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Organochlorine Contaminants in Double-Crested Cormorants from Green Bay, Wisconsin: II. Effects of an Extract Derived from Cormorant Eggs on the Chicken Embryo

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Abstract. White Leghorn chicken (*Gallus domesticus*) eggs were injected prior to incubation with one of four concentrations (0.001, 0.01, 0.1, and 1.0 egg-equivalent) of an extract derived from 1,000 double-crested cormorant (*Phalacrocorax auritus*) eggs collected at Spider Island adjacent to Green Bay in Lake Michigan. One egg-equivalent corresponded to the concentration of contaminants present in an average cormorant egg. This was approximately 322 pg toxic equivalents (TEQs)/g, ww egg with polychlorinatedbiphenyl congener 126 (3,3′,4,4′,5-pentachlorobiphenyl) accounting for over 70% of the TEQs. Injection of 1.0 egg-equivalent resulted in 77% mortality at hatch. The incidence of developmental abnormalities (structural defects or edema) was not affected by injection of the extract. Body weight gain of chicks was reduced in the 1.0 egg-equivalent dose group in the first, second, and third week's post-hatch. Relative brain weights were greater and relative bursa weights were less in the 1.0 egg-equivalent dose group than in the vehicle control at three weeks of age. There were no significant differences in the relative weights of the heart, liver, spleen, testes, or comb among treated and control birds.

Double-crested cormorants (*Phalacrocorax auritus*) are among the species of piscivorous colonial waterbirds residing in the Great Lakes basin that have experienced reproductive problems. Cormorant populations began to decrease in the 1950s and 1960s due to nest destruction by commercial fishermen and egg breakage. They stopped breeding in Lake Michigan by 1963 and in Lake Superior and the Canadian waters of Lake Ontario by the 1970s. Chemical contamination of Great Lakes fish was believed to be a major contributing factor to this problem (Gilbertson *et al.* 1991). Cormorants were the only species of Great Lakes waterbirds to cease breeding which suggests that they may either be more sensitive to or exposed to greater concentrations of some contaminants than other waterbird species (Ludwig 1984; Fox *et al.* 1991c). Initially, DDT/DDE were the contaminants that were having a detrimental effect on cormorant populations (Weseloh *et al.* 1983; Fox *et al.* 1991c), and as their concentrations have decreased in the environment, cormorant populations have been increasing (Ludwig 1984; Fox *et al.* 1991a; Weseloh and Ewins 1994). Despite recovering populations, embryo lethality (Tillitt *et al.* 1992) and congenital malformations have been observed in cormorant populations of the Great Lakes since the late 1970s (Fox *et al.* 1991c). These malformations have included deformed feet and legs, missing or abnormal eyes, and crossed or abnormal bills (Fox *et al.* 1991b). Since such malformations are uncommon in most wild avian populations (Fox *et al.* 1991b), these observations prompted concern as to their etiology. The occurrence of malformations in cormorants and Forster's terns in Green Bay, Lake Michigan, as well as decreased hatchability of Forster's tern eggs, have been correlated with concentrations of polychlorinated biphenyls (PCBs) in the environment (Hoffman *et al.* 1987; Kubiak *et al.* 1989; Gilbertson *et al.* 1991). Larson and associates (1996) demonstrated lower hatchability in Lake Michigan cormorants when compared to a Manitoba reference colony, and Tillitt *et al.* (1991) measured a strong correlation between PCBs and embryotoxicity within cormorant colonies in the Great Lakes. On the basis of such field studies, it has been suggested that polyhalogenated diaromatic hydrocarbons (PHDHs), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and planar PCB congeners, are responsible for these effects (Fox *et al.* 1991a; Giesy *et al.* 1994a,b).

