Accumulation of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Equivalents by Double-Crested Cormorant (Phalacrocorax auritus, Pelecaniformes) Chicks in the North American Great Lakes


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Concentrations of polychlorinated biphenyls (PCBs), and 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQ) were determined in eggs and chicks of double-crested cormorants (DCC) which were collected in 1989 from eight locations in the Laurentian Great Lakes. The mean biomagnification factor (BMF) from forage fish to eggs was found to be 31.3. Absolute and relative concentrations as well as rates of accumulation of total concentrations of PCBs and TCDD-EQ were measurable in all of the samples. The concentrations of both PCBs and TCDD-EQs decreased immediately upon hatching of chicks, due to growth dilution. Initial decreases in absolute masses of TCDD-EQ in chicks were also observed, which indicates that there can be significant elimination of these compounds during early development. The initial rates of accumulation by chicks were dependent only on the mass of fish consumed. After the chicks began thermoregulating, the rates of accumulation, expressed as a concentration, normalized to body weight, became greater. Rates of accumulation of both PCBs and TCDD-EQ were correlated with their respective concentrations in forage fish consumed by the chicks. The relative potency, expressed as the ratio of the concentration of TCDD-EQ to that of total PCBs was calculated to determine if there was significant trophic-level enrichment of the TCDD-EQs, relative to total concentrations of PCBs. A significant enrichment was observed at the more and less contaminated locations, but the degree of enrichment was greater at the less contaminated locations (26 vs 72 µg/g).

INTRODUCTION

Historically, the colonial, fish-eating water birds (CFEWB) of the Great Lakes have been exposed to many toxic, synthetic, halogenated compounds (Gillman et al., 1977; Weseloh et al., 1979, 1983, 1989; Eisler, 1986; Gilbertson et al., 1991). These exposures have been correlated with a number of adverse effects on the reproductive potential of a number of species of birds (Kubiak et al., 1989), which, in turn, caused declines in sizes of populations (Peakall, 1986, 1988; Peakall and Fox, 1987). The most dramatic effect on reproductive performance of wild birds was the result of eggshell thinning, caused primarily by DDE, a degradation product of DDT (Weseloh et al., 1983; Struger et al., 1985; Anderson and Hickey, 1969; Elliott et al., 1988).

Eggs of Great Lakes birds contained many other polychlorinated hydrocarbons (PCH), such as PCBs and polychlorinated dibenz-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) at sufficiently great concentrations to cause adverse effects on the birds and their chicks. Since restriction of the manufacture and use of
many of the persistent PCH, concentrations of these compounds have decreased in the tissues of birds and their eggs (Weseloh et al., 1989) and their food (D'Itri, 1988). Concomitant with these decreases, there have been increases in the populations of most of the colonial fish-eating water birds (CFE WB) on the Great Lakes. Although this trend is encouraging, recent data suggest that the decrease in concentrations of both PCBs and DDE have now leveled off due to internal recycling and continued inputs, primarily from atmospheric deposition (Eisenreich et al., 1981; Arimoto, 1989). Concentrations of PCHs cannot be expected to decrease further very rapidly. In the same time period that concentrations of the routinely measured contaminants were declining effects such as embryo lethality and birth defects persisted in CFE WB, which reproduce on the Great Lakes (Kubiak et al., 1989; Gilbertson, 1988; Gilbertson et al., 1976). These reproductive deficiencies of certain species of CFE WB, in some areas, have been attributed to planar, chlorinated hydrocarbons (pPCH), which are biomagnified by these birds and deposited into their eggs (Kubiak et al., 1989; Gilbertson, 1987, 1988; Gilbertson et al., 1991; Tillitt et al., 1992; Jones et al., 1993a,b). The suite of reproductive anomalies observed includes specific biochemical alterations such as the induction of cytochrome P450 mixed function oxygenase enzymes (MFOs) (Fox et al., 1991; Ludwig et al., 1991), depletion of hepatic reserves of retinoids and vitamin A (Spear et al., 1990), porphyria (Fox and Weseloh, 1987), and wasting syndrome (Ludwig et al., 1993a). These are symptoms of pPCH intoxication (Gilbertson, 1983). These observed effects are similar to those which can be elicited by exposure of birds to pPCH compounds which are similar in structure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) (Cecil et al., 1978; Brunström, 1988; Brunström and Anderson, 1988). In fact, the observed effects are similar to chick edema disease, which can be caused by 2,3,7,8-substituted PCDD and PCDF and structurally similar PCB congeners (Gilbertson, 1983; Gilbertson et al., 1991; Brunström, 1988; Cecil et al., 1974, 1978; Carlson and Duby, 1973). This suite of effects has been named the Great Lakes embryo mortality edema and deformities syndrome (GLEMEDS; Gilbertson et al., 1991).

