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POLYCHLORINATED BIPHENYL RESIDUES AND EGG MORTALITY IN DOUBLE-CRESTED CORMORANTS FROM THE GREAT LAKES

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Abstract—We evaluated the overall potency of polychlorinated biphenyl (PCB)-containing extracts from double-crested cormorant (*Phalacrocorax auritis*) eggs with an in vitro bioassay system, the H4IIE rat hepatoma cell bioassay. Results from the H4IIE bioassay were strongly correlated with the hatching success of eggs in the colonies, whereas conventional methods of PCB analysis correlated poorly with hatching success of eggs from the same colonies. These observations suggest that even though concentrations of total PCB residues have declined in almost all compartments of the environment, their effects are still being observed. The significance of this observation is that the adverse symptoms presently observed in certain Great Lakes fish-eating waterbird populations do not appear to be caused by some as yet unidentified industrial chemical or chemicals and seem not to be the result of pesticides, but rather to the dioxin-like activity of PCBs. Evidence is presented to suggest that the relative enrichment of the potency of PCBs in the environment may play a role in the persistence of the observed adverse symptoms.

Keywords—PCBs TCDD equivalents Reproduction Double-crested cormorant *Phalacrocorax auritis*

INTRODUCTION

Over the past three decades, populations of fish-eating birds in the Great Lakes of North America have exhibited greater than expected mortality rates in chicks and embryos, altered reproductive behavior, and elevated incidences of teratogenesis [1–3].

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Individual birds within these populations have had gross physical and histopathological abnormalities. Although there are many factors that contributed to the symptoms observed in fish-eating bird populations of the Great Lakes, bioaccumulation of persistent toxic chemicals is believed to be a significant factor [4].

Identification of a single contaminant as the causative agent for the observed symptoms has been difficult due to the wide variety of contaminants present in these birds. Persistent agricultural pesticides, such as 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) and its degradation product 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethene (*p,p'*-DDE), and industrial chemicals, such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDGs), are present at elevated concentrations in Great Lakes food chains [5]. However, the concentrations of these chemicals have declined in recent years in most compartments of the Great Lakes aquatic ecosystem [6]. Residual concentrations of DDT and its environmental metabolites, the agents responsible for eggshell thinning and related mortalities of many fish-eating waterbirds, have decreased substantially to an apparent steady state in Great Lakes biota [6]. Concentrations of PCDDs, which are thought to have been responsible for "chick edema" disease in herring gulls from Lake Ontario, have also declined [7]. Similarly, concentrations of total PCBs in some compartments of the food chain are currently 10 to 100 times less than they were in the mid-1960s. However, the adverse symptoms observed in populations of some fish-eating waterbirds have persisted [1–4,7–8].

Great Lakes fish-eating waterbirds exhibit a variety of symptoms similar to those observed when avian species are exposed to planar halogenated hydrocarbons (PHHs) under laboratory conditions [8–14]. PHHs are purported to have the same receptor-mediated mode of action, and the general symptoms of PHH toxicity include altered biochemical homeostasis, gross anatomical deformities, and the reproductive effects of fetotoxicity and teratogenesis [15]. Individually these symptoms could be caused by a variety of chemical, physical, or biological agents, but collectively the same suite of toxic effects are seen across phyla when organisms are exposed to PHHs [16]. Quantitative structure–activity relationships have been demonstrated for PHHs, with the most potent isostereomer in all tested species being 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [15–18].

Evaluation of the toxicological impact of the complex mixtures of PHHs that occur in the environment is difficult due to a number of factors. PHHs are released from a number of regional industrial and urban activities within the Great Lakes basin and are also transported to the region from more remote sources by atmospheric transport [19]. Due to their different chemical structures, the individual PHHs exhibit a wide range of chemical properties and toxicological potencies. Differences in relative volatilities, solubilities, energies of absorption, and rates of degradation cause the relative concentrations of individual congeners to vary both temporally and spatially [20–22]. Recent evidence suggests that there may be selective enrichment of certain PCB homologues and specific congeners in sediments and the aquatic food chain of the Great Lakes [23–25]. The alterations in the relative ratios and patterns of PHH residues in environmental samples further complicate the interpretation and evaluation of their toxicological effects, because the individual PHH isosteres have such a wide range of toxicological potencies and they are known to interact with one another [15–18]. These interactions have been variously observed to be additive, synergistic, and/or antagonistic, depending on the specific PHHs tested and their relative concentration ratios [18,26].