Since PHDHs bind to the aryl hydrocarbon receptor (AhR) and share a common mechanism of toxicity, the toxic equivalent (TEQ) approach has been developed to evaluate the toxicity of complex environmental extracts (Giesy *et al.* 1994a). This approach accounts for the relative contribution of the different chemicals that act through a similar mechanism. Toxic

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equivalency factors (TEFs) are used in estimating the TEQs present in these environmental extracts (Jones *et al.* 1994). PCB, polychlorinated dibenzo-*p*-dioxin (PCDD), and polychlorinated dibenzofuran (PCDF) isomers are assigned TEF values based on their relative toxicity compared to the most toxic of these PHDHs, TCDD. Environmental extracts can be assessed for potential toxicity by multiplying the concentrations of the TCDD-like contaminants by their respective TEFs to obtain toxic equivalents (TEQs), which then are summed to estimate the total potency of the mixture. The toxicity of environmental extracts can also be tested using the H4IIE bioassay. TCDD toxic equivalents (TCDD-EQs) are determined by comparing the hepatic enzyme induction in extract-treated cells with that of TCDD-treated cells (Tillitt *et al.* 1991). High concentrations of TEQs have been reported in the eggs of waterbirds from polluted industrialized areas of the Great Lakes where reproductive impairment has been most severe (Tillitt *et al.* 1991; Yamashita *et al.* 1993). These areas include Green Bay, Lake Michigan; Saginaw Bay, Lake Huron; Hamilton Harbor, Lake Huron; and the Detroit River (Fox 1993).

The domestic chicken (*Gallus domesticus*) embryo was used to examine the potential effects of an extract derived from cormorant eggs collected near Green Bay, Lake Michigan. This is an area of relatively great contamination by PHDHs with a history of compromised reproduction in resident colonies of double-crested cormorants (Tillitt *et al.* 1991; Fox 1993; Yamashita *et al.* 1993; Giesy *et al.* 1994a,b). The chicken embryo was used as a model because it has been shown to be sensitive to these types of compounds (Verrett 1976; Allred and Strange 1977; Brunström 1988, 1990; Brunström and Andersson 1988; Henshel 1993; Sanderson and Bellward 1995).

Materials and Methods

Egg Collection

Unincubated double-crested cormorant eggs were collected in 1988 from Spider Island in Lake Michigan near Green Bay, WI (45°13′N, 86°59′W). All new three, four, and five egg clutches present were collected on May 5, 13, and 19. Eggs were stored under refrigeration for three weeks until they were opened. The few partially incubated clutches (greater than 7 d) were discarded when opened; all other egg contents were pooled and placed into contaminant-free sample containers (I-Chem, Hayward, CA). Samples were then stored frozen at 0°C until transport on dry ice to the Midwest Science Center, National Biological Service (Columbia, MO) on Feb. 26, 1991 where they were processed. Details of the extraction process and analysis of the extract are described in an earlier paper (Meadows *et al.* 1996).

Egg Extract Injection Doses

The doses of cormorant egg extract were 0.001, 0.01, 0.1, and 1.0 egg-equivalent. One egg-equivalent contained the amount of extract present in an average cormorant egg. The contents of 100 cormorant eggs used to prepare the extract weighed 42,127 g, thus, the contents of the average cormorant egg weighed 42 g. The extract was dissolved in methylene chloride and further diluted with cold-filter sterilized triolein (Sigma Chemical Co., St. Louis, MO) which served as the vehicle. Approximately 1.2 µl of the 10 µl injected per egg (vehicle or extract) was methylene chloride. The diluted extract doses and vehicle arrived at Michigan State University (MSU) in July 1994. They were kept frozen between injection days.

Egg Preparation and Injections

The extract was injected into the yolks of White Leghorn chicken eggs prior to incubation (day 0). There were three replications of each group with 20 eggs per replication. Eggs were obtained from the MSU Poultry Science Teaching and Research Center. All eggs were candled to locate the air cell and then weighed. After eggs were weighed and labeled, they were positioned horizontally and placed in a cooler (14.4°C) until injection on the following day. Eggs were laid horizontally overnight to allow the germ spot to float away from the site of injection thus reducing potential injury during injection. Eggs were removed from the cooler and left horizontal until after being injected. A 100 µl syringe (22S gauge; Hamilton Co., Reno, NV) was used for the injections. It was disinfected with 70% ethyl alcohol prior to insertion into the vial and before injection into the egg. The needle length was marked at approximately 29 mm to ensure injection into the yolk (this length was chosen after a series of injections of a colored solution into the yolks of infertile eggs). The blunt end of the egg was disinfected with 70% ethyl alcohol before a small hole was made in the shell over the air cell with a disinfected pin. After horizontal insertion of the needle, the solution was slowly injected, and the needle was left in for a moment following injection. Upon removal of the needle, the hole was sealed with melted paraffin. Following injections, all eggs were placed in the incubator.

