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REPRODUCTIVE SUCCESS, DEVELOPMENTAL ANOMALIES, AND ENVIRONMENTAL CONTAMINANTS IN DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX AURITUS*)

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Abstract—To test an association between environmental contaminants and the prevalence of congenital anomalies in colonial waterbirds, we collected representative eggs for chemical analysis from double-crested cormorant nests at colonies in Lake Michigan, Wisconsin, USA, and Lake Winnipegosis, Manitoba, Canada, and periodically revisited the nests to determine the hatching success, survivorship of hatchlings, and number of deformed hatchlings in the remainder of each clutch. Total concentrations of polychlorinated biphenyls (PCBs) in eggs were determined by capillary gas chromatography. The combined activity of planar chlorinated hydrocarbons (PCHs) in the eggs was measured in an in vitro bioassay based on the induction of ethoxyresorufin-*O*-deethylase (EROD) activity in rat hepatoma cells. The combined EROD induction activity was expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQ). Total concentrations of PCBs and TCDD-EQ were seven to eight times greater in eggs from Lake Michigan (7.8 μg/g and 138 pg/g, respectively) than in those from Lake Winnipegosis (1.0 μg/g and 19 pg/g, respectively). The proportion of eggs hatching at the Lake Michigan colony (59%) was less ($p < 0.05$) than at Lake Winnipegosis (70%), and the prevalence of hatchlings with deformed bills was greater ($p < 0.001$) at Lake Michigan (0.79 vs. 0.06%). However, within the Lake Michigan colony, concentrations of PCBs and TCDD-EQ were not correlated with either hatching success or the occurrence of deformities in nestlings.

Keywords—Polychlorinated biphenyls Ethoxyresorufin-*O*-deethylase Double-crested cormorant Neonatal deformity TCDD equivalents

INTRODUCTION

During the 1970s, double-crested cormorants were nearly extirpated from the Great Lakes in large part because of reproductive failure from egg-shell thinning caused by dichlorodiphenyl-trichloroethane (DDT) [1]. Since that time, DDT use has been restricted, concentrations in tissues and eggs of cormorants have decreased, and their populations have increased [2]. Despite the increase in numbers of cormorants and other waterbirds, biologists remain concerned about the prevalence of deformed embryos and hatchlings in nesting colonies of cormorants and other colonial waterbirds [3]. The developmental abnormalities reported in cormorants, herring gulls (*Larus argentatus*), common terns (*Sterna hirundo*), and Forster's terns (*S. forsteri*) from the Great Lakes [1,4,5] may be a local indicator [6] that pollutants other than DDT are present in the Great Lakes ecosystem at biologically significant concentrations. Developmental anomalies in free-ranging birds are rare (usually <1%) even when they are prevalent enough to attract attention [5,7]. Deformed chicks may represent only a portion of the reproductive problem because some deformed embryos may not hatch and other embryos may not be deformed but may still die from the embryotoxic action of a pollutant. Common manifestations of planar chlorinated hydrocarbon (PCH) exposure in mammals and birds include hepatic and endocrine dysfunction, immune suppression, wasting syndrome, impaired reproduction, and induction of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD) in various tissues [8–13]. Teratological disorders, including limb and bill defects, are also sequelae of exposure [9,14]. Evidence from laboratory studies of planar polychlorinated biphenyls (PCBs) in chickens suggests that the concentrations of PCHs in wild bird eggs in some areas of the Great Lakes may be great enough to cause teratological effects [14–16]. During the 1979–1987 breeding seasons, the prevalence of cormorant chicks with deformed bills (Fig. 1) at Lake Michigan nesting colonies was significantly greater than at nesting colonies in the other Great Lakes or Canada [3]. Over half of the 60 deformed chicks reported in that study were observed on Spider Island in Lake Michigan, and the authors concluded that the bill deformities were caused by PCHs.

