

*Environmental Toxicology*ACCUMULATION OF POLYCHLORINATED BIPHENYLS FROM FLOODPLAIN SOILS
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Abstract—Eggs, nestlings, and adults of the eastern bluebird (*Sialia sialis*) and house wren (*Troglodytes aedon*) were collected at a polychlorinated biphenyl (PCB)-contaminated site and a reference location on the Kalamazoo River (MI, USA). Eggs and nestlings of eastern bluebirds at the more contaminated location contained concentrations of 8.3 and 1.3 mg/kg, respectively, of total PCBs and 77 and 6.3 ng/kg, respectively, of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs). Eggs, nestlings, and adults of house wrens from the contaminated location contained 6.3, 0.77, and 3.2 mg/kg, respectively, of PCBs and 400, 63, and 110 ng/kg, respectively, of TEQs. Concentrations of total PCBs and TEQs in tissues at the more contaminated location were significantly greater than concentrations in tissues at the reference site for all tissue types of both species. Exposures of the two species studied were different, which suggests that terrestrial-based insectivorous passerine species, foraging in the same area, may have differential exposure to PCBs depending on specific foraging techniques and the insect orders that are targeted. Despite the greater accumulation of PCBs at the more contaminated location, the risk of exposure to PCBs did not exceed the threshold for adverse effects at either location.

Keywords—Bioaccumulation Dioxin equivalents House wren Eastern bluebird Kalamazoo River

INTRODUCTION

The Kalamazoo River in southwestern Michigan, USA, was contaminated with polychlorinated biphenyls (PCBs) when carbonless copy paper was inadvertently mixed into the paper recycling process from 1957 to 1971. In 1986, three dams were removed to their sill, which drained approximately 132 ha of formerly impounded sediment to create a large, contiguous landmass of lowland forest and marsh. Previous studies of the Kalamazoo River quantified concentrations of PCBs in multiple matrices, including soil, sediment, plants, mink, and tree swallows [1–3]. Surveys of in-stream surface sediment (depth, 0–10 cm) indicate that concentrations of PCBs range from less than 0.001 to 153 mg/kg dry weight, with a mean PCB concentration of approximately 3.0 mg/kg dry weight [4,5]. Passerine birds with terrestrial diets were identified as receptors of concern because of the contamination in the formerly impounded floodplain soils, but little is known about the trophic transfer and bioavailability of organochlorines to upper-food-web insectivores. Preliminary sampling quantified PCB concentrations in surficial floodplain soils (depth, 0–25 cm) of the former impoundment to be less than 0.001 to as great as 85 mg/kg dry weight, with mean values of approximately 11 mg/kg dry weight [6–8].

The house wren (*Troglodytes aedon*) and eastern bluebird (*Sialia sialis*) were selected as upper-trophic-level monitors of exposure to PCBs derived from contaminated soil at the Kalamazoo River Superfund site. The American robin (*Turdus migratorius*) was identified as a receptor of concern during a baseline ecological risk assessment [1] because of modeled

contamination levels in its omnivorous diet. Likewise, eastern bluebirds, which also belong to the Turdidae family, feed on invertebrates in close contact with contaminated soil during portions of their life cycles [9]. Thus, it was hypothesized that PCB concentrations in bluebird tissue would be analogous to concentrations in American robin tissues. Primarily an insectivorous species, the house wren was used in the present study to determine risk to avian species with entirely insectivorous diets.

Multiple lines of evidence were employed to characterize risk of exposure to PCBs in terrestrial passerine populations. Concentrations of PCBs were significantly greater in the diet of birds at the more contaminated location than in the diet of birds from the upstream reference location [10]. Concentrations of total PCBs were deemed to be less than a threshold at which effects would be expected, but concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) based on avian World Health Organization (WHO-Avian) toxic equivalency factors [11] may exceed a level of effect. Colocated studies (Kalamazoo River) of reproductive performance of passerine species also were conducted during the same time period and applied to the overall evaluation of risk as an ancillary line of evidence [12,13]. These studies of reproductive performance over a two-year period indicated that house wren fledging success was significantly greater at Trowbridge Impoundment (TB; MI, USA) compared to fledging success at Fort Custer State Recreation Area (FC; MI, USA). Eastern bluebirds had significantly decreased productivity at the more contaminated location relative to the reference location during a three-year study, but 30% of the decrease in productivity between locations can be linked to a single female. Although