Incubation

Eggs were incubated in a Petersime model #5 incubator (Petersime Incubator Co., Gettysburg, OH) for up to 24 d with their blunt end up. Conditions in the incubator were standard for commercial operations, 37.5–37.6°C with a relative humidity of approximately 65% (North 1978). Embryo viability was determined by candling on days 4 and 11. On day 18, viability was assessed with an embryo viability detector (EVD) that was provided by the U.S. Fish and Wildlife Service. The EVD detects vibrations within the egg and changes these vibrations to sound waves which can be heard in headphones attached to the EVD (Mineau and Pedrosa, 1986). Any nonviable eggs on days 11 or 18 were opened to estimate the stage of development and to determine the presence of any abnormalities. Viable eggs were transferred to hatching baskets on day 18 of incubation.

Post-Hatch

Upon hatching, each chick was weighed and uniquely identified with a wing band. Chicks were also examined for abnormalities. Once chicks were dry, they were moved to a floor pen bedded with pine and spruce wood chips (Pestell Agri-Products, New Hamburg, ON, Canada) and acclimated to food and water by dipping their beaks in water and feed (Purina Chick Starter, Ralston Purina Co., St. Louis, MO), both of which were provided *ad libitum*. All chicks were raised for three weeks with body weights recorded weekly. In studies involving herring gulls, a three-week survival period was used to estimate reproductive success since mortality of chicks was observed during the first three weeks after hatch (Gilman *et al.* 1977). Any chick which was determined incapable of acquiring food, water, or warmth on its own was euthanized. At the end of the three weeks, chicks were killed by cervical dislocation, decapitated, and subjected to necropsy. The organs taken for weighing included the brain, bursa, heart, liver, spleen, testes, and comb.

Data Analysis

All comparisons were made relative to the vehicle control. Mortality data were evaluated using contingency tables and the Bonferroni Chi-Square table. Eggs classified as containing early dead embryos may have included infertile eggs because it was often difficult to distinguish between the two. The same analysis was used to test the occurrence of developmental abnormalities. Analysis of body weight was conducted using univariate analysis of variance for split-plot repeated measures (Gill 1986) followed by Dunnett's test for comparisons with control. Organ weights were analyzed using a one-way ANOVA and Dunnett's test. Analysis of body and organ weights was performed using the statistical software SAS (SAS Institute Inc. 1990). The level of significance was 0.05, unless otherwise stated.

Results

Concentrations of predominant contaminants and their relative contribution to the calculated toxicity of the cormorant egg extract based on TEFs derived for the chicken (Bosveld *et al.* 1995) are given in Table 1. The majority of the TEQs in the cormorant extract were contributed by PCB congener 126 [3,3′,4,4′,5-PeCB, (73%)]; 1,2,3,7,8-PeCDD (7%), PCB congener 77 [3,3′,4,4′-TCB, (5%)]; 2,3,7,8-TCDD (4%); PCB congener 105 [2,3,3′,4,4′-PeCB, (4%)]; and 2,3,4,7,8-PeCDF (3%).

One egg-equivalent was the only dose to produce significantly greater embryo mortality (77%) than the vehicle control (20%) (Table 2). The 20% mortality at day 21 was considered acceptable since other egg injection studies (Brunström 1988) have reported similar mortality (15%) through day 18 of incubation. Post-hatch survival of chicks in all groups was high. Body weights of White Leghorn chicks were significantly less at the first, second, and third week post-hatch in the 1.0 egg-equivalent dose group (Table 3). Relative brain weights (as percent of body weight) were greater in this group while absolute weights were less than the vehicle control (Table 4). Relative and absolute bursa weights were less when compared to the vehicle control values (Table 5). Relative heart, liver, spleen, and testes weights were not significantly affected by injection of the cormorant egg extract (data not shown). Relative comb weight was also unaffected, although there was a tendency toward lower relative comb weights in the extract-exposed males (Table 6). There was no effect on the sex ratio.