Planar chlorinated hydrocarbons such as the PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) contaminate the biota of the Great Lakes, especially near heavily industrialized areas like Green Bay, Wisconsin, USA [4,17–19]. A composite sample of nine cormorant eggs collected at Spider Island in 1988 contained a total of 5.3 mg/kg PCBs [20]; concurrent analysis of the same extract at a different laboratory revealed a low concentration (<20 ppt) of the most potent other PCH, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [21]. Planar PCBs, PCDDs, and PCDFs all induce

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Fig. 1. Four- to five-week-old (1,110 g) female double-crested cormorant chick with deformed beak found on June 28, 1983, in a nesting colony on Lake Michigan, Door County, Wisconsin, USA.

hepatic EROD activity through a common mode of action. Therefore, it is possible to compare the activity of a complex mixture of these compounds to a standard reference, 2,3,7,8-TCDD, the most toxic of the PCHs [22]. The activity of complex mixtures extracted from biological samples (e.g., from eggs of free-ranging birds) can be expressed relative to the toxicity of 2,3,7,8-TCDD [23] in units of 2,3,7,8-TCDD equivalents (TCDD-EQ). Tissue concentrations of TCDD-EQ (e.g., picograms of TCDD-EQ per gram of tissue) are closely correlated with the induction of several enzymes and with the toxic potency of environmental mixtures of PCDDs, PCDFs, and PCBs [22]. A bioassay procedure based on the correlation between EROD induction in vitro and toxicity has been used to quantify the relationship between hatching success and TCDD-EQ in double-crested cormorants in the Great Lakes [20]. Hepatic AHH and EROD activity in vivo was correlated with the concentrations of PCB, PCDF, and PCDD in the hatchlings of Forster's terns collected in Wisconsin [4,24]. Even though enzyme induction indicates exposure to PCHs, it does not necessarily indicate an adverse biological effect of these compounds.

To test the hypothesis that PCHs were causing reproductive dysfunction in cormorants, we investigated the correlation between concentrations of PCHs and clutch size, hatching success, and the prevalence of chicks with deformities at the Spider Island colony in Lake Michigan, Wisconsin, USA, and at relatively uncontaminated colonies on Lake Winnipegosis, Manitoba, Canada.

STUDY AREAS

Spider Island is approximately 4.0 km east of Rowley's Bay, Door County, Wisconsin, in Lake Michigan (45°13′N, 86°59′W). At Lake Winnipegosis, Manitoba, colonies at Coffee Island (52°0′30″N, 99°49′24″W), Coffee Island Reef (52°0′28″N, 99°49′24″W), Bachelor's Island (51°45′N, 99°54″W), Sugar Island Reef (51°55′30″N, 99°48′10″W), and Hay Island Reef (51°56′36″N, 99°47′30″W) were monitored during 1988, 1989, or 1990.

METHODS

Protocols for studying reproductive success differed between years and sites. In 1988, Spider Island was used in a pilot study to test techniques and gather background data for refinement of methods. All visits to Spider Island were made at night in order to minimize losses of eggs and nestlings to predation by herring gulls that nested among the cormorant nests. Weekly visits to this colony began the first week of May and continued through the third week in July, when all but a few late renesting attempts were complete. A sample of nests was marked during the first visit and revisited each week thereafter until they had either failed or produced fledged chicks. The number of eggs and chicks was recorded at each visit, and, when chicks were large enough (more than approx. 14 d old), serially numbered U.S. Fish and Wildlife Service leg bands were attached to each chick. Deformed or disabled hatchlings were collected and transported to Madison, Wisconsin, to the National Wildlife Health Laboratory or the University of Wisconsin for necropsies and blood collection. Hatchlings were fed beef liver, smelt, and water during transport and euthanized by lethal injection within 24 h of collection. The eyes of two hatchlings were examined histologically.

Full-time incubation begins when the female cormorant lays the third egg even if more eggs are added later [25]. In order to chemically characterize a nest, a minimally incubated egg was randomly chosen from each marked nest when it first contained three eggs. The contents of the individual eggs were stored frozen in chemically clean jars (I-Chem Research, Hayward, CA, USA) for subsequent quantification of organochlorine chemicals and several metals. During this pilot year, composite samples of nine eggs from Spider Island and 10 eggs from Coffee Island were analyzed and compared to verify that

the two sites offered a wide difference in contaminant concentrations in cormorant eggs.

