EFFECTS INDUCED BY FEEDING ORGANOCHLORINE-CONTAMINATED CARP FROM SAGINAW BAY, LAKE HURON, TO LAYING WHITE LEGHORN HENS. II. EMBRYOTOXIC AND TERATOGENIC EFFECTS

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Carp from Saginaw Bay, Lake Huron, MI, was fed to White Leghorn chickens for a period of 8 wk. The diets contained 0.3 (control; 0% carp), 0.8 (3.4% carp), and 6.6 (35% carp) mg polychlorinated biphenyls (PCBs)/kg diet, by wet weight (ww). These concentrations corresponded to 3.3, 26, and 59 pg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents/g diet ww, respectively. Though the diets were not acutely toxic to the adult laying hens, dose- and time-dependent responses were observed in the embryos and chicks. Toxicity was manifested as a dose-dependent increase in embryo mortality and decreased hatching rates. Furthermore, embryos and chicks displayed various deformities, including (1) head and neck edema and hemorrhage, (2) abdominal edema and hemorrhage, (3) foot and leg deformities, (4) skull and brain deformities, (5) yolk-sac deformities, and (6) miscellaneous deformities. The types of deformities observed were similar to those reported for embryos and chicks of colonial waterbirds in Saginaw Bay, as well as in controlled studies where technical mixtures or individual congeners of polychlorinated diaromatic hydrocarbons (PCDAHs) were fed to chickens. Increasing concentrations of carp also significantly affected the various organ weights in 18-d embryos and hatched chicks. At 18 d of incubation, weights of the embryos' livers were directly proportional to the concentration of PCBs in the diets. The weights of the spleens and bursae were inversely proportional to the dietary PCB concentration. After 3 additional days of incubation, significant effects in body, brain, liver, heart, and bursa weights were observed in hatched chicks. The concentrations of total PCBs, as well as 2,3,7,8-TCDD equivalents (TEQs) in the diets, were in the range of those that have been shown to cause similar adverse effects in other species. This study has shown that fish, the primary food source of colonial waterbirds in Saginaw Bay, are capable of causing adverse reproductive effects in a model avian species, the chicken. However, due to differences in the relative potency to cause effects on different endpoints in different species, the results of this study should not be used to predict the threshold for effects in other species.

The impairment of colonial waterbird production in the Laurentian Great Lakes has been linked to elevated concentrations of polychlorinated diaromatic hydrocarbons (PCDAHs) found in the tissues, eggs, and blood of wildlife feeding at the upper trophic levels of the aquatic food chain (Fitchko, 1986; Allan et al., 1991a, 1991b; Giesy et al., 1994a, 1994b, 1994c, 1994d). These adverse effects are manifested by embryotoxicity, lethality, teratogenesis (Colborn, 1988; Ludwig et al., 1990), growth retardation (Kubiak et al., 1989), hepatotoxicity (Fox, 1991), immune suppression (Colborn, 1988), weight loss or wasting syndrome (Kubiak et al., 1989), thymic atrophy, altered thyroid function (Gilbertson et al., 1991; Tillitt et al., 1991a, 1992), and changes in adult breeding behaviors (Fox & Weseloh, 1987; Kubiak et al., 1989). This suite of common toxic effects has been termed Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS; Gilbertson et al., 1991). Similar effects have been observed in other vertebrate fish-eating animals including snapping turtles (*Chelydra serpentina*), osprey (*Pandion haliaetus*), eagles (*Haliaeetus leucocephalus*), herons, colonially nesting terns, gulls, and cormorants (*Phalacrocorax auritus*), and in laboratory studies with mink (*Mustela vison*) (Allan et al., 1991a, 1991b; Giesy et al., 1994a, 1994b, 1994d, 1995; Heaton et al., 1995). A commonality among these species is a dependence on the aquatic food chain, and in particular fish, as a source of food and concomitant exposure to contaminants (Giesy et al., 1994b).

Those PCDAH congeners that form strong complexes with the aromatic hydrocarbon receptor (Ah-r), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the isostereomeric planar polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs), and dibenzodioxins (PCDDs), have been implicated as the causative agents for reproductive impairments in Great Lakes wildlife (Ludwig et al., 1993a, 1993b; Giesy et al., 1994a, 1994b). Once the ligand has bound to the Ah-r, the PCDAH : receptor complex is translocated to the nucleus (Brunström & Reutergardh, 1986; Safe, 1987; Kannan et al., 1988; De Voogt et al., 1990; Tillitt et al., 1991a), where it binds to DNA and alters gene expression (Denison et al., 1985). The results of hazard and risk assessments of these compounds in wildlife indicate that currently TCDD equivalents (TEQs) are the critical measure of contaminants in the Great Lakes ecosystem (Giesy et al., 1994a). That is, they have the greatest potential to cause adverse effects at the least concentrations, and current concentrations seem to be exceeding threshold values in the Great Lakes of North America (Giesy et al., 1994a). Non-*ortho*-substituted (coplanar) PCBs can act through the Ah-r-mediated mechanism (Safe, 1987; De Voogt et al., 1990) and contribute a great proportion of the TEQs (Giesy et al., 1994a; Jones et al., 1993, 1994).

Identifying the probable causative agents of toxicity in wild populations is difficult because no single effect is diagnostic of PCDAH poisoning. Furthermore, these effects may be elicited by other compounds (Fox, 1991; Giesy et al., 1994b). Field studies in which adverse outcomes are observed synoptically with quantification of concentrations of contaminants in tissues of wildlife or their food are correlative and cannot be used alone to demonstrate causality (Fox, 1991). Laboratory studies with wild species are difficult to conduct (Giesy et al., 1994b). Thus, it is difficult to complete Koch's fifth postulate, where the putative causative agent must be reintroduced into the organism and the adverse outcome to which it had been correlated caused.

The present study was designed to test the hypothesis that the same suite of toxic effects observed in Great Lakes fish-eating colonial waterbirds can be elicited in the laboratory by feeding Great Lakes fish to a model avian species, the White Leghorn laying hen (*Gallus domesticus*). Assuming the consumption of Great Lakes fish was the cause of reproductive failures in colonial waterbirds, similar effects on the reproductive dynamics of the laying hen would be expected. Using a control diet allows contamination to be the only variable. There is a great deal more information on the effects caused and the mechanisms of action of contaminants, particularly single compounds or technical mixtures that have not been weathered in the environment, for the chicken than in any wildlife avian species. Furthermore, it was felt that it would be useful to conduct a study of the effects of environmentally weathered and physiologically accumulated mixtures

so that they could be compared to studies with more completely characterized mixtures. Thus, this study becomes a tool to verify the relationship between observations of reproductive anomalies in wild populations of Great Lakes colonial waterbirds and exposure to PCDAH contaminants via the food resource. The relative potencies of PCDAHs are species and endpoint specific (Nosek et al., 1992; Cook et al., 1993; Kennedy et al., 1994). Therefore, the results of this study should not be used to determine thresholds of effects in wild bird populations.

The chicken was chosen as the model avian species because artificial insemination and egg incubation protocols are well defined and because it has a proven sensitivity to PCDAH compounds (Brunström, 1988; Brunström & Andersson, 1988; Henshel, 1993; Henshel et al., 1993; Cook et al., 1993). The chicken embryo is more sensitive to the toxic effects of PCBs than either adults or chicks (Flick et al., 1965; Scott et al., 1971; Britton & Huston, 1972; Platonow & Reinhart, 1973; Cecil et al., 1974; Blazak & Marcum, 1975). Embryotoxicity has been observed in both egg injection and feeding studies with commercial Aroclor mixtures (McLaughlin et al., 1963; Carlson & Duby, 1973; Platonow & Reinhart, 1973; Harris et al., 1976) and individual congeners (Brunström & Darnerud, 1983; Brunström & Reutergardh, 1986; Brunström, 1989, 1990, 1991).

While a number of studies have examined the effects of commercial Aroclor mixtures and individual congeners of PCBs on chickens, none have examined the birds' response to a naturally PCDAH-contaminated environmental food resource. In order to do so, carp (*Cyprinus carpio*) from Saginaw Bay, Lake Huron, MI, was incorporated into 3 experimental diets and fed to laying White Leghorn hens for 8 wk. Each diet contained 30–35% fish meal composed of varying proportions of Saginaw Bay carp and/or ocean fish. The rationale for using carp as a source of naturally contaminated PCDAHs and the procedures employed in collecting and processing the carp and preparing the experimental diets are described in Summer et al. (1996). Here, we report the embryotoxic and teratogenic effects on the embryos and chicks produced by the hens that were fed diets containing PCDAHs assimilated into carp. A companion article describes the effects on biology and reproductive parameters of the adult hens (Summer et al., 1996).

METHODS

A detailed description of the experimental methods is given by Summer et al. (1996). The experimental diets were formulated by mixing various proportions of ocean fish or carp with a standard laying hen diet. The "control" diet contained 30.9% ocean fish and no carp,

the "low-dose" diet contained 28.7% ocean fish and 3.5% carp, and the "high-dose" diet contained 34.5% carp and no ocean fish.

Concentrations of total PCBs and organochlorine insecticides in the diets were determined by gas chromatography with electron-capture detection (Mora et al., 1992; Williams et al., 1992; Giesy et al., 1994c). Approximately 0.5 ml of the PCB extract was used for the determination of ethoxyresorufin *O*-deethylase (EROD) induction potential and calculations of TEQs following methods described in Tillitt et al. (1991b).

Hens

Twenty hens were allocated to each treatment group and 16 roosters (to provide semen for artificial insemination of the hens) were maintained on commercial layer mash. The hens were inseminated once each week with 0.5 ml (approximately 50 million sperm) pooled, freshly collected semen. The hens were brought into egg production by following standard poultry lighting guidelines. Immediately prior to the onset of the study, the 60 hens, each laying a minimum of 6 eggs/hen/wk, were assigned to the study. All hens were fed the control diet for 2 wk to acclimate them to the greater level of fish meal in the diet. After the 2-wk acclimation period, the hens were fed the experimental diets for 8 wk. The roosters were fed the commercial layer mash throughout the study. Food consumption of individual hens was measured weekly and body weights were recorded biweekly.

Eggs

Eggs were collected daily, identified by the date and hen number, and stored temporarily at 13–16°C until placed into an incubator. Each week 10 unincubated eggs were collected from each treatment group and homogenized for analysis of total PCBs, as already described. Eggs were handled three times during the incubation period; once to determine fertility and twice to determine viability. At d 5 of incubation, the eggs were candled to detect the presence of a vascular network, which was indicative of fertilization and the onset of organogenesis. Nonfertile eggs were discarded. At d 11 of incubation, eggs were again candled to determine the viability of the embryos. Dead embryos were removed from the shell and the approximate age at death was determined by the progress of organogenesis with reference to a developmental atlas (Hatchery Systems Co., Mathews, NC). Each embryo was examined for gross pathologies. An embryo viability detector (EVD; Mineau & Pedrosa, 1986) was used on d 18 to determine the viability of late-stage embryos. Embryos found dead at d 18 were aged and examined for deformities. Live eggs were either transferred to hatching baskets and returned to the incubator for hatching or killed for subsequent chemical analyses or histological examination.

Embryos

Each week, 10 live eggs from each treatment were taken at d 18 and carefully opened; the embryo was removed from the egg shell and the yolk sac was detached. Each carcass (head and body) was weighed to the nearest 0.1 g. Embryos were examined for gross deformities, and the brain, heart, liver, spleen, and bursa were removed, trimmed, and weighed to the nearest 0.1 mg. A portion of the liver was preserved in 10% neutral buffered formalin, trimmed, processed, sectioned at 6 μm, and stained with hematoxylin and eosin for histological examination.

Chicks

Hatching ensued on d 21 of incubation, and necropsies were performed on all chicks within 24 h of hatching. Chicks were weighed live and then killed by decapitation. The brain, heart, liver, spleen, and bursa were removed, trimmed, and the weights recorded. Each week, brain and liver tissues were collected randomly from 5 chicks in each treatment group for chemical analyses. Viability of eggs that did not hatch on d 21 was determined by the EVD. Eggs with live embryos were returned to the incubator and those with dead embryos were opened and examined for deformities. The same procedures were carried out daily for all remaining unhatched eggs through d 25 of incubation, at which time incubation was terminated, the unhatched eggs were opened, and the embryos were examined as previously described.

Statistical relationships were analyzed using SAS statistical software (SAS Institute, Inc., 1987). Significance of the main effects, time and treatment, was determined by a two-way analysis of variance (ANOVA). Where significant main effects occurred, the data were further analyzed by Tukey's all possible pairs test statistic. Levels of significance ranging from $p \leq .05$ to $p \leq .0001$ are reported.

RESULTS

Total concentrations of PCBs in the prepared diets, expressed as the sum of Aroclors 1242, 1248, 1254, and 1260, were 0.3, 0.8, and 6.6 mg PCB/kg, ww, for the control, low-, and high-dose diets, respectively. TEQs derived by the H4IIE bioassay were 3.3, 26, and 59 ng/kg, ww, in the control, low-, and high-dose diets, respectively.

Eggs

Concentrations of total PCBs in the egg were initially small and increased as a function of duration of exposure (Table 1). Concentrations of total PCBs in the eggs were greatest after 8 wk (6 wk of exposure) and were approximately proportional to the PCB concentrations in the diets.

The biomagnification factor (BMF) for accumulation of total PCBs in eggs, defined as the ratio of the concentration of PCBs in the eggs to that in the diet, increased throughout the study, with the greatest values observed after 6 wk of feeding the treatment diets (Table 1). The BMFs for total PCBs during the first week were 1.0, 0.3, and 0.3 for the control, low-dose, and high-dose treatments, respectively. BMF values at 6 wk of exposure were 2.3, 5.9, and 5.3 for the control, low-dose, and high-dose diets, respectively. The average BMF for the 3 diets after 6 wk exposure was 4.5.

TABLE 1. Concentrations of total PCBs and biomagnification factors (BMFs) in chicken eggs as a function of treatment and week of exposure

Treatment	Week[a]	Total PCBs[b] (mg/kg, ww)	BMF
Control	1	0.3	1.0
	2	0.5	1.7
	3	0.6	2.0
	4	0.7	2.3
	5	0.5	1.7
	6	1.0	3.3
	7	0.9	3.0
	8	0.7	2.3
	9	1.1	3.7
	10	1.1	3.7
Low-dose	1	0.1	0.3
	2	0.5	1.7
	3	0.9	1.1
	4	2.6	3.3
	5	3.1	3.9
	6	2.7	3.4
	7	3.6	4.5
	8	4.7	5.9
	9	4.9	6.1
	10	4.6	5.8
High-dose	1	0.1	0.3
	2	0.2	0.7
	3	7.5	1.1
	4	10.0	1.5
	5	12.0	1.8
	6	19.0	2.9
	7	26.0	3.9
	8	35.0	5.3
	9	24.0	3.6
	10	26.0	3.9

[a]Acclimation = wk 1 and 2, exposure = wk 3–10.
[b]Analysis by S. Tanabe, Ehime University, Japan.

Hatchability

Full-term hatchability rates decreased with respect to time and were inversely proportional to treatment (Table 2). At the beginning of the trial, 90.2–100% of the eggs hatched, but the hatchability of the eggs laid during wk 2 of the study decreased in all treatment groups. While hatching rates in the control group stabilized, they increased through the remainder of the study in the low-dose group and decreased in the high-dose group. There was no delayed hatching effect in any treatment group, and late-incubation mortality (23–25 d incubation) did not increase significantly with time and was not affected by the treatments.

The period when mortality occurred varied among treatment groups. In the control and low-dose groups, mortality was equal during early and mid-incubation (8–11 d) and late incubation (21–25 d). The early and mid-stage mortality rates were 49.5 and 42.7% for the control and low-dose groups, respectively. Mortality late in incubation was 50.5 and 55.3%, respectively, for these 2 treatment groups. Late-stage mortality, however, was predominant (79% overall) in the high-dose group, and particularly so during the second half of the study (87%).

Embryos

Embryonic liver weights were significantly ($p \leq .0001$) affected by treatment and time of exposure (Table 3). The weights of the livers of 18-d embryos increased with time of exposure throughout the study. The gain was greater in the high-dose group (+19%) than in the control (+2%) or low-dose (+1%) groups. The high-dose group livers always weighed more, on average, than those of the other treatment groups and during the second half of the study were 23–28% greater than those of the control or low-dose groups.

Spleen weights of 18-d embryos remained constant in the control group but decreased by 7–8% during the second half of the study in both the low- and high-dose embryos (Table 3). A significant time effect ($p \leq .05$) was evident, but the differences among treatment groups were variable. During wk 1–5, spleens from the high-dose group weighed 9% more than those of either the control or low-dose groups. However, during the second half of the study, spleens from both the control and high-dose groups weighed 9% more than those from the low-dose group.