Table 1. Concentrations of selected polyhalogenated aromatic hydrocarbons and TEQs in cormorant egg extract

Compound	(pg/g)[a]	TEF[b]	TEQ[c] (pg/g)	% TEQs
2,3,7,8-TCDD	14	1.0	14	4
1,2,3,7,8-PeCDD	18	1.2	22	7
1,2,3,4,7,8-HxCDD	3.4	0.05	0.2	<0.1
1,2,3,6,7,8-HxCDD	22.3	0.01	0.2	<0.1
1,2,3,7,8,9-HxCDD	5.2	0.1	0.5	<1
1,2,3,4,6,7,8-HpCDD	17	0.001	<0.1	<0.1
2,3,7,8-TCDF	0.7	0.9	0.6	<1
1,2,3,7,8-PeCDF	0.3	0.3	0.1	<0.1
2,3,4,7,8-PeCDF	10	1.1	11	3
1,2,3,4,7,8-HxCDF	2	0.01	<0.1	<0.1
2,3,4,6,7,8-HxCDF	1.3	0.1	0.1	<0.1
1,2,3,6,7,8-HxCDF	1.6	0.4	0.6	<1
3,3′,4,4′,5-PeCB (126)	3,367	0.07[d]	236	73
3,3′,4,4′-TCB (77)	840	0.02	17	5
3,3′,4,4′,5,5′-HxCB (169)	363	0.001	<1	<1
2,3,3′,4,4′-PeCB (105)	247,000	5.1×10^{-5}	13	4
2,3′,4,4′,5-PeCB (118)	711,000	4.1×10^{-6}	3	1
2,3,3′,4,4′,5-HxCB (156)	50,000	6.1×10^{-5}	3	1
2,3,3′,4,4′,5′-HxCB (157)	15,000	7.1×10^{-5}	1	<1
2,3′,4,4′,5,5′-HxCB (167)	58,000	3.1×10^{-6}	<1	<1
Total			322	100%

[a] Values are expressed on a wet weight basis
[b] Toxic Equivalency Factor (Bosveld *et al.* 1995) based on cytochrome P450 induction studies in chicken embryos
[c] Toxic Equivalents, relative to 2,3,7,8-TCDD
[d] Toxic Equivalency Factor (Powell *et al.* 1996a) based on LD_{50} values for chicken embryos

Table 2. Hatchability of White Leghorn chicken eggs injected on day 0 of incubation with graded doses of double-crested cormorant egg extract

Dose[a]	Dose: TEQ[b]	TEQs/Egg[c]	# 126/Egg[d]	# Dead/ # Eggs	Mortality (%)
Non-injected control	0	0	0	3/60	5.0
Vehicle control	0	0	0	12/60	20.0
1×10^{-3}	0.322	1.4×10^{1}	1.4×10^{-4}	16/62	25.8
1×10^{-2}	3.22	1.4×10^{2}	1.4×10^{-3}	6/61	9.8
1×10^{-1}	32.2	1.4×10^{3}	1.4×10^{-2}	13/64	20.3
1.0	322	1.4×10^{4}	1.4×10^{-1}	46/60[e]	76.7

[a] Doses expressed in units of egg equivalents/egg (vehicle = triolein)
[b] Doses expressed in Toxic Equivalents (pg/g egg)
[c] Doses expressed in pg TEQ/egg (avg. cormorant egg = 42 g)
[d] Doses expressed in µg PCB congener 126/egg (avg. cormorant egg = 42 g)
[e] Significantly different from vehicle control at $p < 0.05$

Discussion

Toxic Equivalents (TEQs)