Even though the concentrations of PCBs measured in eggs of wild birds which live in some areas of the Great Lakes are great enough to cause adverse effects, such as birth defects and embryo lethality, they are poorly correlated with the total concentrations of PCBs and are not correlated with the concentrations of chlorinated hydrocarbon insecticides in eggs (Tillitt et al., 1992). Furthermore, even though the observed effects are similar to those known to be caused by some congeners of PCDDs and PCDFs, the present concentrations of these congeners are not sufficiently great to be solely responsible for the observed effects. Recently, several authors have suggested that a better predictor of effects than total PCBs, PCDDs, and PCDFs could be made from the concentrations of dioxin-like congeners which bind to the aromatic hydrocarbon receptor (Ah-receptor) through which most of their toxic effects are proposed to be mediated (Safe, 1987; Goldstein, 1980; McFarland and Clarke, 1989). When the concentrations of individual congeners are corrected for their relative potency, much better correlations are observed between lethality of embryos and deformities in embryos and in chicks (Ludwig et al., 1991; Tillitt et al., 1992; Yamashita et al., 1993). Similarly, pPCHs have been linked to impairment of reproduction in salmonid fishes (Ankley et al., 1989; Mac et al., 1985) and in mink (Platanow and Karstad, 1973; Aulerich et al., 1986; Hornshaw et al., 1983) in the Great Lakes.
The potency of individual pPCH congeners to cause toxic effects can be compared to that of the most toxic pPCH, which is 2,3,7,8-TCDD, and toxic equivalency factors (TEF) based on several endpoints, including lethality, deformities, or enzyme induction calculated (Jones et al., 1993a; Safe, 1987; Brunström and Andersson, 1988). These TEFs can then be used to calculate the Ah-1 activity contributed by the concentrations of individual congeners. These can be summed and expressed as a total equivalent concentration of 2,3,7,8-TCDD (see Giesy et al., 1993, for a detailed review of this concept as applied to contaminants in birds). This additive model seems to be effective in predicting the potency to cause both enzyme induction and toxicity endpoints (Safe, 1987). However, this simple additive model does not take into account interactions among Ah-active congeners or among Ah-active and non-Ah-active congeners and other toxic synthetic halogenated compounds which are also present in the eggs (Tillitt et al., 1992; Ludwig et al., 1993b; Giesy et al., 1993). Also, complete and accurate instrumental analyses of the individual congeners is difficult and time-consuming. Furthermore, the absence of a number of the necessary reference standards continues to limit the ability of making comprehensive instrumental analyses.

As an alternative to the additive model, which uses toxic equivalency factors (TEFs) and instrumentally determined concentrations of individual congeners, an in vitro bioassay can be used to determine the biological potency of extracts which contain complex mixtures of pPCH congeners (Bradlaw and Casterline, 1979; Tillitt et al., 1991a,b; Giesy et al., 1993). The assay utilizes the capacity of extracts of tissues, which contain pPCH, to induce specific cytochrome P450-requiring mixed function oxygenase enzymes in cultured rat hepatoma cells (H4IIE). Since the ability to induce these P450-requiring enzymes is correlated with the binding of pPCH to the aryl hydrocarbon receptor (Ah-R) (Goldstein, 1980) and is also correlated to the toxic potency of the congeners (Safe, 1987), it serves as an effective, integrative measure of the toxic potency of complex mixtures. The induction of P450-requiring enzymes is known to be correlated with the potency for causing toxic effects, such as weight loss and thymic atrophy in mammals (Safe, 1987) and mortality and birth defects in bird embryos (Walker, 1990; Brunström, 1988; Brunström and Andersson, 1988).

The H4IIE bioassay has other advantages over the use of standard instrumental techniques of analytical chemistry. Therefore, this bioassay measures potency of exposure at a point close to the site of action. In particular, the bioassay is more rapid and less technically demanding than congener-specific chemical analysis of complex environmental pPCH mixtures. Also, the H4IIE bioassay is useful as a data reduction tool since it provides an integrated estimate of the potency of a mixture of pPCH congeners of widely varying toxicities as well as their interactions with other synthetic, organic chemicals (Safe, 1987). In addition, the bioassay is able to detect Ah-1 activity from compounds which may not be analyzed by instrumental methods. The bioassay does not provide information on the cause of the effects and should be used in concert with instrumental analyses. The bioassay is particularly useful for determining whether all of the TCDD equivalents in an extract have been accounted for and whether there are nonadditive interactions among congeners. The bioassay can also be used with fractionation schemes to guide where to apply more rigorous chemical analyses.

To further assess the ecological significance of Ah-receptor-active pPCH, expressed as TCDD-EQ, we have determined their absolute concentrations and masses, relative concentrations, and rates of accumulation by cormorant chicks during growth and development at several sites in the Great Lakes region. The geographical variation in
the distribution and accumulation of TCDD-EQ and their biomagnification factors (BMF) from forage fish to eggs were also determined.

MATERIALS AND METHODS

Eggs and chicks of double-crested cormorant (DCC; Phalacrocorax auritis) and herring gull (Larus argentatus) were collected in the summer of 1989 from the following locations: Tahquamenon Island, Lake Superior; the Apostle Islands, Lake Superior; the Beaver Islands, Lake Michigan; northern Green Bay, Lake Michigan; St. Martin’s Shoal, northern Lake Huron; Thunder Bay, Lake Huron; North Channel, Lake Huron (Fig. 1). Although collections were to be made in Saginaw Bay, the DCC there failed to reproduce in 1989. Therefore, herring gulls were substituted for DCC at this site. Visits to colonies were scheduled to ensure that young of the desired size classes would be available. Live chicks were collected when they were approximately 100, 350, 700-1000, and 1300-1500 g in weight, which represent approximate age classes of 4, 10, 21, and 32 days of age, respectively. Samples consisted of five individual chicks in the desired size class from each colony. Chicks were euthanized by pressure to the trachea and either placed in glass jars and frozen or large individuals were wrapped in foil and either frozen or stored refrigerated until freezing was possible.

Samples were homogenized by repeated passage through a Hobart brand meat grinder (Hobart Mfg. Co., Troy, OH). Before homogenization individual chicks were dissected to remove the stomach contents (principally stones) and beaks and feet were removed to facilitate grinding. After homogenization sample aliquants were placed in acetonethexane-rinsed glass jars sealed with Teflon-lined lids. Samples were stored at −20°C until extraction.

