CHAPTER 7

# PCBs and Dioxins in Birds

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## INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of synthetic chlorinated aromatic hydrocarbons that were first synthesized in 1881. Since 1930, PCBs have been in general use, having appeared in commercial products including heat transfer agents, lubricants, dielectric agents, flame retardants, plasticizers, and waterproofing materials (Roberts et al., 1978; Eisler, 1986b). However, their predominant use has been as insulating and cooling agents in closed electrical transformers and capacitors because of their low flammability.

PCB residues have been identified throughout the global ecosystem in rivers and lakes, the atmosphere, and fish, birds, and mammals as well as in human adipose tissue, blood, and breast milk (Roberts et al., 1978; Kimbrough, 1980; Safe, 1984; Eisler, 1986b). Such widespread ecosystem contamination has resulted from industrial discharges, leaks, disposal into municipal sewage and landfills, and incomplete incineration in the atmosphere. Between 1930 and 1975, over 630 million kilograms of PCBs were manufactured in the United States (Safe, 1984). As of 1979, a ban on the manufacture of PCBs in the U.S. was implemented.

Prior commercial production of PCBs consisted of chlorination of biphenyls, resulting in complex mixtures of chlorobiphenyls containing a total of 209 theoretically possible isomers but with some less likely to occur than others (Safe, 1984). Ten possible degrees of chlorination resulted in 10 PCB homolog groups, mono- through decachlorobiphenyl, with various positional isomers (congeners) within each group (Figure 1). Congeners have been assigned identification numbers according to the International Union of Pure and Applied Chemistry (IUPAC).

Commercial PCB formulations were sold under a variety of trade names; e.g., in the U.S., Aroclor was the most common. Aroclors are PCB mixtures that were named according to their chlorine content. For example, Aroclor 1254 contains 54% chlorine by weight, and Aroclor 1260 contains 60%.

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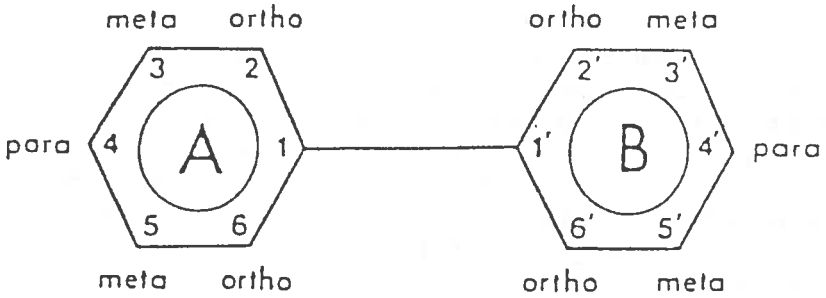


**Figure 1** Generalized structure of biphenyl and possible positions for chlorines (ortho, meta, para) on polychlorinated biphenyl (PCB) congeners.

Aroclors vary in their toxicities according to a number of factors including congener composition and chlorine content. PCB congeners with the chlorine atom in positions 2 and 6 (ortho) are generally more readily metabolized, while those with chlorines in positions 4 and 4′ (para) or positions 3,4 or 3,4,5 on one or both rings tend to be more toxic and are retained in tissues (Eisler, 1986b). Structures of some of the more active PCB congeners are summarized later in this chapter in Table 4.

Polychlorinated dibenzodioxins (PCDDs), unlike PCBs, have not been purposely manufactured, but rather are present as trace impurities associated with chlorophenols and production of herbicides, such as 2,4,5-trichlorophenoxyacetic acid. PCDDs can also be formed by photochemical and thermal reactions during and after incineration, leading to their presence in fly ash and other products of combustion (Eisler, 1986b). PCDDs, like PCBs, are dispersed throughout the global ecosystem, are chemically stable, and bioaccumulate in animal tissues. The number of chlorine atoms in PCDDs can vary from one to eight per molecule, resulting in 75 positional isomers, with the most toxic and widely studied one being 2,3,7,8-tetra-CDD (2,3,7,8-TCDD) (Kimbrough, 1980; Eisler, 1986b) (see Figure 2).

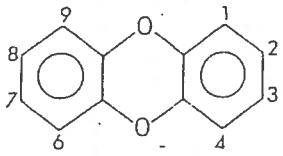


**Figure 2** Generalized structure of dibenzo-*p*-dioxin and possible positions for chlorines.

PCBs and PCDDs may be quite biologically toxic, eliciting a number of common responses including but not limited to thymic atrophy (a wasting syndrome), immunotoxic effects, reproductive impairments, porphyria, and related liver damage (Kimbrough, 1980; Safe, 1984, 1990; Safe et al., 1985). The most toxic PCB congeners, including 3,3′,4,4′,5-penta-CB (PCB 126; IUPAC No. 126), 3,3′,4,4′-tetra-CB (PCB 77), and 3,3′,4,4′,5,5′-hexa-CB (PCB 169), can assume a relatively coplanar conformation generally similar to that of 2,3,7,8-TCDD and are approximate stereo analogs of this compound. Both PCBs and PCDDs are environmentally persistent,

resisting bacterial and chemical breakdown, but are readily absorbed from water into the fats of plankton, thereby entering the aquatic food chain. This process continues as fish, and then piscivorous birds including gulls, cormorants, herons, and terns, accumulate progressively higher concentrations (biomagnification) of these compounds as they become deposited in the fat of the body while natural portions of food items are metabolized for energy or excreted. At the top of the aquatic food chain are bald eagles *(Haliaeetus leucocephalus)* and other raptors that consume gulls and other fish-eating birds as well as fish.

In this review, we have mainly incorporated the findings of interpretive toxicological studies that report PCB or dioxin concentrations in avian tissues and eggs as determined in pen and laboratory studies as well as by field observations. All residues in tissues and eggs are reported in terms of the concentration per wet weight unless specified otherwise. Residues in food are reported as either wet weight or dry weight in the text.

## PCB PEN AND LABORATORY STUDIES

### LETHALITY AND HISTOPATHOLOGY

Adult and juvenile birds of precocial species exhibit varying sensitivities to ingestion of aroclors and other PCB mixtures. In 5-day feeding trials with Aroclor 1254, median lethal concentrations ($LC_{50}$s) in the diet were 604 ppm (dry weight) for northern bobwhite *(Colinus virginianus)*, 1091 ppm for ring-necked pheasants *(Phasianus colchicus)*, 2697 ppm for mallards *(Anas platyrhynchos)*, and 2895 ppm for Japanese quail *(Coturnix japonica)* (Heath et al., 1972). Dahlgren et al. (1972a) administered capsules daily containing 10, 20, or 210 mg of Aroclor 1254 to 11-week-old hen pheasants until death or sacrifice (Table 1). Birds were killed by PCB ingestion in a dose-dependent manner, with one bird dying in less than 2 days after receiving 210 mg and one bird on 10 mg daily surviving for 8 months. The heaviest birds survived the longest. Birds that died from ingesting 210 mg daily had total PCB brain residues ranging from 320 to 770 ppm (520 ± 110, mean ± SD) wet weight. Liver residues were much more variable and ranged from 390 to 9300 ppm (2500 ± 2000), and muscle levels ranged from 51 to 290 ppm (140 ± 53). Treated birds receiving 210 mg daily that were sacrificed at the same time others were dying on this dose had residue levels in tissues overlapping those of birds that died; however, the least overlap occurred in brain tissue, where the levels in sacrificed birds were 370 ± 65 ppm and were 1900 ± 1300 ppm for liver and 83 ± 17 ppm for muscle. The authors concluded that a brain residue level of 300 to 400 ppm was indicative of death due to PCB toxicosis. Pheasants that died had consistently smaller hearts and very small shrunken spleens due to lymphocyte depletion. Weights of kidney and liver proportional to body weight increased with 10- and 20-mg doses but not with 210-mg doses. Hydropericardium and abdominal and subcutaneous edema were not apparent in this species. Brain residue was the only tissue residue that was independent of a number of other parameters when correlations were made;

**Table 1 Effects on Survival, Histopathology and Growth of Aroclors and Other Polychlorinated Biphenyl (PCB) Mixtures**

| Species, age | Concentration in tissue (ppm wet weight) | Effect | Experimental treatment | Ref. |
|---|---|---|---|---|
| Pheasants, 11-week-old hens | 300 to 400 in brain | Death | Aroclor 1254 daily in capsules of up to 210 mg | Dahlgren et al. (1972a) |
| Chickens, cockerels (day-old at start) | 270 to 420 in brain | Death | Aroclor 1260 in diet, up to 600 ppm | Vos and Koeman (1970) |
| Chickens, cockerels (day-old at start) | 120 in brain, 240 in liver, 410 in kidney, 85 in muscle | Death with severe edema and lesions | Aroclor 1254 in diet, 500 ppm | Platonow et al. (1973) |
| Chickens, growing chicks | 20 in adipose tissue | Death in 30% and decreased growth | Aroclor 1254 in diet, 20 ppm | Bird et al. (1978) |
| Chickens, growing chicks | 325 in fat<br>100 in fat<br>21 in fat | Decreased growth<br>Lower hematocrit<br>Lower hemoglobin | Aroclor 1248 in diet, up to 40 ppm | Rehfeld et al. (1972) |
| Japanese quail | 478 in liver | Weight loss and porphyria | Aroclor 1260 oral dose at 100 mg/kg | Vos et al. (1971) |
| Great cormorants, herons | 76-180 in brain, herons with 420-445 in brain | Death | Clophen A60, dosed | Koeman et al. (1973) |
| Common murres, *(Uria aalge)* | >25 in brain | Decreased pituitary and thyroid weights | Aroclor 1254, orally dosed up to 400 mg/kg/day | Jefferies and Parslow (1976) |
| Bengalese finches, adult | 290 in brain, 345 in liver | Death with enlarged kidneys, hydropericardium | Aroclor 1254 in diet, up to 440 ppm | Prestt et al. (1970) |
| Passerine species, (common grackles, red-winged black birds, brown-headed cowbirds, and starlings) | 310 in brain | Diagnostic of death; liver hemorrhagic | Aroclor 1254 in diet at 1,500 ppm | Stickel et al. (1984) |

these parameters included initial weight, the percentage of weight loss, days to death, and the lipid content in brain, liver, and muscle. Therefore, brain residue is considered a desirable tissue residue for diagnostic purposes. Further studies by these authors (Dahlgren et al., 1972b) revealed that periodic food deprivation increased brain residues more rapidly, leading to more rapid death.

More interpretive PCB studies have been conducted with chickens *(Gallus gallus)* than with any other single avian species. Young cockerels (day-old at start) that died on a dosage of 600 ppm (dry diet) of Aroclor 1260 had 270 to 420 ppm of total PCBs in the brain (Vos and Koeman, 1970). Dosage with other commercial PCB mixtures that also contained 60% chlorine resulted in more variable residues. Residues in the brains of five birds that died on Phenoclor DP6 dosage ranged from

70 to 700 ppm and, in four birds that died on Clophen A60 dosage, from 120 to 380 ppm. Liver residues varied more, ranging from 120 to 2900 ppm among 28 birds that died. These two commercial mixtures were more toxic than Aroclor 1260 and produced greater pathological signs. However, both of these mixtures proved to be contaminated with chlorinated dibenzofurans (Vos et al., 1971). In another study, day-old cockerels fed 500 ppm of Aroclor 1254 died with brain residues of about 80 to 190 ppm (Platonow et al., 1973). Tissue concentrations of PCB increased with the duration of exposure. The PCB ratios between liver, kidney, or muscle and brain were constant throughout the study with the duration of exposure. Half of the birds were dead by 43 days with brain residues averaging about 120 ppm; liver, 240 ppm; kidney, 410 ppm; and muscle, 85 ppm. Ratios of the total PCB concentrations in muscle, liver, and kidney to those in brain were 0.7, 2.0, and 3.5, respectively. Pathological examination of these chickens revealed severe edema and lesions, including hydropericardium and subcutaneous edema as well as edema of the liver and muscles. Hemorrhages, myocarditis, and kidney and liver necrosis were apparent.

Studies conducted with altricial species of birds have included observations with cormorants, herons, finches, and blackbirds. Great cormorants *(Phalacrocorax carbo)* dosed experimentally with Clophen A60 died with total PCB brain residues of 76 to 180 ppm (mean, 130 ppm), whereas herons that died on Clophen dosage contained 420 to 445 ppm, suggesting that cormorants were more sensitive than were herons (Koeman et al., 1973); these authors concluded that the survival time in cormorants was related to the capacity of the birds to store the PCBs in adipose and other tissues apart from the brain, indicating that the total body content is not a good criterion for diagnosis of PCB poisoning. Prestt et al. (1970) fed Aroclor 1254 to adult Bengalese finches, a domesticated form of the sharp-tailed finch *(Lonchura striata)*, at dietary levels ranging from 6 to 440 ppm for 8 weeks. The estimated dose rate for 50% mortality at 56 days was 254 mg/kg/day. The mean liver content was 345 ppm (range, 70 to 697 ppm). All birds dying from PCB ingestion had enlarged kidneys, with several birds also showing hydropericardium. Leg paralysis as well as trembling was apparent. In birds dying from PCB exposure, the ratio of total PCB concentration in the liver to that in the brain was 1.2 ± 0.1 (SD), which was 3 times as high as in those birds on similar diets not dying but sacrificed.