Total TEQs present in the cormorant egg extract (Table 1) were approximately 322 pg/g egg using TEFs derived for chickens by Bosveld *et al.* (1995) with the exception of the TEF for PCB 126 which was derived by Powell *et al.* (1996a). This TEF for PCB 126 was chosen since it was derived using nearly the same methods used in the present study. The TEQ of 322 pg/g contrasts with a value of 1,300 pg/g for cormorant eggs collected from Green Bay, Lake Michigan in 1988 by Yamashita *et al.* (1993). The majority of this difference in total TEQs is due to the difference in TEFs used to calculate the TEQ for PCB congener 126. They used a TEF of 0.3 (Yamashita *et al.* 1993) while a TEF of 0.07 (Powell *et al.* 1996a) was used in the present study. Similar to results reported in the present study, Jones *et al.* (1994) reported that total TCDD-EQs in cormorant eggs collected from Green Bay in the summer of 1989 were approximately 382 pg/g, ww. Recently reported results (Williams *et al.* 1995) from a 1989 sample of cormorant

Table 3. Body weights of White Leghorn chickens injected with a double-crested cormorant egg extract on day 0 of incubation

Dose[a]	n[b]	Hatch[c]	1 Week[c]	2 Weeks[c]	3 Weeks[c]
Non-injected control	53	44.4 ± 0.37	75.0 ± 1.05	130.1 ± 2.04	194.3 ± 3.20
Vehicle control	47	43.6 ± 0.39	72.2 ± 1.16	125.6 ± 2.22	187.0 ± 3.47
1×10^{-3}	44	43.9 ± 0.41	72.1 ± 1.20	124.3 ± 2.30	186.4 ± 3.58
1×10^{-2}	54	43.6 ± 0.37	71.4 ± 1.08	124.2 ± 2.07	185.2 ± 3.23
1×10^{-1}	51	44.1 ± 0.38	68.9 ± 1.11	119.4 ± 2.13	181.1 ± 3.33
1.0	13	44.5 ± 0.75	62.8 ± 2.20[d]	106.5 ± 4.22[d]	160.0 ± 6.59[d]

[a] Doses are expressed in units of egg equivalents/egg
[b] Sample size
[c] Data expressed as mean ± standard error (g)
[d] Significantly different from vehicle control at $p < 0.05$

Table 4. Absolute and relative brain weights of three-week-old White Leghorn chicks injected with a double-crested cormorant egg extract on day 0 of incubation

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected control	51	1.69 ± 0.013	0.878 ± 0.0140
Vehicle control	47	1.69 ± 0.014	0.914 ± 0.0149
1×10^{-3}	43	1.67 ± 0.015	0.909 ± 0.0156
1×10^{-2}	54	1.66 ± 0.013	0.908 ± 0.0139
1×10^{-1}	51	1.64 ± 0.013[d]	0.913 ± 0.0143
1.0	13	1.55 ± 0.026[d]	0.994 ± 0.0283[d]

[a] Doses are expressed in units of egg equivalents/egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 5. Absolute and relative bursa weights of three-week-old White Leghorn chicks injected with a double-crested cormorant egg extract on day 0 of incubation

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected control	51	1.17 ± 0.044	0.599 ± 0.0183
Vehicle control	47	1.13 ± 0.047	0.601 ± 0.0202
1×10^{-3}	43	1.09 ± 0.050	0.582 ± 0.0211
1×10^{-2}	54	1.13 ± 0.044	0.600 ± 0.0188
1×10^{-1}	51	1.07 ± 0.046	0.584 ± 0.0194
1.0	12	0.62 ± 0.094[d]	0.385 ± 0.0399[d]

[a] Doses are expressed in units of egg equivalents/egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control at $p < 0.05$

Table 6. Absolute and relative comb weights of three-week-old male White Leghorn chicks injected with a double-crested cormorant egg extract on day 0 of incubation

Dose[a]	n[b]	Weight (g)[c]	% Body Weight[c]
Non-injected control	31	0.282 ± 0.0330	0.139 ± 0.0170
Vehicle control	24	0.376 ± 0.0404	0.193 ± 0.0213
1×10^{-3}	19	0.242 ± 0.0454	0.122 ± 0.0240
1×10^{-2}	26	0.235 ± 0.0388	0.120 ± 0.0205
1×10^{-1}	21	0.254 ± 0.0432	0.135 ± 0.0228
1.0	7	0.192 ± 0.0749	0.115 ± 0.0395