During sample collection freshly regurgitated fish samples were also collected from DCC at each colony. At the end of the collection period the relative abundances of fish species in the diet were determined for each site, based on seasonal records. A single composite sample of regurgitated fish which represented the best field estimate of forage fish species distribution was prepared for determination of TCDD-EQ.

![Fig. 1. Map of sampling locations.](image-url)
Extraction

Extraction of organochlorine compounds for subsequent bioassay was performed according to the methods of Ribbeck et al. (1982) with the following modifications. Samples were dried with four times their weight of anhydrous sodium sulfate and column extracted with 200 ml of methylene chloride. The extract was concentrated to 8 ml, and 5 ml of this was subjected to gel permeation chromatography on a column of Biobeads SX3 (Bio-Rad, Richmond, CA) to remove lipids. The sample was then cleaned on a column of acidic silica gel to remove certain pesticides and residual lipids. However, the fraction used in the assay still contains a number of compounds, such as toxaphene, mirex, chlordane, endrin, and p,p'-DDE.

2,4,6-Trichlorobiphenyl (IUPAC No. 30) was added to each sample before extraction as an internal standard. Previous analyses indicated that, at the doses used, this congener has no effect on the results of the H4IIE bioassay. Extraction efficiency for the procedure has previously been shown to be in excess of 90% for total PCBs (Williams et al., 1992) and 95% for dioxins (Tillitt et al., 1991a; Jones et al., 1993a). Extraction efficiencies for coplanar PCBs are >90% in this procedure (Tillitt et al., 1992). Concentrations of TCDD-EQ in this report have not been corrected for sample-specific extraction efficiencies.

All solvents used in extract preparation were of pesticide grade. Fine biochemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Glassware and equipment used in extract preparation were acetone and hexane rinsed before use.

Bioassay

H4IIE cells were obtained from the American Type Culture Collection (ATCC No. CRL 1548, Washington, DC) and were cultured in Dulbecco's modified Eagle's medium (D-MEM) base (Sigma Cat. No. D5030) and supplemented with 15% fetal bovine serum. Cultures were manipulated and maintained using previously described cell culture procedures (Tillitt et al., 1991a).

Concentrations of TCDD-EQ were measured by the methods described by Tillitt et al. (1991a). Briefly, cells were plated at 0.33 × 10^6 cells/plate in 5-cm-diameter tissue culture plates. After incubation for 24-hr-old cells were dosed with the sample extracts. Cells were exposed to four extract doses, in duplicate, covering three orders of magnitude. Samples were dissolved in isooctane, and the solvent volume was kept constant among doses. A standard dose–response curve for TCDD, against which unknown samples were compared to calculate concentrations of TCDD-EQ, was analyzed simultaneously with the samples. Cells were incubated for 72 hr before harvesting. Cells were harvested by scraping into sucrose-buffered with Tris (Mason et al., 1986) and collected by centrifugation (5200g for 10 min). After resuspension, the protein concentration of each cell suspension was determined by the method of Lowry et al. (1951) by comparison to a bovine serum albumin standard. Cytochrome P450-I1 enzyme activity was measured as ethoxyresorufin-o-deethylase activity (EROD) by the fluorometric method of Pohl and Fouts (1980) on 100 µg of protein from the cell suspension. TCDD-EQ were calculated by comparing the ED₉₀ from individual samples to that for a TCDD standard curve, which was generated at the same time. The potencies of individual extracts were determined according to the method of Sawyer et al. (1984). Variance estimates were calculated according to an additive model of variance (Finney, 1978). The standard deviation (SD) of a given TCDD-EQ
concentration was obtained by multiplying the fractional coefficient of variation by the estimated TCDD-EQ for that sample (Tillitt et al., 1991a). All statistical analyses were performed using the PLOTIT statistical package (Scientific Programming Enterprises, Haslett, MI 48840).

Total concentrations of PCBs were determined by standard methods developed and validated in our laboratory (Williams et al., 1992; Jones et al., 1993b). Quantification was made with a Perkin-Elmer Model PE 8500 gas–liquid chromatograph using a 30-m DB1 fused silica capillary column with 0.25 mm i.d. and 0.25-μm film thickness (J&W Scientific, Folsom CA). Total PCB concentrations were calculated, using the COMSTAR multiple regression analysis program, with PCB congener IUPAC No. 30 as an internal standard (Burkhard and Weininger, 1987).

Quality Assurance and Quality Control

The entire project was conducted under the auspices of a quality assurance officer. A standard operating procedure for the method was prepared and is available from the authors. Blanks of induction by solvents were found to be less than the detection of the assay. The method detection limit was 0.5 ng/kg, w/w. Selected samples were extracted and assayed in duplicate to quantitate assay reproducibility. Duplicate analyses of three samples yielded determinations within 10% of the mean. Reference materials analyzed with each set of samples were within 20% of the median proficiency value.

RESULTS AND DISCUSSION

The concentrations of TCDD-EQ in double-crested cormorant eggs were greatest in Green Bay, exceeded only by concentrations detected in herring gull eggs in Saginaw Bay (Table 1, Fig. 2). Both areas are known to have greater concentrations of pCCHs than most other areas in the Great Lakes (Tillitt et al., 1992). Concentrations of TCDD-EQ were least in eggs from colonies in Lake Superior. The concentrations of TCDD-EQ in herring gulls can be attributed to its year-round residence at the location in addition to the greater concentration of pCCHs at this site (Tillitt et al., 1992). Biomagnification factors (BMF = ratio of concentration of TCDD-EQ in bird eggs to that in forage fish were calculated (Table 1). These values ranged from a minimum of 11.7 at Thunder Bay to a maximum of 56.8 in the Beaver Islands, with a mean BMF of 31.3. These values are similar to those which have been previously reported for pCCH in other birds of the Great Lakes (Ludwig et al., 1993a; Jones et al., 1993a,b).

