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Effects of 3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126) and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Injected into the Yolks of Chicken (*Gallus domesticus*) Eggs Prior to Incubation

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Abstract. The yolks of White Leghorn chicken (*Gallus domesticus*) eggs were injected prior to incubation with either 3,3′,4,4′,5- pentachlorobiphenyl (PCB 126) at doses ranging from 0.1 to 12.8 μg/kg egg or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at doses ranging from 0.04 to 0.64 μg/kg egg. Chicks were subjected to necropsy within 24 h of hatching. The brain, bursa, heart, liver, and spleen were removed and weighed. Assessment of the rate of hatching indicated an LD_{50} ± S.E. of 2.3 ± 0.19 μg/kg egg (7.1 ± 0.58 nmol/kg egg) for PCB 126 and 0.15 ± 0.012 μg/kg egg (0.47 ± 0.037 nmol/kg egg) for TCDD. No significant differences in the incidence of developmental abnormalities (structural defects and edema) were observed in TCDD-exposed embryos, while PCB 126 caused significantly more developmental abnormalities at 3.2, 6.4, and 12.8 μg/kg egg than the vehicle control. PCB 126 caused lower hatchling weights and greater relative brain, heart, and liver weights when compared to the vehicle control group at a dose of 3.2 μg/kg egg which is greater than the LD_{50}. TCDD at 0.08 μg/kg egg caused relative bursa weights to be less than those of the vehicle control. A toxic equivalency factor (TEF) of 0.07 was determined for PCB 126 in relation to TCDD based on overt lethality.

Polychlorinated biphenyls (PCBs) are widespread contaminants in the Great Lakes. Under laboratory conditions they are known to cause a variety of adverse effects in birds; such as embryo lethality, hydropericardium, beak deformities, subcutaneous edema, liver lesions, and induction of cytochrome P-450 enzymes (Brunström 1989, 1991). Non-ortho substituted PCBs are structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and act through the same mechanism of toxicity as TCDD (Giesy *et al.* 1995). This mechanism involves binding of the planar compound with the aryl hydrocarbon receptor (AhR) and subsequent entry of this complex into the nucleus where it interacts with specific DNA sequences located upstream from the cytochrome P4501A1 (CYP1A1) gene. This leads to enhanced expression of CYP1A1 and other genes which in turn leads to the pleiotropic responses (Safe 1990).

Dioxin toxic equivalents (TEQs) provide a total potency estimate for mixtures of Ah-active compounds relative to TCDD, the most potent known polychlorinated hydrocarbon with regard to AhR binding (Safe 1987). Dioxin toxic equivalents are calculated as the sum of the product of the molar concentrations of individual congeners and their toxic equivalency factor (TEF) compared to TCDD which is arbitrarily assigned a TEF of 1.0. Toxic equivalency factors for PCBs, dioxins, and furans can vary depending on the species and end-points used to derive the TEFs (Safe 1990; Bosveld *et al.* 1992).

The most toxic of the planar PCB congeners in avians and most other phyla tested is 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Brunström 1989; Safe 1990). This congener represented approximately 83% of the toxic equivalents in double-crested cormorant (*Phalacrocorax auritus*) eggs collected from Green Bay, Lake Michigan, one of the most contaminated sites in the Great Lakes basin (Yamashita *et al.* 1993).

The objective of this study was to determine the toxicity of PCB 126 and TCDD in White Leghorn chicken (*Gallus domesticus*) embryos. A specific goal was to establish a TEF

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for PCB 126 in the chicken based on biologically relevant parameters.

Materials and Methods

There were eight doses of PCB 126 [0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 μg/kg egg, wet weight (ww)] and five doses of TCDD [0.04, 0.08, 0.16, 0.32, and 0.64 μg/kg egg (ww)]. In addition to those eggs injected with PCB 126 or TCDD, there were eggs which were injected with the respective vehicle (vehicle controls) and eggs which were not injected (non-injected controls). Egg injections were done in a series of three replicates with 20 eggs/group in each replicate.