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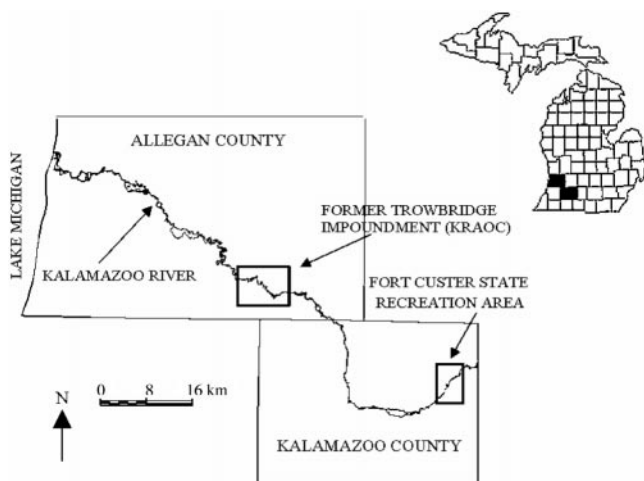


Fig. 1. Map of the Kalamazoo River Area of Concern (KRAOC) and reference site. The inset describes the location of the two counties in Michigan, USA, where studies were conducted. A box designates the boundaries of the upstream reference location, the Fort Custer State Recreation Area, and the Trowbridge Impoundment, located within the KRAOC. (Reprinted from Neigh et al. [3]. Copyright © 2006 Society of Environmental Toxicology and Chemistry. Reprinted by permission of Alliance Communications Group, a division of Allen Press.)

reproductive abnormalities were seen in both species, the cause of the depressed reproductive success was unlikely to be exposure to PCBs and more likely to be differences in habitat suitability, co-contaminants, or prey availability. The final line of evidence examined in the present study was the measured concentration of PCBs in tissues, which was compared to toxicity reference values (TRVs). The primary objectives of the present study were to determine if total PCB and TEQ_{WHO-Avian} concentrations in passerine tissues were different between locations, if all avian species examined in the present study were equally exposed, and if the PCB concentrations in the tissues of passerine birds exceeded concentrations that, based on TRVs, would be expected to result in population-level adverse effects.

MATERIALS AND METHODS

Site details

Eggs, nestlings, and adults were collected from nest boxes between 2001 and 2003 at a location minimally contaminated with PCBs within FC [3] and at TB, a more contaminated location 67 km downstream (Fig. 1). Nest boxes were examined every 1 to 3 d to determine date of clutch initiation and date of hatch. Further physical description of the study sites and nest box locations have been described elsewhere [13].

Tissue sampling

Samples of eggs, nestlings, and adults were collected during the spring and summer of 2001, 2002, and 2003. One pre-determined sample was collected from each nest box for quantification of total PCB and TEQ concentrations. In addition, abandoned and added eggs, dead adults, and dead nestlings were salvaged for additional measurements of PCB and TEQ concentrations. Eggs were sampled 7 to 10 d after laying, and nestlings were sampled 6 to 8 d before fledging. Adult house wrens were sampled during 2002 and 2003 by mist net.

Chemical analysis

The present paper applies a “top-down” methodology based on measured PCB concentrations in eggs, nestlings, and adults to establish exposure in avian species of the Kalamazoo River. Exposure was quantified by congener-specific analysis of approximately 100 PCB congeners, including non-*ortho*-substituted (coplanar) and mono-*ortho*-substituted congeners and was reported as total PCBs. In addition, TEQs were calculated for PCBs based on relative potencies for birds [11]. Concentrations of total PCBs were intended to quantify potential exposure based on all measurable congeners in the environment, whereas concentrations of non-*ortho*-substituted and mono-*ortho*-substituted congeners, reported as TEQ_{WHO-Avian}, evaluated exposure based on the additive toxicity of the PCB congeners known to interact with the aryl hydrocarbon receptor. It has been postulated that the most sensitive measure of toxic effects of PCBs is through the aryl hydrocarbon receptor-mediated pathway and that, because of weathering of the total PCB mixture, the TEQ approach is a more accurate method to estimate exposure and potential toxic effects [14].