Routinely, only total concentrations of PCBs are measured by comparison to commercial technical mixtures as reference standards [27]. This method does not account for different potencies of the individual PCB congeners, the changes in their composition from the technical mixtures, or the interactions that are known to occur among the congeners. Such measurements may be a significant factor in explaining why concentrations of PCBs in fish and wildlife have not been highly correlated with toxicological symptoms, even though qualitatively the symptoms of PHH-like poisoning are greater in those areas of greater contamination [14,28], which in turn has also led to some doubt as to the role of PCBs in the observed adverse effects. A gradient of biological response and PHH chemical contamination has not been previously established in the Great Lakes.

The H4IIE bioassay has been utilized by various researchers to evaluate the relative potency of complex PHH mixtures in environmental samples [29–31]. The H4IIE rat hepatoma cells have the characteristics of low basal P450IA1-associated catalytic activity, yet they are inducible by PHHs [32]. In vitro induction potency of various PHH congeners toward this cell line is correlated with in vivo potency for the toxic end points of body weight loss and thymic atrophy in rats [33]. The

synergistic, antagonistic, and additive interactions that occur in vivo among PHHs may be accounted for by the H4IIE bioassay system. Thus, we used the H4IIE cell line as a biochemical detector system to integrate the effects of active PHHs into a matrix of less active PHHs. Our hopes were that this system would provide a more proximate and predictive measure of toxic potency of PHHs than that obtainable by chemical analysis alone.

Therefore, the purpose of this study was to investigate the relationship between PHHs and adverse symptoms observed in populations of fish-eating birds of the Great Lakes. In particular, we evaluated the overall potency of PCB-containing extracts of double-crested cormorant eggs (*Phalacrocorax auritis*) with the H4IIE rat hepatoma cell bioassay system. Results of enzyme induction in the bioassay were then compared with reproductive success of the cormorant colonies from various regions of the Great Lakes. Additionally, as a measure of environmental enrichment of PCB mixtures we compared the relative potency of PCB residues from the double-crested cormorant eggs with the relative potency of PCB technical standards.

MATERIALS AND METHODS

Double-crested cormorants from 11 colonies representing five regional areas around the Great Lakes and a reference site outside the Great Lakes (Table 1) were monitored for hatching success in 1986, 1987, and/or 1988. Initial visits were designed to census and map the colonies, select nests to be observed (25–50 nests, except where the entire colony was monitored), collect eggs for residue analysis (6–12 eggs per colony), and mark nests and eggs within the experimental zone. We used pie-shaped test areas, which included a portion of the outer border and centered on the middle of the colony so as to normalize edge effects where predation on eggs by gulls could be the greatest. This first visit was staged at a time that 85 to 90% of eggs from the first nesting attempt had been laid. Eggs were weighed, placed in separate solvent-rinsed jars, and stored at −10°C until they were extracted for chemical analyses. There were generally three subsequent visits to the colonies at 10- to 14-d time intervals. Egg viability, hatching success, eggs lost to predation, and new nesting attempts were monitored and recorded. Slight modifications of the above protocol were followed at the Spider Island colony, Green Bay, Lake Michigan. At this site, one egg, randomly selected from each of 64 marked nests, was collected and frozen for subsequent chemical analysis of the composited sample. Thirty-nine of the marked nests were monitored completely through incubation. Hatching success at Spider Island was measured as a percentage of those eggs laid, and no attempt was made to iden-

Table 1. Regional locations, collection years, colony, total PCB, and TCDD-EQ in double-crested cormorant eggs