PCB 126 (AccuStandard, New Haven, CT) was dissolved directly in triolein (Sigma Chemical Co., St. Louis, MO) to make a stock solution (0.2 μg/μL) which was subsequently diluted with triolein to the appropriate dose. Each dose was cold filtered with a 0.22 micron syringe filter to sterilize the solution prior to injection. All injection solutions and stocks of triolein were purged with argon to prevent oxidation of the lipid.

TCDD injection solutions were prepared at the Midwest Science Center, National Biological Service (Columbia, MO). The vehicle was triolein (filter-sterilized) with less than 2% nonane, which was used to aid dissolution of the TCDD. Dosing solutions were sonicated with heating prior to injection to ensure solubilization of the TCDD.

All injections[1] were made directly into the yolk prior to incubation. Before the day of injection, eggs[2] were weighed, candled for the location of the air cell, and allocated to dose groups with each group receiving an equal distribution of eggs weighing from 55 to 70 g. Eggs were subsequently placed on their sides to allow the germ spot to rise to the top and minimize potential injury during injection. Eggs were stored in a cooler at 58°F (14.4°C) until the day of injection. The injection volume was 0.1 μL/g egg (ww). The surface of the egg was sterilized with 70% ethanol and a small hole was made into the air cell with a sterile pin. A sterile microliter syringe (22S gauge; Hamilton Co., Reno, NV) was inserted horizontally through the air cell approximately 30 mm into the egg which was positioned on its side. Following injection of the solution and removal of the needle, the hole was sealed with melted paraffin.

Eggs were incubated in a Petersime model #5 incubator (Petersime Incubator Co., Gettysburg, OH). They were positioned with their blunt end up and incubated according to standard procedures for this species, 99.5–99.75°F (37.5–37.6°C) with a relative humidity of approximately 65% (North 1978). Eggs were candled on days 4, 11, and 18. All nonviable eggs at days 11 or 18 and unhatched eggs at day 24 were opened and the embryos were assessed for developmental abnormalities (structural defects and edema). Hatchling chicks were weighed and killed by decapitation. The brain, bursa, heart, liver, and spleen were removed and weighed.

Categorical data (mortality and incidence of developmental abnormalities) were analyzed using contingency tables and the Bonferroni Chi-square table. The LD_{50}s were calculated using probit analysis on percentages adjusted for mortality in the vehicle control group. Analysis of all other parameters was done using one-way analysis of variance followed by Dunnett's test (SAS Institute Inc. 1990). Relative organ weights, expressed as a percent of body weight, were used for statistical tests in order to correct for effects on overall body size. Actual weights were also analyzed. All comparisons were made with respect to the vehicle control group unless otherwise stated.

[1] This research was approved by Michigan State University's (MSU) All University Committee on Animal Use and Care

[2] Eggs were provided by the MSU Poultry Science Teaching and Research Center

Table 1. Effect of injection of PCB 126 into the yolks of White Leghorn chicken eggs on day 0 of incubation on hatchability and incidence of developmental abnormalities

Dose μg/kg egg (nmol/kg egg)	No. abnormal[a]/ no. eggs (% abnormal)	No. dead/no. eggs (% mortality)
Non-injected	0/53	3/53
(0)	(0)	(5.7)
Vehicle	0/59	5/59
(0)	(0)	(8.5)
0.1	1/60	11/60
(0.3)	(1.7)	(18.3)
0.2	5/59	18/59[b]
(0.6)	(8.5)	(30.5)
0.4	4/59	15/59
(1.2)	(6.8)	(25.4)
0.8	2/59	12/59
(2.5)	(3.4)	(20.3)
1.6	2/60	13/60
(4.9)	(3.3)	(21.7)
3.2	13/60[b]	55/60[b]
(9.8)	(21.7)	(91.7)
6.4	19/59[b]	59/59[b]
(19.6)	(32.2)	(100)
12.8	10/60[b]	60/60[b]
(39.2)	(16.7)	(100)

[a] Any embryo with one or more types of developmental abnormalities (structural defects or edema)

[b] Significantly different from the vehicle control and non-injected control at $p < 0.05$

Results

PCB 126 and TCDD both caused embryo lethality. Mortality was significantly greater in the 0.2, 3.2, 6.4, and 12.8 μg/kg egg dose groups of PCB 126 than that of the vehicle control group (Table 1). TCDD caused significantly greater mortality at doses of 0.16, 0.32, and 0.64 μg/kg egg when compared to the vehicle control (Table 2). LD_{50}s ± S.E. were calculated to be 2.3 ± 0.19 and 0.15 ± 0.012 μg/kg egg for PCB 126 and TCDD, respectively.