Using Aroclor 1254, Stickel et al. (1984) conducted one of the most comprehensive studies designed to establish lethal brain residues of total PCBs in passerine species. These species included immature male common grackles *(Quiscalus quiscula)*, immature female red-winged blackbirds *(Agelaius phoeniceus)*, adult male brown-headed cowbirds *(Molothrus ater)*, and immature female starlings *(Sturnus vulgaris)*. Aroclor 1254 was selected because chromatographic patterns of PCBs in wild birds were found to resemble those of this mixture closely. Dietary concentrations of 1500 ppm (dry weight) were administered until one half of the birds had died. The 50% mortality point for starlings was reached in 4 days, red-winged blackbirds in 6 days, cowbirds in 7 days, and grackles in 8 days. Signs of PCB poisoning began with birds becoming inactive with tremors. At necropsy, the liver frequently had hemorrhagic areas, and the gastrointestinal tract often had blackish

fluid. PCB residues in the brains of birds that died were distinct from those in sacrificed survivors, providing suitable diagnostic criteria. PCB residues varied from 349 to 763 ppm of wet weight in the brains of dead birds and from 54 to 301 ppm in sacrificed birds. The authors considered an approximate level of 310 ppm (3 SDs below the mean) to be diagnostic for a high probability of PCB-induced mortality. PCBs in the brains of dead birds for the three icterine species did not differ significantly from each other (combined mean, 579 ppm), but the average residue levels in starlings (mean, 439 ppm) were significantly lower than those in red-winged blackbirds and grackles. The concentrations in whole bodies and livers were not diagnostic when expressed on a wet-weight basis.

One must be somewhat cautious in utilizing laboratory Aroclor and other commercial PCB mixture feeding studies as absolute predictors of potential avian toxicity in the field, because pattern recognition techniques during analysis of total PCB concentrations and congener patterns suggest that Aroclor mixtures change substantially in the environment and through the food chain (Schwartz and Stalling, 1991).

## REPRODUCTIVE EFFECTS

Reproductive impairment has been reported in at least five species of birds that were given experimental doses of PCBs, including chickens, ringed turtledoves *(Streptopelia risoria)*, Japanese quail, mourning doves *(Zenaida macroura)*, and ring-necked pheasants (Table 2). In one study, laying hens were fed 0, 5, or 50 ppm of Aroclor 1254 for up to 39 weeks (Platonow and Reinhart, 1973). With 5 ppm (10% moisture) in the diet, egg production but not hatchability was reduced; however, after 14 weeks, fertility was lower. The hatchability of fertile eggs was not affected when the total PCB concentration in eggs was below 5 ppm. However, when the concentration exceeded 15 ppm in eggs, embryonic mortality was high. In contrast, Cecil et al. (1972) reported that dietary levels of 20 ppm of dry weight for Aroclor 1254 did not affect the hatching success of chickens at the end of 5 weeks with egg residues of 13.2 ppm. Tumasonis et al. (1973) examined the effect of exposing white leghorn hens for 6 weeks to 50 ppm of Aroclor 1254 in drinking water. Egg weight and fertility were not affected but, as total PCB concentrations increased in the yolk, embryonic development was arrested at progressively earlier stages. Within 2 weeks, hatching success dropped to 34%, and the authors concluded that yolk concentrations greater than 10 to 15 ppm (whole egg concentrations above 4 ppm) were required to affect hatching success. Short bowed legs, clenched toes, and neck deformities were present in some of the chicks that were hatched where yolk PCB levels were 10 to 15 ppm. Hemorrhaging and abnormal livers were apparent. In another study, white leghorn hens received diets containing 0 to 80 ppm of Aroclor 1242 for 6 weeks (Britton and Huston, 1973). Egg production, egg weight, shell thickness, and shell weight were not affected, but hatchability was affected within 2 weeks for hens fed as little as 20 ppm of dry weight. Yolks contained 2.4 ppm (expected whole egg concentration, 0.87 ppm). This conversion is based on the chicken yolk being 0.364 of the whole egg content mass (Sotherland and Rahn, 1987). Even 10 ppm of dry weight in the diet caused a small reduction in hatching at the end of 6 weeks (yolk concentration of 3.7 ppm or expected whole egg concentration of 1.3 ppm). Scott

Table 2 Reproductive Effects of Aroclors and Other Polychlorinated Biphenyl (PCB) Mixtures

| Species, age | Concentration in tissue (ppm wet weight) | Effect | Experimental treatment | Ref. |
|---|---|---|---|---|
| Chickens, laying hens | 5 in eggs | Hatching reduced | Aroclor 1254 in diet, up to 50 ppm | Platonow and Reinhart (1973) |
| Chickens, laying hens | 13.2 in eggs | Did not affect hatching | Aroclor 1254 in diet, 20 ppm | Cecil et al. (1972) |
| Chickens, white leghorn hens | Above 4 in eggs | Embryo mortality and teratogenic | Aroclor 1254 in drinking water at 50 ppm | Tumasonis et al. (1973) |
| Chickens, white leghorn hens | Less than 1 in eggs | Decreased hatching | Aroclor 1242 diet, up to 80 ppm | Britton and Huston (1973) |
| Chickens, fertile white leghorn eggs | 10 in eggs | Embryonic mortality of 64% | Aroclor 1242 injected into air cell of eggs | Blazak and Marcum (1975) |
| Chickens, laying hens | 23 in eggs | Decreased hatching | Aroclor 1248 in diet at 10 ppm | Scott (1977) |
| Chickens, fertile white leghorn eggs | 5 in eggs | Hatching reduced to 17% | Aroclor 1248 injected into yolk sac | Brunstrom and Orberg (1982) |
| Chickens, fertile eggs | 0.05 to 0.1 in eggs | Decreased gluconeogenic enzyme activity | Aroclor 1254 injected into air cell | Srebocan et al. (1977) |
| Ringed-turtle doves | 16 in eggs, 5.5 in adult brain | Embryonic mortality; decreased parental attentiveness | Aroclor 1254 in diet | Peakall and Peakall (1973) |
| Ringed-turtle doves | 2.8 in brain | Depletion of brain dopamine and norepinephrine | Aroclor 1254 in diet | Heinz et al. (1980) |
| Mallard hens | 23 in eggs, 30 in 3-week-old ducklings and 55 in hens | No effects | Aroclor 1254 in diet at 25 ppm | Custer and Heinz (1980) |
| Mallard hens | 105 in eggs | Eggshell thickness decreased; hatching success not affected | Aroclor 1242 in diet | Haseltine and Prouty (1980) |
| Screech owls | 4 to 18 in eggs | No effects | Aroclor 1248 in diet at 3 ppm wet weight | McLane and Hughes (1980) |
| Atlantic puffins | 10 to 81 in eggs, 6 in adults | No effects detected | Aroclor 1254 dosed by implantation of 30-35 mg | Harris and Osborn (1981) |

(1977) reported that hatchability was decreased after 4 weeks of Aroclor 1248 at 10 ppm of dry weight in the diet, with egg residues of 22.7 ppm.

Peakall and Peakall (1973) studied the effects of chronic dietary exposure of 10 ppm (dry diet) of Aroclor 1254 on the reproduction of ringed turtledoves. Embryonic mortality was greatly increased when the eggs were incubated by the parents

but decreased by artificial incubation. Monitoring of egg temperatures suggested that mortality was increased because of decreased parental attentiveness. The mean PCB residues in dove eggs were 16 ppm (wet weight), with mean levels in adult tissues of 736 ppm in fat, 15 ppm in the liver, 8 ppm in muscle, and 5.5 ppm in the brain. An increase in chromosomal aberrations was apparent in 3- to 6-day-old embryos. Further studies with Aroclor 1254 in this species revealed depletions of brain dopamine and norepinephrine that were negatively correlated with brain residues (Heinz et al., 1980). Brain residues of 2.82 ± 0.29 (SE) ppm of wet weight resulted in significant depletions to levels known to cause behavioral impairments, thus supporting the above findings of Peakall and Peakall (1973).

Other species appear to be less reproductively sensitive to PCB exposure. Neither Aroclor 1254 nor Aroclor 1242 affected the reproductive success of mallards. Aroclor 1254 at 25 ppm of dry weight in the diet was fed to 9-month-old mallard hens for at least a month prior to egg laying with no detrimental effect on reproduction or nest attentiveness (Custer and Heinz, 1980). Hatching of ducklings and survival to 3 weeks were unaffected; the mean total PCB residues in eggs were 23.3 ppm (SE, 1.0 ppm); in 3-week-old ducklings, 29.5 ppm (SE, 1.4 ppm); and in hens, 55.3 ppm (SE, 1.9 ppm). Haseltine and Prouty (1980) fed Aroclor 1242 to mallards for 12 weeks at 150 ppm (dry diet) and did not find any differences in hatching success or nest attentiveness, but they found that eggshell thickness decreased by 8.9%; eggs contained an average of 105 ppm of total PCBs (wet weight). Aroclor 1248 fed to screech owls *(Otus asio)* at 3 ppm (wet weight) failed to affect reproduction with egg residues of 3.9 to 17.8 ppm (McLane and Hughes, 1980). When 108 Atlantic puffins *(Fratercula arctica)* were dosed by implantation under the skin along the ribs with 30 to 35 mg of Aroclor 1254, the PCB concentration in fat rapidly increased 10- to 14-fold, remaining there for 4 to 10 months. Survival and breeding performance were not impaired; the egg concentrations of total PCBs of dosed females ranged from 9.6 to 81.3 ppm compared with a mean concentration of 8.4 ppm (SE, 2.6 ppm) for controls exposed to PCBs from the natural environment (Harris and Osborn, 1981). The mean body burdens of dosed adults were 5.99 ppm (SE, 0.93 ppm) and of controls, 0.58 ppm (SE, 0.08 ppm).

## GROWTH AND OTHER SUBLETHAL EFFECTS

Other studies have focused on the sublethal effects, including thyroid and pituitary changes and porphyria. In one study, common murres *(Uria aalge)*, referred to as "guillemots" by the authors, were reared in the laboratory and fed daily doses of Aroclor 1254 for 45 days at dose rates from 12 to 400 mg/kg/day (Jefferies and Parslow, 1976). Brain residues of above 25 ppm were accompanied by dose-related decreases in thyroid weight, follicle size, and colloid area as well as decreased pituitary weight. The authors concluded from these findings that *U. aalge* specimens are at least twice as sensitive to PCBs as are lesser black-backed gulls *(Larus fuscus)*, as judged by their previous findings (Jefferies and Parslow, 1972).

Feeding studies were conducted with Aroclor 1260 in Japanese quail (Vos et al., 1971) and with PCB congeners 2,2',4,4',5,5'-hexa-CB (PCB 153), 2,3,3',4,4'-penta-CB

(PCB 105), and 3,3',4,4',5-penta-CB (PCB 126) to assess porphyria in Japanese quail and American kestrels (Elliott et al., 1990, 1991). Aroclor 1260 at a dose rate of 100 mg/kg of body weight per day for 7 days in Japanese quail resulted in a mean liver residue of 478 ppm accompanied by weight loss, porphyria, and an increase of nearly 20-fold in hepatic mitochondrial δ-aminolevulinic acid (ALA) synthetase activity (Vos et al., 1971). However, as low a dose rate as 1 mg/kg/day increased ALA synthetase activity and resulted in a mean liver residue of 1.4 ppm. The mean liver residue levels (± SD) for PCB 105 of 2.6 ± 1.7 ppm and for PCB 126 of only 0.091 ± 0.08 ppm were associated with porphyria after 2 weeks in Japanese quail (Elliott et al., 1990). PCB 153 at 52.6 ppm in the liver had minimal effects. PCB 126 caused a decrease in thymus weight. All three congeners induced mixed-function oxygenase (MFO) activity as detected by the 7-ethoxyresorufin-*O*-deethylase (EROD) assay. In American kestrels, residue levels in pooled adipose tissue of 182 ppm for PCB 105, 119 ppm for PCB 153, and 3.3 ppm for PCB 126 were not associated with porphyria, but hepatic MFO activities were induced (Elliott et al., 1991).