[a] Doses are expressed in units of egg equivalents/egg
[b] Sample size
[c] Data expressed as mean ± standard error

eggs from Spider Island were in general agreement with this study. However, the mean total TEQs were higher, 552 vs 322 in the present study, when their concentrations were multiplied by the TEFs used in this study and a 14% relative contribution from dioxins and furans was added. The relative contribution of the PCB congeners were in the same rank order in both studies although the numerical contribution in the former study differed somewhat from the extract of the present study because they found higher concentrations of the two most potent congeners, #126 and #77. Of the TEQs reported by Yamashita *et al.* (1993), PCB congener 126 accounted for 83% of the TEQs and PCB congener 105 accounted for 13%. Congener 126 also accounted for the majority of the TEQs in the present cormorant extract (73%). This congener was present at a concentration of 3,367 pg/g, ww egg which is consistent with the concentration (3,600 pg/g egg) determined in cormorant eggs collected by Yamashita *et al.* (1993). Concentrations of other congeners and PHDHs contributing to the toxicity of the extract were also relatively close to those reported by Yamashita *et al.* (1993), except for PCB congener 77 which was nine times higher in their study. However, the relative contribution of this congener was small (less than 2%) in their study due to a low TEF (0.0021). PCB congeners 77 and 105 accounted for approximately 5 and 4%, respectively, of the TEQs in the present cormorant egg extract and 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF accounted for 14% of the TEQs. These differences in results from samples taken at similar times in the same general area demonstrate that the choice of TEFs can be extremely important in the toxicological interpretation of analytical results.

TEQs have been correlated with reproductive and developmental problems in wild avian species. An association has been reported between reproductive impairment in Forster's terns and the occurrence of 2,3,7,8-substituted dioxins and nonortho-, ortho-, monoortho-, and diortho-substituted PCB congeners (Kubiak *et al.* 1989). TCDD-EQs derived through an *in vitro* assay, the H4IIE rat hepatoma bioassay, have been found in the greatest concentrations in the eggs of colonial waterbirds where reproductive impairment has been severe. These birds were also located in areas historically polluted through industrialization (Tillitt *et al.* 1991). TCDD-EQs from contaminated cormorant eggs have been correlated with the reproductive success of cormorants (Larson *et al.* 1996; Tillitt *et al.* 1992). In particular, Tillitt *et al.* (1992) demonstrated a significant relationship between the hatching success of cormorant eggs in the Great Lakes and PCBs.

Mortality

The injection of the cormorant egg extract into White Leghorn chicken eggs in the present study produced increased mortality at 1.0 egg-equivalent, which corresponds to the concentration found in an average cormorant egg from Green Bay, Lake Michigan in 1988. Since PCB congener 126 was present in the extract at 3,367 pg/g egg and an average cormorant egg weighed 42 g, each chicken egg received approximately 141,000 pg of congener 126. This corresponds to an injection dose of approximately 2.4 µg congener 126/kg egg (assuming a chicken egg weighs 60 g) which is essentially the same as the LD_{50} for congener 126 in chickens, 2.3 µg/kg egg (Powell *et al.* 1996a). Since greater than 50% mortality occurred at 2.4 µg/kg egg, it is presumed that the remaining components of the extract contributed to the greater mortality (77%). The LD_{50} for this extract is between 0.2 and 1.0 egg-equivalent which corresponds to 64 to 322 pg TEQ/g [(0.2 to 1.0 egg-equivalent) × 322 pg TEQ/g/egg-equivalent]. The LD_{50} for TCDD in the chicken had been estimated to be 150 pg/g egg (0.15 µg/kg egg; Henshel 1993; Powell *et al.* 1996a). This is within the range of the LD_{50} of the cormorant egg extract, 64 to 322 pg TEQ/g, determined in this study. This supports the additivity assumption which is made when working with TEQs to estimate total potency of an environmental extract.