Bursa weights of the 18-d embryos increased significantly ($p \leq .001$) in the control and low-dose groups, but decreased significantly ($p \leq .001$) in the high-dose group as a function of duration of exposure of the hens (Table 3). On average, bursa weights increased in both the control (+20%) and low-dose (+14%) groups, but decreased by 24% in the high-dose group between the first and second halves

TABLE 2. Hatchability (%) at 21–22 and 23–25 d of incubation and total hatchability for eggs laid by hens fed various concentrations of Saginaw Bay carp for up to 10 wk

	Treatment diet[a]								
	Control			Low-dose			High-dose		
Week	d 21–22	d 23–25	Total	d 21–22	d 23–25	Total	d 21–22	d 23–25	Total
1[b]	98.7	1.3	100	91.8	2.7	94.5	88.8	1.4	90.2
2[b]	87.5	—	87.5	69.0	—	69.0	75.4	—	75.4
3	73.5	2.9	76.4	77.3	—	77.3	87.2	1.3	88.5
4	72.4	3.4	75.8	81.5	—	81.5	95.2	—	95.2
5	84.6	—	84.6	87.6	1.6	89.2	80.2	4.2	84.4
6	85.7	—	85.7	92.6	—	92.6	77.8	2.5	80.3
7	87.0	4.4	91.4	87.9	—	87.9	65.9	—	65.9
8	80.9	4.8	85.7	90.7	—	90.7	69.6	2.2	71.8
9	75.0	2.8	77.8	91.7	1.7	93.4	55.4	2.4	57.8
10	88.3	—	88.3	85.2	—	85.2	73.9	1.1	75.0
Mean (wk 1–5)	83.3	1.5	84.9	81.4	0.9	82.3	85.4	1.4	86.7
Mean (wk 6–10)	83.4	2.4	85.8	89.6	0.3	90.0	68.5	1.6	70.2
Grand means (wk 1–10)	83.4	2.0	85.3	85.5	0.6	86.1	76.9	1.5	78.4

[a]With 20 hens per treatment diet.
[b]All hens fed control diet for 2-wk acclimation period.

TABLE 3. Mean liver, spleen, and bursa weights from 18-d embryos, percent difference among treatments, and percent change over time within the control, low-dose, and high-dose diet groups

	Mean weight (g ± SE)					
	Control (CD)		Low-dose (LD)		High-dose (HD)	
Week	*n*	Weight	*n*	Weight	*n*	Weight
Liver[a]						
1–5	20	0.4307 ± 0.0156	24	0.4486 ± 0.0104	27	0.5094 ± 0.0295
6–10	17	0.4409 ± 0.0066	11	0.4540 ± 0.0143	29	0.6066 ± 0.0276
1–10	37	0.4358 ± 0.0108	35	0.4513 ± 0.0089	56	0.5580 ± 0.0313
Spleen[b]						
1–5	19	0.0069 ± 0.0009	24	0.0069 ± 0.0004	26	0.0075 ± 0.0004
6–10	16	0.0070 ± 0.0004	10	0.0064 ± 0.0002	29	0.0070 ± 0.0003
1–10	35	0.0070 ± 0.0005	34	0.0066 ± 0.0002	55	0.0070 ± 0.0003
Bursa[c]						
1–5	20	0.0267 ± 0.0028	24	0.0256 ± 0.0020	27	0.0275 ± 0.0014
6–10	17	0.0320 ± 0.0021	11	0.0292 ± 0.0024	27	0.0209 ± 0.0016
1–10	37	0.0294 ± 0.0018	35	0.0274 ± 0.0017	54	0.0242 ± 0.0011

[a]Significant time and treatment effects ($p \leq .0001$).
[b]Significant time effect ($p \leq .05$).
[c]Significant time effect ($p \leq .001$).

of the study. Bursa weights of the high-dose group were greatest during wk 1–5, but during wk 6–10, the control group bursa weights were 53% greater and the low-dose group bursa weights were 40% greater than those in the high-dose group.

Nonspecific or background lesions were observed in the heart, spleen, bursa, and liver of some of the 139 18-d embryos collected for histological examination. However, no distinct or constantly occurring lesions were confined to any specific treatment group.

Chicks

Significant treatment effects on total body weights and weights of brains, livers, hearts, and bursae were observed in the 1479 chicks that hatched. Body weights of chicks varied significantly ($p \leq .0001$) with duration of exposure of the hens and treatment. An interaction between the main effects confounded the interpretation, but Tukey's test showed significant ($p \leq .0001$) time and treatment differences consistently during wk 4–10, thus reinforcing the conclusions of the two-way ANOVA. The average body weights for the first and second halves of the study indicated slight increases of 1 and 2% for the control and low-dose chicks and a 9% increase with time in the high-dose group chicks (Table 4). The high-dose chicks always weighed

TABLE 3. *(continued)*

Percent treatment difference			Percent change within treatments over time
HD vs. CD	HD vs. LD	LD vs. CD	
HD = 18% > CD	HD = 14% > LD	LD = 4% > CD	CD = +2%
HD = 38% > CD	HD = 33% > LD	LD = 3% > CD	LD = +1%
HD = 28% > CD	HD = 23% > LD	LD = 3% > CD	HD = +19%
HD = 9% > CD	HD = 9% > LD	LD = CD	CD = ±0%
HD = CD	HD = 9% > LD	LD = 9% < CD	LD = −8%
HD = 3% > CD	HD = 9% > LD	LD = 6% < CD	HD = −7%
HD = 3% > CD	HD = 7% > LD	LD = 4% < CD	CD = +20%
HD = 53% < CD	HD = 40% < LD	LD = 10% < CD	LD = +14%
HD = 21% < CD	HD = 13% < LD	LD = 7% < CD	HD = −24%

the most, and were about 9.5% heavier than the control and low-dose chicks during the second half of the study.

Significant ($p \leq .01$) increases with respect to duration of exposure and treatment were observed in chick brain weights (Table 4). No statistical interaction of the main effects was observed. Brain weights increased from the first to the second half of the study in all treatment groups, though the increases were small (1–2%). Likewise, chicks from the high-dose group always had the greatest brain weights, but were only 2–3% heavier than those in the control and low-dose groups.

Liver weights of chicks increased significantly ($p \leq .001$) as a function of duration of exposure of the hens and treatment (Table 4). A slight interaction between main effects was observed, but there were strong main effects of both treatment and duration of exposure. The weights of the livers in the high-dose group increased by 32% over time, whereas those from chicks in the control or low-dose groups increased by 3–5% (Table 4). The high-dose group chicks always had the heaviest livers. This was particularly evident during the second half of the study when high-dose chick liver weights were 44 and 46% greater than those in the low-dose and control groups, respectively.

Decreases in heart weights ranging from 1 to 3.5% (significance $p \leq .001$) as a function of duration of exposure of the hens were

TABLE 4. Mean body, brain, liver, heart, and bursa weights from hatched chicks, percent difference among treatments, and percent change over time within the control, low-dose, and high-dose diet groups

	Mean weight (g ± SE)					
	Control (CD)		Low-dose (LD)		High-dose (HD)	
Week	*n*	Weight	*n*	Weight	*n*	Weight
Body[a]						
1–5	206	34.21 ± 0.9362	239	33.94 ± 0.9001	315	34.65 ± 1.0441
6–10	164	34.49 ± 0.3447	247	34.49 ± 0.3447	304	37.81 ± 0.2483
1–10	370	34.35 ± 0.5008	486	34.34 ± 0.5008	619	36.23 ± 0.7330
Brain[b]						
1–5	206	0.8177 ± 0.0096	239	0.8182 ± 0.0070	315	0.8325 ± 0.0066
6–10	165	0.8302 ± 0.0042	247	0.8273 ± 0.0027	304	0.8523 ± 0.0050
1–10	371	0.8240 ± 0.0056	486	0.8228 ± 0.0040	619	0.8424 ± 0.0052
Liver[c]						
1–5	206	0.6898 ± 0.0125	239	0.6910 ± 0.0122	315	0.7926 ± 0.0526
6–10	165	0.7138 ± 0.0068	247	0.7262 ± 0.0031	304	1.0437 ± 0.0054
1–10	371	0.7018 ± 0.0081	486	0.7086 ± 0.0084	619	0.9182 ± 0.0477
Heart[d]						
1–5	206	0.2157 ± 0.0036	238	0.2124 ± 0.0057	315	0.2233 ± 0.0123
6–10	165	0.2112 ± 0.0046	247	0.2051 ± 0.0018	304	0.2215 ± 0.0037
1–10	371	0.2134 ± 0.0030	485	0.2088 ± 0.0032	619	0.2224 ± 0.0064
Bursa[e]						
1–5	206	0.0359 ± 0.0015	239	0.0372 ± 0.0016	315	0.0383 ± 0.0020
6–10	165	0.0369 ± 0.0004	247	0.0354 ± 0.0006	303	0.0323 ± 0.0005
1–10	371	0.0364 ± 0.0008	486	0.0363 ± 0.0009	618	0.0353 ± 0.0014

[a]Significant time and treatment effects ($p \leq .0001$).
[b]Significant time and treatment effects ($p \leq .01$).
[c]Significant time and treatment effects ($p \leq .001$).
[d]Significant time effect ($p \leq .001$).
[e]Significant time ($p \leq .0001$) and treatment ($p \leq .05$) effects.

observed in all treatment groups (Table 4). Chicks from the high-dose group had the heaviest hearts (5–8% greater than the control or low-dose chick heart weights) throughout the study and had the smallest decreases in heart weights over time.

Bursa weights of the chicks showed a strong ($p \leq .0001$) effect of duration of exposure of the hens and a weaker ($p \leq .05$) treatment effect (Table 4). An interaction between main effects was also observed. Bursa weights averaged over the first and second halves of the exposure were 3% greater during the second half of the exposure in the control group, while bursa weights were 4 and 16% less during the second half of the exposure in the low- and high-dose groups, respectively. Initially, the high-dose chicks had the greatest bursa weights, but by the second half of the study, both the control

TABLE 4. *(continued)*

Percent treatment difference			Percent change within treatments over time
HD vs. CD	HD vs. LD	LD vs. CD	
HD = 1% > CD	HD = 2% > LD	LD = 1% < CD	CD = +1%
HD = 10% > CD	HD = 9% > LD	LD = 1% > CD	LD = +2%
HD = 5% > CD	HD = 5% > LD	LD = CD	HD = +9%
HD = 2% > CD	HD = 2% > LD	LD = CD	CD = +2%
HD = 3% > CD	HD = 3% > LD	LD = CD	LD = +1%
HD = 2% > CD	HD = 2% > LD	LD = CD	HD = +2%
HD = 15% > CD	HD = 15% > LD	LD = CD	CD = +3%
HD = 46% > CD	HD = 44% > LD	LD = 2% > CD	LD = +5%
HD = 31% > CD	HD = 30% > LD	LD = 1% > CD	HD = +32%
HD = 4% > CD	HD = 5% > LD	LD = 1% < CD	CD = −2%
HD = 5% > CD	HD = 8% > LD	LD = 3% < CD	LD = −4%
HD = 4% > CD	HD = 6% > LD	LD = 2% < CD	HD = −1%
HD = 7% > CD	HD = 3% > LD	LD = 4% > CD	CD = +3%
HD = 14% < CD	HD = 10% < LD	LD = 4% < CD	LD = −5%
HD = 3% < CD	HD = 3% < LD	LD = CD	HD = −16%

and low-dose chicks had bursa weights 14 and 10% greater than those in the high-dose group, respectively.

Deformities

Two thousand eighty-one embryos and chicks were examined for abnormalities. The occurrence of deformities was significantly influenced by both duration of exposure of the hens and treatment. Over the course of the study, deformities were observed in 607 (29%) of the embryos and chicks examined and were found in 17% of the control, 24% of the low-dose group, and 40% of the high-dose group embryos and chicks. Of the 607 malformed embryos and chicks, 363 (60%) were in the high-dose group, 154 (25%) were in the low-dose group, and 90 (15%) were in the control group. The majority of

abnormalities (82–93%) occurred in the last half of the study, which correlates with the increased concentrations of PCBs in the eggs during the last half of the study, as shown in Table 1.

A shift from single to multiple occurrences of deformities was evident and followed a time- and dose-dependent pattern. Of all the embryos/chicks that had deformities during the first half of the study, the proportion of embryos/chicks with a single deformity ranged from 73 to 85% in all treatment groups (Table 5). Cases of single deformities decreased in the last half of the trial and individuals with multiple deformities (ranging from 2 to 7 separately identifiable deformities per individual) became more prevalent and were found in 49% of the control, 45% of the low-dose, and 68% of the high-dose embryos/chicks that had deformities.

Grouped into 6 categories and tabulated across all treatment groups for the entire study (Table 6), edema of the head/neck was the most prevalent teratogenic effect (64%) followed by abdominal edema (15%), foot/leg deformities (14%), miscellaneous abnormalities (2.9%), skull/brain malformations (2.6%), and yolk-sac anomalies (1.6%).

Edema and hemorrhage of the head/neck included intramuscular and/or subcutaneous edema and hemorrhage of the "pipping muscle" and cranial regions. Intramuscular edema was characterized by fluid infiltration into the muscle tissue causing the affected muscle tissue to enlarge and appear yellowish in color compared to nonedematous

TABLE 5. Number of embryos and chicks with either a single or multiple incidence of deformities from hens fed various concentrations of Saginaw Bay carp

Parameter	Treatment diet[a]		
	Control	Low-dose	High-dose
Total number embryos/chicks examined for deformities	520	655	906
Wk 1–5			
Single deformity	13 (81%)	11 (73%)	22 (85%)
Multiple deformities	3 (19%)	4 (27%)	4 (15%)
Total	16 (100%)	15 (100%)	26 (100%)
Wk 6–10			
Single deformity	38 (51%)	76 (55%)	107 (32%)
Multiple deformities	36 (49%)	63 (45%)	230 (68%)
Total	74 (100%)	139 (100%)	337 (100%)
Grand total (wk 1–10)			
Single deformity	51 (57%)	87 (56%)	129 (36%)
Multiple deformities	39 (43%)	67 (44%)	234 (64%)
Total	90 (100%)	154 (100%)	363 (100%)
Overall deformity rate	17%	24%	40%

[a]Control, low-, and high-dose diets contained 0, 3.4, or 35% carp, respectively.

FIGURE 1. Dorsal view of a normal chick (left) and a chick from the high-dose group (right) with "moderate" subcutaneous and intramuscular head and neck edema and hemorrhage.

muscle tissue (Figures 1 and 2). Subcutaneous edema (Figures 1 and 2) was characterized by the presence of a gelatinous mass of yellowish fluid on the surface of the muscle tissue immediately below the skin. Edema/hemorrhage of the neck/head was the most prevalent teratogenic effect in all treatment groups, particularly so during the second half of the study, and accounted for 51.7, 52.3, and 70.0% of

FIGURE 2. Chick from the high-dose group with "severe" subcutaneous and intramuscular head and neck edema and hemorrhage.

all the deformities in the control, low-, and high-dose groups, respectively. This type of deformity was the most prevalent in both live and dead chicks but was more prevalent in dead chicks (56–78%) than in live chicks (48–67%). Eighteen-day embryos were the only group that showed a greater occurrence of other types of abnormalities.

Abdominal edema and hemorrhage were either intramuscular or subcutaneous. This classification also included "blister edemas," which consisted of a subcutaneous accumulation of clear fluid on the back (Figures 3 and 4). "Blister edemas" were observed in chicks from each treatment group, including a live chick in the control group, 2 live chicks in the low-dose group, and 5 of the 18-d high-dose embryos, 10 dead chicks in the high-dose group, and 4 live chicks in the high-dose group. Abdominal edema and hemorrhage, the second most frequent class of deformities overall (Table 6), occurred primarily during the second half of the study and accounted for 20.1, 24.4, and 10.7% of the deformities in the control, low-, and high-dose groups, respectively. Most of the abdominal deformities were found in live chicks (wk 6–10) in the control and low-dose groups, but accounted for the greatest proportion of deformities in high-dose group 18-d embryos.

Deformities of the feet and legs consisted of curled, clenched, or splayed toe(s) and feet that rotated inward, appearing clubbed (Figure

FIGURE 3. Hatched chick from the high-dose group with swelling of the lower back due to an accumulation of subcutaneous edema ("blister edema").

FIGURE 4. Chick from the high-dose group that died at 20 d of incubation with marked subcutaneous edema due to an accumulation of clear fluid ("blister edema") over the entire lower back region.

TABLE 6. Number of incidences of the 6 major classes of deformities in all 18-d embryos, dead embryos/chicks, and hatched chicks

Terata	18-d Embryos	Dead embryos/chicks	Hatched chicks	Total
Number of embryos/chicks examined	139	463	1479	2081
Head/neck edema/hemorrhage	5	322	449	776
Abdominal edema/hemorrhage	6	24	149	179
Foot/leg deformities	1	55	113	169
Miscellaneous	0	15	21	36
Skull/brain deformities	6	14	12	32
Yolk-sac deformities	0	19	1	20
Total	18	449	745	1212

5). In some cases, the legs were splayed so that the chick was unable to stand erect with its legs directly underneath its body (Figure 6). Occasionally, bilaterally malformed femurs (femurs with a 90° angle beginning in the center of the length of the bone) were observed (Figure 7). All of the femoral deformities occurred in dead embryos in the high-dose group from 21 to 25 d incubation. Early stages of femoral deformity were observed in one 18-d embryo in the high-dose group. Foot and leg deformities accounted for 14% of the abnormalities that occurred in the 3 treatment groups and were more prevalent in live chicks than in dead chicks. In all groups, a time-dependent trend was evident with more foot-leg deformities occurring during the second half of the study.