Aroclor 1254 was more toxic than was Aroclor 1248 and affected growth more readily in chicks. Feeding 10 and 20 ppm of Aroclor 1254 (dry weight) to growing chicks resulted in decreased growth accompanied by 10 and 30% mortality, respectively, by 8 weeks of age, with approximate adipose tissue residues of 10 to 20 ppm (Bird et al., 1978). Feeding 40 ppm of Aroclor 1248 (dry weight) decreased the growth of chicks and resulted in 325 ppm (wet weight) in adipose tissue fat (Rehfeld et al., 1972). Feeding 20 ppm of dry weight decreased the hemoglobin concentration, and 30 ppm decreased hematocrit, with residues of 21 ppm and 100 ppm in the fat, respectively.

Other studies have examined the effects of specific PCB congeners on growth. The effects of feeding 400 ppm (dry weight) of five hexachlorobiphenyl congeners, including 2,2',4,4',6,6'-hexa-CB (PCB 155), 2,2',3,3',6,6'-hexa-CB (PCB 136), 2,2',4,4',5,5'-hexa-CB (PCB 153), 2,2',3,3',4,4'-hexa-CB (PCB 128), and 3,3',4,4',5,5'-hexa-CB (PCB 169), were examined in growing chicks for 21 days (McKinney et al., 1976). PCB 169 was the most toxic and caused complete mortality, general edema, marked thymic involution, and the highest accumulation (mean, 203 ppm) of all congeners in the liver. Growth was reduced by all congeners except PCB 155. The liver/body weight ratio was increased by all congeners, with the largest increase produced by PCB 155 and the smallest by PCB 136, with mean liver residues of 24 and 9 ppm, respectively. Pathological changes in the liver, including necrosis and fatty infiltration, were marked for PCB 155 and moderate for PCB 128.

Another study examined the effects of PCB congeners 126, 77, and 105 on the growth and development of American kestrel nestlings by daily dosing over the first 10 days posthatching (Hoffman et al., 1993a). Dosing with PCB 126 in the amount of 50 ng/g of body weight per day resulted in a geometric mean liver concentration of 156 ppb (range, 68 to 563 ppb) (wet weight), with pronounced liver enlargement and some mild coagulative necrosis of the liver, some colloid depletion of the thyroid, and lymphoid depletion of the spleen. Other effects at this dose level included a marginal decrease in body and bone lengths. Increasing the dose to 250 ng/g resulted

in a mean liver concentration of 380 ppb (218 to 666 ppb); intensification of the above effects was seen, as well as decreased spleen weight and further lymphoid depletion of the spleen and bursa. Dosing at 1000 ng/g resulted in a mean liver concentration of 1098 ppb (652 to 4478 ppb), with decreased bursa weight and body weight in addition to the above effects. Higher doses and resulting liver concentrations of congeners 77 and 105 were required to produce any of the above effects; dosing with 1000 ng/g of PCB 77 resulted in a liver concentration of 892 ppb and the onset of coagulative liver necrosis, whereas dosing with 4000 ng of PCB 105 per gram resulted in a liver concentration of 1677 ppb, with liver necrosis and mild depletion of thyroid colloid.

## EGG INJECTION STUDIES

The utility of egg injection studies for predicting potential embryotoxicity of PCBs and tetrachlorodibenzodioxin (TCDD) compares favorably with that of feeding studies. In instances where the same chemicals have been administered by both methods, the egg concentrations and effects are quite similar.

Several egg injection studies have been conducted with Aroclor mixtures, all having used the chicken egg (Table 2). Blazak and Marcum (1975) injected Aroclor 1242 into the air cell of fertile white leghorn eggs and then incubated them for a brief observation period of 4 to 5 days. Both 10 and 20 μg/g of egg (ppm) caused 64 to 67% embryonic mortality but did not result in chromosomal breakage, as had been reported in ringed turtledoves by Peakall et al. (1972). Srebocan et al. (1977) injected Aroclor 1254 (doses of 0, 0.05, 0.1, 0.5, and 5 μg/g of egg; ppm) into the air cell of chicken eggs on day 0 and incubated the eggs until day 14. The effects on survival were not reported, but decreased activity in key gluconeogenic enzymes was found to occur, starting at the lowest dose. Brunstrom and Orberg (1982) injected Aroclor 1248 into the yolk sacs of white leghorn eggs after 4 days of incubation to attain egg concentrations of 0, 1, 5, and 25 ppm. Hatchability was 96, 92, 17, and 0%, respectively, with mortality occurring the earliest in the 25-ppm group (days 6 to 12).

Considerably more egg injection studies have been conducted with specific PCB congeners, studying the effects in chickens and other species (Table 3). Rifkind et al. (1985) injected chicken eggs (unspecified location of injection) at 10 days of incubation with 5 to 1000 nmol per egg for each of three PCB congeners, including 3,3′,4,4′-tetra-CB (PCB 77), PCB 169, and PCB 136. PCB 77 caused dose-related decreases in survival from days 10 to 19 of exposure at 100 to 1000 nmol per egg (584 to 5840 ppb, assuming a 50-g constant mass of egg), as did PCB 169 at 500 to 1000 nmol per egg (3610 to 7220 ppb). These decreases in survival were accompanied by decreased thymus weight and increased pericardial and subcutaneous edema in surviving embryos. These authors reported that the dose-response relations for lethality and for hepatic MFO induction, including both aryl hydrocarbon hydroxylase (AHH) and EROD activities, were dissociated and that the maximal induction levels were not correlated with the extent of lethality. Another study by these authors revealed that hepatocyte swelling, the major histopathological change, was apparent

**Table 3 Egg Injection and Other Laboratory Studies with Planar Polychlorinated Biphenyls (PCBs) and Dioxin**

| Species, age | Compound, concentration | Effect | Ref. |
|---|---|---|---|
| Chicken, white leghorn embryo | 2,3,7,8-TCDD[a] | | |
| | 10 ppt | 2-fold AHH induction | Poland and Glover (1973) |
| | 10-20 ppt | Onset of embryotoxicity | Verrett (1970) |
| | 40-50 ppt | Mortality, edema, hemorrhaging over surface | Verrett (1976) |
| | 63 ppt | $ED_{50}$ for AHH induction | Poland and Glover (1973) |
| | 147 ppt | $LD_{50}$ (air cell injection) | Verrett, 1976 |
| | 115 ppt | $LD_{50}$ (yolk sac injection) | Henshel (1993) |
| | 180 ppt | $LD_{50}$ (air cell injection) | Henshel (1993) |
| | 240 ppt | $LD_{50}$ (air cell injection) | Allred and Strange (1977) |
| | 302 ppt | $ED_{50}$ for AHH induction | Sawyer et al. (1986) |
| | 1 ppb | 100% mortality | Higginbotham et al. (1968) |
| | 3,3′,4,4′5-PeCB (PCB 126) | | |
| | 0.4 ppb | $LD_{50}$ (air cell injection), day 4 through hatching | Hoffman et al. (1995) |
| | 3.1 ppb | $LD_{50}$ (air cell injection), day 7 through day 10 | Brunstrom and Andersson (1988) |
| | 3,3′4,4′-TeCB (PCB 77) | | |
| | 2.6 ppb | $LD_{50}$ (air cell injection) | Hoffman et al. (1995) |
| | 8.6 ppb | $LD_{50}$ (air cell injection) | Brunstrom and Andersson (1988) |
| | 40 ppb | $LD_{50}$ (air cell injection) | Vos et al. (1982) |
| | 2,3,3′4,4′-PeCB (PCB 105) | | |
| | 2,200 ppb | $LD_{50}$ (air cell injection) | Brunstrom (1990) |
| | 2,3,3′4,4′5-HxCB (PCB 157) | | |
| | 2,000 ppb | $LD_{50}$ (air cell injection) | Vos et al. (1982) |
| | 1,500 ppb | $LD_{50}$ (air cell injection) | Brunstrom (1990) |
| Pheasant, embryo | 2,3,7,8-TCDD | | |
| | 1.4 ppb | $LD_{50}$ (albumin) | Nosek et al. (1993) |
| | 2.2 ppb | $LD_{50}$ (yolk) | |
| Bobwhite, embryo | 3,3′,4,4′,5′-PeCB (PCB 126) | | |
| | 24 ppb | $LD_{50}$ (air cell injection), through hatching | Hoffman et al. (1995) |
| Common tern, embryo | 3,3′,4,4′,5′-PeCB (PCB 126) | 35% embryo mortality (air cell injection), through hatching | Hoffman et al. (1995) |
| | 45 ppb | | |
| American kestrel, embryo | 3,3′,4,4′,5′-PeCB (PCB 126) | $LD_{50}$ (air cell injection), through hatching | Hoffman et al. (1995) |
| | 65 ppb | | |
| American kestrel, nestling | 3,3′,4,4′,5′-PeCB (PCB 126) | Histopathology of liver, thyroid, and spleen | Hoffman et al. (1995) |
| | 156 ppb (68-563) in liver | | |

Table 3 (continued) Egg Injection and Other Laboratory Studies with Planar Polychlorinated Biphenyls (PCBs) and Dioxin

| Species, age | Compound, concentration | Effect | Ref. |
|---|---|---|---|
| Mallard, embryo; goldeneye, embryo | 3,3'4,4'-TeCB (PCB 77) 5,000 ppb | No effects (air cell injection) | Brunstrom (1988) |

[a] 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzodioxin; AHH, aryl hydrocarbon hydroxylase; $ED_{50}$, 50% effective dose; $LD_{50}$, 50% lethal dose; 3,3',4,4',5-PeCB, 3,3',4,4',5-pentachlorobiphenyl; 3,3',4,4'-TeCB, 3,3',4,4'-tetrachlorobiphenyl; 2,3,3',4,4'-PeCB, pentachlorobiphenyl; 2,3,3',4,4',5-HxCB, hexachlorobiphenyl.

within 24 hours after doses as low as 5 nmol per egg (29 ppb) for PCB 77 and PCB 169 but only at doses of 5,000 nmol per egg (36,100 ppb) and higher for 2,4,5,2',4',5'-hexa-CB (PCB 153), whereas PCB 136 was inactive (Rifkind et al., 1984). The same relation as for hepatocyte swelling held for induction of MFO (AHH and EROD assays).

Comparison of the toxicity of PCB congeners injected into the yolk sac of chicken eggs at an earlier stage of development (4 days of incubation) revealed much greater toxicity, where PCB 126 was the most toxic and also the most potent inducer of MFO (EROD) in chick embryo liver (Brunstrom, 1989). The MFO (EROD)-inducing potencies correlated well with embryolethality. With air cell injections on day 7 of incubation, 50% lethal doses ($LD_{50}$s) (72 hr later) for PCB congeners 126, 77, 169, and 105 were 3.1, 8.6, 170, and 2200 ppb or 9.4, 29, 480, and 6700 nmol/kg, respectively (Brunstrom and Andersson, 1988; Brunstrom, 1990; Brunstrom et al., 1990). When injections were administered at the earlier stage of incubation (day 4) via the yolk sac and eggs were incubated until day 18, the above congeners caused higher embryonic mortality; the congener PCB 126 was approximately fivefold more toxic than was PCB 77, and analogs of PCB 77 (chlorinated at one ortho position) were three to four orders of magnitude less toxic. Hoffman et al. (1995) examined the effects of PCB congeners 126, 77, 105, and 153 in chickens, bobwhite, American kestrels, and common terns through hatching following air cell injections on day 4. The $LD_{50}$s for these congeners were approximately 0.4 ppb, 2.6 ppb, 3326 ppb, and greater than 14,000 ppb, respectively, in chickens; low-effect levels (10 to 20% embryonic mortality) were 0.2, 2000, and 14,000 ppb, respectively. The difference between these results, especially for PCB 126, and those of Brunstrom and Andersson (1988) is probably due to two important factors: (1) Hoffman et al. (1995) used day 4 of incubation, an earlier and more sensitive stage of embryonic development; and (2) permitted eggs to hatch, also a critical stage for survival; whereas Brunstrom and Andersson (1988) used day 7 embryos for dosing and recorded $LD_{50}$s 72 hours later. Indeed, when Brunstrom and Danerud (1983) injected PCB 77 into the yolk sac of chicken eggs at 4 days of incubation and permitted the eggs to hatch, they reported that 4 ppb decreased hatching success by 40%, in very close agreement with the findings of Hoffman (1994).

Non-ortho-chlorinated (coplanar) congeners were more potent inhibitors than were mono-ortho-chlorinated congeners of lymphoid development in the embryonic bursa (Brunstrom et al., 1990). Andersson et al. (1991) compared the numbers of lymphoid cells in the thymus and in the bursa after chicken eggs were treated with coplanar PCB congeners by air cell injection on day 13; here, the values for 50% of maximum inhibition ($ED_{50}$) of bursal development were 4 μg/kg for PCB 126, 50 for PCB 77, and 300 for PCB 169. The most immunotoxic of the mono-ortho-chlorinated analogs of PCB 77 and PCB 126 were about 1000 times less potent than was PCB 126.