No deformed embryos or hatchlings were observed in the vehicle control group while doses of 3.2, 6.4, and 12.8 μg PCB 126/kg egg caused a significant increase in the incidence of developmental abnormalities (Table 1). The types of developmental abnormalities observed included skull, eye, beak, and toe deformities as well as subcutaneous edema. TCDD at 0.16 and 0.32 μg/kg egg caused a significant increase in the incidence of developmental abnormalities when compared to the vehicle control. None of the dose groups were statistically different from the non-injected control group (Table 2).

PCB 126 and TCDD both caused sublethal effects such as changes in body weights and various organ weights. Body weights (Table 3) of chicks receiving 3.2 μg/kg egg PCB 126 were significantly less than those in the vehicle control group. Doses greater than 3.2 μg/kg egg resulted in 100% embryo

Table 2. Effect of injection of TCDD into the yolks of White Leghorn chicken eggs on day 0 of incubation on hatchability and incidence of developmental abnormalities

Dose μg/kg egg (nmol/kg egg)	No. abnormal[a]/ no. eggs (% abnormal)	No. dead/no. eggs (% mortality)
Non-injected	3/52	12/52
(0)	(5.8)	(23.1)
Vehicle	0/56	13/56
(0)	(0)	(23.2)
0.04	1/54	19/54
(0.12)	(1.9)	(35.2)
0.08	1/56	16/56
(0.25)	(1.8)	(28.6)
0.16	7/54[b]	47/54[bc]
(0.50)	(13.0)	(87.0)
0.32	7/55[b]	55/55[bc]
(0.99)	(12.7)	(100)
0.64	2/56	56/56[bc]
(1.99)	(3.6)	(100)

[a] Any embryo with one or more types of developmental abnormalities (structural defects or edema)
[b] Significantly different from the vehicle control at $p < 0.05$
[c] Significantly different from the non-injected control at $p < 0.05$

Table 3. Effect of PCB 126 on the hatch body weights of White Leghorn chickens

Dose[a]	n[b]	Body weight[c] (g)
Non-injected	50	43.0 ± 0.49
Vehicle	54	41.4 ± 0.47
0.1	49	42.4 ± 0.50
0.2	41	42.4 ± 0.54
0.4	42	41.9 ± 0.54
0.8	45	41.6 ± 0.52
1.6	47	41.8 ± 0.51
3.2	8	36.1 ± 1.23[d]

[a] Doses expressed as μg/kg egg
[b] Sample size
[c] Data expressed as mean ± standard error
[d] Significantly different from vehicle control and non-injected control at $p < 0.05$

mortality. There were no differences in body weights between chicks exposed to TCDD and those exposed to the vehicle only (data not shown), even at the greatest dose not resulting in 100% embryo mortality (0.16 μg/kg egg). Relative brain and liver weights (Table 4) were both significantly greater at 3.2 μg/kg egg of PCB 126 than the values of the same parameters in the vehicle control group. Relative heart weights were less than the heart weights of the vehicle control group at 0.1 μg/kg egg and greater at 3.2 μg/kg egg. There were no significant differences in actual or relative spleen weights when compared to the vehicle control group (data not shown). TCDD caused relative bursa weights to be significantly less (Table 5) and heart weights to be significantly greater than bursa and heart weights of vehicle controls (Table 5) at 0.08 μg/kg egg. However, heart weights in the vehicle control group were significantly less than those of the non-injected controls.