Some miscellaneous deformities that were occasional in nature were observed. Eye deformities consisted of embryos and chicks that had one or both eyes missing, enlarged, or reduced in size. Beak abnormalities included missing, shortened, or crossed beaks. Incomplete feathering, weak chicks, fluid-filled yolk sacs, and liver hemorrhages were incidental. Chicks described as weakened were unable to raise their heads or straighten their bodies from the curled, embryonic position. These occasional teratogenic effects occurred in each treatment group and accounted for about 3–4% of the deformities in each group.

Skull and brain deformities were characterized by missing or softened skulls covering the top of the brain. Malformed brain cases that were too small to accommodate the brain were occasionally observed.

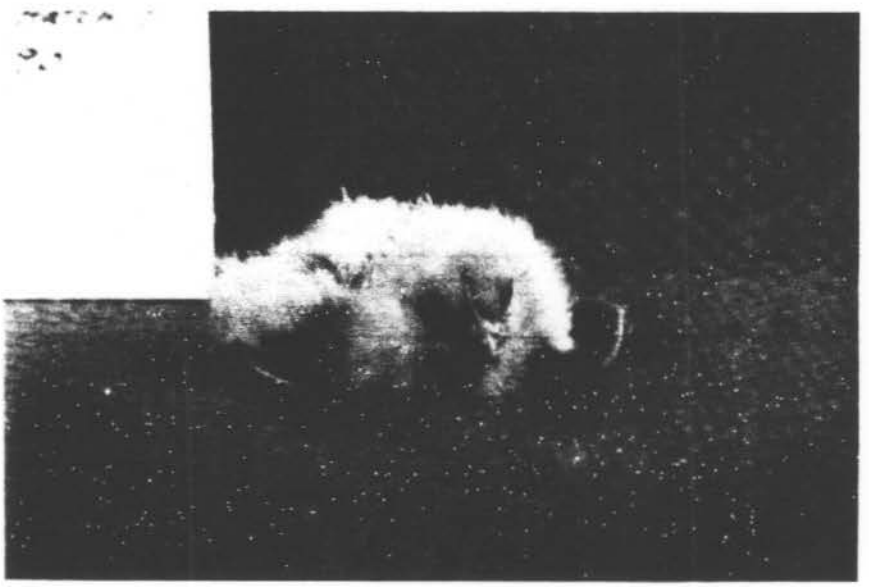

FIGURE 5. Chick from the high-dose group that was unable to support its body weight. Note contraction of the digits.

FIGURE 6. Chick from the low-dose group that was unable to stand erect.

Every incidence of a skull deformity was accompanied by edematous brain tissue, but edematous brain tissue also occurred in other embryos and chicks with normal skulls. Occasional hemorrhaging of the brain tissue was also included in this category. The greatest proportion of skull and brain deformities occurred in 18-d embryos, all of which were in the high-dose group. These types of deformities

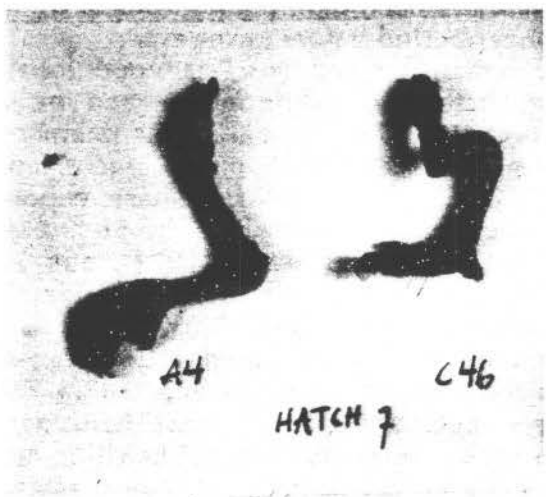

FIGURE 7. Normal leg from a control group chick (left) and leg from a high-dose chick (right) with a femoral bone deformity characterized by abnormal curvature.

were also observed in dead chicks in the low-dose group and in high-dose group chicks (dead and live).

Unabsorbed yolk sacs were observed in "full-term" chicks, as were edematous and missing sphincters, which resulted in incomplete abdominal closure. These abnormalities were infrequent and were most common in dead chicks.

DISCUSSION AND CONCLUSIONS

A complete account of hen feed consumption, hen body weights, egg production, and egg weights pertaining to this study is presented in Summer et al. (1996). As described in that article, the occurrence of fatty liver hemorrhagic syndrome (FLHS) in both the control (78%) and low-dose (75%) treatment groups may have contributed to the effects seen in these parameters. Only 15% of the high-dose hens were afflicted with FLHS due to PCB-induced protection of the liver from excessive lipid accumulation (Hansen, 1975). Here we discuss the embryotoxicity and teratogenicity associated with consumption by the hens of diets containing carp from Saginaw Bay contaminated with PCBs and other PCDAHs.

The BMF for total PCBs in the eggs from hens in all the groups averaged 4.5 after 6 wk of exposure. This value is less than the values for wild birds, which have been reported to range from 30–35 (Braune & Norstrom, 1989). This may be attributed to the fact that the hens were continually transferring PCBs into eggs so that the quantity of PCBs would not accumulate to as great a degree as in the birds that lay eggs only once or twice in a season. Thus, in this study, the PCB concentrations measured in the eggs did not reach the concentrations that have been reported for wild birds consuming similar diets for a longer period before laying eggs.

In this study, mortality of embryos was proportional to exposure of the hens to carp in the diet, while fertility and the number of eggs produced were not affected by the carp diets (Summer et al., 1996). Other studies have found that hatchability is more sensitive than egg production to PCBs (Peakall, 1986). Dietary exposure to 20 mg Aroclor 1242/kg, ww, reduced hatchability by 50% by wk 2 of feeding in a study by Briggs and Harris (1973), and 20 mg Aroclors 1232, 1242, 1248, or 1254/kg, ww, in the diet for 9 wk decreased hatching rates to 44.5, 11.0, 1.8, and 69.0%, respectively (Lillie et al., 1974). A concentration of 2.4 mg Aroclor 1242/kg, ww, in the egg yolk reduced hatchability (Britton & Huston, 1972). Fifteen milligrams Aroclor 1254/kg of egg yolk, ww, decreased hatching, but concentrations less than 5 mg/kg resulted in normal hatching rates (Platonow & Reinhart, 1973). Reduced hatchability was noted when chickens were fed diets containing 10 mg Aroclors 1232, 1242, or 1248/kg, ww, for

6 wk, but not at dietary concentrations of 5 mg/kg (Britton & Huston, 1972). This reduced hatchability was associated with concentrations greater than 3 mg total PCB/kg of egg yolk, ww (about 1 mg/kg, whole egg concentration) from hens fed diets containing 10 mg Aroclor 1242/kg (Britton & Huston, 1972), and with a whole-egg concentration of about 5 mg/kg in eggs from hens fed diets containing 50 mg Aroclor 1254/kg (Platonow & Reinhart, 1973). Similarly, hatching rates were reduced to 50% by exposure to 10 mg Aroclor 1248/kg, ww, and to 2.4% by a dietary concentration of 20 mg Aroclor 1248/kg, ww for 8 wk, (Scott et al., 1971), but feeding 0.5 or 1.0 mg Aroclor 1248/kg, ww, of diet to hens did not affect hatching rates. In our study, exposure to 0.8 mg PCBs/kg diet, ww (low-dose group), also did not have an adverse effect on hatchability, although noticeable reductions in hatchability in the high-dose group (6.6 mg PCB/kg diet, ww) started during wk 5 of exposure when the hens had consumed about 20.4 mg PCBs. The proportionally greater embryo mortality caused by the high carp diet in our study compared to the commercial Aroclor PCB mixtures may have been due to the fact that the more toxic PCB congeners in the carp had been enriched relative to the original technical mixtures (Giesy et al., 1994a).

As has been shown in other studies (Scott et al., 1971; Lillie et al., 1974; Ax & Hansen, 1975; Harris et al., 1976), most of the mortality in this study, and particularly in the high-dose group, occurred in the late stages of incubation (d 21–25). Briggs and Harris (1973) found that the greatest proportion of mortality occurred during the mid-stages of incubation (d 7–15) when they fed Aroclor 1242 to hens. The timing of mortality may be influenced by the timing and intensity of exposure in relation to organogenesis. In an egg injection study, embryo mortality occurred at early developmental stages, but if injection occurred after organogenesis was complete (d 9), there was no increase in embryo lethality (Carlson & Duby, 1973). A similar pattern was seen when hens were exposed to 50 mg Aroclor 1254/L drinking water for a period of 6 wk. Mortality of chicken eggs has been found to be directly proportional to the concentration of PCBs in egg yolks, and mortality occurred at progressively earlier stages of incubation as the concentration of PCBs increased (Tumasonis et al., 1973). The low-dose group in our study did have an increasing percentage of mortality occurring earlier in incubation as exposure time progressed, but this was not true for the high-dose group. Late-stage mortality was predominant during the second half of the exposure period in the high-dose group, but decreased in the low-dose group.

FLHS may also play a role in the interpretation of the embryo and chick organ weights. Egg weights in the low-dose and control groups were, on average, 4.7 g less than those in the high-dose

group (Summer et al., 1996). The lesser weights of eggs in the high-dose group were only temporary and a function of the dietary change at the initiation of the study.

The consistently smaller eggs in the low-dose and control groups, however, may confound the interpretation of the effects observed in the organ weight data. Those weights that had only slightly significant differences among treatments, 18-d embryo body weights, chick brain weights, and chick heart weights, might not have been significant if the difference in egg weights did not exist, and the levels of significance might have been reduced in the 18-d embryo and chick liver weights. Similarly, those organ weights that had higher levels of significance coupled with decreases in weight in the low- and high-dose groups, namely, 18-d embryo spleen and bursa weights and chick bursa weights, might have shown even greater levels of significance had egg weights been unaffected. Organ weights normalized to either body or brain weights were not reported, due to the occurrence of significant time or treatment effects found in body and brain weights.

Organs, including the liver, spleen, bursa, and thymus, have been reported to be sensitive to PCBs. In chicks fed 400 ppm Aroclor 1260 for 60 d (Vos & Koeman, 1970) or 5 different hexachlorobiphenyls (McKinney et al., 1976), liver weights were significantly greater and spleen weights were significantly less than those of controls. When 3,4,3',4'-tetrachlorobiphenyl (TCB; IUPAC number 77) was injected into chicken eggs at d 9 of incubation, Rifkind and Muschick (1983) noted that the weights of livers and spleens from the 18-d embryos had significantly increased to 117 and 122% of controls. Thymus weights were 79% of controls and bursa weights were 90% of control weights, but were not significantly different. Weights of the spleen and bursa were also less in chicks produced by hens fed various levels of Aroclors 1232, 1242, and 1248 (Harris et al., 1976). The bursa is the site of B-cell development and differentiation in the chick and is responsible for establishing competency in immunoglobulin synthesis and producing the antibody response (Harris et al., 1976; Nikolaidis et al., 1988, 1989). In the present study, bursa weights were reduced significantly in both 18-d embryos and chicks. However, no assessment of potential immune suppression was made.

Most of the teratogenic effects observed in this study were similar to those reported by other researchers in laboratory studies of exposures to mixtures of PCBs and TCDDs and wild bird flocks in the Great Lakes region of North America (Giesy et al., 1994a). Edema and hemorrhaging of the head/neck region was the most predominant deformity observed in our study. Similar types of effects have been observed in controlled experiments of the effects of PCBs on chicken eggs (Cecil et al., 1974; Lillie et al., 1974). Subcutaneous edema was a predominant abnormality observed in chicks of double-crested cor-

morants and Caspian terns (*Hydroprogne caspia*) (Kurita & Ludwig, 1988; Yamashita et al., 1992). Edema has also been reported in herring gulls (*Larus argentatus*) of the Great Lakes (Fox & Weseloh, 1987; Gilbertson, 1989; Gilbertson et al., 1991). In our study, edema was observed only in the later stages of incubation (18–25 d). This is similar to the findings for naturally incubated double-crested cormorant eggs (Kurita & Ludwig, 1988). Furthermore, in our study head/neck edema was found in over half of the dead embryos and chicks of all groups (57.4, 56.2, and 77.6% in the control, low-, and high-dose groups, respectively). If the edema is severe enough, it may restrict the ability of chicks to pip the shell or hinder the action of the pipping muscle. Double-crested cormorants in the Great Lakes have been observed to have subcutaneous edema so severe (two to three times the normal size) that chicks were unable to pip (Ludwig et al., 1990).

The abdominal edema observed in our study was always subcutaneous in nature. Generally, it occurred over the breast area but sometimes extended down to the inguinal region.

The "blister edemas" observed in our study have been observed by other investigators when chickens were exposed to PCBs (Lillie et al., 1974; Brunström & Darnerud, 1983). This type of deformity was observed in both dead and live chicks in our study. If not too severe, the "blister edema" did not appear to be debilitating to the chick. However, very large "blister edemas" were observed in certain dead embryos, the largest of which contained 7.2 g fluid.

Deformities of the legs and feet similar to those observed in our study have been observed in both laboratory exposures of chickens to PCBs and surveys of wild bird populations that were known to be contaminated by PCBs. Chicks that hatched with short, bowed legs and crooked, clenched toes were reported by Tumasonis et al. (1973). Chicks with rotated ankles have been reported (Lillie et al., 1974; Cecil et al., 1974). Clubbed feet, a condition characterized by the inward rotation of the tarsometatarsus or femur, were reported to be common in Caspian terns but infrequent in double-crested cormorant chicks in contaminated regions of the Great Lakes (Kurita et al., 1987; Ludwig et al., 1990).

In addition to these abnormal leg conditions, some chicks in this study had seemingly normal legs, yet they were unable to stand erect. A similar condition was observed in ring-billed gulls (*Larus delawarensis*) in 1972 and 1973 (Gilbertson et al., 1976). The ring-billed gulls, like the chicks, were unable to stand or walk normally and resorted to the use of their wings to move themselves about on the ground. This condition, termed "perosis" in poultry and generally attributed to a choline or manganese deficiency, occurs when the birds have no support at the tibiotarsal tarsometatarsal joint.

Bilaterally malformed femurs that occurred in dead embryos of the

high-dose group have not, to our knowledge, been previously described. The first of these deformities occurred during wk 4 of exposure to the treatment diets. The early stages of this malformation were seen in one 18-d embryo. The rest of the chicks survived until d 20–25 d of incubation, although all eventually died in the shell without pipping. The physiological basis for this deformity is unknown.

Deformities related to the incomplete absorption of the yolk sac were relatively uncommon in our study. Only one chick hatched with an unabsorbed yolk sac, and the remaining occurrences were seen in dead chicks. In some cases, the organs were outside the body cavity. Most of the chicks with this condition were in the treatment groups; however, it was also found in three dead chicks in the control group. Cases of unabsorbed yolk sacs have been reported in chicks from PCB-fed chickens (Lillie et al., 1974), as well as in Caspian terns (Kurita et al., 1987), double-crested cormorants, herring gulls, ring-billed gulls, common terns (*Sterna hirundo*), and red-breasted mergansers (*Mergus serrator*) (Ludwig et al., 1990). The syndrome termed gastroschisis ("split-belly"), described by Ludwig et al. (1990), included enlarged yolk sac attachments, organs that remained outside the body, and a thin body wall.

The remainder of the deformities that were observed in this study, namely, deformities of the eye, beak, skull/brain, and abnormal feathering patterns, were rarely observed. However, they are mentioned by other researchers (McLaughlin et al., 1963; Carlson & Duby, 1973; Platonow & Reinhart, 1973; Lillie et al., 1974; Brunström & Darnerud, 1983; Brunström, 1988, 1989, 1990; Kubiak et al., 1989; Ludwig et al., 1990; Fox et al., 1991). Hydropericardium as reported in other chicken studies with PCBs (Brunström, 1988, 1989, 1990; McCune et al., 1962) and ascites as found in double-crested cormorant embryos (Kurita et al., 1987; Ludwig et al., 1990) were not observed in this study.