Concentrations and total masses (Table 2) of TCDD-EQ initially decreased as the DCC chicks grew. This initial decrease was evident by the time the chicks weighed 100 g and the magnitude paralleled concentrations of TCDD-EQ. The major portion of the decrease in concentrations can be attributed to growth dilution. However, at some locations the decreases in concentrations of TCDD-EQ were accompanied by decreases in the total mass of TCDD-EQ in the chicks relative to masses observed in eggs. The decrease in total mass of TCDD-EQ was 55% at Green Bay, 79% at St. Martin’s Shoal, 25% in the North Channel, and 40% at the Apostle Islands. Apparently there was significant elimination of TCDD-EQ during early growth, during the period when the concentration of TCDD-EQ in the chicks was greater than that in their food.

The initial decreases in concentrations of TCDD-EQ were followed by a less rapid decrease (Tahquamenon Island and the Apostle Islands), a slow increase (Beaver Islands
and Thunder Bay), or a more rapid increase in concentrations of TCDD-EQs (Green Bay). In two areas, North Channel of Lake Huron and St. Martin's Shoal, the initial decrease in the concentration of TCDD-EQ was followed by increases in concentrations and then slow but steady decreases in concentrations of TCDD-EQ. When TCDD-EQ accumulation was evaluated in relation to total masses of TCDD-EQ, two distinct patterns were observed (Table 3 and Fig. 3). At all locations the total mass of TCDD-EQ increased in rapidly and linearly (Fig. 3).

Accumulation of TCDD-EQ, expressed as picograms, TCDD-EQ per gram, wet body weight gained, were calculated from total masses of TCDD-EQ and growth rates (Table 4). Due to the biphasic nature of accumulation at some locations, rates were compared during different growth phases (Table 4). Three different rates of accumulation were calculated: The overall rate was calculated as the increase in total mass of TCDD-EQ from eggs to the largest chicks; the final accumulation rate was the difference in total mass of TCDD-EQ between the two largest size classes; the maximal uptake rate was calculated as the difference between total mass of TCDD-EQ between the largest chick size class and the size class with the least total mass of TCDD-EQ. The

### TABLE 1

**Concentrations of TCDD-EQ in Double-Crested Cormorant or Herring Gull Eggs and Samples of Forage Fish Samples from Locations around the Great Lakes**

<table>
<thead>
<tr>
<th>Location and species</th>
<th>TCDD-EQ (pg/g), wet wt (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg samples</td>
<td></td>
</tr>
<tr>
<td>GB, Little Gull</td>
<td>382.3 (16.5)</td>
</tr>
<tr>
<td>LM, Beaver Is.</td>
<td>329.4 (41.5)</td>
</tr>
<tr>
<td>LH, St. Martins</td>
<td>329.8 (40.6)</td>
</tr>
<tr>
<td>LH, Thunder Bay</td>
<td>211.5 (13.6)</td>
</tr>
<tr>
<td>LH, North Channel</td>
<td>325.2 (57.8)</td>
</tr>
<tr>
<td>LH, Tahquamenon Is.</td>
<td>155.4 (10.4)</td>
</tr>
<tr>
<td>LS, Apostles Is.</td>
<td>141.7 (16.1)</td>
</tr>
<tr>
<td>LH, Saginaw Bay (HG)</td>
<td>557.2 (10.4)</td>
</tr>
<tr>
<td>Forage fish samples</td>
<td></td>
</tr>
<tr>
<td>Green Bay</td>
<td>24.4 (5.1)</td>
</tr>
<tr>
<td>Beaver Islands</td>
<td>5.8 (0.6)</td>
</tr>
<tr>
<td>Thunder Bay</td>
<td>18.1 (1.5)</td>
</tr>
<tr>
<td>North Channel</td>
<td>7.6 (0.9)</td>
</tr>
<tr>
<td>St. Martins Shoal</td>
<td>11.1 (1.0)</td>
</tr>
</tbody>
</table>

*Note.* Subsamples of composite homogenates of 12 eggs were used for the determination of concentrations of TCDD-EQ. Values represent individual determinations with standard deviations calculated by the additive variance method. All concentrations are pg TCDD-EQ/g wet wt of sample (ppt). Regurgitated fish samples were collected during visits to the colonies and composited (see Fig. 1 for locations).

*GB, Green Bay; LH, Lake Huron; LM, Lake Michigan; LS, Lake Superior; HG, herring gulls.*
 maximal uptake rate represented the rate of accumulation independent of the mass of TCDD-EQ in the eggs.

The overall rates of accumulation of TCDD-EQ by double-crested cormorant chicks varied from 14.3 pg/g, w/w (i.e., body weight) gained in Lake Superior to 162.4 pg/g, w/w gained in Green Bay. The differences between the three rates of uptake analyzed are of interest. At the lesser contaminated sites, such as Thunder Bay, St. Martin’s Shoal, and North Channel, the final uptake rate was less than the overall and maximal rates of uptake which suggests that at these locations the rates of accumulation became less as the chicks matured. In contrast, at the more contaminated sites, such as Green Bay and the Beaver Islands, the final rate of uptake rate was greater than the overall rate of uptake, which suggests that at these locations the rate of uptake became greater as the chicks matured. The reason for this difference is not clear but may reflect differential accumulation, elimination efficiencies, or more energy allocated to movement than growth as chicks became independent. Alternatively, it may indicate temporal differences in the availability of TCDD-EQ from forage at the locations sites.