	Egg mortality (%)	Total PCBs (μg/g)	(SD)	H4IIE TCDD-EQ (pg/g)	(SD)
Green Bay, Lake Michigan					
1986 Little Gull Island	32	14.8	(0.1)	344	(26)
Snake Island	22	10.8	(0.7)	201	(13)
1987 Gravelly/Little Gull islands	39	12.3	(0.6)	299	(32)
1988 Spider Island	37	5.3	(0.3)	344	(36)
North central Lake Michigan					
1986 Hat Island	30	8.1	(0.6)	248	(16)
Pismire Island	25	6.8	(0.1)	95	(8)
West Grape Island	23	6.6	(0.7)	157	(9)
Northwestern Lake Huron					
1986 St. Martin's Shoal	21	0.1	(<0.01)	85	(6)
1987 St. Martin's Shoal	24	9.8	(0.6)	206	(16)
Southeastern Lake Superior					
1986 Tahquamenon Island	24	9.0	(0.2)	192	(14)
1987 Tahquamenon Island	26	4.4	(0.2)	103	(6)
Lake Ontario					
1988 Pigeon Island	27	5.5	(0.5)	217	(20)
Lake Winnipegosis, Manitoba, Canada					
1988 Hay Reef	8	0.8	(0.1)	35	(3)

tify eggs lost to predation or other potential types of noncontaminant related losses.

Composites of 5 to 12 eggs were extracted [34], and the extracts were used to dose the H4IIE cells as previously described [35–36]. The extraction procedures used in these studies contained a Florisil gel cleanup step and resulted in an extract that contained PCBs without PCDDs or PCDFs [37–39]. Ethoxyresorufin-*O*-deethylase (EROD) induction potency by the PCB-containing extracts in the H4IIE cells was calibrated against a TCDD standard dose–response curve to determine TCDD equivalents (TCDD-EQs) in the samples. Total concentrations of PCBs in the samples were analyzed by GC/electron capture detector and quantitated as described previously [27].

RESULTS AND DISCUSSION

Concentrations of H4IIE bioassay–derived TCDD-EQ in cormorant eggs were in the range of 35 to 344 pg TCDD-EQ/g wet weight, whereas total PCBs ranged between 0.05 and 14.8 μg/g wet weight (Table 1). Mortality of cormorant eggs was between 8 and 39%. There was a significant correlation ($p = 0.045$) between total concentrations of PCBs in composites of cormorant eggs from a colony and egg mortality rates from the same colony, as would be expected if PCBs were exerting an influence on hatching success (Fig. 1). However, the correlation between total concentrations of PCBs and egg mortality rates had a coefficient of determination (r^2) of only 0.319; thus, much of the variance in egg mortality was unexplained and prediction of effects from this general linear model would have been inaccurate. In contrast, not only was the correlation between concentrations of H4IIE bioassay–derived TCDD-EQ in cormorant egg composites and egg mortality rates from these colonies statistically more significant ($p \leq 0.0003$), but also the total concentration of TCDD-EQ was a much better predictor of egg mortality rates ($r^2 = 0.703$) than the total concentration of PCBs (Fig. 2). Therefore, there is an apparent dose–response relationship between the bioassay-derived TCDD-EQ in the cormorant eggs and egg mortality rates at these colonies, which supports the hypothesis that these dioxin-like compounds cause reproductive impairment in double-crested cormorants in the Great Lakes [3–4]. A relationship such as this had not been established previously.

The relative potency of PCB residues in the cormorant eggs was three- to fourfold greater than that of PCB technical standards when tested in the H4IIE bioassay (Fig. 3). The bioassay-derived potencies of PCB-containing extracts taken from the cormorant eggs had a mean value of 28.0 pg TCDD-EQ/μg PCB. Patterns of relative concentrations of PCB congeners in environmental sam-