The expected rate of occurrence per 1000 chicks for the 6 major categories of teratogenic effects for the control, low-, and high-dose diet groups, based on the number of terata observed in each group, was calculated (Table 7). Expected rates for the more serious (severe head/neck edema and hemorrhage) and the more unusual ("blister edema" and fermoral bone) deformities are also presented (Table 7). The ratio of head/neck edema to severe head/neck edema is less in the high-dose group (4.6 : 1) than in the low-dose (18.7 : 1) or control (14.8 : 1) groups, which indicates that if a high-dose chick has head/neck edema, it is 3–4 times more likely to have a severe edema than either the control or low-dose group chick. The ratios for abdominal edema to "blister edema" were even larger (4.5 : 1, 32 : 1, and 29 : 1 for the high-dose, low-dose, and control groups, respectively). The increased likelihood of "blister edemas" in the high-dose group

TABLE 7. Expected rate of occurrence (per 1000 chicks) of deformities observed in chicks from hens fed various concentrations of Saginaw Bay carp

	Treatment diet[a]		
Terata	Control	Low-dose	High-dose
Head/neck edema/hemorrhage	148	206	623
Severe head/neck edema/hemorrhage	10	11	136
Abdominal edema/hemorrhage	58	96	95
Blister edema	2	3	21
Foot/leg deformities	54	67	107
Femoral bone deformities	0	0	14
Miscellaneous	12	11	25
Skull/brain deformities	10	6	25
Yolk-sac deformities	6	8	13

[a]Control, low-, and high-dose diets contained 0% (0.3 ppm PCBs; 3.3 ppb TEQs), 3.4% (0.8 ppm PCBs; 26 ppb TEQs), or 35% (6.6 ppm PCBs; 59 ppb TEQs) carp, respectively.

probably is not as critical as the increased likelihood of severe head/neck edema because of its interference with hatching.

Also critical to the survival probabilities of the chicks is the shift from single occurrences of deformities to multiple deformities occurring in individual chicks. In the control and low-dose groups, the trend shifted between the first and second halves of the study from about 70–80% single deformity to 50–55% single deformity. An even greater increase (53%) in multiple terata occurred during the second half of the study in the high-dose group. Chicks that are afflicted with a single deformity are, intuitively speaking, more likely to have better survival probabilities than those chicks that are affected with multiple (up to seven terata per individual in this study) deformities. This was not examined as the chicks were sacrificed within 24 h of hatching. In the stressor-filled natural environment, however, chicks that are weakened by the extra effort to hatch with edema of the pipping muscle, crippled by clubbed or otherwise deformed feet, or left unable to forage due to shortened or deformed beaks have virtually no chance of survival.

The results of this study have demonstrated a dose-dependent positive correlation of the number of deformities with the concentration of PCBs in the diet that is similar to those observed in studies with technical mixtures of PCBs. This indicates that the current concentrations and relative proportions of PCDAHs, such as PCBs and TEQs in fishes of Saginaw Bay, can cause the same types of deformities as the technical mixtures of PCDAHs and that the types of deformities observed in wild populations of colonial, fish-eating waterbirds of Saginaw Bay are consistent with those observed in this study and are

most likely caused by PCDAHs in the fish that make up the diets of these birds.

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EFFECTS INDUCED BY FEEDING ORGANOCHLORINE-CONTAMINATED CARP FROM SAGINAW BAY, LAKE HURON, TO LAYING WHITE LEGHORN HENS. I. EFFECTS ON HEALTH OF ADULT HENS, EGG PRODUCTION, AND FERTILITY

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*This study was conducted to determine the effects of consumption of halogenated hydrocarbon compounds, primarily polychlorinated biphenyls (PCBs), contained in Great Lakes fish by the domestic chicken (*Gallus domesticus*). In this article we report the results of feeding White Leghorn hens for a period of 8 wk diets that contained 31–35% ocean fish and/or carp (*Cyprinus carpio*) from Saginaw Bay, Lake Huron, MI, which provided 0.3 (control), 0.8 (low-dose group), or 6.6 (high-dose group) mg PCB/kg, wet weight (ww). These concentrations were analogous to 3.3, 26, or 59 pg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents (TEQs)/g diet, ww, respectively. There were no significant effects on feed consumption among the groups. An unexpectedly high incidence of fatty liver hemorrhagic syndrome (FLHS) was observed in hens from the control (78% FLHS) and low-dose (75% FLHS) groups when compared to the high-dose group (15% FLHS). Birds in the control and low-dose groups had a significant increase in liver and body weights. Significant decreases in egg production, weight, and fertility were immediate in all dose groups, with the effect being permanent in the control and low-dose groups. Although the incidence of FLHS was an unexpected complication, the fact that there were no significant effects on egg production, egg weights, or fertility in the high-dose group suggests that the no-observable-adverse-effect concentration (NOAEC) for these parameters is in excess of 26 mg total weathered PCBs/kg egg, ww. This value was the average concentration of PCBs in the high-dose group eggs during the last week of the study.*

Exposure of wildlife in the Great Lakes region to halogenated hydrocarbons has resulted in adverse effects (Fitchko, 1986; Allan et al., 1991a, 1991b; Giesy et al., 1994a, 1994b, 1995). These effects, including declines in populations, have been best documented for colonial, fish-eating water birds (Peakall, 1988; Gilbertson et al., 1991). Since the more recent cessation of the manufacture and use of the most persistent and widespread contaminants, concentrations of these compounds in fish and birds have decreased (Allan et al., 1991b). Subsequently, populations of many of the fish-eating water birds have increased (Price & Weseloh, 1986). However, other adverse effects such as localized impairment of reproductive performance (Kubiak et al., 1989; Fox et al., 1991; Tillitt et al., 1991a, 1991b; Giesy et al., 1994a), anatomical defects, and embryo lethality (Gilbertson et al., 1976; Gilbertson, 1983; Yamashita et al., 1992), have become apparent. This suite of effects has been described as Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) (Gilbertson et al., 1991). The clinical signs of GLEMEDS are similar to those associated with "chick edema disease," which was first described in commercial poultry flocks in 1957. Chick edema disease occurred when broiler chickens were fed polychlorinated biphenyl- (PCB) and dioxin-contaminated feed (Firestone, 1973; Gilbertson, 1983; Gilbertson et al., 1991).

Field researchers have linked reproductive anomalies observed in fish-eating wildlife of the Great Lakes to contaminants found in fish, which is their primary food source. Correlations of contaminant concentrations in the tissues of fish and birds indicate that contaminants in fish, and in particular certain PCB congeners, are the likely cause of reproductive anomalies in wild piscivorous birds (Gilbertson et al., 1991). Laboratory studies in which contaminated fish have been fed to

mink (*Mustela vison*) have shown some Great Lakes fish to be extremely toxic (Hornshaw et al., 1983; Heaton et al., 1995). However, the question of causality in birds has not been addressed in a controlled laboratory setting.

In this study, fish from the Great Lakes were fed to a model avian species, the domestic chicken (*Gallus domesticus*), to determine if the contaminants in the fish would induce GLEMEDS-like symptoms in a laboratory trial. Contaminants in fish represent a unique mixture of organochlorine compounds derived from individual congeners from many parent compounds. Weathering, selective accumulation, and metabolism of environmental contaminants make it difficult to prepare cocktails representative of complex environmental mixtures with the appropriate proportions of congeners. Therefore, we chose to use a diet that contained a natural physiologically selected mixture of contaminants, including those that could be quantified as well as those that could not.

The chicken was chosen as the model avian species because it is easily maintained in captivity and protocols for artificial insemination and egg incubation are well defined. In addition, as demonstrated in egg injection studies (Verret, 1976; Brunström & Andersson, 1988; Brunström & Lund, 1988; Bosveld et al., 1992) and feeding studies (Platonow & Reinhart, 1973; Lillie et al., 1974; Hansen, 1975; Hansen et al., 1976), the chicken is sensitive to commercial mixtures and individual congeners of PCBs and especially to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and similar polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and non- and mono-*ortho*-substituted PCB congeners.

Although small forage fish, such as alewife (*Alosa pseudoharengus*) and smelt (*Osmerus mordax*), are the staple dietary species of colonial waterbirds, carp (*Cyprinus carpio*) from Saginaw Bay, Lake Huron, Michigan, were selected as a representative, naturally contaminated food resource because (1) they were known to contain "high" concentrations of polychlorinated diaromatic hydrocarbons (PCDAHs), which permitted their incorporation into the chicken diets at reasonably low levels, and (2) they tended to concentrate at the mouth of the Saginaw River in the late fall, which allowed for relatively easy collection of large quantities of fish.

The primary objective of this study was to ascertain the biological and reproductive effects of environmental contaminants, primarily PCBs, contained in Great Lakes fish and consumed by the White Leghorn chicken.

MATERIALS AND METHODS

The carp used in the diets were collected by electroshocking from the mouth of the Saginaw River (Lake Huron, Michigan) in November

1988. The fish were ground, blended into a homogeneous mixture, and stored frozen at –17°C. Ocean fish scraps (cod, haddock, pollack, and flounder; Boston Feed Supply, Natick, MA) were ground and blended as already described and incorporated into the control and low-dose diets as a source of "clean" fish to provide 31–35% fish in each diet (Table 1).

Before preparing experimental diets, ground ocean fish and carp were cooked at 82°C for approximately 2 h to dehydrate the fish and inactivate any thiaminase activity in the carp (Gnaedinger & Krzeczkowski, 1966). Ground corn was added to the cooked fish to absorb the remaining moisture and provide a "semi-dry" product for mixing with the other dietary ingredients. The diets (Table 1) were formulated based on a nutrient analysis of the cooked fish samples in an attempt to meet the nutrient requirements of the laying hens (National Research Council, 1984). Samples of the hens' diets were collected for nutrient analysis and quantification of total PCBs and TCDD equivalents (TEQs) (Table 2). Concentrations of total PCBs (measured as the sum of Aroclors 1242, 1248, 1254, and 1260) were determined by gas chromatography with electron-capture detection (Mora et al., 1992; Williams et al., 1992; Giesy et al., 1994b). Concentrations of TEQs were determined using two different approaches. One involved determining concentrations of individual PCDD, PCDF, and PCB congeners in the fish (adjusting for the percentage of fish in each diet), multiplying these concentrations by appropriate toxic equivalent factors (TEFs), and summing the individual TEQs as described in Giesy et al. (1994a). The second approach involved measuring the

TABLE 1. Composition (%) of laying hen diets

Dietary components	Diets		
	Control	Low-dose	High-dose
Carp	—	3.4	34.5
Ocean fish	30.9	28.7	—
Corn, number 2 yellow	37.7	39.9	45.9
Oat hulls	6.7	6.1	11.1
Soybean meal (44%)	11.4	10.4	1.5
Isolated soy protein (90%)	3.0	2.1	—
Alfalfa meal (17%)	2.0	1.8	—
Vitamin premix	0.3	0.3	0.3
Mineral premix	0.3	0.3	0.3
Selenium premix	0.06	0.05	0.05
Limestone	7.5	6.8	6.3
Salt	0.06	0.05	—
Ethyoxyquin (g/45.4 kg diet)[a]	5.67	5.67	5.67

[a]Purity = 90%; Roche Animal Nutrition, Hoffman-LaRoche, Inc., Nutley, NJ.

TABLE 2. Nutrient analysis and concentrations of total PCBs and TEQs in the hens' diets

	Diets		
Nutrient analysis (ww)[a]	Control	Low-dose	High-dose
Moisture, %	10.8	11.3	8.4
Fat, %	11.5	13.6	15.4
Crude protein, %	20.0	22.5	20.6
Crude fiber, %	6.3	5.3	6.5
Calcium, %	4.1	3.4	4.1
Phosphorus, %	1.1	0.8	1.3
Potassium, %	0.7	0.6	0.6
Magnesium, %	0.1	0.1	0.1
Sodium, %	0.2	0.2	0.1
Ash, %	11.1	12.0	9.6
Iron, ppm	393	415	431
Manganese, ppm	357	414	357
Copper, ppm	38.4	59.4	45.8
Zinc, ppm	305	407	469
Total PCB[b]	0.3	0.8	6.6
TEQ (H4IIE)[c,d]	3.3	26	59
TEQ (calculated)[c,e]	0.4	4.5	45

[a]Analysis by Litchfield Analytical Services, Litchfield, MI.
[b]As mg/kg diet, ww.
[c]As pg/g diet, ww.
[d]Determined by H4IIE bioassay.
[e]Calculated from molar concentrations and TEFs from H4IIE assay.

induction of ethoxyresorufin *O*-deethylase (EROD) activity in the H4IIE rat hepatoma cell line by extracts of the diets to determine the total concentration of H4IIE bioassay-derived TEQs (Tillitt et al., 1991a, 1991b). The prepared diets were stored at –17°C until fed to the chickens.

Ninety 18-wk-old Babcock White Leghorn pullets and 16 roosters were allocated to the study on April 6, 1990. The birds were leg banded and placed in individual cages in three-tiered layer batteries. Prior to initiation of the study, all birds were fed ad libitum a commercial layer mash (Purina Accu-Line Breeder 121; Purina Mills, Inc., St. Louis, MO), with the roosters remaining on the commercial diet for the duration of the study. A standard poultry lighting schedule was implemented to bring the hens into egg production, which was monitored daily. Peak egg production was achieved at 27 wk of age (June 13). By 35 wk of age, the birds were receiving 15.75 h of light per day and were maintained on that photoperiod throughout the remainder of the study.

The hens were artificially inseminated twice a week with 0.5 ml

(approximately 50 million sperm) of pooled, freshly collected semen from the 16 roosters. During the second week, egg production decreased markedly and, in response to a concern that handling stress associated with artificial insemination might be impacting production, it was decided to inseminate the hens only once each week. No loss of fertility was recorded with the weekly inseminations.

Prior to the onset of the definitive study, the 60 top-producing hens (each laying a minimum of 6 eggs/hen/wk) were assigned to 3 dietary groups of 20 hens per group. The hens in each group were housed individually in a three-tiered laying battery in separate but identical animal rooms. All hens were fed the control (31% ocean fish) diet for 2 wk to acclimate them to the "high" level of fish in the diet and acclimate them to their cages prior to the definitive trial. Feed and water were available to the birds ad libitum throughout the study. Following the acclimation period, the treatment diets were fed to the hens for 8 wk. Food consumption of individual hens was measured each week and body weights were recorded biweekly. Eggs were collected daily, identified, weighed, and stored in a cooler (12.8–15.6°C) for subsequent incubation and analyses.

At the termination of the trial, blood samples were collected from the hens and hematocrit values were determined. The hens were then euthanized, necropsies were performed, and the brain, liver, and spleen weights were recorded. Portions of the spleen and liver were preserved in 10% neutral buffered formalin, trimmed, processed, sectioned at 6 μm, and stained with hematoxylin and eosin for histopathologic examination.

The data were analyzed using SAS statistical software (SAS Institute, Inc., 1987). Significance of the main effects, time and treatment, was determined by a two-way analysis of variance (ANOVA). The parameters that had significant ($p \leq .05$) main effect interactions were then analyzed by a one-way ANOVA to determine the validity of the main effects. Where significant main effects occurred, the data were further analyzed by Tukey's all possible pairs test statistics. Levels of significance ranging from $p \leq .05$ to $p \leq .0001$ are reported.

RESULTS

Concentrations of total PCBs in the diets were 0.3 (control; 0% carp), 0.8 (3.5% carp), and 6.6 (34% carp) mg PCB/kg diet, ww (Table 2). Concentrations of TEQs measured with the H4IIE assay were higher than the concentrations predicted from the toxic equivalent factor (TEF) values and molar concentrations of PCDD, PCDF, and non- and mono-*ortho*-substituted PCB congeners (Table 2). The concentrations of total PCBs in the eggs were initially low in all the groups (0.3, 0.1, and 0.1 mg/kg, ww for the control, low-dose, and high-dose

groups, respectively) and increased as a function of duration of exposure (see Summer et al., 1996).

Food consumption did not differ significantly among treatment groups, although during the second half of the study, hens in the high-dose group ate 1.7–8.5 g/hen/d more, on average, than hens in the control group and 0.1–1.4 g/hen/d more than hens in the low-dose group (Table 3). As the study progressed, food consumption in all experimental treatments decreased overall by approximately 20% after fluctuating widely during the early part of the study.

Biweekly body weight gains averaged 53.4, 46.8, and 22.8 g for the control, low-dose, and high-dose group hens, respectively (Table 4). Both time and treatment had significant ($p \leq .0001$) effects on body weight. Most hens in each group gained weight over the course of the trial, but more hens gained weight during each observation period in the control and low-dose groups (79 and 81%, respectively) than in the high-dose group (63%). Thus, an inverse relationship existed, particularly in the second half of the study, between the control and high-dose group hens with respect to food consumption and body weight. While the high-dose group hens consumed more feed, they weighed less and fewer hens gained weight. Those high-dose hens that did gain weight did so at a slower rate than hens in the control group.