Hatching success of a nest was defined as the number of hatched eggs divided by the total number of incubated eggs present when the first egg hatched; this compensated for the removal of eggs for chemical analysis and predation. Eggs that failed to hatch by July 24 were recorded as unsuccessful. By 3 weeks of age the birds were ambulatory and considered successfully fledged because we could no longer accurately determine which nest they came from. Reoccupied nests had eggs or young during one visit, were empty the next visit, and contained one or more eggs the following visit. Data from reoccupied nests were not included in the analyses.

Large numbers of hatchlings must be examined to yield statistically adequate samples for comparison of the incidence of rare phenomena like hatchling deformities. However, the cost of quantifying PCHs precludes chemical examination of large numbers of individual eggs. Therefore, we decided to use the sample egg technique [26–30] in 1989 and 1990 for hypothesis testing. Briefly, this technique involves removing a single egg from each nest at a site for subsequent retrospective analysis of only the nests that exhibit a property of concern. In this case, we were interested in nests that were successful, those that failed, and those that contained a deformed nestling. We collected and froze the contents of a single egg (the sample egg) from each clutch at Spider Island and from a subset of the large number of clutches available at Lake Winnipegosis. We then revisited the nests and determined the fate of the remaining eggs in each clutch. If a developmental anomaly occurred in any of the embryos or hatchlings from a nest, we were able to determine the chemical content of the sample egg. The utility of this technique depends on the relative variability of contaminant concentrations between eggs within a clutch compared to the variation between clutches. In a concurrent study at Spider Island, 85% of the variability was between clutches and only 15% was within clutches [31].

In 1989 and 1990, visits to Spider Island began with a daylight trip in mid-April before the initiation of nesting. This visit was made to mark nest structures that survived the winter and to reduce the time required for marking nests during the active nesting period. Subsequently, all visits to the Spider Island colony during the nesting season were made at night. In 1989, investigators entered the colony 13 times on a weekly basis (May 3–August 1) to record the number of eggs and hatchlings in marked nests, to collect eggs from three-egg clutches, and to band the young chicks. Marking of nests and egg collection were ongoing processes as the cormorants initiated new nests. All hatchlings were examined for deformities each week. On average, investigators spent 3 h in the colony each visit. Following the 1989 nesting season, nest markers were removed from Spider Island in September, and the locations of nests that had contained deformed young were recorded because these instances appeared to be spatially clumped. As a rapid a posteriori test for clumping, the colony was divided into 16 segments, each approx. 30 m long, and the total number of nests and the number of nests with deformed young were recorded for each segment. In 1990, after the first visit in mid-April, seven subsequent nighttime visits were made on May 8, May 30, June 14, June 20, June 26, July 10, and July 24.

Coffee Island Reef in Lake Winnipegosis was visited less frequently than Spider Island in 1989 (May 15, June 7, June 15, and July 3). Only active nests were marked during the first visit, and the hatchlings were not banded; otherwise, procedures were similar to those used at Spider Island. On July 3, 1989, when the majority of the hatchlings were ambulatory, four additional nesting colonies (Bachelor's Island, Sugar Island Reef, Hay Island Reef, and Coffee Island) on Lake Winnipegosis were visited to increase the number of hatchlings checked for deformities. In 1990, the Hay Island Reef colony was visited on May 29, June 19, and July 5 to record reproductive success. On July 5, 1990, the colony at Sugar Island Reef was visited to increase the number of hatchlings examined for deformities.

Sample eggs from nests with poor reproductive success (the entire remaining clutch of two, three, or four eggs failed to hatch), with good reproductive success (all chicks fledged from the entire remaining clutch of three or four eggs), and that contained a deformed chick were selected randomly for chemical and enzymatic analysis. We also analyzed a randomly selected set of sample eggs obtained in 1989 from Coffee Island Reef nests with good reproduction. Insufficient numbers of other nesting outcomes were available at Lake Winnipegosis to allow statistical analysis.