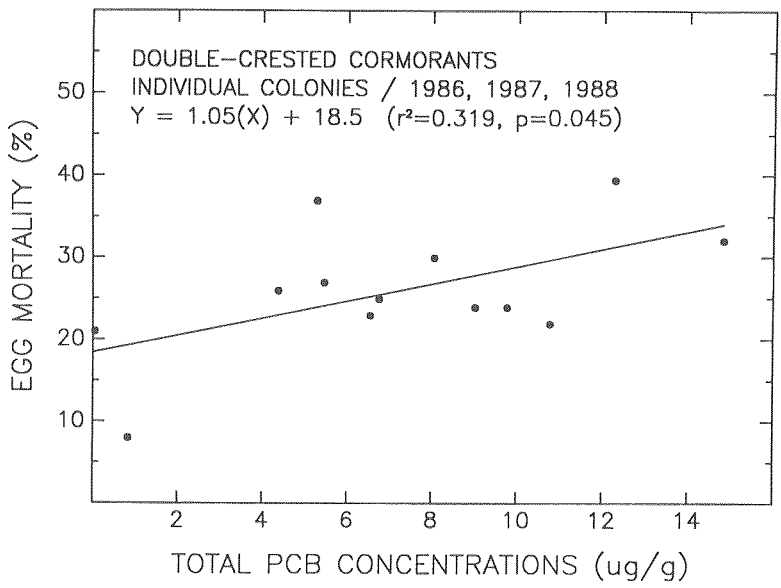

Fig. 1. Correlation between concentrations of total PCBs in double-crested cormorant eggs and egg mortality rates from various Great Lakes colonies.

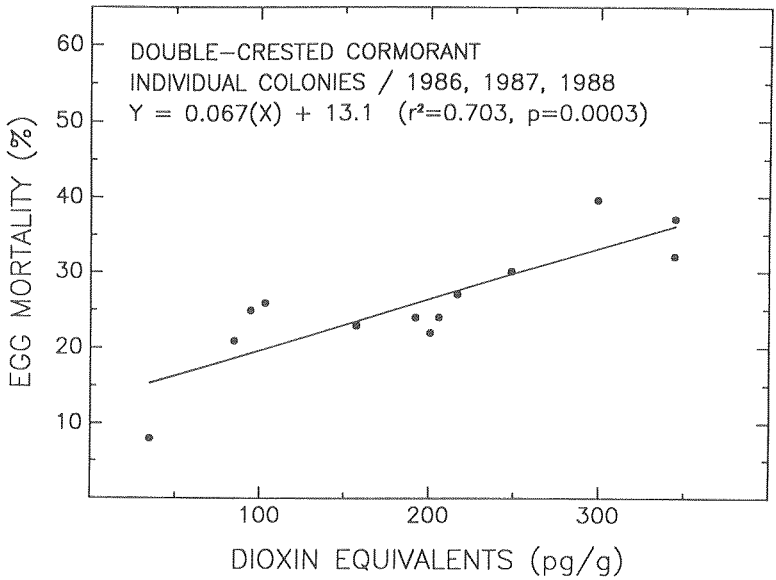

Fig. 2. Correlation between concentrations of H4IIE bioassay-derived TCDD-EQs in double-crested cormorant eggs and egg mortality rates from various Great Lakes colonies.

ples are often different from technical standards [20–22], and selective enrichment of some of the more toxic PCB congeners has been demonstrated in a few cases [40]. However, the interpretation of this information has been unclear due to the multiplicity of toxic interactions known to occur among various PHHs. This is the first clear demonstration of the relative enrichment of the overall potency of PCB mixtures in the environment. The fact that the mixture of PCB residues in the cormorant eggs is three- to fourfold more potent in the H4IIE bioassay than it is in the PCB technical

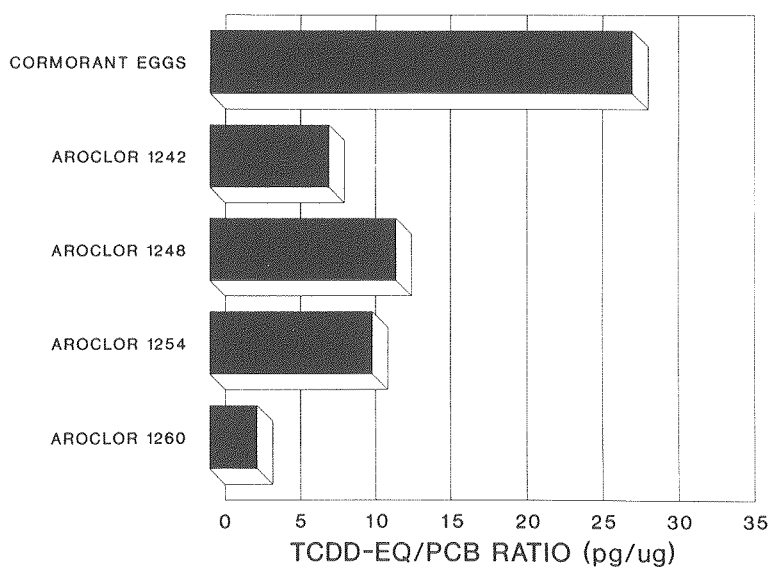

Fig. 3. H4IIE bioassay-derived TCDD-EQs (normalized to total PCB concentration) in double-crested cormorant eggs and technical Aroclor standards.