During the 14 d prior to the acclimation period, the hens were laying 6.2–6.3 eggs/hen/wk (89–90% egg production; Table 5). Egg production decreased immediately after the hens were placed on the control fish diet during acclimation and continued to be less in the control and low-dose groups throughout the treatment period. The differences in egg production were highly significant ($p \leq .0001$) with respect to treatment. Overall, egg production averaged 4.2, 5.2, and 5.8 eggs/hen/wk for the control, low-, and high-dose groups, respectively (Table 5). During the last 5 wk of the study, the control hens continued to lay fewer eggs (3.8 eggs/hen/wk) while the high-dose hens returned to pre-trial production levels (6.2 eggs/hen/wk). Egg production in the low-dose hens stabilized around wk 3 of the study and averaged about 5.2 eggs/hen/wk through the remainder of the trial. Control hens consistently laid fewer eggs than the low- or high-dose hens. Thirty percent of the control hens laid 0–3 eggs/hen/wk during the first 5 wk of the study, while only 15 and 13% of the low- and high-dose hens, respectively, laid 0–3 eggs/hen/wk during that same period. Forty-one percent of the control, 47% of the low-dose, and 56% of the high-dose hens laid 6–7 eggs/hen/wk during wk 1–5. The divergence in egg production was more pronounced during the second half of the study, when the proportion of hens laying 0–3 eggs/hen/wk increased to 42% in the controls and 20% in the low-dose group, but decreased to only 1% of hens in the high-dose group. The number of hens laying 6–7 eggs/hen/wk during the second

TABLE 3. Feed and PCB consumption of hens fed various concentrations of Saginaw Bay carp

	Diet[a]					
	Control, mean daily consumption		Low-dose, mean daily consumption		High-dose, mean daily consumption	
Week	Food (g/hen/d)[b]	PCB[c] (μg/hen/d)	Food (g/hen/d)[b]	PCB[c] (μg/hen/d)	Food (g/hen/d)[b]	PCB[c] (μg/hen/d)
1[d]	106 ± 3.4	31.9	103 ± 3.2	31.9	107 ± 2.5	33.0
2[d]	101 ± 3.6	31.3	92.6 ± 3.7	28.7	90.1 ± 2.3	27.9
3	90.7 ± 4.1	28.1	85.5 ± 2.9	64.1	75.9 ± 1.8	500
4	83.6 ± 3.8	25.9	89.7 ± 2.4	67.3	80.0 ± 2.9	527
5	92.4 ± 3.8	28.6	94.8 ± 2.8	71.1	87.7 ± 2.3	578
6	90.5 ± 4.1	28.1	92.1 ± 3.0	69.1	92.2 ± 2.7	608
7	85.7 ± 4.1	26.6	89.7 ± 3.8	67.3	91.2 ± 2.0	601
8	81.9 ± 3.8	25.4	90.0 ± 3.5	67.3	90.4 ± 2.2	596
9	82.3 ± 4.2[e]	25.5	87.1 ± 2.2	65.3	88.5 ± 2.3	583
10	83.3 ± 4.2[f]	25.8	87.4 ± 3.5	65.6	88.5 ± 2.7	583
Mean (wk 1–5)	94.7	29.2	93.1	52.6	88.0	333
Mean (wk 6–10)	84.7	26.3	89.3	67.0	90.2	594
Grand mean (wk 1–10)	89.7	27.7	91.2	59.8	89.1	570
Mean total food (g/hen) and PCB (mg/hen) consumption	6282	1.9	6383	4.2	6238	39.9

[a]With 20 hens per dietary group.
[b]Mean ± SE.
[c]PCB consumption based on dietary PCB concentration and food consumption.
[d]All hens were fed the control diet for a 2-wk acclimation period.
[e]n = 19.
[f]n = 18.

TABLE 4. Body weight, percentage of hens gaining or losing weight, and body weight gain or loss of hens fed various concentrations of Saginaw Bay carp

	Diet								
	Control[a] (0% carp)			Low-dose[a] (3.4% carp)			High-dose[a] (34.5% carp)		
Week	Mean body weight[b] (g/hen)	Percent hens gain/loss[c]	Mean gain/loss (g)	Mean body weight[b] (g/hen)	Percent hens gain/loss[c]	Mean gain/loss (g)	Mean body weight[b] (g/hen)	Percent hens gain/loss[c]	Mean gain/loss (g)
1[d]	1506 ± 34.2	—	—	1468 ± 28.1	—	—	1502 ± 17.6	—	—
3	1613 ± 35.4	95/5	+106	1556 ± 27.8	95/5	+87.9	1604 ± 27.9	95/5	+103
5	1630 ± 37.3	60/40	+17.1	1561 ± 30.4	50/50	+13.5	1528 ± 29.8	10/90	−76.2
7	1711 ± 45.4	90/10	+81.4	1625 ± 34.3	95/5	+63.7	1583 ± 29.7	85/15	+55.3
9	1734 ± 47.8[e]	75/25	+35.8	1655 ± 34.4	80/20	+29.8	1595 ± 32.8	55/45	+11.8
10	1763 ± 55.9[f]	75/25	+26.5	1694 ± 35.2	85/15	+39.1	1611 ± 35.7	70/30	+20.8
Mean		79/21	+53.4		81/19	+46.8		63/37	+22.8

[a]With 20 hens per dietary group.
[b]Mean ± SE.
[c]The percentage of hens experiencing a gain or loss of body weight.
[d]All hens were fed the control diet for a 2-wk acclimation period.
[e]n = 19.
[f]n = 18.

TABLE 5. Daily egg production and egg weights of laying hens fed diets containing various concentrations of Saginaw Bay carp

	Diet								
	Control[a] (0% carp)			Low-dose[a] (3.4% carp)			High-dose[a] (34.5% carp)		
Week	Mean number of eggs/week[b]	Percent production[c]	Egg weight (g/egg)[b]	Mean number of eggs/week[b]	Percent production[c]	Egg weight (g/egg)[b]	Mean number of eggs/week[b]	Percent production[c]	Egg weight (g/egg)[b]
14-d Pretrial average	6.2 ± 0.17	89	—	6.2 ± 0.15	89	—	6.3 ± 0.18	90	—
1[d]	5.3 ± 0.25	76	52.6 ± 0.46	4.9 ± 0.27	70	51.6 ± 0.49	4.8 ± 0.27	69	51.8 ± 0.59
2[d]	5.0 ± 0.41	71	47.3 ± 0.58	4.9 ± 0.40	70	45.1 ± 0.55	5.1 ± 0.42	73	45.3 ± 0.43
3	4.2 ± 0.44	60	46.9 ± 0.41	5.3 ± 0.41	76	46.7 ± 0.47	6.1 ± 0.29	87	46.5 ± 0.47
4	3.8 ± 0.50	54	46.9 ± 0.44	5.6 ± 0.28	80	47.0 ± 0.43	5.9 ± 0.22	84	50.4 ± 0.46
5	4.2 ± 0.57	60	47.4 ± 0.46	5.3 ± 0.35	76	47.1 ± 0.40	5.7 ± 0.41	81	51.4 ± 0.36
6	3.8 ± 0.78	54	47.0 ± 0.40	5.4 ± 0.43	77	47.9 ± 0.38	6.2 ± 0.15	89	52.8 ± 0.54
7	4.1 ± 0.55	59	48.1 ± 0.49	5.1 ± 0.41	73	47.7 ± 0.34	6.1 ± 0.18	87	53.1 ± 0.43
8	3.9 ± 0.58	56	48.1 ± 0.46	5.4 ± 0.43	77	48.1 ± 0.32	6.3 ± 0.19	90	53.6 ± 0.40
9	3.7 ± 0.57[e]	53	49.0 ± 0.56	5.3 ± 0.46	76	48.3 ± 0.35	6.1 ± 0.17	87	54.2 ± 0.45
10	3.5 ± 0.63[f]	50	50.0 ± 0.59	4.6 ± 0.46	66	49.7 ± 0.50	6.1 ± 0.26	87	54.1 ± 0.36
Mean (wk 1–5)	4.5	64	48.2	5.2	74	47.5	5.5	79	49.1
Mean (wk 6–10)	3.8	54	48.4	5.2	74	48.3	6.2	88	53.6
Grand mean (wk 1–10)	4.2	59	48.3	5.2	74	47.9	5.8	83	51.3

[a]With 20 hens per dietary group.
[b]Mean ± SE.
[c]Percent production = (number of eggs/wk)/(7 eggs/wk) where 7 eggs/wk = maximal egg production.
[d]All hens fed the control diet during a 2-wk acclimation period.
[e]n = 19.
[f]n = 18.

half of the study decreased to 36% in the control group, but increased to 58 and 82% of the hens in the low- and high-dose groups.

Significant differences ($p \leq .0001$) in egg weights were observed as a function of treatment and time. At the beginning of the study, the mean weight of eggs from all treatment groups was 52 g (Table 5) but decreased to 46 g during wk 2. Egg weights then gradually increased throughout the remainder of the study, with the high-dose group eggs increasing the most to a final mean weight of 54 g. During wk 6–10, eggs from the control and low-dose groups were comparable in weight, but after wk 4, the high-dose group eggs were consistently heavier than those from the other groups by an average of 4.7 g.

Fertility initially ranged from 88 to 92%, but decreased to 64–81% in all treatment groups by wk 2 (Table 6). Fertility rates continued to be depressed throughout the course of the study in both the control and low-dose groups, but returned to an average of 87% in the high-dose group hens during the last 4 wk of the trial.

Two hens from the control group died during wk 8 and 9. The principal postmortem examination findings in both birds were diffuse hepatic lipidosis and hepatic rupture with intra-abdominal blood and carcass pallor due to blood loss. These findings and histopathologic alterations, including hepatic necrosis, hemorrhage, moderate heterophilic infiltration, capsular fibrosis, and diffuse hepatocellular vacuolar changes, are consistent findings of the fatty liver hemorrhagic syn-

TABLE 6. Fertility (%) of eggs laid by hens fed diets containing various concentrations of Saginaw Bay carp

	Diet		
Week	Control (0% carp)	Low-dose (3.4% carp)	High-dose (34.5% carp)
1	89.5	91.7	88.4
2	81.2	64.3	76.5
3	62.2	70.9	83.3
4	61.1	67.0	84.7
5	65.4	74.8	77.7
6	64.8	65.7	81.1
7	72.8	67.6	87.6
8	65.4	65.7	89.7
9	61.1	70.9	84.2
10	71.0	63.3	86.0
Mean (wk 1–5)	71.9	73.7	82.1
Mean (wk 6–10)	67.0	66.6	85.7
Grand mean (wk 1–10)	69.5	70.2	83.9

drome (FLHS). The principal findings of postmortem and histopathologic examination on the remaining 58 hens at the termination of the trial were confined to the liver and included hepatocellular lipidosis, necrosis, and hemorrhage consistent with the FLHS. These findings were present in 14 hens from the control group (70%), 15 hens from the low-dose group (75%), and 3 hens in the high-dose groups (15%). Hens in all groups appeared to be in good physical condition prior to necropsy.

Body weights of the control group and liver weights of the control and low-dose groups were significantly greater than those of the high-dose group (Table 7). Liver weights of the control hens were highly variable (range = 90 g), while the high-dose hens' liver weights were within a 37-g range. The livers of the control group hens weighed an average of 12 and 19 g more than the livers of the low- and high-dose group hens, respectively.

DISCUSSION

The concentrations of neither total PCBs nor TEQs fed in this study were expected to cause mortality in the adult hens since the concentrations of both were less than those that have been demonstrated to be lethal. The dietary concentrations of total PCBs required to cause acute or chronic lethality in birds range from 750 mg/kg body weight in the bobwhite quail (*Colinus virginianus*) to greater than 4000 mg/kg for the ring-necked pheasant (*Phasianus colchicus*) (Heath et al., 1972).

Clinical signs and lesions observed in many of the hens in this study, particularly in the control and low-dose groups, closely resembled those described for FLHS by other researchers (Couch, 1956;

TABLE 7. Body and organ weights, hematocrit values, and incidence of fatty liver hemorrhagic syndrome (FLHS) for laying hens fed diets containing various concentrations of Saginaw Bay carp

	Diet		
	Control[a] (0% carp)	Low-dose (3.4% carp)	High-dose (34.5% carp)
Body weight (g ± SE)	1763 ± 55.9[b]	1694 ± 35.3	1611 ± 35.7
Brain weight (g ± SE)	3.23 ± 0.053	3.13 ± 0.036	3.18 ± 0.038
Liver weight (g ± SE)	63.4 ± 5.76[b]	51.3 ± 2.80[b]	44.6 ± 1.71
Spleen weight (g ± SE)	1.22 ± 0.064	1.16 ± 0.082	1.05 ± 0.081
Hematocrit (% ± SE)	27.9 ± 0.95	26.2 ± 0.85	32.2 ± 0.75
Number of hens with FLHS	14 (78%)	15 (75%)	3 (15%)

[a]Values for the 2 hens that died during the study not included; thus in control group $n = 18$ while $n = 20$ for the low-dose and high-dose groups.

[b]Significantly different ($p < .05$) from high-dose group.

Ringer & Sheppard, 1963; Tudor, 1967; Wolford & Polin, 1972) and the pictorial description by Wolford et al. (1971). Couch (1956) was the first to characterize the syndrome and reported that hens afflicted with FLHS appeared healthy but had excess abdominal fat, fatty livers, liver capillary hemorrhages, liver hematomas, and increased mortality. A 25–30% increase in body weight and 33% drop in egg production were described as typical. It has been noted by Ringer and Sheppard (1963) that immediately prior to death the comb, face, and wattles were pale and the comb was cool to the touch but no decrease in egg production was observed.

Although the hens in this study appeared to be in good physical condition prior to necropsy, those with suspected FLHS were readily identified by the condition of the liver at necropsy. The severity of the syndrome, which ranged from livers that were only slightly hemorrhagic and mahogany in color to livers that were yellowish-brown, extremely friable (to the point that the liver could not be removed from the hen in one piece), tumorous, and hemorrhagic, was similar to that reported by Tudor (1967) and Wolford et al. (1971). The relationship between the incidence of FLHS and lesser hematocrit values of the hens was probably attributable to liver hemorrhaging (Table 7). A "normal" mean (±SE) hematocrit value for laying White Leghorn hens of 31.3 ± 1.01% has been reported (Sturkie & Textor, 1960). The two hens in the control group that died during the study had lesions consistent with those of FLHS. In FLHS, hepatocellular lipidosis results in a friable liver (Tudor, 1967). Mortality results from blood loss due to hemorrhage into the abdominal cavity when the liver capsule (capsule of Glisson) ruptures (Wolford et al., 1971).

The prevalence of FLHS in the control (80%) and low-dose (75%) dietary groups seemed remarkable when compared to only a 15% occurrence in the high-dose group. However, a similar pattern of FLHS was noted by Hurley et al. (1975) when they fed hens a commercial poultry feed or a commercial feed with the addition of 2,3′,4,4′,5-pentachlorobiphenyl. The livers of the PCB-treated chickens were visibly less fatty and weighed less than those of the control hens throughout the 14-wk study. The same pentachlorobiphenyl was compared to 2,2′,3,3′,6-pentachlorobiphenyl, which was rapidly metabolized and actually depressed hepatic microsomal oxidase activity (Hansen, 1975, 1987); the potent inducer again reduced liver fat content, while the liver fat levels in the hens receiving the noninducer were the same as controls. Similar results were obtained with former commercial PCB mixtures. Aroclor 1254, a potent inducer, was found to provide protection against FLHS; Aroclor 1242, a less potent inducer that has the potential to lower the rate of lipid metabolism through competitive inhibition by more readily metabolized congeners, had a more severe effect on FLHS (Hansen, 1975). It was concluded by

Hansen (1975) that PCB-related microsomal induction may offer some protection against FLHS in confined laying hens by increasing the rates of steroid and fatty acid oxidation. Thus, it would appear that the high-dose group hens in this study were experiencing a PCB-related protection against FLHS.

The unusual food consumption, body weight, and egg production trends observed in this study may be explained, as least in part, by the occurrence of FLHS. The control hens consistently weighed the most throughout the study, yet ate the least amount of feed over the last half of the study. Part of the weight differential may be attributed to the manifestations of FLHS in the control group, including heavier livers, presumably due to hepatic fat infiltration, and excess abdominal fat (this was not quantified, however). Livers of the control hens averaged 12 and 19 g more than the livers of the low- and high-dose group hens, respectively. A greater energy demand in the high-dose group, leading to greater food consumption and lesser body weights, may have occurred in response to the higher rate of egg production. The 30% decline in egg production of the control group would be expected in hens with FLHS (Couch, 1956). The initial decrease in egg production in all groups was probably a result of the sudden change in the hens' diet during the acclimation period. The hens in the low- and high-dose groups quickly acclimated to the "high" fish diets and began laying more eggs. The high-dose hens, however, made the quickest and most complete recovery and, by the end of the study, had returned to pretrial egg production levels. But, as expected, due to the high occurrence of FLHS in the low-dose group, pretrial egg production was not achieved by these birds.

In a review of the effects of PCBs on birds, Peakall et al. (1990) reported the "best estimate" of the critical concentration of PCBs for reproductive effects in bird eggs to be 40 mg PCB/kg, ww. In more traditional PCB-chicken feeding studies, that is, studies without the FLHS factor, feed consumption and body weights were usually not significantly affected by exposure to PCBs at sublethal concentrations in the diet (Scott et al., 1971; Britton & Huston, 1973; Stendell, 1976). Feeding 2 or 20 mg Aroclors 1242, 1248, 1254, or 20 mg Aroclors 1221, 1232, 1268, or 5442/kg diet had no effect on body weight of laying hens after 9 wk of treatment, but 20 mg Aroclors 1242, 1248, or 1254/kg diet significantly reduced food consumption (Lillie et al., 1974). Hens in our study experienced about a 20% reduction in food consumption over time, but the differences among the treatment groups were not significant. Body weights, however, differed by 9% among groups and were influenced by the effects of both time and treatment.