Comparative avian egg injection studies by Brunstrom and co-workers have shown that chickens are more sensitive than turkeys *(Meleagris gallopavo)*, pheasants, ducks (mallards and goldeneyes; *Bucephala clangula*), domestic geese *(Anser anser)*, herring gulls, and black-headed gulls *(Larus ridibundus)*, at a dose rate of 20 ppb for PCB 77 mortality in chicken embryos of 70 to 100% that occurred by 18 days of incubation with malformations. Yet, 5000 ppb to ducks and 1000 ppb to geese and herring gulls had no effects (Brunstrom, 1988). However, gallinaceous birds were more sensitive, where 1000 ppb in pheasant eggs resulted in complete mortality (Brunstrom and Reutergardh, 1986). In turkeys, 200 to 1000 ppb caused 17 to 60% mortality (Brunstrom and Lund, 1988). Other species studied have included bobwhite and American kestrels by Hoffman et al. (1995). The $LD_{50}$ for PCB 126 was approximately 0.4 ppb in chickens, whereas the $LD_{50}$ for this congener was 24 ppb for bobwhite and 65 ppb for American kestrels. Forty-five ppb caused a decrease of 35% in hatching success for common terns.

## USE OF TOXIC EQUIVALENCY FACTORS FOR "TCDD EQUIVALENTS" IN QUANTIFYING PCB AND DIOXIN TOXICITY

Toxic equivalency factors (TEFs) express the relative potency of dioxin-like compounds including coplanar PCBs relative to 2,3,7,8-TCDD. Related compounds, acting through the same "mode of action," should produce the same effects as actual TCDD but at different concentrations to account for potency differences. The toxicity of commercial PCB mixtures has been associated with the presence of certain PCB congeners having four or more chlorine atoms in both the para and metapositions of the biphenyl rings but no chlorine atoms in the orthoposition (hence, "non-ortho-PCBs"); these congeners are thought to adopt a more coplanar structure than their ortho-substituted analogs, thus attaining more frequent isostereomerism with the highly toxic 2,3,7,8-TCDD. Biological responses are similar between coplanar PCBs (such as 77, 126, and 169) and 2,3,7,8-TCDD; these responses include edema, weight loss, hepatic and thymic changes, embryotoxicity, teratogenicity, and immunotoxicity (Tanabe, 1989). All of these isosteres have high binding affinity to hepatic cytosolic receptor protein (Ah receptor) and can thereby readily induce hepatic microsomal MFO enzymes including AHH and EROD. Collectively, these chemicals

are referred to as "planar halogenated hydrocarbons" (PHHs) including the proximate isostereomers for PCBs, PCDDs, and polychlorinated dibenzofurans (PCDFs), as well as certain others.

Because of the generally accepted common mode of action for PHHs, biological potencies have been theoretically calculated for complex mixtures by expressing the potency of individual congeners relative to the most toxic PHH (2,3,7,8-TCDD) (Bradlaw and Casterline, 1979; Safe, 1987) and then summing them. Therefore, one approach has been to perform congener-specific analysis and then to calculate the total potency of the mixture by multiplying the concentration of each congener by its toxic equivalency factor (TEF) and summing the products, assuming an "additive model" (Eadon et al., 1986; Safe, 1987, 1990; Tanabe et al., 1987; Kannan et al., 1988; Kubiak et al., 1989; Kutz et al., 1990; Ahlborg et al., 1992, 1994). The total potency is expressed in units of an equivalent quantity of 2,3,7,8-TCDD (hereinafter referred to as "TCDD-EQs").

A number of TEF schemes have been developed for dioxins and related compounds based on AHH or EROD enzyme induction potency (Safe, 1987; Tanabe et al., 1987; Kannan et al., 1988; Kubiak et al., 1989; Smith et al., 1990) or in vivo and in vitro effects (Kutz et al., 1990, Safe, 1990). Others have attempted to assign TEFs on the basis of acute toxicity (Eadon et al., 1986). TEFs have been derived by several means including the ability of each congener to induce cytochrome P-450-dependent AHH or EROD activity in H4IIE rat hepatoma cell culture (Niwa et al., 1975; Sawyer and Safe, 1982). Mammalian toxicity studies as well as chicken egg injection studies suggest enough correspondence in potency to warrant the use of this mammalian culture system (Kubiak et al., 1989; Ludwig et al., 1993; Tillitt et al., 1993). H4IIE rat hepatoma cells have low basal AHH and EROD enzyme activities, yet they are highly inducible by PHHs. The U.S. Food and Drug Administration (FDA) has used this bioassay to evaluate complex mixtures of PHHs from environmental samples and in food (Bradlaw and Casterline, 1979; Trotter et al., 1982). Kubiak (1991) proposed TEFs for avian embryotoxicity based on either $LD_{50}$ or $LD_{85\text{-}90}$ potency values from various chicken egg injection studies. Current understanding of this dioxin-like class of compounds suggests that the suite of acute effects known as the Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) (Gilbertson et al., 1991) are currently the most sensitive endpoints of exposure to the avian embryo. A TEF scheme based on a single endpoint, embryo-mortality, seems the most realistic. The bases for these TEFs are included in Table 4. Recent work by Nosek et al. (1992a, 1992b, 1993) has shown little toxicological difference related to the source (injection or maternal deposition) of TCDD contamination in pheasant eggs, thus giving further credibility to injection study-derived TEFs. The Netherlands has adopted a TEF scheme for PCB congeners (van Zorge, 1990, reported in Beurskens et al., 1993) that is very similar to that in Table 4. Other in ovo and in vitro studies of the chicken by Vos et al. (1982), Yao et al. (1990), and Bosveld et al. (1992) generally support these TEFs (all potencies are consistent within an order of magnitude). The use of any TEF scheme should be approached with an understanding of the utility and limitations of selecting one scheme over another.

Table 4 Toxic Equivalency Factors (TEFs) for Avian Embryotoxicity Based on Egg Injection Studies[a]

| Compound (IUPAC[b] no.) | $LD_{50}$ concentration (pg/g) | $LD_{50}$ TEF (TCDD/PCB ratio) | $LD_{85\text{-}90}$ concentration (pg/g)[c] | $LD_{85\text{-}90}$ TEF (TCDD/PCB ratio) |
|---|---|---|---|---|
| 2,3,7,8-TCDD | 147[d] | 1 | 1,000[c] | 1 |
| 3,3',4,4',5-PeCB (PCB 126) | 3,100[e] | 0.05 | 8,000[e] | 0.125 |
| 3,3',4,4'-TeCB (PCB 77) | 8,600[e] | 0.02 | 22,000[e] | 0.045 |
| 3,3',4,4',5,5'-HxCB (PCB 169) | 170,000[e] | 0.001 | 330,000[e] | 0.003 |
| 2,3,3',4,4',5-HxCB (PCB 156) | 1,400,000[f] | 0.0001 | 2,500,000[f] | 0.0001 |
| 2,3,3',4,4'-PeCB (PCB 105) | 2,200,000[f] | 0.00007 | 2,500,000[f] | 0.0001 |
| 2,3',4,4',5-PeCB (PCB 118) | >5,000,000[f] | <0.00003 | >5,000,000[f] | <0.00001 |

[a] Adapted from presentation of Kubiak (1991). It should be noted that $LD_{50}$ values for PCB congeners 126 and 77 are lower with treatment on day 4 and survival measured through hatching as shown in Table 3; respective TEFs are 0.25 and 0.04.
[b] IUPAC, International Union of Pure and Applied Chemistry; $LD_{50}$, 50% lethal dose, TCDD, tetrachlorodibenzodioxin; PCP, polychlorinated biphenyl; $LD_{85\text{-}90}$, 85-90% lethal dose; 3,3',4,4',5-PeCB, pentachlorobiphenyl (other PeCBs defined similarly); 3,3',4,4'-TeCB, tetrachlorobiphenyl; 3,3',4,4',5,5'-HxCB, and 2,3,3',4,4',5-HxCB, hexachlorobiphenyls.
[c] Higginbotham et al. (1968); the $LD_{85\text{-}90}$ is defined as the net toxicity over control mortality.
[d] Verrett (1976).
[e] Brunstrom and Andersson (1988).
[f] Brunstrom (1990).

Although analytical techniques for all congeners of these compounds exist, they are extremely time consuming and costly; samples may theoretically contain up to 209 different PCB, 75 PCDD, and 135 PCDF congeners (Safe, 1987). Furthermore, it is presently difficult, if not impossible, to predict the biological effects of these mixtures with any certainty, because of the many possible combinations of congeners with many potential interactions among them that may be synergistic, additive, or antagonistic (Birnbaum et al., 1985; Weber et al., 1985).

Tillitt et al. (1991) examined the overall potencies of PCB mixtures by the ability of each mixture to induce cytochrome P-450-associated EROD activity after it was added to the rat hepatoma cell culture. These overall potencies were compared with potencies derived from summing the components of each mixture as TCDD-EQs. This method was applied to PCB-containing extracts from colonial waterbird eggs collected from the Great Lakes and revealed that the greatest concentrations of TCDD-EQs were found in the most polluted colonies where reproductive impairment was most severe. However, discrepancies between egg extract, rat hepatoma-derived TCDD-EQs, and the "additive model" based upon calculated TCDD-EQs (chemical residue analysis-summed TCDD-EQs) occurred. Hoffman et al. (1995) has been conducting bobwhite and chicken egg injection studies using combinations of two or three congeners, including PCB 126, PCB 105, PCB 77, and PCB 153. These

findings suggest less-than-additive and possibly antagonistic interactions. This type of interaction is thought to be due to binding competition for Ah receptors, reducing the receptor-binding probability of congeners that are more active inducers (Safe, 1990). When studying the effects of PHH on the reproductive success of fish-eating birds, Forster's terns *(Sterna forsteri)* in the Great Lakes, Kubiak et al. (1989) converted analytical residue determinations of TCDD and PCB congeners into TCDD-EQs. Here, the summed TCDD-EQ values of individual congener residues resulted in total TCDD-EQs nearly an order of magnitude greater than the egg extract, H4IIE-derived TCDD-EQs (Tillitt et al., 1993). It was thought that these differences may have been due to less-than-additive or antagonistic effects that would only be assessed in the H4IIE assay (Bannister et al., 1987), indicating the merits of the H4IIE method or measured TCDD-EQs over calculated TCDD-EQs. Therefore, the H4IIE extract bioassay, direct injection of dioxin-like extract into fertile eggs of the chicken or other appropriate species, or adult feeding studies with environmentally derived mixtures should be used to measure embryotoxicity and confirm the relative potency of mixture exposure in the environment.

## PCB EFFECTS IN THE FIELD

### HERRING GULLS

Some of the most thoroughly documented studies linking PCBs to avian mortality, reproductive failure, and population declines have been conducted in the Great Lakes region. PCBs were the probable cause of mortality of many ring-billed gulls *(Larus delawarensis)* that died in southern Ontario in the late summer and early fall of 1969 and 1973 (Sileo et al., 1977) as supported by the laboratory data of Stickel et al. (1984). Among 54 gulls for which no disease-related cause of death could be determined, residues of PCBs in the brain exceeded 300 ppm (310 to 1110 ppm) in 33 specimens and were above 200 ppm in an additional 16. 1,1'-Dichloroethenylidene-bis(4-chlorobenzene) (DDE) residues in all but one of these were well below lethal levels, and dieldrin levels were 5 ppm or higher in only six specimens. Therefore, the concentrations of PCBs alone in most samples were sufficiently high, based upon experimental studies, to have caused mortality (Stickel et al., 1984).

Keith (1966) and Ludwig and Tomoff (1966) reported low reproductive success and eggshell damage in association with high organochlorine residues in Lake Michigan herring gulls *(Larus argentatus)*. Extended studies with Lake Ontario herring gulls documented similar effects, including embryo mortality, reduced hatching success, and high chick mortality (Gilbertson, 1974; Gilbertson and Hale, 1974a, 1974b). These effects were associated with high PCB (550 ppm of dry weight) and DDE (140 ppm) levels that were 10 to 100 times higher than levels in eggs from other North American colonies. Gilbertson and Fox (1977) collected herring gull eggs in 1974 from contaminated colonies on eastern Lake Ontario and from relatively uncontaminated colonies in New Brunswick and Alberta, Canada and incubated them in laboratory incubators. Hatching success for Lake Ontario gulls was 60%

less than that in controls, and subcutaneous edema, hepatomegaly, impaired bone growth, and congenital anomalies were present in hatchlings along with liver porphyria and microsomal AHH induction. Organochlorines may also have reduced reproductive success by altering parental behavior; the total organochlorine (OC) content was correlated with the total time eggs were unattended in the nest by the parent (Fox et al., 1978; Peakall et al., 1980).

