	**35.8**	201	215	**-6.7**
Royal R at Yarmouth	STB	152			152		
York R at York	STB	147			147		
	YRY-STB-05	104	84.5	**20.7**	104	123	**-16.7**
Old Orchard Beach	BLF	161			161		
	OOB-BLF-01	154	109	**34.2**	154	174	**-12.2**
AVE				**28.7**			**-10.2**

PCB LR= low resolution GC/MS
PCB HR= high resolution GC/MS

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