Egg production has been reported to be decreased by exposure to PCBs. Dietary concentrations of 20 mg Aroclors 1232, 1242, 1248, or

1254/kg caused significantly less egg production by White Leghorn hens (Lillie et al., 1974). In a study by Platonow and Reinhart (1973), 50 mg Aroclor 1254/kg diet fed to White Leghorn hens for 14 wk significantly decreased egg production. Following removal of PCBs from the diet at wk 14, the decline in egg production continued for another 2 wk before there was a gradual increase in production. No effects on egg production were observed, however, when up to 80 mg Aroclor 1242/kg diet was fed to hens for 6 wk (Britton & Huston, 1973). Similarly, Briggs and Harris (1973) did not find significant changes in egg production when they fed 20 or 50 mg Aroclor 1242/kg diet to hens for 2 wk. In our study, egg production was significantly less in the control group compared to the low- and high-dose diet groups. Two factors probably contributed to the decreased egg production. The sudden change to the "high" fish diets may have led to the initial drop in egg production in all groups. The consistently lower rate of egg production by the control and low-dose group hens, however, may be attributed to FLHS, since the high-dose group hens did not have a long-term treatment-induced change in egg production.

A similar explanation may account for the trends observed in egg weights. Decreased egg weights were observed throughout the study in both the control and low-dose groups—the two groups most afflicted with FLHS. The initial decrease in egg weights in the high-dose group was only temporary.

No treatment-induced reduction in egg fertility was observed in the high-dose group. This is in agreement with the findings of other studies where chickens were fed diets containing PCBs (Briggs & Harris, 1973; Tumasonis et al., 1973; Ax & Hansen, 1975; Hansen, 1987). Both the control and low-dose groups, however, experienced lesser fertility rates, probably due to FLHS-induced physiological changes in the hens. The mean concentration of total PCBs in eggs laid after 8 wk exposure to the high-dose diet was 26 mg PCB/kg egg, ww (Summer et al., 1996), which is greater than current concentrations in eggs of most colonial fish-eating water birds from most regions of the Great Lakes (Giesy et al., 1994a, 1995). Thus, based on total concentrations of PCBs as the measure of exposure and number and weight of eggs laid and fertility of eggs, the no-observable-adverse-effect concentration (NOAEC) was determined to be greater than 26 mg total weathered PCBs/kg egg, ww.

Recently, several authors have suggested that a better predictor of effects than total concentrations of PCBs, PCDDs, and PCDFs could be made from the concentrations of dioxin-like congeners that bind to the aromatic hydrocarbon receptor (Ah-receptor), through which their better documented toxic effects are proposed to be mediated (Goldstein, 1980; Safe, 1987; McFarland & Clarke, 1989; Nebert, 1989). The potency of individual PCDAH congeners to cause toxic

effects can be compared to that of the most potent known PCDAH, which is 2,3,7,8-TCDD. This is done by the use of toxic equivalency factors (TEF), based on several toxic effects including lethality, deformities, or enzyme induction (Jones et al., 1993, 1994). These TEFs can then be used to calculate concentrations of TEQs that are contributed by individual congeners. The TEQs can be summed and expressed as a total equivalent concentration of 2,3,7,8-TCDD (see Giesy et al., 1994a, 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 selected 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 that are also present (Giesy et al., 1994a).

The greatest concentration of TEQs fed in the diet was 59 ng TEQ/kg, ww. Information on total concentrations of TEQs in the eggs was not available (samples for residue analyses lost in shipment), but if the biomagnification factor (BMF) was similar to that for total PCBs in the high-dose group (Giesy et al., 1994a), a total concentration of approximately 255 ng TEQ/kg egg, ww, would be expected. This would be more than the LD50, which has been determined to be 147 ng 2,3,7,8-TCDD/kg egg, ww, in egg injection studies with chickens (Verret, 1976). Since no studies where 2,3,7,8-TCDD was fed to chickens in the diet and no measure of TEQs in the eggs were available, it is difficult to compare the results obtained here to those of injection studies in the literature. However, it can be concluded that the dietary NOAEC for TEQ in the presence of environmentally weathered complex mixtures of chlorinated hydrocarbons, as determined by the H4IIE assay, is greater than 59 ng TEQ/kg diet, ww.

Since the H4IIE assay is a mammalian cell line, it may respond differently to complex mixtures than avian cells (Kennedy et al., 1994). Therefore, as more information becomes available on the responsiveness of avian cells to 2,3,7,8-TCDD and similar types of compounds, the results of the H4IIE assay will need to be calibrated to those results.

The results presented here indicate that the concentrations of total PCBs and TEQs fed in the diet were less than the concentrations required to have significant effects on the number or weight of eggs produced or egg fertility. However, they did affect the growth and survival of chicks and incidence of birth defects (Summer et al., 1996).

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ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY
ETAP

Retinoids in eggs and embryos of birds fed fish from the Great Lakes

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Abstract

Retinoids were analyzed in 11-day chick embryos and eggs from white Leghorn hens (*Gallus domesticus*) fed environmentally-derived polychlorinated biphenyls (PCB) in carp (*Cyprinus carpio*) from Saginaw Bay. The yolks and the embryos contained all-*trans*-retinol, 3,4-didehydroretinol and retinyl esters. There was no significant difference in the total retinoid content in the yolks of 11-day incubated eggs among hens fed for 7 week diets containing 0.5–6.6 mg PCB/kg diet. However, the proportional amount of retinols in the high (6.6 mg) PCB group was significantly less than that in low (0.5 mg) PCB controls, while the amount of retinyl palmitate in the high PCB group was significantly greater than that in the controls. Retinoids in the embryos were not affected by any of the PCB levels fed to hens for 7 weeks prior to laying the eggs. The 50% reduction in the molar ratio of retinols to retinyl palmitate in the yolks of eggs as the result of the high PCB level fed to hens for 7 weeks can serve as an indicator for chronic exposure to PCB contamination at the level of 6.6 mg or higher PCB/kg diet. © 1997 Elsevier Science B.V.

Keywords: Retinoids; Chick embryo; PCB

1. Introduction

The continuous global exposure to persistent organohalogens (Zile, 1992; Vanden Heuvel and Lucier, 1993; Mennear and Lee, 1994) has impacted the health and reproductive performance of some marine mammals and of fish and wildlife populations that inhabit contaminated environments such as the Great Lakes region in the USA (Gilbertson et al., 1991; Peterson et al., 1993; Kamrin and Ringer, 1994; Sanderson et al., 1994). Elevated environmental levels of certain persistent organohalogens, particularly polychlorinated-biphenyls (PCBs), -dibenzofurans (PCDF) and -dibenzo-*p*-dioxins (PCDD) have been linked to embryo mortality and abnormalities observed in fish-eating birds in the Great Lakes basin (Gilbertson and Schneider, 1991; Gilbertson et al., 1991; Weseloh et al., 1991). Studies with wildlife suggest a cause–effect linkage between environmental contamination and reproductive failure and increased mortality (Fox et al., 1991a,b,c; Gilbertson et al., 1991; Tillitt, 1991; Summer, 1992; Zile, 1992; Giesy, 1994a,b; Sanderson et al., 1994). Although the concentrations of most of the persistent organohalogens in the Great Lakes have declined since the 1960s, as estimated from analysis of various ecosystem compartments (Bishop and Weseloh, 1990; Bishop et al., 1991), a significant further decline in the near future is not expected (Giesy, 1991; Tillitt, 1991). Thus, the impact of the polluted environment on the various animal species, including humans that inhabit the Great Lakes basin continues to be a concern (Summer, 1992; Zile, 1992).

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The toxicity and mechanism of action of persistent organohalogens has been the subject of investigation by many laboratories (Safe, 1986; Rozman, 1992; Kohn et al., 1993; Nebert et al., 1993; Whitlock, 1993). It is widely accepted that persistent organohalogens structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have similar mechanisms of action mediated through binding to the Ah-receptor (Safe, 1986; Kohn et al., 1993; Nebert et al., 1993; Whitlock, 1993; Sanderson et al., 1994). This mechanism results in a number of responses, including induction of CytP-450 isoenzymes, uridinediphosphate glucuronosyl transferases, aldehyde dehydrogenases and other proteins.

Exposure to persistent organohalogens can cause effects similar to vitamin A deficiency (Akoso et al., 1982; Darjano et al., 1983; Kimbrough, 1984; Thunberg, 1984; Hakansson, 1988; Brouwer, 1991) due to effects on vitamin A metabolism (Zile, 1992). The effects could be the result of an induction of enzymes specific for retinoid catabolism which result in changes in the relative proportions and amounts of retinoid metabolites in tissues (Zile, 1992). In ring doves, reproductive performance was impaired as a result of exposure to tetrachlorobiphenyl (Spear et al., 1989). In that study 43% of the embryos died before hatching. After 3 days of incubation egg yolks from exposed birds contained lesser amounts of retinol and retinyl palmitate compared to controls and there was an increase of the ratio of yolk retinol to retinyl palmitate. Total concentrations of retinoids in the eggs of herring gulls in the Great Lakes were unrelated to concentrations of persistent organohalogens in eggs. However, there was a positive correlation between the ratio of yolk retinol to retinyl palmitate with several indices of environmental toxicants (Spear et al., 1990). In a comprehensive study of biomarkers for exposure of common tern to polyhalogenated aromatic hydrocarbons (PHAH), Murk et al. (1994) observed lower levels of yolksac retinyl esters in tern eggs from colonies exposed to an increased level of PHAH pollution. These researchers as well as Spear et al. (1990) have suggested that yolk retinoids may serve as biomarkers for PHAH in environment. While species display a wide range of symptoms and diversity in their sensitivity to different persistent organohalogens, the embryo is particularly sensitive to the exposure of some PHAH (Brunstrom and Reutergardh, 1986; Hoffman et al., 1987; Brunstrom, 1988; Zile, 1992; Summer et al., 1996a,b). Since the reproductive performance of birds in the wild is known to be impaired as a result of exposure to polyhalogenated aromatic hydrocarbons, and since it is known that retinoid metabolism can be altered by PHAH (Spear et al., 1990; Zile,' 1992), we wanted to further examine the hypothesis that an altered retinoid composition in the eggs may serve as a useful marker for effects of persistent environmental organohalogens in birds. Impaired vitamin A metabolism may affect vitamin A function, which is essential for normal reproductive performance and for embryonic development (Mason, 1935; Thompson, 1969; Dersch and Zile, 1993).

The available baseline information concerning vitamin A compounds in the avian species is conflicting. We describe here the vitamin A levels and metabolite patterns in eggs and 11-day embryos of domestic birds fed fish-containing diets that contain graded amounts of PCB and, in part, mimic the diets consumed by waterfowl inhabiting the environmentally polluted Great Lakes basin. This model also allows for the testing of the hypothesis that egg retinoids may serve as biomarkers for aquatic environmental contamination.

2. Materials and methods

2.1. Composition of diets; PCB content

The carp used in the diets were collected by electroshocking from the mouth of the Saginaw River (Lake Huron, MI, USA) in November, 1988. The fish were ground, blended into a homogeneous mixture, and stored frozen at −17°C. Ocean fish scraps (cod, haddock, pollack, and flounder trimmings; Boston Feed Supply, Natick, MA) were ground and blended as described above and incorporated into the control and low-dose diets as a source of 'clean' fish to provide 31–35% fish in each diet (Tables 1 and 2).

Table 1
Major dietary components of the control, low-dose, and high-dose diets fed to white Leghorn laying hens

Dietary components	Treatment diet		
	Control	Low-dose PCB	High-dose PCB
Carp	—	3.4	34.5
Ocean fish	30.9	28.7	—
Corn, #2 yellow	37.7	39.9	45.9
Oat hulls	6.7	6.1	11.1
Soybean meal (44%)	11.4	10.4	1.5
Isolated soy protein (90%)	3.0	2.1	—
Alfalfa meal (17%)	2.0	1.8	—
Vitamin premix	0.3	0.3	0.3
Mineral premix	0.3	0.3	0.3
Selenium premix	0.06	0.05	0.05
Limestone	7.5	6.8	6.3
Salt	0.06	0.05	—
Ethoxyquin[a] (g/100 lb diet)	5.67	5.67	5.67

[a] 90% pure, Roche Animal Nutrition, Hoffmann-LaRoche, Inc., Nutley, NJ.

Table 2
Nutrient analysis[a] of the diets of adult hens

Dietary component	Treatment diet					
	Control		Low-dose		High-dose	
	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.
Fat (%)	11.5	12.9	13.6	15.3	15.45	16.87
Crude protein (%)	20.0	22.4	22.5	25.4	20.63	22.52
Crude fiber (%)	6.3	7.0	5.3	6.0	6.50	7.10
Calcium (%)	4.1	4.6	3.4	3.8	4.13	4.51
Phosphorus (%)	1.1	1.2	0.8	0.9	1.25	1.36
Potassium (%)	0.7	0.7	0.6	0.7	0.57	0.62
Magnesium (%)	0.1	0.2	0.1	0.2	0.12	0.14
Sodium (%)	0.2	0.4	0.2	0.2	0.13	0.14
Iron (ppm)	392.6	440.1	415.4	468.3	430.6	470.0
Manganese (ppm)	357.1	400.3	414.1	466.9	357.0	389.7
Copper (ppm)	38.4	43.0	59.4	67.0	45.8	50.0
Zinc (ppm)	304.8	341.6	406.5	458.3	469.1	512.1
Ash (%)	11.1	12.4	12.0	13.5	9.60	10.48

[a] Wet weight analysis provided by Litchfield Analytical Services, 535 Marshall St., P.O. Box 457, Litchfield, MI.

Before preparing experimental diets, ground ocean fish and carp were cooked at 82°C for approximately 2 h to dehydrate the fish and inactivate any thiaminase present in the carp (Gnaedinger and Krzeczkowski, 1966). Ground corn was added to the cooked fish to absorb the remaining moisture and provide a 'dry' product for mixing with the other dietary ingredients. The diets (Table 1), were formulated based on a nutrient analysis of the cooked fish samples in an attempt to meet the nutrient requirements of the laying hen (National Research Council, 1984). Samples of the diets were collected for nutrient analysis and total PCB and TCDD-EQ analyses (Table 2). The prepared diets were stored at −17°C until fed to the chickens.

Total concentrations of total PCBs were 0.31, 0.75, and 6.59 mg PCB/kg (w/w; measured as the sum of Aroclors 1242, 1248, 1254, and 1260). Concentrations of individual PCDD, PCDF and PCB congeners were determined in the carp and each of the diets (Tillitt et al., 1995). Equivalency factors for the induction of EROD in the H4IIE rat hepatoma cell line (Tillitt et al., 1995) were used to determine the total concentration of H4IIE bioassay-derived TCDD-EQS (TEq) (Table 3). Concentrations of TEq measured with the H4IIE assay were similar to but less than the concentrations predicted from the TEF values and molar concentrations of PCDD, PCDF and non- and mono-*ortho*-substituted PCB congeners.

2.2. Birds, eggs and embryos

Mature white Leghorn hens (*Gallus domesticus*) were exposed to environmentally-derived planar halogenated hydrocarbons in Saginaw Bay carp (*Cyrinus carpio*) through a synthetic diet containing either 35% ocean fish (control, 0.5 mg PCB/kg) or 28% ocean fish and 3.4% Saginaw Bay carp (low dose, 0.9 mg PCB/kg) or 34.5% Saginaw Bay carp (high-dose, 6.6 mg PCB/kg). See detailed discussion in Summer (1992); and in Summer et al. (1996a,b). All hens were fed the control (31% ocean fish) diet for 2 weeks to accustom them to the 'high' level of fish in the diet and acclimate them to their cages prior to the definitive trial. Feed and water were available to the birds ad libitum throughout the study. Six hens were kept as replacements during the acclimation period in the event that some of the hens did not adjust to the 'high' level of fish in the diets. These hens were treated in the same manner as the acclimated hens. Five hens were culled from the study and replaced during the acclimation period. Eggs obtained at the end of this 2-week adjustment period were incubated for 11 days and served as initial controls (week 0) for all dietary groups (Table 4). Following the

Table 3
Concentrations of total PCBs and TEqs in diets of hens

Compound	Treatment diet		
	Control	Low-dose PCB	High-dose PCB
Total PCB[a]	0.3	0.8	6.6
TEq (H4IIE)[b,c]	1.0	6.6	59.0
Teq (calculated)[b,d]	0.4	4.5	45.0

[a] mg PCB/kg (w/w).
[b] ng Teq/kg (w/w).
[c] Determined by H4IIE bioassay.
[d] Calculated from molar concentrations and TEF from H4IIE assay.