Observations of other sublethal effects in herring gulls have included histopathology of the thyroid in adult birds collected from the Great Lakes between 1974 and 1983. Compared with those of a control colony in the Bay of Fundy, the thyroids from Great Lakes gulls had a greater mass and were microfollicular and frequently hyperplastic (Moccia et al., 1986). These effects were found to be consistent with the presence of polyhalogenated hydrocarbons, including PCBs. Porphyria was reported in adult Great Lakes herring gulls collected from 1980 to 1985, with the highest levels in gulls from the lower Green Bay (Lake Michigan), Saginaw Bay (Lake Huron), and Lake Ontario (Fox et al., 1988; Kennedy and Fox, 1990). Concentrations of highly carboxylated porphyrins (HCPs) in herring gulls from Saginaw Bay were significantly correlated with residues of hexachlorobenzene (HCB) ($r = 0.612$, $P < 0.05$), total PCBs ($r = 0.594$, $P < 0.05$), and DDE ($r = 0.588$, $P < 0.05$). DDE is not known to induce porphyria, but concentrations of DDE were highly correlated with total PCBs and PCDD residues in liver.

Gilbertson et al. (1991) reviewed the history of reproductive problems and their classification as GLEMEDS. Some of this material is addressed in more detail below.

## TERN STUDIES

In 1983, the reproductive success of a Green Bay colony of state-endangered Forster's terns *(S. forsteri)* was compared with that of a successful inland colony (Hoffman et al., 1987; Kubiak et al., 1989). The hatching success in a laboratory incubator of eggs collected from Green Bay was only 52% of that for eggs from the inland control colony. Green Bay hatchlings weighed less and had an increased ratio of liver weight to body weight, shorter femur length, edema, and malformations. Hepatic MFO activity (AHH) was 3-fold higher in Green Bay hatchlings. Green Bay eggs contained a median concentration of 23 ppm of total PCBs and 37 ppt of 2,3,7,8-TCDD compared with 3.2 ppm and 8 ppt, respectively, for the control colony. The median total PCDD concentrations in eggs from Green Bay and the control area were 102 and 25 ppt, respectively. On the basis of relative AHH induction, Kubiak et al. (1989) estimated the potencies of individual PCB congeners as TCDD-EQs, using the data of Sawyer and Safe (1982); the TEFs used were published in Smith et al. (1990). Kubiak et al. (1989) concluded that two PCB congeners, PCB 105 and PCB 126, accounted for over 90% of the toxicity (2175 ppt vs. 201 ppt of the total median estimated TCDD-EQs). However, studies by Brunstrom et al. (1990) and Hoffman et al. (1995) have revealed the PCB congener 77 to be more embryotoxic than previously thought, thereby accounting for some of the toxicity reported in tern eggs. Additionally, PCB 77 was not recovered efficiently, further diminishing its relative importance (Kubiak et al., 1989). Data from 1982 on concentrations of

PCB 77 in Forster's tern eggs showed this mixture to be of substantially greater importance (Smith et al., 1990). An important extrinsic effect that further reduced the hatching success of field eggs from the Green Bay region was decreased parental nest attentiveness; hatchability was improved when eggs from the Green Bay region were exchanged and incubated by foster parents from the control location. As a sequel to the above study, Harris et al. (1993) reported greater hatching success, number of young fledged, and length of incubation in Forster's terns at Green Bay in 1988. The median total PCB residue (7.3 ppm) was 67% lower in 1988 than in 1983 and corresponded to a 42% reduction in TCDD-EQs from 1983. The authors suggested that contaminant reduction and improved reproductive performance were due to low river flows in 1988 and associated reduced PCB loading to Green Bay. Nevertheless, 42% of the chicks that were monitored died before fledging, and their body weight growth curves deviated from normal, showing signs of a wasting syndrome with loss of soft tissue, primarily pectoral muscle. The young were found to accumulate total PCBs at a rate of 18 μg/day. A no-observable-adverse-effects level (NOAEL) of 40 to 84 μg/kg/day for reproductive success was estimated from the 2-year results.

Schwartz and Stalling (1991) have assessed eggs of Forster's terns from the above study sites through pattern recognition techniques and concluded that total PCB concentrations and congener patterns support the view that Aroclor mixtures change substantially in the environment. It is therefore prudent to be cautious in the use of Aroclor matching as a means for showing a relation between source, biotic exposure, and effects. Technical PCB mixture sources should be linked to biotic exposures and accumulation/biomagnification by use of congener-specific analysis of escape pathways. This will identify which sources contribute to environmental contamination by the specific congeners of interest in organisms.

Ankley et al. (1993) and Jones et al. (1993a, 1993b) have demonstrated the uptake of specific congeners and TCDD equivalents quantified by the H4IIE rat hepatoma cell extract bioassay in Forster's terns by examining the total pollutant mass instead of the concentration. In this way, the effects of growth dilution and metabolism in hatchlings would be minimized in determining uptake/retention of these compounds in chicks and in comparisons with sibling eggs.

The H4IIE extract bioassay (Tillitt et al., 1993) resembled the TEF study approach (Kubiak et al., 1989) in predicting toxic differences between sites, but the bioassay estimated toxicity at only about a tenth of that estimated by the corresponding congener-specific analysis approach using TEFs and an additive model of toxicity used by Kubiak et al. (1989).

Other species of terns studied in the Great Lakes region have included the common tern *(Sterna hirundo)* and the Caspian tern *(Sterna caspia)*. In a study on common terns conducted in 1984 and 1985, the hatching success was determined for eggs from industrialized locations, including Green Bay and Saginaw Bay, and for eggs from several reference locations (Hoffman et al., 1993b). Hatching success was lowest for eggs from the Saginaw Bay (24% for one colony and 60% for another colony), 71% for eggs from Green Bay, and 85% for controls, corresponding to mean total PCB concentrations of 7.6 and 8.5 ppm for Saginaw Bay colonies,

10.0 ppm for Green Bay colonies, and 4.7 ppm for colonies from reference locations. Elevated levels of PCDDs were considered to be a contributing factor to the toxicity at Saginaw Bay. Nevertheless, the log-transformed total PCB content of eggs was related to the femur length/body weight ratio of hatchlings ($r = -0.70$, $P < 0.05$) and to liver microsomal AHH activity ($r = 0.71$, $P < 0.05$).

Concentrations of total PCBs as great as 18.5 to 39.3 ppm in eggs did not seem to reduce the productivity of Caspian terns during 1980 to 1981 (Struger and Weseloh, 1985). This may indicate that Caspian terns are less sensitive to the effects of PCBs than are common and Forster's terns, possibly because of their larger size and slower metabolic rate. However, in the Caspian terns studied at Saginaw Bay colonies, the PCB levels were higher by 2.1-fold as TCDD-EQs (using Brunstrom-derived TEFs) in second-clutch eggs (2800 ppt) than in first-clutch eggs (1300 ppt), and the frequency of deformed embryos was greater in the second clutches (Ludwig et al., 1993; Yamashita et al., 1993). The abnormalities, hatch rate, productivity, and fledging rates all appeared to be related to a large flood in the Saginaw River watershed that mobilized large amounts of contaminated sediment and delivered it to Saginaw Bay (Ludwig et al., 1993). Mora et al. (1993) found that plasma concentrations of total PCBs in adult Caspian terns were greatest in Green Bay and Saginaw Bay, where banding studies showed that fewer birds returned to their natal region, suggesting poorer survival in locations with an increasing plasma PCB concentration. Total PCB concentrations in the plasma of adults were 2.5 to 3.5 ppm in those locations, and only 20% of the birds banded as chicks were observed to return to these sites. At other sites where plasma PCB concentrations were below 1.5 ppm, 70% or more terns returned to the natal sites. Although productivity did not appear to be affected at Green Bay and Saginaw Bay, it is possible that post-fledging survival may have been lower in those locations, affecting the number capable of returning.

## CORMORANTS

Double-crested cormorant *(Phalacrocorax auritus)* populations were probably adversely affected by PCBs as well as by DDE; no cormorants were known to have fledged from any of the colonies in the Canadian waters of Lake Ontario from 1954 to 1977 (Price and Wesloh, 1986). Since the late 1970s, there has been a marked expansion of the breeding population. However, congenital anomalies and embryonic death are currently associated with PCBs in certain colonies (Fox et al., 1991b, 1991c).

Fox et al. (1991a) found that the probability of observing a malformed (specifically, bill defects) double-crested cormorant chick on a visit to a colony in Green Bay (Lake Michigan) was 10 to 32 times greater than on a visit to a colony in reference areas located in the Canadian prairies and northwestern Ontario. The prevalence of malformed chicks in the Green Bay region (52.1/10,000) was markedly greater than in all other regions during the 1979 through 1987 period of study. Indeed, data from Tillitt et al. (1992) clearly show that the highest TCDD equivalents determined from the rat hepatoma cell extract bioassay similarly occur in Green Bay

and are lowest in the Canadian prairies. In that study, only PCB-fraction extracts from eggs were assessed, not the dioxin or furan fraction.

Yamashita et al. (1993) reported the highest frequencies of deformities in live cormorant embryos from colonies with the highest PCB concentrations in eggs collected in 1988. Total PCB concentrations of 7.3 ppm and TCDD-EQs of 1200 to 1300 ppt were found in eggs from Green Bay and Beaver Islands on Lake Michigan, where the frequency of deformed, live embryos was 6 to 7%. In contrast, on Tahquamenon Island on Lake Superior, the deformity frequency was 2%, the total PCB concentration was 3.6 ppm, and TCDD-EQs were 350 ppt. Embryo mortality was highest in Green Bay colonies (22 to 39%) during 1986 through 1988 (Tillitt et al., 1992). TCDD-EQs derived from the H4IIE rat hepatoma assay using extracts from Green Bay cormorant eggs were 201 ± 13 (SD) to 344 ± 36 ppt, whereas TCDD-EQs using extracts from eggs from Lake Winnipegosis, where embryo mortality was only 8%, were 35 ± 3 ppt. Analysis of data on total PCBs revealed that PCBs at 7 to 9 ppm were associated with approximately 25% embryo mortality. However, the relation for H4IIE-derived TCDD-EQs and embryo mortality was statistically stronger ($r^2 = 0.703$, $P = 0.0003$), where TCDD-EQs at 150 to 250 ppt were associated with 25% embryo mortality.

Work by van den Berg et al. (1992) examined great cormorants *(P. carbo)* from two colonies in the Netherlands. They found yolk sac concentrations of total mono-ortho-PCBs and total PCDD(F)s between 10 and 250 ppm, and 1 to 8 ppb (lipid weight) could produce alterations in EROD liver activity, free plasma thyroxine ($T_4$) content, head length, size of the yolk sac, and relative liver weight. These effects are consistent with those described as GLEMEDS by Gilbertson et al. (1991). The lipid-basis hatchling yolk sac concentrations reported by van den Berg and co-workers, when converted to fresh wet weight concentrations based on a mean lipid egg content of 5.5% for great cormorants (Sotherland and Rahn, 1987), would yield considerably higher concentrations for PCBs, PCDDs, and PCDFs than reported by Yamashita et al. (1993) for Great Lakes double-crested cormorant eggs exhibiting GLEMEDS effects. Unfortunately, the lipid percentages in hatchling yolk sacs were not reported by van den Berg et al. (1992), so this comparison is illustrative and not directly comparable.

## EAGLES

Total PCB concentrations in bald eagle (*H. leucocephalus*) eggs were reported to be as high as 40 to 100 ppm (fresh weight) in 1986 on Lake Erie, Lake Huron, and Lake Michigan (Colborn, 1991). Colborn concluded that, although a strong association between dichlorodiphenyltrichloroethane (DDT)/DDE and impaired bald eagle reproductive success has been provided, the high levels of PCBs and associated toxicity in other species implicate PCBs as possible agents of embryotoxicity in eagles. Kozie and Anderson (1991) reported decreased productivity in bald eagles that were nesting along the Wisconsin shoreline of Lake Superior during 1983 to 1988 compared with inland Wisconsin. PCB concentrations in the brains of nestlings varied from 2.9 to 42 ppm compared with nondetectable to 0.42 ppm for controls

from inland locations. DDE concentrations in brains varied from 1.5 to 16 ppm compared with nondetectable to 0.09 ppm for controls from inland locations. Schwartz et al. (1993) reported on congener-specific analysis of an addled bald eagle egg from the Thunder Bay area of Lake Huron, North America. This addled egg contained the highest known concentration of PCB 126 and other PCB congeners in a wild bird egg when normalized to fresh wet weight. The analyzed and reported concentration was 71 ng/g for PCB 126. Normalization to fresh wet weight produced a concentration of approximately 42 ng/g (Bowerman et al., 1994b). This egg was reported to have an embryo with a "beak skewed to the right" that died about day 19-20 of incubation. This concentration was 13.5-fold higher than the $LD_{50}$ in the chicken egg (Brunstrom and Andersson, 1988) and one that causes embryonic mortality in American kestrel embryos (Hoffman et al., 1995). Jones et al. (1993a) cite this egg in their study of biomagnification of dioxin-like compounds in the Thunder Bay ecosystem using the rat hepatoma cell extract bioassay. The egg contained 1,065 pg/g of TCDD-EQs (fresh wet weight), two orders of magnitude above the alewife and smelt concentration of TCDD-EQs from Thunder Bay waters.