Egg contents were homogenized with a Polytron blender before removal of portions for extraction of chlorinated hydrocarbons. A known mass of 2,4,6-trichlorobiphenyl (International Union of Pure and Applied Chemistry [IUPAC] PCB 30) in isooctane was added to each portion as an internal standard for the quantitation of total PCB concentrations and extraction efficiencies. Background concentrations of congener 30 in cormorant eggs from both locations were negligible according to previous analyses [32]. Extraction of the eggs was performed according to the method of Ribick et al. [33] with the following modifications. After drying the sample with four mass equivalents of anhydrous sodium sulfate, lipophilic compounds were eluted from the sample with 200 ml methylene chloride. The extract was then concentrated and subjected to automated gel permeation chromatography on a column of Biobeads SX-3 (BioRad, Rockville Centre, NY, USA) to remove lipids and lipophilic xenobiotics. Additional cleanup and separation of contaminants were performed on a column of acid-silica gel in 1:1 methylene chloride:hexane. Extracts were solvent-exchanged into isooctane. The efficiency of the extraction procedure previously was shown to be in excess of 90% for total PCBs and 95% for PCDDs [23]. The efficiencies of the extraction for individual planar PCB congeners for this procedure were estimated to lie between the efficiencies for total PCBs and dioxins. Since extraction efficiencies were in excess of 90%, the data were not corrected for compound- or congener-specific extraction efficiencies.

Total concentrations of PCBs were determined by quantifying individual PCB congeners by capillary gas chromatography with electron capture detection (ECD) [34]. Quantification was based on peak areas relative to the area of the internal procedural standard (PCB 30). Response factors for individual PCB congeners were previously described [35]. These 63 Ni-ECD response factors were linear over the range of concentrations studied. Total PCBs were quantified with the assistance of the COMSTAR multilinear regression program [36]. Final PCB concentrations were expressed on a fresh wet weight basis; results were not corrected for moisture loss because we collected fresh eggs and removed and froze the contents promptly in airtight containers.

The TCDD-EQ were quantified by EROD induction of the extracts of eggs with the H4IIE assay system by the methods described by Tillitt et al. [23]. The cells were dosed with egg extracts in 100 μl isooctane at four dilutions covering two

Table 1. Reproductive parameters and bill deformities in double-crested cormorant chicks from Spider Island, Lake Michigan, Wisconsin, USA, and Lake Winnipegosis, Manitoba, Canada, during the 1988–1990 breeding seasons

	Spider Island			Lake Winnipegosis	
	1988	1989	1990	1989	1990
Clutch size	3.24 ± 0.70	3.62 ± 0.74	3.57 ± 0.65	4.25[a] ± 0.81	3.71[a] ± 0.75
Nests	34	466	953	52	161
Hatching success[b] (%)	65.4	55.2	57.7	75.7[c]	64.1[c]
Nests	39	463	921	52	157
Chicks examined	1,548	1,511	2,700	11,736	~13,000
Bill defects	13	13	18	12[d]	2[d]

[a]Lake Winnipegosis differs from Spider Island in each year ($p < 0.01$).
[b]Hatching success = mean number of hatchlings per number of incubated eggs present when the first egg hatched (chicks per total eggs incubated in nest).
[c]Lake Winnipegosis differs from Spider Island in each year ($p < 0.05$).
[d]Lake Winnipegosis differs from Spider Island in each year ($p < 0.001$).

orders of magnitude. After dosing, the tissue cultures were incubated for 72 h and then harvested for determination of EROD activity by the spectrofluorometric method of Pohl and Fouts [37]. Protein was determined by the method of Lowry et al. [38] with bovine serum albumin used as a standard. The TCDD-EQ in the extract were initially determined according to the methods of Sawyer et al. [39]. Concentrations of TCDD-EQ in samples were calculated from final extract volume and the weight of sample extracted. Relative potency was calculated as micrograms TCDD-EQ per gram total PCBs. To determine the precision of the assay, the contents of five eggs were extracted and analyzed twice.