standards may offer a suggestion as to why the adverse symptoms in Great Lakes waterbirds have persisted whereas the total concentrations of PCBs have decreased. It is still unknown whether the enrichment of PCB mixtures demonstrated here is a matter of food chain enrichment, changes in source patterns, the inclusion of yet unidentified dioxin-like compounds in the egg extracts, or a combination of these effects. The inclusion of yet unidentified coextractants within the tested fraction that have significant dioxin-like activity is a possible reason for the observed enrichment. The complete residue characterization required to confirm or deny the presence of such compounds was not included in our investigations. However, it is known from previous characterizations that the extraction protocols used in this study result in an extract that contains PCBs, without PCDDs or PCDFs [37–39]. Additionally, there were no significant spatial differences in the PCB-normalized relative potencies detected in these samples from five Great Lakes locations, suggesting that the enrichment process is a phenomenon related to the species and not the source of contaminants.

The significance of the relationship between H4IIE bioassay-derived TCDD-EQ and reproductive success in cormorants has implications for three aspects of the environmental contamination issues in the Great Lakes. First, it suggests that even though concentrations of total PCBs and other persistent compounds have decreased in virtually all compartments of the environment, it appears they continue to elicit adverse effects. Concentrations of PCBs in Great Lakes biota are believed to have reached an apparent steady state, due to continued inputs from long-range atmospheric transport and internal recycling, and they are not expected to decrease significantly for some time [6].

Second, it appears from this work that the reproductive symptoms reported in cormorants from the Great Lakes are due to PHHs, and largely PCBs, but not pesticides. In the 1960s and 1970s concentrations of both PCBs and *p,p'*-DDE in cormorants and other compartments of the Great Lakes environment were much greater than they are now. The decline of double-crested cormorant populations seen during this period was largely attributed to eggshell thinning by *p,p'*-DDE [1–3]. Mean concentrations of *p,p'*-DDE in cormorant eggs from Lake Michigan colonies from 1977 to 1978 were 3 to 5 ppm (milligrams per kilogram), and these colonies had 7 to 14% eggshell thinning [41]. A concentration of 10 ppm DDE is associated with 20% eggshell thinning, an amount believed to cause severe and possibly complete reproductive failure in cormorants [42]. The great concentrations of DDE found in Great Lakes cormorant eggs during the 1960s and 1970s might have masked the effects of PCBs in those same eggs. Now that concentrations of DDE are much lower and seem to be below the threshold concentration for eggshell thinning, there appears to be expression of the embryotoxic and teratogenic effects attributable to certain PCBs and other PHHs. The H4IIE bioassay-derived TCDD-EQs measured in the cormorant eggs from this study explain a major portion of the variance observed in reproductive success within these colonies. These effects could also be enhanced due to the enrichment of the PCB mixtures to more toxic combinations.

The third implication of this work is that because the extracts tested in the H4IIE bioassay contained PCBs and not PCDD or PCDF-type PHHs, we suggest that PCBs and not PCDDs or PCDFs are the major contaminant influence on cormorant reproductive success in the Great Lakes. A similar conclusion was reached in a study of Forster's tern reproduction on Green Bay, Lake Michigan [14]. Based on chemical analysis and an additive model of toxicity, the planar PCBs accounted for over 90% of the dioxin equivalents in those bird eggs [14].

In conclusion, we have demonstrated a dose-response relationship between PCBs, as measured by the H4IIE bioassay, and mortality of eggs in double-crested cormorants in the Great Lakes. We also clearly have demonstrated an increased potency of PCBs taken from double-crested cormorant eggs as compared to that of PCB technical standards. The ecological significance of our observations is not completely understood. Currently the number of double-crested cormorants in the Great Lakes is increasing, and concentrations of total PCBs have decreased [43–44]. The proportion of egg mortality attributable to PCBs suggests that they are eliciting effects at the organismal level and that similar effects could occur in other species at the top of the aquatic food chain.

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