This possibility is supported by the observation that the final uptake rate at Tahquamenon Island, where concentrations of TCDD-EQ in fish are less, is greater than the overall uptake rate. The greatest rates of accumulation which were observed in this study were for herring gull chicks in Saginaw Bay, which had an overall rate of 384.7 pg/g body wt gained and the final uptake rate was 921.8 pg/g body wt gained.

The coefficient of determination for the correlation between the concentrations of TCDD-EQ in eggs and larger chicks from the same site was 0.956 when the relationship was approximated by an exponential model \( y = a + bx + cx^2 \). The strong correlation between these two variables indicates that the equivalents accumulated at both life stages came from a common source, which implies that the TCDD-EQ deposited in the eggs were accumulated by the adults after they returned to the Great Lakes. The exponential nature of the relationship observed in several areas may indicate that
### Table 2
Concentrations of TCDD-EQ in Double-Crested Cormorant or Herring Gull Chicks

<table>
<thead>
<tr>
<th>Location</th>
<th>Size (g)</th>
<th>Mean wt (g)</th>
<th>TCDD-EQ, pg/g (SD)</th>
<th>TCDD-EQ (pg/chick)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green Bay</td>
<td>100</td>
<td>103.58</td>
<td>111.97 (16.40)</td>
<td>11,598.0</td>
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<tr>
<td></td>
<td>350</td>
<td>281.60</td>
<td>23.66 (2.75)</td>
<td>6,663.0</td>
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<tr>
<td></td>
<td>700–1000</td>
<td>644.80</td>
<td>84.06 (4.28)</td>
<td>54,200.0</td>
</tr>
<tr>
<td></td>
<td>1300–1500</td>
<td>1,428.20</td>
<td>168.34 (22.14)</td>
<td>240,421.0</td>
</tr>
<tr>
<td>Beaver Islands</td>
<td>100</td>
<td>117.60</td>
<td>124.26 (18.00)</td>
<td>14,613.0</td>
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<td></td>
<td>350</td>
<td>320.54</td>
<td>69.53 (6.86)</td>
<td>22,287.0</td>
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<td></td>
<td>700–1000</td>
<td>653.20</td>
<td>83.14 (10.79)</td>
<td>54,310.0</td>
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<tr>
<td></td>
<td>1300–1500</td>
<td>1,389.80</td>
<td>106.86 (12.79)</td>
<td>148,521.0</td>
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<tr>
<td>St. Martins Shoal</td>
<td>100</td>
<td>98.38</td>
<td>27.55 (3.22)</td>
<td>2,710.0</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>281.64</td>
<td>86.24 (9.47)</td>
<td>24,289.0</td>
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<tr>
<td></td>
<td>700–1000</td>
<td>1,013.60</td>
<td>72.70 (10.34)</td>
<td>73,691.0</td>
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<td>1300–1500</td>
<td>1,423.78</td>
<td>55.38 (7.55)</td>
<td>78,474.0</td>
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<td>Thunder Bay</td>
<td>100</td>
<td>114.44</td>
<td>149.62 (18.35)</td>
<td>17,123.0</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>356.40</td>
<td>20.85 (1.37)</td>
<td>7,431.0</td>
</tr>
<tr>
<td></td>
<td>700–1000</td>
<td>876.80</td>
<td>55.84 (4.56)</td>
<td>48,962.0</td>
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<tr>
<td></td>
<td>1300–1500</td>
<td>1,187.20</td>
<td>45.01 (4.18)</td>
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<td>North Channel</td>
<td>100</td>
<td>183.16</td>
<td>83.78 (9.53)</td>
<td>15,346.0</td>
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<td>350</td>
<td>388.80</td>
<td>24.86 (2.17)</td>
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<td>700–1000</td>
<td>552.80</td>
<td>85.22 (9.64)</td>
<td>47,109.0</td>
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<td></td>
<td>1300–1500</td>
<td>1,294.2</td>
<td>37.32 (4.79)</td>
<td>48,301.0</td>
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<tr>
<td>Tahquamenon Island</td>
<td>100</td>
<td>96.18</td>
<td>95.03 (9.55)</td>
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<td></td>
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<td>222.98</td>
<td>91.08 (8.9)</td>
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<td></td>
<td>700–1000</td>
<td>898.20</td>
<td>17.90 (1.02)</td>
<td>16,078.0</td>
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<tr>
<td></td>
<td>1300–1500</td>
<td>1,398.96</td>
<td>27.38 (1.62)</td>
<td>38,309.0</td>
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<tr>
<td>Apostle Islands</td>
<td>100</td>
<td>126.00</td>
<td>26.86 (2.88)</td>
<td>3,384.0</td>
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<tr>
<td></td>
<td>1300–1500</td>
<td>1,432.50</td>
<td>17.80 (1.41)</td>
<td>25,500.0</td>
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<tr>
<td>Saginaw Bay</td>
<td>100</td>
<td>261.20</td>
<td>226.49 (24.32)</td>
<td>59,158.0</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>499.38</td>
<td>96.85 (11.74)</td>
<td>48,367.0</td>
</tr>
<tr>
<td></td>
<td>700–1000</td>
<td>787.86</td>
<td>398.92 (39.99)</td>
<td>314,295.0</td>
</tr>
</tbody>
</table>

Note. Five chicks of each size at each location were collected and composite samples were prepared. “Size” indicates approximate size of chicks. Values represent individual determinations on samples with the standard deviation (SD) calculated using the linear model of variance. All concentrations are based on wet weight of sample.