Portions of the same egg contents were analyzed at the Plant and Soils Analysis Laboratory, University of Wisconsin–Madison, for the following 12 metals (detection limit, ppm): cadmium (0.01), chromium (0.017), copper (0.025), nickel (0.037), zinc (0.01), lithium (0.025), cobalt (0.018), iron (0.011), manganese (0.003), arsenic (0.279), lead (0.111), and selenium (0.19). The contents of the eggs were mixed with 3:1 nitric : perchloric acid, digested with heat for 4 h, and diluted with water. Metal concentrations were determined with inductively coupled plasma spectrophotometry and reported on a wet weight basis. Portions of the same eggs were also analyzed for mercury by cold vapor generation at Hazelton Laboratories in Madison, Wisconsin.

Arcsine transformations of the proportions of eggs hatching were associated with clutch size by analysis of covariance (ANCOVA) (SAS Institute, Cary, NC, USA) and SYSTAT® (SYSTAT, Evanston, IL, USA). Distributions of hatching success against clutch size at Spider Island and Lake Winnipegosis were compared by ANCOVA. The prevalences of deformities were compared with a χ^2 test. Differences in concentrations of TCDD-EQ, total PCBs, and relative potency among productivity groups at Spider Island were compared by Kruskal-Wallis analysis of variance (ANOVA). Mann-Whitney *U* tests were used for comparisons of the same parameters between colonies. Bartlett's test of homogeneity was used to examine variances in concentrations of PCBs, TCDD-EQ, and potency. Fisher's least-significant-difference procedure for multiple comparisons was used for testing differences in hatching success by clutch size. A Runs test was used to test for a spatial pattern in the locations of nests with deformed chicks, and *t* tests with equal variances were used to test differences in clutch sizes at the two locations.

RESULTS

The total concentration of PCBs in a composite of 10 cormorant eggs collected at Coffee Island in 1988 was 0.9 ppm (Midwest Ecological Science Center, unpublished data), compared to 5.3 ppm at Spider Island [20].

Hatching success was greater in larger clutches ($p = 0.04$) and ranged from an average of 30% in 37 one-egg clutches to 67% in 15 five-egg clutches. For comparisons between the two study sites, hatching success was compared by ANCOVA with clutch size as the covariate. Both clutch size and hatching success were lower ($p = 0.01$ and $p < 0.05$, respectively) at Spider Island in both 1989 and 1990 but did not differ between the years ($p = 0.12$ and $p > 0.05$, respectively, Table 1).

Nestlings with bill defects were more prevalent ($p < 0.001$) at Spider Island than at Lake Winnipegosis during both years (Table 1). The most common distortion was a lateral and ventral deflection of the upper bill. Sequential inspection of banded hatchlings showed that the twisted bills were first evident at 1 to 2 weeks of age. Two hatchlings with upper bills foreshortened approx. two-thirds were found on Spider Island in 1989. The pathogenesis of the deformations was not determined.

Five chicks at Spider Island (two in 1989 and three in 1990) with limb defects involving abnormal rotation of joints and hypoplastic femoral musculature or polydactyly were discovered during the banding process. We never found more than one deformed chick per nest. A few (four in 1989 and seven in 1990) cormorant hatchlings near fledging age at Spider Island had exudative conjunctivitis. These chicks were normal except for the eye lesions. The eyes of two were examined histologically. One had a congenital malformation of the Descemet's membrane and a dearth of lens material. The other had primary conjunctivitis associated with inflammatory changes suggestive of an infection.

At Spider Island, cormorants nesting earliest used the north and south ends of the island. Cormorants nesting later used sites on the perimeter or between the regions occupied by the early-nesting pairs. No pattern was detected in the location of nests with deformed chicks ($p > 0.05$).