Table 4
Retinoids of 11-day embryos and yolk/albumen of eggs from hens fed control fish diet for 2 weeks

	Yolk/albumen	Embryos
All-*trans*-retinol		
nmol/g[a], dry weight	16.5 ± 3.6[b]	4.3 ± 0.8
nmol/egg	100.9 ± 17.3	1.0 ± 0.4
3,4-Didehydroretinol		
nmol/g	(included with all-*trans*-retinol)	7.2 ± 0.9
nmol/egg		1.9 ± 0.6
Retinyl palmitate		
nmol/g	7.3 ± 1.8	48.5 ± 14.3
nmol/egg	45.8 ± 13.8	13.6 ± 5.3
Retinyl linoleate		
nmol/g	ND	47.8 ± 17.0
nmol/egg	ND	12.7 ± 14.3
Molar ratio		
RP/ROH	0.5	
+ ddROH[c]		
ddROH/ROH	—	1.9
RL+RP/ROH	—	9.1
+ ddROH		

[a] Dry weight basis.
[b] Values are means ± SEM; $n = 9$. ND, not detected.
[c] ROH, All-*trans*-retinol; ddROH, 3,4-didehydroretinol; RP, retinyl palmitate; RL, retinyl linoleate.

acclimation period, birds were assigned to three dietary treatment groups of 20 hens/group, and the treatment diets fed for 7 weeks. Eggs were collected daily and incubated for 11 days, at which time the embryos and the egg contents were examined for various developmental and biochemical parameters.

2.3. Preparation of samples

Three eggs were randomly chosen from each of the groups, i.e. the control group, the low PCB group, and the high PCB group, for each time point corresponding to 0, 2, 4 or 7 weeks of dietary treatment, and stored in liquid N_2 until analysis. At that time the yolk and albumen portion were allowed to thaw and the frozen embryos dissected away from the yolk and albumen mixture, which could not be separated. Since yolk is the major component of this mixture and since the albumen does not contain any retinoids (Plack, 1960; Zile, unpublished data), the retinoids in this mixture reflect the retinoid content of the yolk. Embryos were thawed in saline (0.9% NaCl) and rinsed 4 × with ice cold saline. The yolk/albumen mixture and each embryo were homogenized separately, then lyophilized. The dried samples were weighed and stored at −80°C until extraction.

2.4. Extraction of retinoids

Retinoids were extracted according to a combination of the methods of Spear et al. (1989), Cullum and Zile (1985) and Zile et al. (1989). Lyophilized yolk/albumen, 0.1 g of dry material was first extracted with 3 ml of methanol containing 0.001% butylated hydroxytoluene, then with 1.5 ml of methanol and 1.5 ml of hexane and finally with 3 ml of hexane. Supernatants were pooled, evaporated with a stream of nitrogen and the dried sample reconstituted with 0.3 ml of methanol; 25–50-μl aliquots were used for analysis of retinoids. Extractions of lyophilized embryonic tissues were done in the same manner with the following modifications: 50 mg of lyophilized whole embryo was used, the volume of each solvent was reduced by 50%, the residue was redissolved in 200 μl of methanol; 70-μl aliquots were used for retinoid analysis.

2.5. Retinoid analysis by HPLC

The HPLC system used was as described earlier (Cullum and Zile, 1985, 1986). Retinoids were analyzed using a C_{18}-reversed-phase column (Whatman, Partisil 10 ODS-3, 25 cm × 4.6 mm) with Guard-PAK precolumn module containing μBondapak-C_{18} precolumn cartridge (Millipore). Step gradient elution of retinoid standards and biological samples was accomplished with mixtures of water and methanol (MeOH), (v/v) and mixtures of $CHCl_3$ and MeOH, with a flow-rate 2.0 ml/min at room temperature (Fig. 1A). For analysis of retinoids in the yolk/albumen extracts the solvent sequence was as follows: MeOH:0.1 M ammonium acetate (75:25), 10 min; MeOH:H_2O (88:12), 15 min; and MeOH:$CHCl_3$ (88:15), 13 min. For extracts of embryo samples the solvent sequence was as follows: MeOH:0.1 M ammonium acetate (65:35), 15 min; MeOH:H_2O (88:12), 12 min; and MeOH:$CHCl_3$ (85:15), 8 min. Absorbance was monitored at 340 nm. All-*trans*-retinol and all-*trans*-retinal were separated on a 5μ Zorbax ODS column, 25 cm × 4.6 mm (DuPont Instruments), using MeOH:0.1 M ammonium acetate (65:35), followed by MeOH:H_2O (88:12), and MeOH:$CHCl_3$ (85:15) (Fig. 1B). Retinoids were identified by coelution with authentic standards. No attempt was made to separate the A_1-all-*trans*-retinyl esters from the all-*trans*-didehydroretinyl (A_2) esters.

2.6. Quantitation of retinoids

An external standardization procedure for all-*trans*-retinol, all-*trans*-retinoic acid and all-*trans*-retinyl palmitate was as described by Cullum and Zile (1986). Retinoids were added to lyophilized samples from yolk/albumen and embryonic tissue obtained from vitamin A-deficient quail eggs incubated for 2–3 days at 38°C

and 98% R.H. in an incubator with automatic rotation (Dersch and Zile, 1993). HPLC standard curves for retinoids were established as follows: all-*trans*-retinoic acid, range 5–100 ng, detection limit 2 ng; all-*trans*-retinol, range 5–200 ng, detection limit 2 ng; all-*trans*-retinyl palmitate, range 5–20 000 ng, detection limit 5 ng. Recoveries were as follows: all-*trans*-retinoic acid (0.1–0.2 μg/g lyophilized material), 74%; all-*trans*-retinol (2.3–4.6 μg/g), 72%; and retinyl-palmitate (4.5–9.0 μg/g), 43%. All-*trans*-retinyl acetate was added as internal standard to samples prior to extraction to assess recoveries; 3,4-didehydroretinol was quantitated using the HPLC standard curve for all-*trans*-retinol. Retinyl linoleate and retinyl palmitate were assessed by the standard curve for all-*trans*-A_1-retinyl palmitate. The amount of each retinoid was computed using a HP 3393A Computing Integrator according to (Eq. (1)).

$$\text{AMT(i)} = \text{A(i)/A(istd)} \times \text{RRF(i)} \times \text{AMT(istd)} \times \text{MUL FACTOR} \quad (1)$$

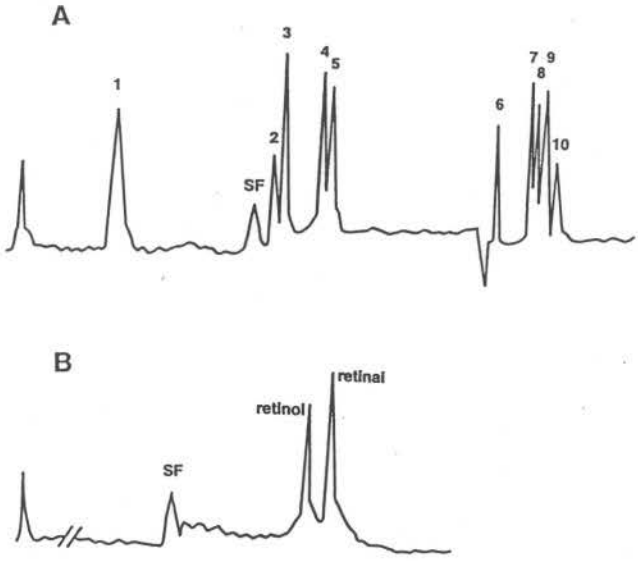

Fig. 1. (A) Separation of retinoid standards by reverse-phase HPLC on Partisil 10 ODS-3 column. Standards and their respective retention times are as follows: (1), all-*trans*-retinoic acid, 46 ng, 7.5 min; (2), 3,4-didehydroretinol, 29 ng, 16.5 min; (3), all-*trans*-retinol, 24.5 ng, 17.5 min; all-*trans*-retinal, 100 ng, 17.5 min; (4), all-*trans*-retinyl acetate, 26.5 ng, 20.4 min; (5), methylretinoate, 40 ng, 20.9 min; (6), *trans*-β-apo-8′-carotenal, 100 ng, 31.3 min; (7), all-*trans*-retinyl linolenate, 50 ng, 33.3 min; (8), all-*trans*-retinyl linoleate, 40 ng, 33.7 min; (9), all-*trans*-retinyl palmitate, 28.4 ng, 34.2 min; all-*trans*-retinyl oleate, 25 ng, 34.2 min; (10), all-*trans*-retinyl stearate, 40 ng, 35.0 min. Solvent sequence was MeOH:0.1 M ammonium acetate (75:25), 10 min; MeOH:H_2O (88:12), 15 min; and MeOH:$CHCl_3$ (88:15), 13 min; SF, solvent front. (B) Separation of all-*trans*-retinol and all-*trans*-retinal standards by reverse-phase HPLC on Zorbax ODS 5μ column, 25 cm × 4.6 mm (DuPont). Retinol, 54 ng, 28.9 min; all-*trans*-retinal, 222.3 ng, 30.3 min. Solvent sequence was MeOH:0.1 M ammonium acetate (65:35), 15 min; MeOH:H_2O (88:12), 15 min; and MeOH:$CHCl_3$ (85:15), 8 min. SF, solvent front.

AMT(i) = amount of retinoid i in the sample run; A(i) = area of retinoid i measured in the sample run; RRF(i) = relative response factor determined for retinoid i through calibration run (Eq. (2)); A(istd) = area of the internal standard peak measured in the sample run; AMT(istd) = internal standard amount for the sample run; MUL FACTOR was equal to 1 in our experimental system.

$$\text{RRF(i)} = \frac{\text{AMT(i)/A(i)}}{\text{AMT(istd)/A(istd)}} \quad (2)$$

Concentrations of retinoids in samples of egg yolk/albumen and embryos were calculated as nmoles of retinoid/g of lyophilized dry powder of yolk/albumen and embryo or per whole egg and per whole embryo, and expressed as mean ± SD for each dietary group; the number of determinations ranged from three to nine.

The following vitamin A_1 retinoid standards were used: all-*trans*-retinol, all-*trans*-retinoic acid, *trans*-β-apo-8′-carotenal (Sigma); 9-*cis*-retinoic acid, all-*trans*-retinal, all-*trans*-retinyl acetate, all-*trans*-retinyl palmitate, all-*trans*-retinyl linoleate, all-*trans*-retinyl linoleate, all-*trans*-retinyl stearate, all-*trans*-retinyl oleate (all from Hoffmann-LaRoche, Inc.); and 3,4-didehydroretinol (vitamin A_2, Dr. Burri, USDA). Retinoid purity was checked by HPLC prior to use.

2.7. Statistics

Significance of differences between retinoid concentrations in control and exposed groups was evaluated by Student's *t*-test for unpaired samples (Statgraphics city and state).

3. Results

3.1. Initial amounts of retinoids in yolk/albumen and embryos

A typical HPLC profile of retinoids in yolk/albumen is shown in Fig. 2A. The yolk/albumen mixture contained all-*trans*-retinol, 3,4-didehydroretinol and retinyl palmitate (Fig. 2, Table 4); retinoic acids and other polar retinoids were not detected. All-*trans*-retinol represented the main form (67–75%) of vitamin A in the yolk/albumen extracts; 3,4-didehydroretinol represented 5–8% of the total retinoids (Fig. 2B); retinal was not detected (Fig. 2C,D). The concentration of all-*trans*-retinol in the yolk/albumen extracts includes 3,4-didehydroretinol (Table 4). The only retinyl ester observed in the yolk/albumen mixture eluted in the area of all-*trans*-retinyl palmitate (Fig. 2A,B, peak 4), and represented 20–25% of the total retinoids. This area of elution may also contain didehydroretinyl palmitate.

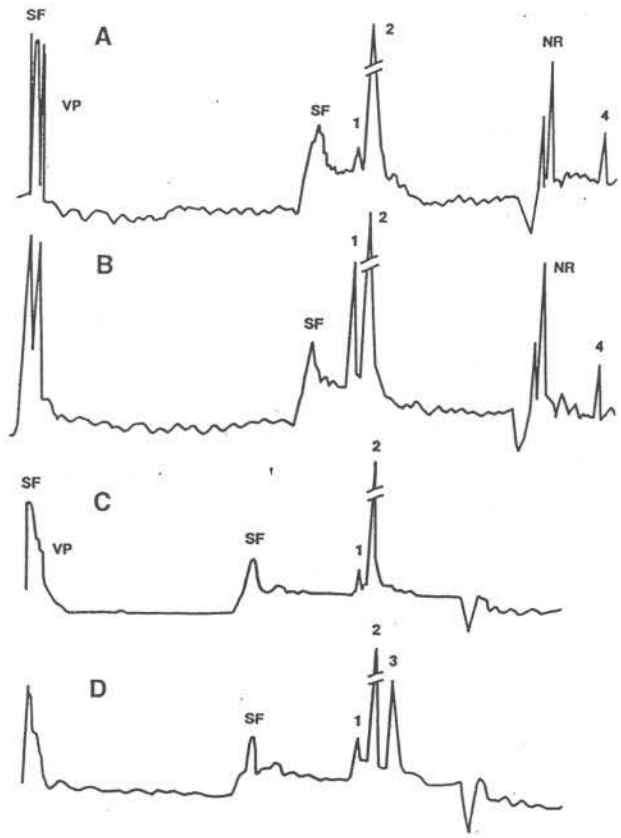

Fig. 2. Separation of retinoids in yolk/albumen from eggs after the incubation of eggs for 11 days. (1) 3,4-Didehydroretinol; (2) all-*trans*-retinol; (3) all-*trans*-retinal; NR, non-retinoids; (4) all-*trans*-retinyl palmitate. VP, very polar metabolites; SF, solvent front. (A) HPLC on Partisil 10 ODS-3 column. Solvent sequence was as for Fig. 1B. (B) HPLC as in (A) except that 100 ng of 3,4-didehydro-retinol (peak 1) was added to the yolk/albumen extract. (C,D) HPLC of yolk/albumen extract on Zorbax ODS 5μ analytical column without (C) and with (D) added all-*trans*-retinal standard, 97 ng (peak 3). Solvent sequence was as for Fig. 1B. No precolumn was used. Peak 1 coeluted with 3,4-didehydroretinol and peak 2 coeluted with all-*trans*-retinol. VP, very polar metabolites; SF, solvent front.

The absorbance eluting with the solvent front in the non-polar region (Fig. 2A,B, peak NR) was not associated with retinoids.

Embryos from the control eggs incubated for 11 days contained all-*trans*-retinol, 3,4-didehydroretinol, and esters eluting with all-*trans*-retinyl palmitate and all-*trans*-retinyl linoleate (Fig. 3A). Retinal was not detected (Fig. 3B,C). The peaks eluting in the polar region were not characterized. Retinyl palmitate and retinyl linoleate were present in equal amounts in the embryos and represented the major form (91%) of retinoids in the 11-day chick embryo (Table 4).

3.2. Retinoids in yolk/albumen mixture of 11-day incubated eggs

Prior to feeding contaminated diets to hens, the retinoid content of 11-day incubated eggs was analyzed for each dietary group; the initial values varied widely within treatment groups (Table 5, week 0). At this time Group A (control) was continued on the control diet, while Groups B (low PCB) and C (high PCB) were switched to the PCB-containing fish diets. Over the experimental period of 7 weeks, the values for retinol in the yolk/albumen of eggs from the control Group A ranged from 13.6 ± 3.8 to 20.4 ± 4.5 nmol/g or from 64.4 ± 6.9 to 131.3 ± 34.9 nmol/egg (Tables 4 and 5). Retinyl palmitate in these samples ranged from 5.4 ± 1.7 nmol/g or 26.9 ± 14.4 nmol/egg to 11.6 ± 3.8 nmol/g or 64.8 ± 26.0 nmole/egg (Tables 4 and 5).

Dietary treatment of hens fed fish containing a low level of PCB (0.9 mg of PCB/kg of diet) for 2, 4 or 7 weeks did not significantly alter the amounts or propor-

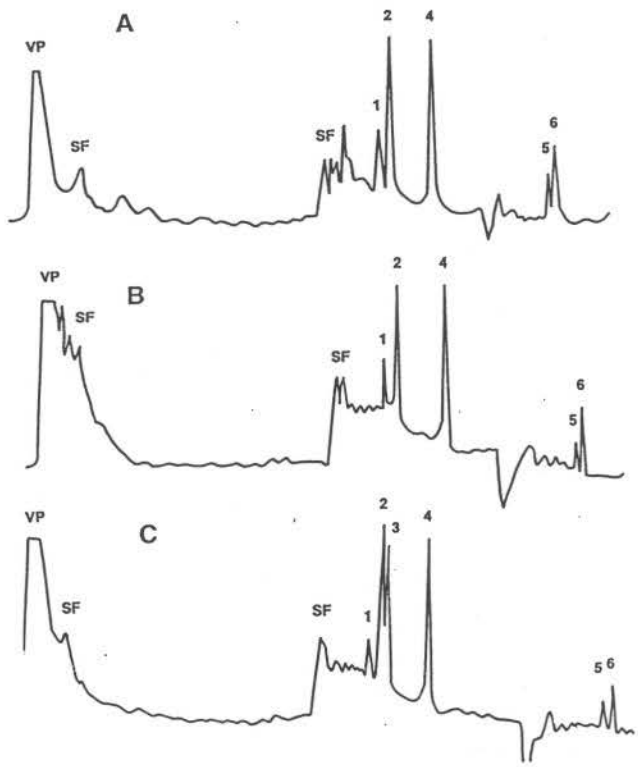

Fig. 3. Separation of retinoids from 11-day embryos. (A) Typical elution profile on Partisil C_{18} ODS 10 reverse phase column, using step gradient elution with MeOH:0.1 M ammonium acetate (65:35), MeOH:H_2O (88:12), and MeOH:$CHCl_3$ (85:15). (1) 3,4-Didehydroretinol; (2) all-*trans*-retinol; (3) all-*trans*-retinal standard; (4) all-*trans*-retinyl acetate internal standard; (5) all-*trans*-retinyl linoleate; and (6) all-*trans*-retinyl palmitate. VP, very polar metabolites; SF, solvent front. (B,C) Separation of retinoids from 11-day embryos on Excalibar Spherisorb ODS 5μ column, 25 cm × 4.6 mm. (B) embryo extract; (C) embryo extract with added all-*trans*-retinal standard, 97 ng. (1) 3,4-Didehydroretinol; (2) all-*trans*-retinol; (3) all-*trans*-retinal standard; (4) all-*trans*-retinyl acetate; (5) all-*trans*-retinyl linoleate; and (6) all-*trans*-retinyl palmitate. VP, very polar metabolites; SF, solvent front.