Chicks accumulate TCDD-EQ more rapidly than adults. This may be due to the lesser energy expenditure of the chicks or lesser enzyme p450IA1 activities in the chicks, which results in less metabolic degradation and subsequent elimination of pPCB congeners.

The correlations between rates of TCDD-EQ accumulation and concentrations of TCDD-EQ in fish were difficult to interpret. There was a significant correlation between the concentrations of TCDD-EQ in forage fish samples and the overall uptake rate ($r^2 = 0.284$). However, the value for the Beaver Islands seems to be aberrant; with this value removed, the correlation was stronger ($r^2 = 0.828$). Unfortunately, the number
TABLE 3
TOTAL MASSES OF TCDD-EQ AND PCBs IN INDIVIDUAL CHICKS WERE CALCULATED FROM WEIGHT AND CONCENTRATION DATA

<table>
<thead>
<tr>
<th>Location</th>
<th>Size</th>
<th>Weight (g)</th>
<th>Tot PCBs (μg)</th>
<th>TCDD-EQ (ng)</th>
<th>Potency (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tahquamenon Island</td>
<td>Egg</td>
<td>96.18</td>
<td>361.0</td>
<td>6,150.74</td>
<td>17.04</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>96.18</td>
<td>204.9</td>
<td>9,140.15</td>
<td>44.61</td>
</tr>
<tr>
<td>350</td>
<td></td>
<td>222.98</td>
<td>428.1</td>
<td>20,307.68</td>
<td>47.43</td>
</tr>
<tr>
<td>700</td>
<td></td>
<td>898.20</td>
<td>485.0</td>
<td>16,077.78</td>
<td>33.15</td>
</tr>
<tr>
<td>&gt;1000</td>
<td></td>
<td>1,398.50</td>
<td>531.5</td>
<td>38,308.65</td>
<td>72.08</td>
</tr>
<tr>
<td>Green Bay</td>
<td>Egg</td>
<td>770.0</td>
<td>323.0</td>
<td>11,597.68</td>
<td>35.91</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>103.58</td>
<td>770.0</td>
<td>14,845.13</td>
<td>19.28</td>
</tr>
<tr>
<td>350</td>
<td></td>
<td>281.60</td>
<td>408.3</td>
<td>6,663.37</td>
<td>16.32</td>
</tr>
<tr>
<td>700</td>
<td></td>
<td>644.80</td>
<td>858.5</td>
<td>54,200.28</td>
<td>6.33</td>
</tr>
<tr>
<td>&gt;1000</td>
<td></td>
<td>1,428.20</td>
<td>9254.7</td>
<td>240,421.23</td>
<td>25.98</td>
</tr>
</tbody>
</table>

Note: The potency of the extracts was calculated as μg TCDD-EQ/g total PCBs.

of data points for this analysis is small and removal of one point can have a major influence on the interpretation of the relationship. Also, the assumption that the small fish samples collected (1300–1500) are representative of the fish fed to the young over the entire growing season is weak. Although an effort was made to collect fish during the whole season and to collect a representative sample of this fish for analysis, it is not possible to determine if these samples were representative of the forage fish consumed.

![Fig. 3](image-url) Average total mass of TCDD-EQ in individual double-crested cormorant chicks as a function of chick size (age). Symbols: triangles, Tahquamenon Island, Lake Superior; squares, Thunder Bay, Lake Huron; circles, Green Bay, Lake Michigan. See Fig. 1 for locations.
TABLE 4
RATES OF ACCUMULATION OF TCDD-EQ (pg TCDD-EQ
ACUMULATED PER GRAM OF BODY WEIGHT
GAINED ON A WET WEIGHT BASIS)

<table>
<thead>
<tr>
<th>Location</th>
<th>Rate (pg TCDD-EQ/g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximal</td>
</tr>
<tr>
<td>Green Bay</td>
<td>203.9</td>
</tr>
<tr>
<td>Beaver Islands</td>
<td></td>
</tr>
<tr>
<td>Thunder Bay</td>
<td>53.1</td>
</tr>
<tr>
<td>St. Martins Shoal</td>
<td>57.4</td>
</tr>
<tr>
<td>North Channel</td>
<td>42.7</td>
</tr>
<tr>
<td>Apostle Islands</td>
<td>16.9</td>
</tr>
<tr>
<td>Tahquamenon Island</td>
<td></td>
</tr>
</tbody>
</table>

* Burden at highest concentration minus burden at lowest concentration divided by body weight gained for respective samples.
* Final body burden minus egg burden divided by overall body weight gain.
* Body burden of >1000g chicks minus burden of 750g chicks divided by body weight gain for respective samples.
* Indicates that value is the same as overall uptake rate.
* Indicates that appropriate samples not available.

Most information on the toxicity of pPCH, especially PCBs, is based on laboratory studies with technical Aroclor mixtures. However, if the relative proportions of the different congeners with different toxic potencies change during the bioaccumulation process, this change must be accounted for in a hazard assessment. It is difficult to determine safe exposures if one cannot compare concentrations observed in the field with the results of controlled laboratory studies.