Total concentrations of PCBs and TCDD-EQ were less ($p < 0.01$) at Lake Winnipegosis than in any of the groups at Spider Island (Table 2). The PCB-normalized relative potency did not differ between the two sites ($p = 0.91$, Table 2). Neither the total concentration of PCBs ($p = 0.87$), EROD activity ($p = 0.85$), nor relative potency ($p = 0.90$) differed among the

Table 2. Concentrations (X ± SD) of total polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQ), and potency (TCDD-EQ per total PCBs) in extracts of individual reference eggs collected to represent clutches of double-crested cormorants from Spider Island, Lake Michigan, Wisconsin, USA, and Lake Winnipegosis, Manitoba, Canada

Location	Nest group[a] [*N*]	Total PCBs (μg/g) [range]	TCDD-EQ (pg/g) [range]	Potency (μg/g) [range]
Spider Island	Deformed [6]	7.34 ± 3.28 [2.85–10.84]	152.75 ± 109.75 [61.34–366.20]	23.84 ± 17.72 [9.63–54.76]
	High [10]	7.60 ± 2.33 [2.76–11.19]	134.31 ± 29.43 [104.11–202.78]	21.72 ± 18.38 [11.69–73.47]
	Low [10]	8.22 ± 4.39 [3.46–18.70]	134.19 ± 71.57 [45.40–305.28]	20.08 ± 12.20 [3.62–45.29]
Lake Winnipegosis	High [5]	1.03[b] ± 0.31 [0.61–1.44]	18.95[b] ± 12.61 [5.98–35.57]	19.41 ± 13.21 [4.14–39.52]

[a]Deformed = one of the chicks from the clutch had a deformed bill; high = all of the eggs produced fledged chicks; low = none of the eggs hatched.
[b]Lake Winnipegosis differs from all Spider Island groups ($p = 0.01$).

productivity groups within the Spider Island colony. However, the variances in TCDD-EQ were different between the groups at Spider Island ($p < 0.05$). This difference resulted from three extremely high TCDD-EQ measurements, two in the deformed group and one in the low productivity group. By visual inspection, the other TCDD-EQ measurements were approximately equal. The mean coefficient of variation (C.V.) for the replicate samples was 33.7% ± 16.8, and the range was 6.2 to 49.4%.

Concentrations (ppm) of cadmium, chromium, nickel, lithium, arsenic, lead, and selenium were less than detection limits in all samples. Concentrations of detectable metals (copper, zinc, cobalt, iron, manganese, and mercury) did not differ among reproductive groups or sites. Means (ppm) and standard deviations of the six detectable metals in 31 eggs were as follows: copper, 0.78 ± 0.12; zinc, 6.78 ± 1.16; cobalt, 0.13 ± 0.17; iron, 17.73 ± 3.21; manganese, 0.23 ± 0.08; and mercury, 0.16 ± 0.08.

DISCUSSION

Decreased hatchability was associated with PCB concentrations of 3 to 14 ppm in eggs produced by chickens fed PCBs [40], 5 ppm of injected PCBs caused embryo mortality in chickens [41], and a lowest observed adverse effect level of 3 to 5 ppm has been estimated for egg concentrations in free-ranging birds [42]. Some bird species seem more resistant to PCBs than others; neither egg production nor hatchability of screech owls (*Otus asio*) was influenced by diets that produced 3.9 to 17.8 ppm in eggs [43], and mallard (*Anas platyrhynchos*) hatching success was not affected in clutches with mean concentrations of 105 ppm [44]. The relative sensitivity of double-crested cormorants to PCBs is unknown.

The mean concentrations of PCBs we found in cormorant eggs (1 to 8 ppm wet weight) were at the lower end of the range reported for double-crested cormorants on the Great Lakes (4.3 to 75.3 ppm dry weight by Gilbertson and Reynolds [45], 23.8 ppm wet weight by Weseloh and Teeple [46], and 2 to 16.5 ppm wet weight by Heinz et al. [47]). This was expected because our study was more recent than the others and because organochlorine chemicals are continuing to decrease in Great Lakes biota generally. Both Fox et al. [3] and Tillitt et al. [20] showed an association between PCH contaminants and mortality of embryos of double-crested cormorants on the Great Lakes at PCH levels similar to those we measured at Spider Island. In fact, our 1988 range-finding results were included by Tillitt et al. [20] and were at the higher end of the ranges reported there. Therefore, at least a part of the reduced hatchability at Spider Island was likely caused by the PCHs at that site.