Bowerman et al. (1994b) reported on the historic, documented cases of bill defects in bald eagle nestlings of the Great Lakes region. Using banding records to determine sample size ($n$ = 9444), these authors found that the prevalence of bill defects in eaglets was comparable to the prevalence of bill deformities in double-crested cormorants in the Great Lakes Basin. During the period of bill defects in the eaglets, concentrations of PCBs in addled eggs ranged from 19 to 98 ppm (fresh wet weight) during 1976 to 1978 (Wiemeyer et al., 1984) and from 3.4 to 119 ppm (fresh wet weight) during 1985 to 1990 (Kubiak and Best, 1991; D. Best, U.S. Fish and Wildlife Service, unpublished data, 1993). Kubiak and Best (1991), Best et al. (1994), and Bowerman et al. (1994b) related nest productivity to concentrations in addled bald eagle eggs from the Great Lakes. Productivity curves developed by Kubiak and Best (1991) look virtually the same as those developed for the white-tailed sea eagle by Helander et al. (1982). Healthy productivity (one young per active nesting pair of adults) associates with 5 to 10 ppm (fresh wet weight) of total PCBs in eagle eggs. Bowerman et al. (1990) analyzed blood plasma from Great Lakes Basin eaglets. They found that plasma PCB concentrations, on an arithmetic mean basis, were greater than 8-fold higher (183 vs. 24 μg/l) in Great Lakes eaglets than in eaglet plasma collected from interior areas away from Great Lakes-contaminated forage.

Anthony et al. (1993) analyzed fresh bald eagle eggs from the Columbia River estuary, North America, and compared the residues to productivity. Productivity averaged 0.56 young/occupied nesting site, and total PCB concentrations averaged 12.7 ppm (fresh wet weight) and ranged from 4.8 to 26.7 ppm. High DDE concentrations in these eggs and both DDE and PCB association with egg shell thinning and productivity did not allow for the putative organochlorine to be clearly determined.

The Baltic Sea is known for significant contamination from PCBs and related compounds (Helander et al., 1982). Tarhanen et al. (1989) analyzed addled white-tailed sea eagle eggs from the Baltic Sea and from interior Lapland. The Baltic Sea

eagle egg contained 48.5 ppm of total PCBs, whereas the Lapland eagle egg contained 5.9 ppm. These investigators also determined coplanar PCB congeners in both eggs. Concentrations of coplanar, dioxin-like congeners, PCB 77, PCB 126, and PCB 169, were 21, 20.6, and 6 ppb (fresh wet weight), respectively, for the Baltic Sea eagle egg and 9.8, 0.95, and not detectable for the Lapland eggs. The Baltic Sea egg concentrations of PCBs 77 and 126 were approximately 2- and 7-fold higher, respectively, than the $LD_{50}$ in the domestic chicken egg (Brunstrom and Andersson, 1988), whereas the Lapland egg was equal to the $LD_{50}$ for PCB 77 and 33% of the $LD_{50}$ for PCB 126. Analyses of the muscle and liver of dead Baltic Sea white-tailed sea eagles were also conducted, and the residues were generally of the same order of magnitude as were those in Baltic Sea eggs.

Helander et al. (1982) studied the white-tailed sea eagle of the Baltic Sea coast and Lapland. Total PCB concentrations of approximately 5 to 10 ppm were associated with "healthy" reproductive success of one young produced/active nesting territory. Similar analysis of bald eagle eggs from North America produced similar results. Helander (1983) documented the occurrence of bill deformities in white-tailed sea eagle nestlings in Sweden. These defects were specifically linked to PCB contamination in the Baltic Sea between the study years 1965 and 1978 (Helander et al., 1982). Two nestlings of the 115 Baltic Sea eaglets examined were documented with bill deformities. Although the sample size of Helander and co-workers was small, the prevalence of bill defects per 10,000 observations would have been 173.9, considerably higher than for bald eagles from the Great Lakes region (Bowerman et al., 1994b) or the highest prevalence in double-crested cormorants from the Great Lakes, 52.1 (Fox et al., 1991c). The PCB content of eagle eggs from the Baltic Sea region during the time bill defects were recorded ranged from 18.7 to 159 ppm (fresh wet weight) in comparison with that of eagle eggs from Lapland, containing 8.8 to 11.1 ppm, where no deformities were documented in the 60 nestlings examined.

## BLACK-CROWNED NIGHT HERONS

In the San Francisco Bay area (San Francisco Bay National Wildlife Refuge, SFBNWR), Hoffman et al. (1986) revealed a negative correlation ($r = -0.61$, $P < 0.05$) between body weight at hatching (pipping) of black-crowned night herons *(Nycticorax nycticorax)* and log-transformed PCB residues in eggs from the same nest. DDE was not significantly correlated with body weight at hatching. The geometric mean for the total PCB concentration of SFBNWR eggs was 4.1 ppm (range, 0.8 to 52.0 ppm). The yolk-free body weight (internally absorbed yolk sac removed) was lower when SFBNWR embryos were compared with control embryos from a captive colony at the Patuxent Wildlife Research Center. In another study comparing multiple geographical areas, the total PCB concentrations in black-crowned night heron embryos were positively correlated with cytochrome P-450 parameters, including AHH and EROD activities (Rattner et al., 1993); the highest PCB concentrations and MFO induction were found in birds from Green Bay.

Inasmuch as DDE and other "hard organochlorines" do not cause the suite of effects seen in GLEMEDS, the above reproductive problems and presence of bill

defects in eagles and other species of birds exhibiting these effects strongly point out the limitations of statistical association of single contaminants in the investigation of chemically induced epizootics involving reproductive impairment or other toxicological endpoints. Future efforts, similar to the GLEMEDS investigations that involved "practical causal inference" (Fox, 1991), will be necessary to more fully interpret field exposures. Highly cocorrelated compounds will have to be assessed relative to their ability to produce specific effects.

## LABORATORY STUDIES OF TCDD

Hudson et al. (1984) reported 37-day $LD_{50}$s of 15,000, >108,000, and >810,000 pg/g for bobwhite quail, mallards, and ringed turtledoves, respectively, following a single oral dose of TCDD. Grieg et al. (1973) reported that chickens given single oral doses of TCDD at 25,000 to 50,000 pg/g died within 12 to 21 days posttreatment. In a more comprehensive study, TCDD was orally administered to 3-day-old white leghorn chickens for 21 days at doses of 0, 10, 100, 1,000, and 10,000 pg/g/day with a NOAEL for mortality of 100 pg/g/day (Schwetz et al., 1973). However, none of the above studies determined residues. Nosek et al. (1992a) treated ring-necked pheasant hens with single doses of TCDD by i.p. injection (6.25, 25, or 100 μg/kg); i.p. injection was favored by the authors over oral dosing for the administration of known quantities of TCDD. The lowest single dose of TCDD to produce a delayed onset of body weight loss and mortality (wasting syndrome) was 25 μg/kg, which resulted in 25% loss in body weight and in approximately 80% mortality at the end of 12 weeks. When hen pheasants were treated weekly with lower doses of TCDD (0.01 to 1.0 μg/kg/week for 10 weeks), signs of the wasting syndrome and mortality were also produced with a cumulative dose of 10 μg/kg at the end of 10 weeks. Egg production at this cumulative dosage level was reduced as was hatchability (egg concentration of approximately 3300 ppt). A cumulative dose of 1 μg/kg resulted in approximately 1% of the cumulative dose being transferred to each of the first 15 eggs laid (Nosek et al., 1992b). The percentage for each egg was not affected by the order in which the eggs were laid. Greater than 99% of all TCDD within the egg was found in the yolk. Injection of 1099 ppt into the egg via the albumin showed a $t_{1/2}$ of 13 days for whole-body elimination of $^{3}H$-labeled TCDD in the chicks that hatched. The authors estimated that wild hen pheasants laying two clutches totaling 20 eggs may eliminate 33% of the body burden of TCDD into the eggs.

In another study, Nosek et al. (1993) injected TCDD mixed in 1,4-dioxane into the yolk or the albumin of pheasant eggs prior to incubation. The TCDD doses that were calculated to cause 50% mortality above that in controls ($LD_{50}$) when injected into the egg albumin or into the yolk were 1354 and 2182 ppt (pg of TCDD/g of egg), respectively. Administration of the vehicle alone resulted in 37.5% mortality when in the albumin and 50% mortality when injected into the yolk. However, the authors stated that this range of mortality was within the historical range for pheasant eggs that had not received injection. Embryo mortality above that in the vehicle control group began to occur at a TCDD dose of 1000 ppt per egg. However, this

dose and lower doses had no effect on the growth in hatchlings, edema, or histopathology of the liver, spleen, heart, bursa, or thymus. Cardiac malformations were not apparent in day-old hatchlings, and antibody-mediated immunity was not affected in 28-day-old chicks. The authors concluded that embryo mortality was the most sensitive sign of TCDD toxicity. The $LD_{50}$ of TCDD was similar to the TCDD dose calculated by these authors to be naturally deposited by TCDD-exposed hen pheasants into eggs that failed to hatch (3300 ppt). Induction of hepatic EROD activity in day-old hatchlings was considered to be the most sensitive indicator of exposure. The dose causing half the maximum induction ($ED_{50}$) was 312 ppt, and the dose causing maximum induction (over 5-fold) was 1000 ppt.

Earlier studies have shown chicken embryos to be considerably more sensitive to TCDD egg injections than are pheasant embryos. Flick et al. (1965) first injected the unsaponifiable fraction of fat-containing PCDDs at 0.9, 1.8, or 4.5 ng per egg, which decreased hatching success by 60 to 100% and resulted in multiple malformations of the brain, legs, and beak, and in stunted growth. Verrett (1970) reported that as little as 10 to 20 ppt of 2,3,7,8-TCDD injected into chicken eggs produced embryonic mortality, edema, and malformations. Verrett (1976) determined $LD_{50}$ concentrations for a variety of dioxins and furans in the chicken egg following injection into the air cell at 96 hours of development through hatching; 2,3,7,8-TCDF was the only compound as toxic as 2,3,7,8-TCDD. The estimated $LD_{50}$ was 0.007 μg of 2,3,7,8-TCDD per egg (personal communication by Verrett in Bradlaw and Casterline, 1979, and in Goldstein, 1980); assuming a 50-g egg content weight, this corresponds to approximately 140 pg/g (ppt). The actual $LD_{50}$ reported by Verrett (1976) was 147 ppt. Allred and Strange (1977) injected TCDD into the air cell of unincubated white leghorn eggs and estimated the $LD_{50}$ by 18 days of incubation to be approximately 240 ppt. An increased liver/egg weight ratio occurred. Higginbotham et al. (1968) determined that 1000 ppt of TCDD injected into the chicken egg produced total mortality. Rifkind et al. (1985) injected TCDD (0.0001 to 12 nmol per egg or 0.65 to 78 ppb) into chicken eggs, with the exact location of injection unspecified, at 10 days of incubation. However, in this study, perhaps because of the late stage of exposure and location of injection, an exceptionally high TCDD concentration was required to affect survival where 6 nmol per egg (39 ppb) caused only 30% embryo mortality. Surviving embryos exhibited decreased thymus weight and increased pericardial and subcutaneous edema. The dose-response relation for lethality and for hepatic MFO induction, including both AHH and EROD activities, was dissociated, and the maximal induction levels were not correlated with the extent of lethality. On day 0, Henshel (1993) injected TCDD into the yolk sac or air cell of chicken eggs and found that the $LD_{50}$s through hatching were approximately 115 and 180 ppt, respectively. The hatching weight was lower for embryos exposed to higher concentrations. Cheung et al. (1981) injected TCDD doses of 0.009 to 77.5 pmol per egg (0.0585 to 504 ppt) on day 0 into the albumin of white leghorns and examined them on day 14. The subcutaneous edema observed in some embryos was independent of dose. Mortality from treatment was minimal, and control mortality was 21%. However, a dose-dependent increase in cardiovascular malformations was apparent, where 6 ppt caused a 20% increase above the unexpectedly high

control incidence of 29% and a doubling at 65 ppt. Other deformities, including malformed legs and crossed beaks associated with microphthalmia, occurred at lower frequencies of 1 to 3%.

Martin et al. (1989) estimated the $LD_{50}$ for eastern bluebirds (*Sialia sialis*) following TCDD injections into the albumin of eggs; the $LD_{50}$ was greater than 1 but less than 10 ng of TCDD/g of egg (ppb), with embryo mortality being the most sensitive manifestation of toxicity. Eye and beak malformations, such as those seen in chicken embryos, were not apparent, nor was there edema or any effects on posthatching growth or histopathology of tissues in 8-day-old bluebird nestlings.