Chemical weathering due to differential solubilities, volatilities, adsorption constants, and degradation rates can result in relative concentrations of pPCH congeners different from the technical mixtures and also different from one location to another. Furthermore, these patterns can change over time (Oliver et al., 1989; Jones et al., 1993a,b). Also, there can be changes in the relative pattern of accumulation in the ecosystem as biomagnification occurs (Oliver and Niimi, 1988).

Selective accumulation of the more toxic PCB congeners can result in a mixture in the tissues of target animals, which is more toxic than would be predicted from an estimate of the original Aroclor mixture (Tillitt et al., 1992). This enrichment of the more toxic, non-ortho-substituted PCB congeners results in a relative toxic potency of the mixture which is from four to six times greater than the original technical mixture before selective weathering and selective biomagnification (Oliver et al., 1989; Burkhard et al., 1985) and bioaccumulation (Kubiak et al., 1989; Yamashita et al., 1993). The enrichment of specific pPCH congeners has previously been demonstrated in waterbirds (Boon et al., 1989; Borlakoglu et al., 1988; Walker, 1990). This enrichment was greatest for the 2,3,7,8-substituted PCDD and PCDF and non-ortho-substituted PCB congeners which have a relatively great biomagnification potential and are poorly metabolized by most species (Broman et al., 1992). These congeners tend to accumulate in tissues of marine organisms (Tanabe et al., 1987). PCB congeners
with vicinal hydrogen atoms in the ortho and meta positions with more than one ortho-chlorine atom are also resistant to metabolism (Boon et al., 1989).

One way to estimate the relative toxicity of mixtures is to calculate the ratio of TCDD-EQ, which measures the total concentration of congeners from PCDD, PCDF, and PCBs and divide this by the total concentration of PCBs (Jones et al., 1993a,b; Tillitt et al., 1992). This will account for different contributions from the three major classes of congeners and relate it to the PCB fraction, which is thought to account for most of the toxic potency. The concentration of H411E-derived TCDD-

EQ in each sample was normalized to the total concentration of PCBs measured in the same sample by dividing the concentration of TCDD-EQ by the total concentration of PCBs. Calculation of this ratio allows one to determine if the concentrations of TCDD-EQ were enriched relative to technical Aroclor mixtures. Since PCBs are currently the primary source of TCDD-EQ in tissues of animals in the Great Lakes, this normalization allows one to calculate the relative toxic potency in mixtures from different locations (Giesy et al., 1993). If greater than that observed in technical Aroclor mixtures, this ratio may indicate that there may be enrichment of coplanar PCB congeners or sources of TCDD-EQ, which are from sources other than PCBs (Giesy et al., 1993; Jones et al., 1993a).

The relative potencies observed in this study are similar to those observed in other studies, where the average relative potencies have been found to be approximately 37.0 µg/g, which is approximately 10 times as great as that for the Aroclor 1242.

### TABLE 5

<table>
<thead>
<tr>
<th>Location</th>
<th>Total PCB (mg/kg)</th>
<th>TCDD-EQ (ng/kg)</th>
<th>Relative potency (ng/kg TCDD-EQ)/(mg/kg PCB)</th>
<th>Potency ratio to Aroclor 1242</th>
<th>Egg lethality % at 23 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigeon Is., Lk. Ontario</td>
<td>5.5*</td>
<td>217*</td>
<td>39.5</td>
<td>3.99:1</td>
<td>28*</td>
</tr>
<tr>
<td>Spider Is., Green Bay</td>
<td>6.5*</td>
<td>337*</td>
<td>51.9</td>
<td>5.34:1</td>
<td>37*</td>
</tr>
<tr>
<td>Little Gull Is., Green Bay</td>
<td>7.3*</td>
<td>277*</td>
<td>37.9</td>
<td>3.83:1</td>
<td>34*</td>
</tr>
<tr>
<td>Gull Is., N. Lk. Michigan</td>
<td>6.7*</td>
<td>175*</td>
<td>26.1</td>
<td>2.64:1</td>
<td>27*</td>
</tr>
<tr>
<td>St. Martins, Lk. Huron</td>
<td>5.7*</td>
<td>145*</td>
<td>25.4</td>
<td>2.57:1</td>
<td>20*</td>
</tr>
<tr>
<td>Tahquamenon Is., Lk. Superior</td>
<td>3.5*</td>
<td>146*</td>
<td>41.7</td>
<td>4.21:1</td>
<td>19*</td>
</tr>
<tr>
<td>Lk. Winnipegosis, Manitoba</td>
<td>0.9*</td>
<td>35*</td>
<td>38.5</td>
<td>3.89:1</td>
<td>8*</td>
</tr>
<tr>
<td>Mean potency</td>
<td></td>
<td></td>
<td>37.3</td>
<td>3.77:1</td>
<td></td>
</tr>
</tbody>
</table>

technical mixture (Table 5) (Sawyer et al., 1984). The toxic potencies of extracts of
double-crested cormorant eggs from other locations on the Great Lakes were 2.5 to
5.24 (mean = 3.77) times greater than technical mixtures of Aroclors, which were also
measured in the eggs (Tillitt et al., 1992; Jones et al., 1993a,b).
The relative potencies in DDC chicks were approximately threefold greater in the
more remote areas than in Green Bay. The relative potencies (EC50 for EROD in-
duction) of extracts from Green Bay ranged from 6 to 56 μg TCDD-EQ/g PCB (Jones
et al., 1993a), which indicates that the total PCB content of these samples was a poor
indicator of the biological potency of the toxicity mediated through the Ah-receptor.
even though the measured concentrations of TCDD-EQ were correlated with the total
concentration of PCBs. If all of the TCDD-EQ could be attributed to PCB congeners
and these congeners were sorted equally in the environment and assimilated and
metabolized equally, there would be no significant difference in relative potency among
samples.