Discussion

Mortality

LD_{50} values of 2.3 ± 0.19 and 0.15 ± 0.012 μg/kg egg for PCB 126 and TCDD reported here are very similar to the results reported by others. Earlier research by Brunström and Andersson (1988) indicated an LD_{50} of 3.1 μg/kg egg for PCB 126 in chickens. However, exposure to this congener did not occur until day 7 of incubation and assessment of mortality was made only at 72 h post-injection. In addition, the site of exposure was the air cell as opposed to the yolk which was the site in the present study. When Brunström (1991) injected 0.2 μg PCB 126/kg egg into the yolk on day 4, he observed 10% mortality by day 18 while injection of 2 μg/kg egg of PCB 126 resulted in 90% mortality by day 18. In the present study, a dose of 0.2 μg PCB 126/kg egg resulted in 30.5% mortality, however, the data in Table 1 indicate that the real beginning of a response is between 1.6 and 3.2 μg/kg egg, so the marginally significant response at 0.2 μg/kg egg is not of biological significance. The 3.2 μg/kg egg dose resulted in approximately 92% mortality which is reasonably close to the results reported by Brunström (1991). The LD_{50} for TCDD has been reported to be 0.15 μg/kg egg when injected into the yolk on day 0 (Henshel 1993) which is the same as the LD_{50} reported here. Injection of TCDD into the air cell of fertile eggs resulted in an LD_{50} value of 0.24 μg/kg egg (Allred and Strange 1977) when viability was assessed on day 18 of incubation which is only slightly greater than the LD_{50} value reported in this study. In another study where TCDD was injected into the air cell on day 4 of incubation, the LD_{50} was calculated to be 0.15 μg/kg egg (Verrett 1976). Despite differences in laboratories, injection sites, and chronology of exposure across studies, it is apparent that the LD_{50} is a repeatable measure of toxicity in avian egg injection studies involving PCBs and dioxins.

The vehicle and/or injection volume may significantly affect the lethality of a given compound. In a previous study (Powell *et al.* 1996), the LD_{50} for PCB 126 was determined to be 0.6 μg/kg egg, which is 25% of the value reported in this study (2.3 μg/kg egg). In the previous trial, the vehicle used was an emulsion of lecithin, peanut oil, and water described by Brunström and colleagues (Brunström and Örberg 1982; Brunström and Darnerud 1983) and the injection volume was 1 μL/g egg. In the present study, the vehicle was triolein and the volume injected was 0.1 μL/g egg which presumably led to less mortality in the vehicle control eggs in this study. Therefore, the LD_{50} for PCB 126 in the chicken egg reported here is more appropriate than the LD_{50} reported in our previous study (Powell *et al.* 1996).

Teratogenicity

A significantly greater number of developmental abnormalities was observed in eggs injected with PCB 126 at 3.2 μg/kg egg and above. Edema, microphthalmia (reduced eye size), and beak deformities were the most frequent types of abnormalities observed. The teratogenic capabilities of PCB 126 in the chicken have also been reported in other studies (Brunström and Andersson 1988; Powell *et al.* 1996). Brunström and Andersson (1988) observed microphthalmia, subcutaneous edema, and beak de-

Table 4. Effect of PCB 126 on relative (actual) organ weights of hatching White Leghorn chickens

Dose (μg/kg egg)	n[a]	Brain[b]	Bursa[b]	Heart[b]	Liver[b]
Non-injected	50	2.0 ± 0.03	0.14 ± 0.006	0.61 ± 0.011	2.0 ± 0.04
		(0.87 ± 0.009)	(0.061 ± 0.0025)	(0.26 ± 0.004)	(0.87 ± 0.015)
Vehicle	54	2.1 ± 0.03	0.14 ± 0.006	0.60 ± 0.011	2.1 ± 0.04
		(0.85 ± 0.009)	(0.059 ± 0.0024)	(0.25 ± 0.004)	(0.86 ± 0.014)
0.1	49	2.0 ± 0.03	0.13 ± 0.006	0.55 ± 0.011[cd]	2.1 ± 0.04
		(0.85 ± 0.009)	(0.055 ± 0.0025)	(0.23 ± 0.005)[d]	(0.88 ± 0.015)
0.2	41	2.0 ± 0.03	0.14 ± 0.007	0.56 ± 0.012[d]	2.1 ± 0.04
		(0.85 ± 0.010)	(0.060 ± 0.0028)	(0.24 ± 0.005)[d]	(0.88 ± 0.016)
0.4	42	2.0 ± 0.03	0.14 ± 0.006	0.58 ± 0.012	2.1 ± 0.04
	(43)	(0.85 ± 0.010)	(0.059 ± 0.0027)	(0.24 ± 0.005)	(0.88 ± 0.016)
0.8	45	2.0 ± 0.03	0.13 ± 0.006	0.60 ± 0.012	2.1 ± 0.04
		(0.84 ± 0.009)	(0.053 ± 0.0026)	(0.25 ± 0.005)	(0.88 ± 0.016)
1.6	47	2.1 ± 0.03	0.13 ± 0.006	0.59 ± 0.011	2.1 ± 0.04
		(0.85 ± 0.009)	(0.055 ± 0.0026)	(0.25 ± 0.005)	(0.89 ± 0.015)
3.2	8	2.4 ± 0.07[cd]	0.11 ± 0.015	0.70 ± 0.028[cd]	2.7 ± 0.10[cd]
	(10)	(0.85 ± 0.020)	(0.036 ± 0.0058)[cd]	(0.25 ± 0.010)	(0.90 ± 0.033)