## FIELD STUDIES OF TCDD

Retrospective analysis of herring gull eggs from the Great Lakes has revealed that TCDD concentrations averaged 500 ppt during 1974 but were as high as 1200 ppt in earlier years (Gilbertson, 1988), which was probably a factor contributing to reproductive failure in addition to other documented organochlorines, including PCBs and DDT (Gilbertson et al., 1991) (Table 5). These high concentrations of TCDD, as well as of other organochlorines, declined to about 160 ppt by 1976 in gull eggs, and gull reproduction improved dramatically. More recently, Spear et al. (1990), concerned with vitamin A imbalance, examined egg yolk retinoids in this species and found that the molar ratio of retinol to retinyl palmitate was different among colonies from the Great Lakes and correlated with several indices of PCDD and PCDF concentrations in gull eggs from those sites. The molar ratio of retinol to retinyl palmitate was positively correlated with TCDD-EQs of PCDDs and PCDFs ($r = 0.866, P < 0.01$) and with the sum of PCDD and PCDF concentrations ($r = 0.759$, $P < 0.05$). Excess retinoic acid itself is experimentally teratogenic to avian embryos. The median retinol and retinyl palmitate concentrations in the livers of herring gulls in 1982 were 131 and 231 ppt, respectively, on Lake Ontario; 289 and 377 ppt on Lake Michigan; 382 and 562 ppt on Lake Superior; and 864 and 1737 ppt in New Brunswick (Spear et al., 1986). Corresponding 2,3,7,8-TCDD concentrations in eggs were 90, 10, 13, and 3 ppt, showing an inverse relation between liver retinoid levels and dioxin contamination.

Observations of other sublethal effects in herring gulls have included histopathology of the thyroid in adult birds collected from the Great Lakes between 1974 and 1983. Compared with a control colony in the Bay of Fundy, the thyroids from gulls from the Great Lakes had a greater mass and were microfollicular and frequently hyperplastic (Moccia et al., 1986). These effects were found to be consistent with the presence of polyhalogenated hydrocarbons, including PCBs. Porphyria was reported in adult herring gulls from the Great Lakes collected from 1980 to 1985, with the highest levels in gulls from the lower Green Bay (Lake Michigan), Saginaw Bay (Lake Huron), and Lake Ontario (Fox et al., 1988; Kennedy and Fox, 1990). Concentrations of HCPs in herring gulls from Saginaw Bay were significantly correlated with residues of total 2,3,7,8-substituted tetra- through heptachloro dibenzo-*p*-dioxins ($r = 0.786, P < 0.05$) and DDE ($r = 0.588, P < 0.05$). DDE is not

**Table 5 Field Studies Measuring Exposure and Effects Consistent with Planar Polychlorinated Biphenyls (PCBs) and Dioxins**

| Species, tissue | Concentration | Effects | Ref. |
|---|---|---|---|
| Herring gull,[a] eggs | 2,3,7,8-TCDD = 3 > 1200 ppt<br>Total PCBs = 6 > 180 ppm | GLEMEDS consistent,[b] embryo mortality, impaired reproductive success, beak defects, AHH induction, vitamin A depletion, porphyria | Gilbertson et al. (1991); Norstrom et al. (1982); Ellenton et al. (1985); Fox et al. (1988); Spear et al. (1990); Kennedy and Fox (1990) |
| Forster's tern, eggs | Total PCBs = 6-26 ppm<br>3,3',4,4',5-PeCB = 540-9,100 ppt<br>2,3,3',4,4'-PeCB = 330-730 ppb<br>2,3,7,8-TCDD = 14-105 ppt<br>TCDD equivalents from congener chemistry[d] = 618-7,366 ppt<br>TCDD equivalents from H4IIE extract bioassay = 90-339 ppt | Embryo mortality, impaired reproductive success, subcutaneous edema of head and neck, AHH induction, hard tissue deformities, mostly beaks[b] | Kubiak et al. (1989); Hoffman et al. (1987); Tillitt et al. (1993) |
| Common tern, eggs | Total PCBs = 5-24 ppm | General reproductive impairment, deformities, edema and AHH induction[b] | Hoffman et al. (1993b) |
| Caspian tern, eggs | Total PCBs = 4-18 ppm<br>TCDD equivalents from congener chemistry[c] = 1,300-2,800 ppt<br>TCDD equivalents from H4IIE extract bioassay = 50-416 ppt<br>2,3,7,8-TCDD = 8-22 ppt<br>3,3',4,4'-TeCB = 15,000-23,000 ppt<br>3,3',4,4',5-PeCB = 3,300-7,900 ppt<br>2,3,3',4,4'-PeCB = 140-370 ppb | Multiple deformities, edema, embryo mortality, impaired reproductive success[b] | Ludwig et al. (1993); Yamashita et al. (1993); Tillitt et al. (1991) |
| Caspian tern, adult blood plasma | Total PCBs = 1-14 ppm | General inverse relationship between population exposure and adult breeder numbers as measured by natal site tenacity suggested lower post-fledging survival to adult breeding age | Mora et al. (1993) |
| Double-crested cormorant,[a] eggs | TCDD equivalents from H4IIE extract bioassay = 85-413 ppt<br>2,3,7,8-TCDD = 5.3-22 ppt | Embryonic mortality, beak deformities, club foot[b] | Fox et al. (1991a,b,c); Tillett et al. (1991); Yamashita et al. (1993) |

**Table 5 (continued) Field Studies Measuring Exposure and Effects Consistent with Planar Polychlorinated Biphenyls (PCBs) and Dioxins**

| Species, tissue | Concentration | Effects | Ref. |
|---|---|---|---|
| | Total PCBs = 3.6-6.8 ppm<br>3,3',4,4',5-PeCB = 800-7,900 ppt<br>2,3,3',4,4'-PeCB = 110-370 ppb<br>TCDD equivalents from congener chemistry[c] = 350-1,300 ppt | | |
| Cormorant, hatchling yolk sac | Total mono-ortho PCBs = 10-250 ppm of lipid weight<br>Total PCDD/PCDF ratio = 1-8 ppb of lipid weight | Concentration-dependent alterations in EROD activity, free T4 plasma content, head length, yolk sac size, and relative liver weight | van den Berg et al. (1992) |
| Bald eagle, eggs | Total PCBs = 8-77 ppm (All below 1 sample)<br>3,3',4,4'-TeCB = 25 ppb<br>3,3',4,4',5-PeCB = 71 ppb<br>Total PCBs = 99.8 ppm<br>TCDD equivalents from H4IIE extract bioassay = 1,065 ppt | General reproductive impairment, deformities of the beak; 50- to 100-fold magnification of TCDD equivalents (H4IIE extract bioassay from small fish to eggs of fish-eating birds; fractional composition of PCB 126 enriched compared with any source Aroclor mixture | Kubiak and Best (1991); Jones et al. (1993a); Schwartz et al. (1993); Bowerman et al. (1994a,b); Bowerman et al. (1995) |
| White-tailed sea eagle (Sweden), eggs | Total PCBs = 20-159 ppm | General reproductive impairment, deformities of the beak | Helander et al. (1982); Helander (1983) |
| Black-crowned night heron, eggs | TCDD equivalents from H4IIE extract bioassay = 221 ppt | Hepatomegaly, AHH induction, subcutaneous edema of neck and throat | Hoffman et al. (1993b); Rattner et al. (1993); Tillitt et al. (1991) |
| Great blue heron, eggs | 2,3,7,8-TCDD = 211 ± 34 ppt<br>TCDD equivalents from congener chemistry[c] = 227 ± 36 | Altered embryonic growth, shortened beak, scarcity of down follicles, subcutaneous edema, MFO induction, and intercerebral asymmetry | Hart et al. (1991); Bellward et al. (1990); Henshel et al. (1995) |
| Wood duck, eggs | 2,3,7,8-TCDD = 2-482 ppt, 36 ppt (geometric mean)<br>TCDD equivalents from congener chemistry[d] = 3-611 ppt, 52 ppt (geometric mean) | General reproductive impairment, deformities of the beak, subcutaneous edema of head and neck | White and Seginak (1994); White and Hoffman (1995) |

**Table 5 (continued) Field Studies Measuring Exposure and Effects Consistent with Planar Polychlorinated Biphenyls (PCBs) and Dioxins**

| Species, tissue | Concentration | Effects | Ref. |
|---|---|---|---|
| Peregrine falcon, eggs | 2,3,7,8-TCDD = 4.7-9 ppt<br>Total PCBs = 1.4-13 ppm<br>3,3',4,4'-TeCB = 170-3300 ppt<br>3,3',4,4',5-PeCB = 80-2600 ppt<br>TCDD equivalents from congener chemistry[d] = 120 ppt (geometric mean) | PCB 126 approached the $LD_{50}$ in chicken eggs of 3,100 ppt but considerably lower than the kestrel $LD_{50}$ of 70-100 ppb | Jarman et al. (1993) |

[a] Studies cited for certain species involve multiple biological and chemical samples that may not always be of exactly the same year of study or exact site of collection. We have endeavored to screen these citations for temporal and geographic consistency for the identified species. Readers are encouraged to review each citation from an individual species to gain additional appreciation of sample associations.

[b] 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzodioxin; AHH, aryl hydrocarbon hydroxylase; 3,3',4,4',5-PeCB, pentachlorobiphenyl (2,3,3',4,4'-PeCB defined similarly); 3,3',4,4'-TeCB, tetrachlorobiphenyl; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofuran; EROD, 7-ethoxyresocifin-*O*-deethylase; MFO, mixed-function oxygenase; $LD_{50}$, 50% lethal dose.

[c] GLEMEDS, Great Lakes Embryo Mortality, Edema, and Deformities Syndrome as reviewed by Gilbertson et al. (1991).

[d] Readers are cautioned that chemistry-derived TCDD equivalents are a function of the toxic equivalency factors that are used in that study and as such are relative indicators of exposure to actual 2,3,7,8-TCDD, assuming an additive model of toxicity. Various sets of TEFs have been used for this type of data interpretation. Some of these references are identified in the text.

known to induce porphyria, but concentrations of DDE were highly correlated with total PCBs and PCDD residues in liver.

Elliot et al. (1989) reported TCDD concentrations in great blue heron *(Ardea herodias)* eggs from four colonies on the coast of British Columbia. PCDD levels in eggs were significantly elevated at a colony near a Kraft paper mill at Crofton on Vancouver Island in 1986. In 1987, the colony failed to raise any young, with 2,3,7,8-TCDD levels nearly 3 times higher than in 1986. Other contaminants, including PCBs, organochlorine pesticides, and mercury, were generally low. In 1986, the mean 2,3,7,8-TCDD level was 66 ppt (range, 8 to 218 ppt) (wet weight), and that of 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) was 2 ppt (not detectable [ND] to 14 ppt). The total TCDD-EQs calculated according to the method of Mason et al. (1986) were 79 ppt (19 to 272 ppt). In 1987, although the concentrations of 2,3,7,8-TCDD and of total TCDD-EQs had tripled, the authors felt that predation had played a role in the poor productivity. Here, 2,3,7,8-TCDD accounted for 82% of the total TCDD-EQs. In 1988 and further back, eggs were collected and allowed to hatch in a laboratory incubator (Bellward et al., 1990; Hart et al., 1991). Although hatching success was not significantly affected in the incubator with 2,3,7,8-TCDD egg concentrations of 211 ppt, subcutaneous edema was apparent in four of 12 chicks but absent in control chicks. TCDD concentrations in eggs from the same clutch were inversely correlated ($P < 0.01$) with measures of growth, including yolk-free

body weight, tibia length, and organ weights. Liver microsomal EROD activity in hatchlings was positively correlated ($r = 0.572$, $P < 0.001$) with 2,3,7,8-TCDD concentrations in eggs from the same clutch. Brains from highly contaminated colonies (Crofton) in 1988 exhibited a high frequency of intercerebral asymmetry that decreased in subsequent years as levels of TCDD decreased (Henshel et al., 1995). The asymmetry was significantly correlated with the level of TCDD in eggs taken from the same nest.

White and Seginak (1994) studied the reproductive success in wood ducks *(Aix sponsa)* in nest boxes downstream from Bayou Meto, a major drainage system in central Arkansas contaminated with PCDDs and PCDFs from a former chemical plant that manufactured the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Based on TEFs, the residues in eggs of wood ducks were 50-fold higher in eggs near the point source than in those from an uncontaminated reference area. The geometric mean and range for 2,3,7,8-TCDD were 36 ppt and 1.6 to 482 ppt, respectively, and were 26 ppt and 2.4 to 244 ppt for 2,3,7,8-TCDF at the most contaminated site. The TCDD-EQs were 52 ppt (range, 3.7 to 611 ppt), with 70% accounted for by 2,3,7,8-TCDD. Overall productivity (nest success, hatching success, and duckling production) was suppressed ($P < 0.05$) at nest sites 9 and 17 km downstream from the source, as was hatching success as far as 58 km downstream compared with that from a reference site. Egg TCDD-EQs were inversely correlated ($P < 0.0001$) with productivity in corresponding nests; i.e., as egg TCDD-EQs decreased, productivity increased. In addition, teratogenesis and oxidative stress were documented at the more contaminated sites (White and Hoffman, 1995). The threshold range of toxicity, based on TCDD-EQs where reduced productivity was evident in wood ducks, was 20 to 50 ppt. Concentrations of PCBs and DDE were virtually absent and therefore not a factor. It was concluded that the wood duck is particularly sensitive to PCDD/PCDF exposure and might serve as a good model for monitoring.