Teratogenicity

There is an association between abnormalities in live cormorant chicks and areas with eggs contaminated with 2,3,7,8-TCDD, 2,3,7,8-TCDF, and planar PCBs (Larson *et al.* 1996; Yamashita *et al.* 1993). However, there were no significant differences in the incidences of abnormalities among any of the dose groups in the present study. The rate of occurrence of bill defects in cormorant chicks from Green Bay was 0.5% (Fox *et al.* 1991a). With such a low rate of occurrence, a much larger sample size may be needed to detect significant differences among treatment groups. The greatest dose (1.0 egg-equivalent) delivered in this study contained a lower concentration of congener 126 (2.4 µg/kg egg) than did doses of congener 126, which caused an increased incidence of abnormalities in a previous injection study, (3.2–12.8 µg/kg egg; Powell *et al.* 1996a). In another study, 0.9 µg/kg egg of congener 126 caused a significant increase in the incidence of abnormalities (Powell *et al.* 1996b). However, differences in solvents used suggest that direct quantitative comparisons of these two studies are not appropriate. In each of these studies, significantly increased incidences of abnormalities were seen only at doses greater than the calculated LD_{50}s in those studies. It is also possible that the presence of less toxic PCB congeners in combination with congener 126 may have been antagonistic (Hoffman *et al.* 1996) thus explaining the lack of an increase in the incidence of abnormalities.

Body Weights

The cormorant extract had a significant effect on body weights at the first, second, and third weeks post-hatch. Chicks from eggs injected with PCB congener 126 (0.9, 2.7 µg/kg egg) were also reported to have lower body weights at the second and third week of rearing (Powell *et al.* 1996b). Lower body weights have also been observed in chicks exposed *in ovo* to PCB congener 77 (Brunström and Darnerud 1983) and Aroclor® 1242 (Carlson and Duby 1973). Three-week-old chicks of hens fed 20 ppm Aroclor® 1242 and 1248 for eight weeks were significantly lighter than chicks of hens fed control feed (Harris *et al.* 1976).

Organ and Comb Weights

Relative brain weights were greater in the 1.0 egg-equivalent dose group, however, this was probably a result of the significantly lower body weights also observed in chicks of this dose group. The average relative weight of the bursa was significantly less in chicks exposed to 1.0 egg-equivalent prior to incubation compared to control chicks. Chicks exposed to PCB congener 126 prior to incubation also had lower relative bursa weights at three weeks post-hatch (Powell *et al.* 1996b). Relative heart, liver, spleen, and testes weights were not significantly different when compared to the vehicle control chicks. While not significantly different, it is of interest that comb weight, a secondary sex characteristic, was lower in the 1.0 egg-equivalent dose group when compared to the vehicle control group. This suggests that the extract may have had an estrogenic or anti-androgenic effect. Organochlorine compounds have been reported to cause feminization of male germ cells in the testes (Fry *et al.* 1987). When one-month-old cockerels were fed 500 ppm Aroclor® 1254 over a 12 week period, they showed no comb growth and their combs weighed significantly less than controls. These PCB-fed birds also had reduced gain in testes weight (Platonow and Funnell 1971). Cockerels fed 500 ppm of the same Aroclor® for 30 d also had lower comb and testes weights than the control birds (Cecil *et al.* 1978). In this study, the lack of an effect on testes weights may be due to the relatively young age of the birds and the small size of the testes at this age when compared to older birds for which there have been reported changes in testes weight.

Conclusions

The hepatic cytosolic AhR of the chicken has an affinity for TCDD which is approximately 15 times greater than the AhR affinity of the cormorant (Sanderson and Bellward 1995). The LD_{50} for this cormorant egg extract in the chicken is between 0.2 and 1.0 egg-equivalent. Thus, the LD_{50} of the cormorant egg extract in the cormorant may be estimated to be between 3 and 15 egg-equivalents [15 × (0.2 to 1.0 egg-equivalent)]. In addition, using total TEQs from the cormorant egg extract, the predicted LD_{50} for this extract in the cormorant would be 966 to 4,830 pg TEQ/g. The estimated LD_{50} for the cormorant based on field studies, 1,000 pg TCDD-EQ/g (Tillitt 1989), is within the range of the predicted LD_{50} determined in this study. Thus, 3 to 15 times the concentration of PHDHs found in 1988 cormorant eggs would be required to cause 50% mortality in the cormorant. Since egg PCB concentrations in the 1960s and 1970s were twenty times greater than that of eggs collected in 1988, it is possible that these compounds could account for a great deal, if not all, of the mortality (or lack of breeding success) observed in the 1960s and 1970s (Tillitt *et al.* 1996).

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