[a] Sample size [0.2 μg/kg egg, n = 40 for bursa; 0.4 μg/kg egg, n = 41 (42) for bursa; and 3.2 μg/kg egg, n = 7 (9) for bursa]
[b] Data expressed as mean ± standard error, relative weight [actual weight (g)]
[c] Significantly different from vehicle control at $p < 0.05$
[d] Significantly different from non-injected control at $p < 0.05$

Table 5. Effect of TCDD on relative (actual) organ weights of hatching White Leghorn chickens

Dose (μg/kg egg)	n[a]	Brain[b]	Bursa[b]	Heart[b]	Liver[b]
Non-injected	39	2.01 ± 0.031	0.12 ± 0.005	0.61 ± 0.010	1.88 ± 0.036
		(0.872 ± 0.0082)	(0.053 ± 0.0020)	(0.27 ± 0.004)	(0.82 ± 0.018)
Vehicle	43	2.05 ± 0.027	0.11 ± 0.004	0.57 ± 0.010[d]	1.83 ± 0.034
		(0.873 ± 0.0070)	(0.049 ± 0.0019)	(0.24 ± 0.004)[d]	(0.78 ± 0.017)
0.04	34	2.06 ± 0.030	0.11 ± 0.005	0.57 ± 0.011	1.93 ± 0.039
	(35)	(0.848 ± 0.0078)	(0.045 ± 0.0021)[d]	(0.24 ± 0.005)[d]	(0.80 ± 0.019)
0.08	39	2.02 ± 0.028	0.10 ± 0.005[cd]	0.61 ± 0.011[c]	1.93 ± 0.036
		(0.849 ± 0.0074)	(0.041 ± 0.0020)[cd]	(0.26 ± 0.005)	(0.82 ± 0.018)
0.16	6	1.95 ± 0.071	0.09 ± 0.012[d]	0.61 ± 0.027	1.96 ± 0.092
	(7)	(0.838 ± 0.0175)	(0.038 ± 0.0046)[d]	(0.26 ± 0.011)	(0.86 ± 0.043)

[a] Sample size
[b] Data expressed as mean ± standard error, relative weight [actual weight (g)]
[c] Significantly different from vehicle control at $p < 0.05$
[d] Significantly different from non-injected control at $p < 0.05$

formities at 2 μg/kg egg, which is similar to results reported here. While there was no statistically greater incidence of developmental abnormalities observed as a result of TCDD exposure when compared to the non-injected control group, edema of the head and neck region and abnormally small embryos occurred more often in the TCDD treatment groups. Other observed abnormalities included shortened upper beak, microphthalmia, and stunted legs. These types of developmental abnormalities have been observed in other studies involving *in ovo* exposure to TCDD (Cheung *et al.* 1981; Sanderson and Bellward 1995; Henshel, personal communication). When 0.009 to 77.5 pmol TCDD/egg (0.005 to 0.45 μg/kg egg) was injected into the albumen of chicken eggs on day 0, leg, beak, and eye malformations were observed, however the occurrence of these abnormalities was only 1–3% (Cheung *et al.* 1981). Subcutaneous edema in chicken embryos exposed to TCDD at a dose of 0.01 μg/kg egg was reported by Sanderson and Bellward (1995). Since this dose was less than the dose required to induce liver enzyme activity, they suggested that edema may be a more sensitive response to TCDD exposure.