Table 5
Retinoids in the yolk/albumen fraction of 11-day incubated eggs from hens fed PCB-contaminated Great Lakes fish

Group[a]	Week[b]	Retinols[c] (nmol)		Retinyl palmitate (RP) (nmol)		Retinols[d]/RP
		/g	total	/g	total	
A	0	14.9 ± 2.5[5]	104.3 ± 10.5	4.1 ± 1.1	46.3 ± 21.9	4.3 ± 1.6
B		13.6 ± 3.8	64.4 ± 6.9	5.4 ± 1.7	26.9 ± 14.4	2.9 ± 1.3
C		20.4 ± 4.5	131.3 ± 34.9	8.0 ± 3.3	53.4 ± 20.0	2.7 ± 0.6
A	2	18.8 ± 6.8	105.4 ± 49.0	11.6 ± 3.8	64.8 ± 26.0	1.6 ± 0.4
B		17.9 ± 3.5	104.1 ± 30.2	7.7 ± 2.6	44.5 ± 16.8	2.5 ± 1.4
C		21.5 ± 3.3	112.9 ± 67.4	9.3 ± 1.9	46.6 ± 18.8	1.9 ± 0.5
A	4	18.7 ± 3.6	144.9 ± 16.8	5.6 ± 2.5	42.8 ± 8.4	3.6 ± 0.8
B		20.5 ± 2.8	160.4 ± 26.9	10.4 ± 4.6	72.3 ± 30.0	2.4 ± 0.8
C		20.9 ± 1.8	146.6 ± 41.9	8.3 ± 2.5	57.2 ± 24.7	2.7 ± 0.9
A	7	19.9 ± 1.9	139.0 ± 8.1	5.4 ± 1.9	31.7 ± 18.5	4.3 ± 1.8
B		19.6 ± 1.2	125.8 ± 29.4	7.2 ± 1.8	48.4 ± 22.2	3.0 ± 0.6
C		16.1 ± 2.9*	77.9 ± 31.2*	11.5 ± 4.6	62.5 ± 4.6*	2.0 ± 0.8*

[a] A, control, 0.5 mg PCBs/kg diet; B, low PCBs, 0.9 mg PCBs/kg diet; C, high PCBs, 6.6 mg PCBs/kg diet. Other experimental details are in Section 2.
[b] Weeks fed experimental diets. All groups were acclimated for 2 weeks on the control fish-containing diet prior to assigning them to different diets. Data for week 0 represent the initial values for each group immediately prior to switching groups B and C to different diets.
[c] Combined all-*trans*- and 3,4-didehydroretinols.
[d] Molar ratio of total retinoids in the yolk/albumen fraction of egg.
[e] Mean ± SEM; $n = 3$. *Significant differences at $0.05 < p < 0.1$ between groups A and C at 7 weeks.

tions of retinoids in the yolk/albumen of eggs incubated for 11 days (Table 5, Group B) as compared to the control Group A. Similarly, feeding hens fish diet containing a high amount of PCB (6.6 mg of PCB/kg of diet) for 2 or 4 weeks did not have any effect on the yolk/albumen retinoids (Table 5, Group C). However, feeding high PCB diet for 7 weeks markedly and significantly altered retinol and retinyl palmitate levels in the yolk/albumen of 11-day incubated eggs from the hens receiving this diet. The total mass of retinol was decreased 50%, while that of retinyl palmitate was doubled, as compared to that of the control group. The ratio of retinol to retinyl palmitate was only 50% of that of the control Group A (Table 5, Groups A and C at 7 weeks). The total mass of retinoids in yolk/albumen varied greatly and ranged from 91.3 to 232.7 nmol, but was not significantly different from the controls at any time (Table 8).

3.3. Retinoids in embryos of 11-day incubated eggs

The embryos contained all-*trans*-retinol and 3,4-didehydroretinol (Fig. 3, Table 6). Didehydroretinol was the predominant retinol in the 11-day chick embryo. Retinyl palmitate and retinyl linoleate were present in equal amounts and constituted the major retinoids in embryos (Table 7). These esters may include both the A_1 and A_2 series of retinoids. The amounts of individual as well as total retinoids (17.7–54.7 nmol/embryo) varied among treatments throughout the 7-week period of study (Table 8). No significant differences were observed in the total or relative concentration of retinoids in embryos from eggs of hens fed PCB-contaminated fish diets for 2, 4 or 7 weeks, relative to the respective controls. Similarly, the ratio of retinols to retinyl esters in the embryos was not significantly different from the controls during the 7-week period of dietary treatment of birds with contaminated fish (Table 7).

3.4. Retinoids in eggs after 11 days of incubation

Most of the vitamin A (76–88%) in 11-day incubated eggs was in the yolk/albumen fraction (Table 8). Total masses of retinoids in the yolk/albumen fraction and embryos of these eggs were not significantly affected by feeding PCB-containing fish to hens (Table 8).

3.5. Food consumption of hens

Food consumption did not differ significantly among treatment groups although on the average hens in the high-dose group ate 1.7–8.5 g/hen/day more than hens in the control group and 0.1–1.5 g/hen/day more than hens in the low-dose group (Summer et al., 1996a). As the study progressed, food consumption in all experimental treatments decreased overall by approximately 20% after fluctuating widely during the early part of the study.

Table 6
Retinols in 11-day chick embryos from eggs of hens fed PCB-contaminated Great Lakes fish

Group[a]	Week[b]	Retinol (ROH) (nmol)		3,4-Didehydroretinol (ddROH) (nmol)		ddROH[c]/ROH in embryo
		/g	/embryo	/g	/embryo	
A	0	3.1 ± 1.2[d]	0.6 ± 0.3	7.0 ± 2.0	1.5 ± 0.4	2.4 ± 0.3
B		6.1 ± 0.9	1.8 ± 0.5	8.7 ± 0.6	2.8 ± 0.6	1.5 ± 0.3
C		3.6 ± 0.2	0.7 ± 0.3	6.0 ± 0.1	1.3 ± 0.7	1.7 ± 0.2
A	2	5.3 ± 1.4	1.1 ± 0.3	7.4 ± 0.5	1.6 ± 0.4	1.7 ± 0.5
B		5.2 ± 0.9	1.4 ± 0.2	7.6 ± 0.5	2.1 ± 0.2	1.5 ± 0.3
C		4.3 ± 0.5	1.2 ± 0.2	7.5 ± 0.7	2.2 ± 0.3	1.7 ± 0.5
A	4	6.7 ± 0.5	1.5 ± 0.6	7.1 ± 0.4	1.6 ± 0.8	1.0 ± 0.2
B		5.9 ± 1.0	1.6 ± 0.6	6.7 ± 1.0	1.8 ± 0.7	1.2 ± 0.1
C		4.0 ± 0.2	1.2 ± 0.1	8.8 ± 0.7	2.6 ± 0.3	2.2 ± 0.2
A	7	4.7 ± 0.9	1.3 ± 0.4	7.9 ± 0.8	2.2 ± 0.3	1.7 ± 0.3
B		6.1 ± 2.4	1.2 ± 0.3	8.7 ± 0.3	1.9 ± 0.5	1.6 ± 0.5
C		6.3 ± 0.6*	1.9 ± 0.2	8.3 ± 0.8	2.4 ± 0.2	1.2 ± 0.1

[a] A, control; B, low PCB; C, high PCB. See Table 4 and Section 2 for experimental details.
[b] Weeks fed experimental diets. See Table 4 and Section 2.
[c] Molar ratio.
[d] Mean ± SEM; $n = 3$.

4. Discussion

Studies with polyhalogenated aromatic hydrocarbons (PHAH) have previously established that these environmental pollutants adversely affect vitamin A homeostasis that can cause enhanced catabolism and loss of vitamin A (Zile, 1992), and have led to well-founded concerns that wildlife exposed to environmental contaminants may be at risk from impaired vitamin A nutrition. Thus, vitamin A deficiency may be a contributing factor to the observed reproductive failures and embryonal abnormalities in wildlife. This possibility has been indicated in a recent analysis of data obtained from studies with the common tern (Murk et al., 1996).

The molar ratio of retinol to retinyl palmitate in the egg yolks of herring gulls from the Great Lakes basin was found to be inversely correlated with several indices of polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentrations in these eggs (Spear et al., 1990). Similarly, Murk et al. (1994) reported an inverse relationship between yolksac retinyl esters and PHAH pollution in eggs of common tern. These authors suggested the use of retinoids as a functional biomarker of exposure of birds to polychlorinated diaromatic hydrocarbons. Therefore, we tested this hypothesis under controlled laboratory conditions, using domestic fowl. This species is sensitive to the effects of halogenated aromatic hydrocarbons and is commonly used as a surrogate organism in testing protocols. Furthermore, the sensitivity of the chicken to polyhalogenated aromatic hydrocarbons relative to other piscivorous birds has been determined (Lorenzen et al., 1997; Sanderson et al., 1997). Carp (*Cyprinus carpio*) from Saginaw Bay, Michigan, were selected as a representative, naturally-contaminated food resource because (a) they were known to contain 'high' concentrations of polychlorinated diaromatic hydrocarbons (PCDAHs) which permitted their incorporation into the chicken diets at reasonably low levels and (b) they tended to concentrate at the mouth of the Saginaw River in the late fall allowing for relatively easy collection of large quantities of fish. Although small forage fish, such as alewife (*Alosa pseudoharengus*) and smelt (*Osmerus mordax*) are the staple dietary species of colonial waterbirds, the concentrations of PCBs in the carp are environmentally relevant and would represent a worst case situation.

Another aspect of this study was to obtain baseline data concerning retinoids in hen eggs and chicken embryos since recent data on avian retinoids (Spear et al., 1989, 1990; Murk et al., 1994), appear to be in conflict with older literature (Plack, 1960, 1963, 1964; Plack and Kon, 1961; Plack et al., 1964; Joshi et al., 1973).

After an 11-day incubation of white Leghorn hen eggs, the yolk retinoids are mostly (67–75%) in the form of all-*trans*-retinol, with retinyl palmitate (20–25% of total), and 3,4-didehydroretinol (5–8% of total) as minor retinoids, the yolk composition most likely reflecting that of circulatory retinoids of the hen at the time of egg formation. The same retinoids were recently demonstrated in quail eggs (Dong and Zile, 1995). The findings agree, in part, with those of previous investigators (Plack, 1960, 1964; Plack and Kon, 1961; Joshi et al., 1973), although, similarly to Spear et al. (1990), we did not find significant amounts of retinal in the yolks. Similar to the findings of Plack et al. (1964), but in contrast to Joshi et al. (1973), Spear et al. (1990), and Murk et al. (1994), we observed 3,4-didehydroretinol in the yolk. These differences may be due to differences

Table 7
Retinyl esters in 11-day chick embryos from eggs of hens fed PCB-contaminated Great Lakes fish

Group[a]	Week[b]	Retinyl linoleate (RL) (nmol)		Retinyl palmitate (RP) (nmol)		RL+RP[c]/retinols in embryo
		/g	/embryo	/g	/embryo	
A	0	65.1 ± 22.9[d]	13.0 ± 8.8	31.7 ± 23.1	6.3 ± 2.5	9.2
B		65.8 ± 20.3	22.7 ± 11.8	82.2 ± 12.9	27.4 ± 11.2	10.9
C		12.5 ± 7.8	2.4 ± 1.4	31.6 ± 7.0	7.0 ± 2.2	4.7
A	2	48.4 ± 9.9	10.0 ± 0.6	60.4 ± 23.4	12.4 ± 5.4	8.3
B		72.5 ± 6.6	19.6 ± 1.6	50.1 ± 21.5	13.7 ± 7.1	9.5
C		76.2 ± 24.5	24.6 ± 1.9	55.8 ± 23.4	16.4 ± 6.7	12.1
A	4	32.7 ± 9.2	6.1 ± 2.3	56.9 ± 6.4	10.1 ± 6.9	5.2
B		57.4 ± 19.5	14.4 ± 4.5	71.1 ± 10.8	19.1 ± 1.8	9.9
C		62.2 ± 19.5	18.2 ± 5.5	93.9 ± 18.5	27.9 ± 6.5	12.1
A	7	79.4 ± 19.0	25.3 ± 2.7	79.8 ± 9.7	17.5 ± 7.2	12.2
B		44.4 ± 11.5	9.2 ± 3.4	63.5 ± 29.8	13.9 ± 6.3	7.5
C		65.5 ± 22.8	20.0 ± 7.6	68.0 ± 4.9	21.5 ± 1.5	9.7

[a] A, control; B, low PCB; C, high PCB. See Table 5 and Section 2 for experimental details.
[b] Weeks fed experimental diets. See Table 5 and Section 2.
[c] Molar ratios; values for retinols are from Table 6.
[d] Mean ± SEM; $n = 3$.

among species or the inability of some chromatographic systems to resolve the two retinols and retinal. A standardized method of analysis such as that described by Murk et al. (1994) and Dong and Zile (1995) will be required if retinoids are to be used for monitoring environmental pollution.

The total mass of combined retinoids in the yolks did not change significantly during the treatment period nor as the result of a two-fold increase in the daily consumption of PCB by the birds. This is in contrast to the results of Spear et al. (1989) who found a decrease in retinoid content of eggs from hens that had been exposed to tetrachlorobiphenyl. However, in the 11-day incubated eggs laid by hens that had been exposed for 7 weeks to the greatest concentration of PCBs (6.6 mg/kg of diet) we observed a shift in the yolk retinoid metabolite profile. The molar ratio of retinols to retinyl palmitate was reduced by 50%. Our results thus appear to be in conflict with those of Spear et al. (1990), who report a positive correlation in the yolk retinol to retinyl palmitate ratio and the levels of polychlorinated dibenzo-*p*-dioxin and dibenzofuran in herring gull eggs. While Murk et al. (1994) did not detect measurable amounts of retinols in the yolksacs of tern eggs, they found retinyl esters in these yolksacs to be inversely related to PHAH pollution at the sites of the breeding colonies of tern from which the eggs were obtained. The yolks from eggs incubated for 11 days contained both retinols and retinyl esters, but the amount of esters was directly related to the PCB level in the diet of birds from which these eggs were obtained. These discrepancies may be due to species differences as well as to the differences in the incubation times of the eggs, since these researchers themselves (Spear et al., 1989, 1990) as well as others (Joshi et al., 1973) have demonstrated that the proportion of the various yolk retinoids changes constantly during the incubation time and the development of the embryo. While in our studies the data on yolk retinoids was obtained exclusively from 11-day incubated eggs, the eggs analyzed by Spear et al. (1990) were obtained at various incubation times and those of Murk et al. (1994) were obtained at hatching. Furthermore, among the studies differences exist in the exposure times of the birds to the PHAH. Thus, a direct comparison is not possible.

The retinoid profiles in the 11-day white Leghorn chick embryos reflected, in general, those of the yolk. Two esters, retinyl palmitate and retinyl linoleate were present in equal amounts and represented the major form of retinoids (91%) in embryos. All-*trans*-retinol and 3,4-didehydroretinol constituted the remainder of the retinoids; retinal was present at very low levels. It is, however, likely that retinoic acids and their isomers represent some of the unidentified minor components eluting in the polar region; we did not characterize these metabolites. We have shown that the 24-h normal quail embryo has all the above retinoids (Dong and Zile, 1995) and that the 32-h quail embryo is capable of generating these retinoids, including didehydroretinol, from administered all-*trans*-retinol-A_1 (unpublished). The retinoid profiles and levels of retinoids in the 11-day embryos remained unaltered by the PCB feeding to the parent birds. Teratogenic effects observed in the embryos of this study as well as a complete description of the impact of environmentally persistent organohalogens on avian reproduction have been published elsewhere (Summer, 1992; Summer et al., 1996a,b).

In summary, we have characterized the retinoid composition in the yolk and the 11-day embryo of eggs from white Leghorn hens fed diets similar to those