## ESTIMATION OF EGG AND BODY RESIDUES FROM FORAGE

Concentrations of PCBs and dioxin-like congeners in target organs, tissues, or whole bodies cannot always be determined at the most opportune time because of numerous factors. Braune and Norstrom (1989) published an eloquent study on apparent biomagnification factors in herring gulls from Lake Ontario. Adult herring gulls of the Great Lakes can be assumed to be at "dynamic steady-state equilibrium" with its main forage food, because herring gull adults do not venture far from their breeding colonies and because they remain on the Lakes virtually year round. Biomagnification factors from forage to egg were generated by Kubiak and Best (1991) from the data of Braune and Norstrom (1989). Because the most sensitive endpoint for PCB- and dioxin-like toxicity appears to be reproductive impairment associated with egg residues, Table 6 depicts the biomagnification factors (BMFs) from alewife, as forage, to herring gull egg, as target organ. Additional BMF data were communicated by R. Norstrom (Environment Canada, 1993) beyond those

Table 6 Biomagnification Factors from Alewife to Herring Gull Egg for Dioxin-like and Other Organochlorine Compounds

| Compound | Biomagnification factor |
|---|---|
| 2,3,7,8-TCDD[a] | 21 |
| 1,2,3,7,8-PeCDD | 10 |
| 1,2,3,6,7,8-HxCDD | 16 |
| 1,2,3,4,6,7,8-HpCDD | >6 |
| OCDD | >8 |
| 2,3,7,8-TCDF | <0.65 |
| 2,3,4,7,8-PeCDF | 4 |
| 1,2,3,4,7,8-, 1,2,3,4,6,7-HxCDF | >4 |
| 1,2,3,6,7,8-HxCDF | >4 |
| Total PCBs | 32 |
| 2,3,3',4,4'-PeCB | 20 |
| 2,3',4,4',5-PeCB | 31 |
| 2,2',3,4,4',5-HxCB | 42 |
| 3,3',4-TrCB | 0.8* |
| 3,3',4,4'-TeCB | 1.8* |
| 3,3',4,4',5-PeCB | 29* |
| 3,3',4,4',5,5'-HxCB | 46* |
| Hexachlorobenzene | 20 |
| DDE | 34 |
| Mirex | 30 |
| Photomirex | 34 |
| β-HCH | 10 |
| Octachlorostyrene | 8 |
| Oxychlordane | 60 |
| *trans*-Nonachlor | 3 |
| *cis*-Nonachlor | 5 |
| DDT | 2 |
| Heptachlor epoxide | 30 |
| Dieldrin | 7 |

[a] 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzodioxine; 1,2,3,7,8-PeCDD, pentachlorodibenzodioxin; 1,2,3,6,7,8-HxCDD, hexachlorodibenzodioxin; 1,2,3,4,6,7,8-HpCDD, heptachlorodibenzodioxin; OCDD, octachlorodibenzodioxin; 2,3,7,8-TCDF, tetrachlorodibenzofuran; 2,3,4,7,8-PeCDF, pentachlorodibenzofuran; 1,2,3,4,7,8-HxCDF, hexachlorodibenzofuran(1,2,3,4,6,7-HxCDF and 1,2,3,6,7,8-HxCDF defined similarly); total PCBs, total polychlorinated biphenyls; 2,3,3',4,4'-PeCB, pentachlorobiphenyl (2,3',4,4',5-PeCB and 3,3',4,4',5-PeCB defined similarly); 2,2',3,4,4',5-HxCB, hexachlorobiphenyl (3,3',4,4',5,5'-HxCB defined similarly); 3,3',4-TrCB, trichlorobiphenyl; 3,3',4,4'-TeCB, tetrachlorobiphenyl; DDE, 1,1'-(dichloroethenylidane)-bis(4-chlorophenzane); β-HCH, β-benzenehexachloride; DDT, 1,1'-(2,2,2-tetrachloroethylidene)-bis(4-chlorobenzene).

Data adapted from Braune and Norstrom, 1989;* provided by Ross Norstrom, personal communication to T. J. Kubiak.

published. Therefore, it is possible to estimate egg concentrations from adult forage, with the assumption that the forage is the predominant food eaten and that pharmacokinetic differences between carnivorous species are not large. Differences are most likely in species size, which affects metabolism and thus the mass of food ingested per unit of body weight. There may be other differences associated with birds, as specialized feeders are inferior to nonspecialized feeders in their capacities for metabolizing organochlorines (Walker et al., 1987). Nonetheless, this procedure has direct benefit, in that an egg concentration (or lipid concentration in the adult and adult whole-body concentration) can be estimated before the egg is analyzed. This allows for some estimation of the potential risk of exposure, based on the known effect levels discussed in this review. Of course, species-specific and site-specific BMFs would be preferred if available. This approach has been used recently in various ways to estimate the concentrations for risk assessment purposes (Sullivan et al., 1987; Thiel, 1990; Kubiak and Best, 1991; Bowerman et al., 1995). A similar approach using the H4IIE rat hepatoma cell extract bioassay appears to be practical as well, since TCDD-EQs generated from this extract bioassay clearly show biomagnification from forage, such as with the alewife, to predacious fish and fish-eating birds (Jones et al., 1993a, 1993b). The estimated concentrations of TCDD-EQs in eggs could be compared with the threshold effect level of choice or a NOAEL concentration for the actual TCDD in the avian egg.

## SUMMARY

PCB residue in the brain was generally found to be the most diagnostic tissue residue for lethality in adult birds. For Aroclor 1254, brain residues of total PCBs of approximately 300 ppm are diagnostic of lethality in pheasants and passerines (Table 7). For Clophen A60, lethal brain residues were 76 to 180 ppm in great cormorants but 420 to 445 ppm in herons. In a field die-off of ring-billed gulls, total PCB brain residues for two thirds of the dead birds exceeded 300 ppm and exceeded 200 ppm for the remainder. However, one must be somewhat cautious in relating Aroclor and other commercial PCB mixture feeding studies to field observations, because pattern recognition techniques during analysis of total PCB concentrations and congener patterns suggest that Aroclor mixtures change substantially in the environment and through the food chain.

The most sensitive functional endpoint for PCB- and dioxin-mediated toxicity appears to be reproductive impairment as associated with egg residues. Chickens are the most sensitive species with respect to PCB effects on reproduction. For Aroclor 1254 and Aroclor 1242, the hatchability of chicken eggs was reduced when residues were above 4 and 1 ppm, respectively. Aroclor 1254 decreased hatching success and parental attentiveness of ringed turtledoves during incubation; the mean total PCB residues in dove eggs were 16 ppm and, in adult brains, 5.5 ppm. Brain residues of 3 ppm resulted in significant depletion of brain dopamine and norepinephrine. Mallards, screech owls, and Atlantic puffins appeared to be more resistant to PCB exposure.

Table 7 Summary of Polychlorinated Biphenyl (PCB) and Tetrachlorodibenzodioxin (TCDD) Effect Levels

| Concentration | Effect |
|---|---|
| 20 to 50 ppt of TCDD in eggs | Embryo mortality and teratogenesis in chickens, decreased productivity and teratogenesis for wood ducks |
| 90 to 339 ppt of TCDD equivalents (H4IIE bioassay of egg extract) | Embryotoxicity in Forster's tern |
| 150 to 250 ppt of TCDD in eggs | Decreased embryonic growth, edema in herons |
| 618 to 7,366 ppt of TCDD equivalents (congener chemistry) | Embryotoxicity in Forster's tern |
| 1,000 ppt of TCDD in eggs | Embryo mortality in pheasants |
| <10,000 ppt of TCDD in eggs | Embryo mortality in bluebirds |
| 1 to 5 ppm of total PCBs in eggs | Decreased hatching success for chickens |
| 8 to 25 ppm of total PCBs in eggs | Decreased hatching success for terns, cormorants, doves, eagles |
| 75 to 300 ppm of total PCBs in brain | Lethality in great cormorants, gulls, passerines, and pheasants |

Several developmental and physiological studies have revealed coplanar PCB 126 to be the most toxic of all PCB congeners, causing porphyria in Japanese quail (liver residues under 0.1 ppm) and liver enlargement with coagulative necrosis, colloid depletion of the thyroid, and lymphoid depletion of the spleen in American kestrel nestlings (liver residue of 150 ppb). The utility of studies of injections into eggs for predicting the potential embryotoxicity of PCBs and TCDD compares favorably with feeding studies. In instances in which the same chemicals, aroclors or TCDD, have been administered by both methods, the concentrations in eggs and the effects are quite similar. Comparison of PCB congeners by injection into eggs has revealed chickens to be the most sensitive species and PCB 126 to be the most toxic congener; with air cell injections on day 7 of incubation, $LD_{50}$s (72 hours later) for PCB congeners 126, 77, 169, and 105 were 3.1, 8.6, 170, and 2200 ppb, respectively, but lower at earlier exposure. For PCB 126, the $LD_{50}$ was between 40 and 70 ppb for bobwhite and between 70 and 100 ppb for American kestrels. For PCB 77 in pheasant eggs, 1000 ppb resulted in complete mortality and, in turkeys, 1000 ppb caused 60% mortality. However, 5000 ppb in ducks and 1000 ppb in geese and herring gulls had no effects. The $LD_{50}$s for TCDD when injected into the egg albumin or yolk of pheasants were 1354 and 2182 ppt per egg, respectively, and were similar to the dose calculated to be naturally deposited by TCDD-exposed hen pheasants into eggs that failed to hatch (3300 ppt). Chicken embryos are considerably more sensitive to TCDD than are embryos of other species. As little as 10 to 20 ppt of 2,3,7,8-TCDD injected into chicken eggs produced embryonic mortality, edema, and malformations, and $LD_{50}$s (through hatching) were approximately 150 ppt (yolk sac) and 250 ppt (air cell). For the eastern bluebird, the $LD_{50}$ for injection into egg albumin was between greater than 1000 and 10,000 ppt, with embryo mortality being the most sensitive manifestation of toxicity with little evidence of other effects.

Because of a common mode of action for PHHs including PCBs and PCDDs, biological potencies may be calculated by expressing the potency of individual PCB congeners identified in the sample using a TEF relative to the most toxic PHH (2,3,7,8-TCDD) and then summing them in an "additive" fashion to produce a total

potency as TCDD equivalents (TCDD-EQs). For assessing potential avian embryotoxicity, it is best that TEFs be derived from studies of injections into eggs, as presented in Table 4. Another approach assesses the overall potency of PCB-containing extracts from tissues to directly induce cytochrome P-450-dependent EROD activity in H4IIE rat hepatoma cells as compared with the standard 2,3,7,8-TCDD. Discrepancies between the additive model and egg extract, H4IIE-derived TCDD-EQs exist. However, the H4IIE assay has the benefit of directly assessing any nonadditive overall effects that other studies have revealed do occur. Therefore, the H4IIE extract bioassay, direct injection of dioxin-like extract into fertile eggs of the chicken or other appropriate species, or adult feeding studies with environmentally derived mixtures should be used to measure embryotoxicity and confirm the relative potency of mixture exposure in the environment.

When Forster's tern eggs in the Great Lakes region contained a median concentration of 23 ppm of total PCBs and 37 ppt of 2,3,7,8-TCDD (2175-ppt total median-estimated TCDD-EQs), hatching success was only 50%. With a median concentration of 7.3 ppm of total PCBs but approximately 1250 ppt of total TCDD-EQs, 42% of the chicks monitored died before fledging, and their body weight growth curves deviated from normal. Mean total PCB levels of above 7.5 ppm for common tern eggs were associated with decreased hatching success. In Caspian terns, egg concentrations in the 20- to 40-ppm range did not seem to alter productivity, possibly because of the larger size and slower metabolism of this species in contrast to the other terns. In double-crested cormorants, a concentration of 7 to 9 ppm of PCB in eggs was associated with approximately 25% embryo mortality.

In great blue heron eggs collected in the field, TCDD at 211 ppt did not decrease hatching success, but edema was apparent and growth reduced. Mean TCDD-EQs of 52 ppt in wood duck eggs, with 70% accounted for by 2,3,7,8-TCDD, were related to suppressed overall productivity in the field and teratogenesis.

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