Body Weight

Chickens hatching from the 3.2 μg/kg egg dose group of PCB 126 had significantly lower body weights than those in the vehicle control group. This is in contrast to results of a previous experiment in which body weights of hatchling chicks from

eggs injected with PCB 126 on day 0 of incubation were not different when compared to the vehicle control group (Powell *et al.* 1996). However, the greatest dose at which hatching occurred in the first experiment was 2.7 μg/kg egg, which was less than the dose which caused a significant change in hatch body weights in the present trial. It is unclear if this effect on hatchling body weight is a real effect on body weight or an overall toxic reaction just short of death. Chickens exposed to TCDD in this study had no significant differences in body weights. In contrast, Henshel (personal communication) found that chicken embryo body weight upon hatching decreased with increasing concentrations of TCDD. These effects were observed at concentrations above 100 pg/g egg (0.1 μg/kg egg).

Brain

Relative brain weights were greater in chicks hatching from the 3.2 μg/kg egg dose group of PCB 126. However, this group of chicks also had lower body weights at hatch. Thus, the apparent effect observed in relative brain weight was due to the change in body weight. Actual brain weights were not different when compared to the vehicle control group. There were also no differences in actual or relative brain weights of chickens exposed *in ovo* to TCDD which is probably due to the lack of an effect on body weight.

Bursa of Fabricius

The bursa, a specialized organ in young birds involved in B-cell development and differentiation (Nikolaidis *et al.* 1988, 1990), was significantly affected by TCDD but not PCB 126. Injection of PCB 126 caused significantly smaller actual bursa weights (3.2 μg/kg egg), but not significantly smaller relative weights. Since this dose of PCB 126 caused a significant decrease in body weights, the low bursa weights are presumably a reflection of this effect since relative weights, which are corrected for body weight, are not significantly different. Injection of TCDD resulted in a reduction of both actual and relative bursa weights at 0.08 μg/kg egg. The same trend in weight reduction was observed in the 0.16 μg/kg egg dose group, but the small sample size in this dose group reduced the statistical power and probably obscured real changes already demonstrated at the 0.08 μg/kg egg dose group. Previous studies have shown a PCB-induced decrease in bursa weights (Harris *et al.* 1976) and TCDD-induced inhibition of lymphoid development in the bursa (Nikolaidis *et al.* 1990).

Heart

The effects of PCB 126 and TCDD were difficult to interpret. For example, PCB 126 caused relative heart weights to be less at 0.2 μg/kg egg and greater at 3.2 μg/kg egg than heart weights in the vehicle control group. There were no biologically relevant changes in the heart weights of TCDD-exposed chicks since the vehicle control heart weights were significantly less than those of the non-injected group. Perhaps variations in tissue trimming around the vessels altered the results. Other studies with chlorinated biphenyls have reported pericardial edema in exposed chicks (McCune *et al.* 1962; Flick *et al.* 1963; Brunström 1988, 1990; Brunström *et al.* 1990). Brunström and Andersson (1988) reported incidences of hydropericardium in chicks exposed to 2 μg PCB 126/kg egg on day 4 and examined on day 18 of incubation. No obvious signs of hydropericardium were observed in this study.

Liver

Both PCB 126 and TCDD caused a trend of increasing actual liver weights. A dose of 3.2 μg PCB 126/kg egg caused relative liver weights to be significantly greater than the vehicle control, however, body weights were also lower at this dose group. Increases in liver weight have been associated with induction of detoxifying enzymes (Cecil *et al.* 1978). PCB 126 induces hepatic 7-ethoxyresorufin *O*-deethylase (EROD) activity in chicken embryos (Brunström and Andersson 1988; Brunström 1990; Sanderson and Bellward 1995). An ED_{50} of 0.3 nmol/kg egg (0.1 μg/kg egg) for PCB 126's ability to induce EROD activity was determined by Brunström and Andersson (1988). They also noted liver lesions at 2 μg/kg egg in 18-day-old embryos exposed on day 4 of incubation. Relative liver weights were not significantly greater in TCDD-exposed chicks when compared to the vehicle control in the present study. Sanderson and Bellward (1995) observed increases in liver weights in chicken hatchlings exposed to 3 μg/kg egg of TCDD on day 16 of incubation.