If chemical contamination caused the smaller clutches, poorer hatching success, and higher frequency of deformed beaks at Spider Island compared to Lake Winnipegosis, then why was the same association not apparent among the experimental groups at the Spider Island colony where extraneous variables were presumably relatively constant? A possible explanation for this observation is that biological responses to toxicants depend on both the exposure of an animal and its individual susceptibility. Within the Spider Island colony, we may have measured predominantly the individual susceptibility component because our chemical analysis sample sizes were necessarily extremely small. With a C.V. of 20% for concentrations of TCDD-EQ, 39 eggs from both the successful group of nests and the group with deformed chicks would have been required to detect a true difference of 15% in concentrations of TCDD-EQ [48]. The actual C.V.s were greater than 20%. Even though we checked virtually every nest in the colony at Spider Island, our samples of nests with poor hatching success or deformed hatchlings were small. The fact that variances in TCDD-EQ were significantly different between groups at Spider Island further confounds this situation. Both the highest and lowest TCDD-EQs were found in the deformed group, and, while the highest might be expected, the lowest measurements are somewhat difficult to reconcile with a dose-related phenomenon. Simply, it may not be feasible to obtain enough affected nests to correlate reproductive responses with concentrations of TCDD-EQ within a single colony.

Alternatively, of course, the possibility that either deformity incidence or reproductive differences were independent of exposure to the contaminants measured in this study cannot be discarded. Given the empirical results reported here, further examination of this causal link using a refined approach to the problem of measuring and controlling dose is certainly needed. Differences between geographic locations necessarily confounded unknown and uncontrolled sources of variation with the parameter of interest, contaminant exposure.

Some of the known but unmeasured sources of variation include predation by gulls, latitude, and disturbance by investigators. We observed predation by gulls despite our efforts to minimize it by working after dark when they were relatively

inactive. Clutches of many avian species are larger at higher latitudes [49], but the influence of the 7° difference between Spider Island and Lake Winnipegosis is unknown. There was certainly more investigator disturbance at Spider Island than at Lake Winnipegosis because we visited the former colony weekly during the entire nesting season, whereas the latter was visited only four times each year. In addition, the Spider Island colony was larger, and more time was required to complete our measurements there than at Lake Winnipegosis. The quantitative effect of these differences is unknown.

Unknown but potential sources of variation include disease, nutrition, other contaminants, and genetics. In 1990, Newcastle disease occurred in cormorants nesting in western Canada (National Wildlife Research Center, unpublished data), but there was no clinical evidence of this disease at either Lake Winnipegosis or Spider Island. In 1992, the disease was identified in cormorants from Green Bay and Spider Island in particular. No virological or bacteriological pathogens were isolated from 10 cormorant eggs collected from Spider Island in 1989 [50]. Disease does not seem a likely factor in the etiology of the reproductive responses. Bill deformities similar to those observed have been reported in captive cormorants as a result of vitamin D deficiencies in artificial diets [51]. We measured various heavy metals in reference eggs, and they were similar to those reported by Haseltine et al. [52] from Spider Island and nearby islands in Lake Michigan. It also is possible that a genetic factor such as inbreeding contributed to the responses seen, but no data bearing on this were obtained.

In conclusion, we were unable to either accept or reject the hypothesis that environmental chemicals caused the observed embryo mortality and hatchling deformities in cormorants at the Spider Island site based on the data reported here. Our results are consistent with the interpretation that PCBs reduced reproduction at this site based on observed correlations and laboratory studies of PCH effects. However, a variety of unmeasured variables likely interfered with demonstrating a clear causal relationship. Limited sample sizes compromised the power of a retrospective approach for controlling many of the unmeasured variables by working within the contaminated colony. More direct experimental approaches may be necessary to overcome these difficulties.

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