The relative potency ratio is indicative of the source of PCBs and other pPCH and
their environmental weathering. The ratio was used to determine if the more potent
PCB congeners were preferentially accumulated by chicks. The relative potency of the
extracts of samples of eggs from Tahquamenon Island and Green Bay was similar
(17.04 and 19.28 μg/g, respectively; Table 3). However, by the time chicks were near
fledging, the potencies of samples from Tahquamenon were almost threefold greater
than those of samples from Green Bay (25.98 vs 72.08 μg/g) (Table 3). Thus, the
extracts from Tahquamenon Island, where the concentration of both TCDD-EQ and
total PCBs were less, exhibited greater relative potencies than those from Green Bay,
where the concentrations of both total PCBs and TCDD-EQs were greater. To further
elucidate this phenomenon, ratios were calculated between initial and final masses of
total PCBs and TCDD-EQs in chicks. At Tahquamenon Island the total mass of PCBs
in the chicks increased 1.47-fold, while the mass of TCDD-EQ increased 6.22-fold.
At Green Bay the total mass of PCB in chicks increased 12.02-fold, while the total
mass of TCDD-EQ in the chicks increased 16.19-fold. These observations indicate
that Tahquamenon Island TCDD-EQs were biomagnified to a greater extent than
were total concentrations of PCBs. A similar pattern was observed in Green Bay.
However, the difference between the rates of biomagnification was not as great. This
indicates that the lesser potency of the extract from chicks in Green Bay, when expressed
on a PCB basis, was due to the accumulation of greater concentrations of the lesser
toxic PCB congeners. The relative potency of pPCH extracts, normalized to total
concentrations of PCBs, at the two sites exhibited similar patterns as the chicks grew
(Table 3 and Fig. 4). There was an initial increase in the potency of the PCB fraction
followed by a steady decrease and then a dramatic increase in the potency of the
extract as the chicks approached fledging. The decrease in potency in mid-development
is accompanied by a decrease in the total mass of TCDD-EQs, which suggests that at
that time elimination of pPCHs may be active. The rapid increase in accumulation of
pPCH near the time of fledging may be attributed to the greater expenditure of
energy by chicks after they begin thermoregulation and the rate of growth declines.
Since more energy is expended, less biomass is accumulated as cormorant tissue. If
the efficiency of accumulation remained similar, then the accumulation of both TCDD-
EQ and total PCB concentrations relative to increases in biomass would both be
greater, which would not result in selective enrichment. Thus, the selective enrichment
observed was most likely due to differential metabolism and greater retention of the
coplanar congeners than of the mono- and di-ortho-substituted PCB congeners and possibly other compounds, which can cause induction in the bioassay.

CONCLUSIONS

Concentrations of TCDD-EQ were detectable in all fish-eating water bird eggs from the Great Lakes. Concentrations of TCDD-EQs in samples of eggs from Lake Superior were approximately one-half to one-third as great as those observed in samples of eggs collected from Green Bay, Wisconsin, an area known to be more greatly contaminated with pPCHs than most other areas of the Great Lakes (Kubiak et al., 1989). However, concentrations of TCDD-EQs in chicks collected near fledging were 8- to 11-fold greater in Green Bay than those in Lake Superior. These observations, along with the similar potency of the extracts, when normalized to total PCB concentration, found in egg samples from the two sites indicate that pPCHs in eggs from both locations were accumulated from a common food source. It is hypothesized that when adult birds distribute through the Great Lakes in the spring they utilize a common food source with similar concentrations of TCDD-EQ and that TCDD-EQ from this source are deposited into the eggs. At the different colonies, the chicks are fed from distinct local food sources. Therefore, differences in the concentrations of TCDD-EQ in the chicks become apparent. If one is primarily interested in the concentrations of pPCH which may be causing effects on the embryos, then monitoring of concentrations in the egg would be the most appropriate. However, if one is interested in monitoring for accumulation from the diet, then it would be more pertinent to monitor concentrations of TCDD-EQ in larger chicks rather than eggs since the pPCH measured in the larger chicks are virtually all of local origin. In addition, the greater total masses of pPCH accumulated by chicks represent a more integrated measure of local
contamination due to the extended period of time over which accumulation occurs (Fig. 3).

Comparison of biomagnification of both TCDD-EQ and total PCBs at two sites indicates that the biological potency of pPCH mixtures can be significantly altered in a time span of only a few weeks. This is of particular concern since the concentration of total PCBs is still commonly used as a regulatory tool and indicator of environmental contamination. This study has demonstrated that the biological potency of a PCB mixture can change between 5- and 10-fold in as little as 30 days. Clearly total concentrations of PCBs would be a poor indicator of toxicological responses.

There is a need for the analysis of archived samples and for ongoing monitoring of fish-eating bird colonies to provide sufficient information for determining temporal trends of concentrations of pPCH in these species. It would be difficult to establish management, rehabilitation, or remediation strategies without an adequate understanding of temporal trends in pPCH contamination. The H4IIIE bioassay can be used as an effective supplemental tool to instrumental analyses for monitoring the accumulation of Ah-active pPCHs in environmental samples; and since they are more often correlated with the adverse effects currently observed in CFEWB of the North American Great Lakes, they may be more useful in hazard assessments and monitoring programs.

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REFERENCES


DOXIN EQUIVALENTS IN DOUBLE-CRESTED CORMORANTS