Toxic Equivalency Factors (TEFs)

TEFs for PCB 126 in the chicken were determined for endpoints in which there were statistical differences from the vehicle control. The TEF based on the LD_{50} values ($LD_{50\text{-}TCDD}$/$LD_{50\text{-}C126}$) was 0.07. The relative potency of PCB 126 was also determined to be 0.07 using EROD induction in cultured hepatocytes from chicken embryos (Yao *et al.* 1990). The estimated TEF using LOAELs ($LOAEL_{TCDD}$/$LOAEL_{C126}$) with mortality as the endpoint was 0.05. These LOAELs were calculated by taking the geometric mean between the lowest dose at which there was a statistical difference from the control and the dose below it. There were no biologically relevant changes in the other parameters for both compounds from which TEFs could be derived. Based on the following TEF values for PCB 126; 0.015, 0.12, 0.1, 0.008, 0.30, and 0.01 calculated using body weight loss in the rat, thymic atrophy in the rat, fetal mouse thymic lymphoid development, hepatic AHH induction in the rat, AHH induction in rat hepatoma H4IIE cells, and AHH induction in chick hepatocytes, respectively, Safe (1990) concluded that a TEF of 0.1 was appropriate for PCB 126. A chicken egg injection study in which various polyhalogenated aromatic hydrocarbons, including PCB 126 and TCDD, were injected into the air cell on day 17 and hepatic EROD induction was assessed 24 h later resulted in a TEF of 0.1 (Bosveld *et al.* 1992). The TEFs calculated in the present study were very similar to those reported previously despite the differences in exposure times and the parameters examined. Our data support the use of a TEF for PCB 126 of 0.1 for acute mortality/embryo lethality. However, the data also suggest that an even lower TEF, perhaps 0.05, might better reflect conditions often used

in ecological risk assessment where the intent is to estimate NOAEL or LOAEL exposures.

Conclusions

The LD_{50} values obtained for PCB 126 and TCDD, 2.3 ± 0.19 and 0.15 ± 0.012 μg/kg egg, respectively, from this study provide a good estimate for the sensitivity of the chicken to these compounds in terms of mortality. This study has also shown an effective method for exposing chicken embryos to lipophilic chemicals on day 0 of incubation. Triolein injected at 0.1 μL/g egg provided early exposure via the yolk with minimal embryo mortality. Of all the parameters examined, the embryo mortality data provided the clearest dose-response relationship. Thus, based on the LD_{50} values, a TEF of 0.07 was determined for PCB 126 in the chicken embryo, and this TEF is similar to the TEF value of 0.1 which is commonly used in the literature for PCB 126.

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References

Allred PM, Strange JR (1977) The effects of 2,4,5- trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin on developing chicken embryos. Arch Environ Contam Toxicol 6:483–489

Bosveld ATC, Van den Berg M, Theelen RMC (1992) Assessment of the EROD inducing potency of eleven 2,3,7,8-substituted PCDD/Fs and three coplanar PCBs in the chick embryo. Chemosphere 25:911–916

Brunström B (1988) Sensitivity of embryos from duck, goose, herring gull, and various breeds to 3,3′,4,4′-tetrachlorobiphenyl. Poult Sci 67:52–57

——— (1989) Toxicity of coplanar polychlorinated biphenyls in avian embryos. Chemosphere 19:765–768

——— (1990) Mono-ortho-chlorobiphenyls: toxicity and induction of 7-ethoxyresorufin *O*-deethylase (EROD) activity in chick embryos. Arch Toxicol 63:188–192

——— (1991) Toxicity and EROD-inducing potency of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in avian embryos. Comp Biochem Physiol 100:241–243

Brunström B, Andersson L (1988) Toxicity and 7-ethoxyresorufin O-deethylase-inducing potency of coplanar polychlorinated biphenyls (PCBs) in chick embryos. Arch Toxicol 62:263–266

Brunström B, Darnerud PO (1983) Toxicity and distribution in chick embryos of 3,3′,4,4′-tetrachlorobiphenyl injected into eggs. Toxicology 27:103–110

Brunström B, Örberg J (1982) A method for studying embryotoxicity of lipophilic substances experimentally introduced into hens' eggs. Ambio 11:209–211

Brunström B, Broman D, Näf C (1990) Embryotoxicity of polycyclic aromatic hydrocarbons (PAHs) in three domestic avian species, and of PAHs and coplanar polychlorinated biphenyls (PCBs) in common eider. Environ Pollut 67:133–143

Cecil HC, Harris SJ, Bitman J (1978) Liver mixed function oxidases in chicken: induction by polychlorinated biphenyls and lack of induction by DDT. Arch Environ Contam Toxicol 7:283–290

Cheung MO, Gilbert EF, Peterson RE (1981) Cardiovascular teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the chick embryo. Toxicol Appl Pharmacol 61:197–204

Flick DF, Douglass CD, Gallo L (1963) Studies of chick edema disease. Poult Sci 42:855–862

Giesy JP, Ludwig JP, Tillitt DE (1994) Dioxins, dibenzofurans, PCBs and colonial, fish-eating water birds. In: Schecter A (ed) Dioxins and Health. Plenum Press, NY. pp 249–307

Harris SJ, Cecil HC, Bitman J, Lille RJ (1976) Antibody response and reduction in bursa of Fabricius and spleen weights of progeny of chickens fed PCBs. Poult Sci 55:1933–1940

Henshel DS (1993) LD50 and teratogenicity studies of the effects of TCDD on chicken embryos. Soc Environ Toxicol Chem Abstr 14:280

McCune EL, Savage JE, O'Dell BL (1962) Hydropericardium and ascites in chicks fed a chlorinated hydrocarbon. Poult Sci 41:295–299

Nikolaidis E, Brunström B, Dencker L, Veromaa T (1990) TCDD inhibits the support of B-cell development by the bursa of Fabricius. Pharmacol Toxicol 67:22–26

Nikolaidis E, Brunström B, Dencker L (1988) Effects of TCDD congeners 3,3′,4,4′-tetrachlorobiphenyl and 3,3′,4,4′-tetrachloroazoxybenzene on lymphoid development of the bursa of Fabricius of the chick embryo. Toxicol Appl Pharmacol 92:315–323

North, MO (1978) Commercial Chicken Production Manual. AVI Publishing Company, Westport, CT, 692 pp

Powell DC, Aulerich RJ, Stromborg KL, Bursian SJ (1996) The effects of 3,3′,4,4′-tetrachlorobiphenyl, 2,3,3′,4,4′-pentachlorobiphenyl, and 3,3′,4,4′,5-pentachlorobiphenyl on the developing chicken embryo when injected prior to incubation. Toxicol Environ Health (in press)

Safe S (1987) Determination of 2,3,7,8-TCDD toxic equivalent factors (TEFs): support for the use of the in vitro AHH induction assay. Chemosphere 16:791-802

——— (1990) Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins, and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 21:51–88

Sanderson JT, Bellward GD (1995) Hepatic microsomal ethoxyresorufin-*O*-deethylase-inducing potency *in ovo* and cytosolic Ah receptor binding affinity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin:comparison of four avian species. Toxicol Appl Pharmacol 132: 131–145

SAS Institute Inc (1990) SAS/STAT User's Guide, Version 6, 4th ed. SAS Institute Inc, Cary, NC. 1686 pp

Verrett MJ (1976) Investigation of the toxic and teratogenic effects of halogenated dibenzo-p-dioxins and dibenzofurans in the developing chicken embryo. Memo Rept, U.S. Food and Drug Admin, Washington, DC, 14 pp

Yamashita N, Tanabe S, Ludwig JP, Kurita H, Ludwig ME, Tatsukawa R (1993) Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. Environ Pollut 79:163–173

Yao C, Panigrahy B, Safe S (1990) Utilization of cultured chick embryo hepatocytes as in vitro bioassays for polychlorinated biphenyls (PCBs): quantitative structure-induction relationships. Chemosphere 21:1007–1016