Eggs were prepared by removing the eggshell; the yolk and albumen remained for chemical analyses. Nestlings and adults were processed before chemical analyses by removing feathers, beaks, wings, legs, and stomach contents and then homogenizing the whole body in a solvent-rinsed grinder.

Concentrations of PCB congeners and *o,p'*- and *p,p'*-isomers of DDT were determined by previously described methods [3,15]. All samples were analyzed for PCB congeners, and a randomly selected subset of egg samples ($n = 5$ samples/species/site) were analyzed for DDT and DDT isomers. Chemical analyses included pertinent quality-assurance practices, including surrogate spikes, blanks, and duplicates. Acceptable surrogate recoveries ranged from 65 to 135%. Congeners were Soxhlet-extracted and separated by liquid chromatography. Total concentrations of PCBs were quantified by a Perkin-Elmer AutoSystem (Boston, MA, USA) and a Hewlett-Packard 5890 series II gas chromatograph (Wilmington, DE, USA) equipped with ⁶³Ni electron-capture detectors (5% phenylpolysiloxane; length, 30 m; inner diameter, 0.25 mm; film thickness 0.25 μm; Zebtron ZB-5, Phenomenex, Torrance, CA, USA). The method detection limit for PCBs was estimated to be 1×10^{-3} mg/kg wet weight. Total PCB concentrations were calculated as the sum of all resolved PCB congeners and coeluting congeners. Non-*ortho*-substituted PCB congeners (International Union of Pure and Applied Chemistry nos. 77, 81, 126, and 169) were separated from coeluting congeners and interferences by carbon-column chromatography (30-cm \times 15-mm glass columns). Extracts containing the non-*ortho*-substituted (coplanar) congeners were analyzed by a gas chromatograph mass-selective detector (Hewlett-Packard 5890 series II gas chromatograph) equipped with a Hewlett-Packard 5972 series detector. Detection limits varied among samples, but the mean detection limit for all samples was less than 100 ng/kg wet weight.

TEQ computation

Toxic equivalents in bird tissues were calculated by multiplying individual concentrations of aryl hydrocarbon receptor-active PCB congeners by their respective, bird-specific World Health Organization toxic equivalence factors [11]. Total TEQ_{WHO-Avian} concentrations were calculated as the sum of detectable individual non-*ortho*-substituted and mono-*ortho*-substituted PCB congeners (PCBs 77, 81, 105, 118, 126, 156,

157, 167, and 169). When at least one of the coplanar congeners (except for PCB 169) could not be quantified because of interfering compounds, the sample was removed from statistical analyses. Congener 169 was not regularly detected in the samples. For congeners that occurred at concentrations less than the limit of quantification, a proxy value of half the limit of quantification was assigned to report a conservative estimate of exposure. The true value of the congeners below the limit of quantification lies somewhere between zero and the detection limit, which was determined based on a congener-specific basis. For the present study, all but eight samples had at least one congener with a proxy value assigned. This method had minimal effects on samples taken from TB, but because more congeners were below the limit of quantification at the reference area, FC, the effect was greater [16]. Some mono-*ortho*-substituted congeners coeluted with other congeners, so to report the most conservative estimate, the total concentration of the coelution group was considered to belong to its respective mono-*ortho*-substituted congener.

Statistical analyses

For statistical analyses, each nest box was treated as a separate experimental unit, and values were reported on a per-box basis. All nesting attempts were treated as separate and individual observations. Normality was assessed with the Kolmogorov-Smirnov one-sample test with Lilliefors transformation, and homogeneity of variance was verified by the *F* test. All concentration data were log-transformed before analyses. All parametric data were analyzed by Student's *t* test or two-way analysis of variance (ANOVA), and nonparametric data were analyzed by Mann-Whitney *U* or Kruskal-Wallis tests. The criterion for significance used in all tests was $p < 0.05$.

Biomagnification and accumulation rates

Biomagnification factors and accumulation ratios were calculated for total PCBs and TEQ_{WHO-Avian} as the lipid-normalized concentration in the upper trophic level divided by the lipid-normalized concentration in the lower trophic level. Accumulation was calculated based on live-sampled adults and nestlings and fresh sampled eggs.

Accumulation rates were calculated to determine the mass of PCBs gained by the nestlings over the nestling period through the diet. The mass of PCBs in the egg was subtracted from the total mass in the nestlings. The accumulation rate was calculated as the difference in total mass of PCBs and TEQ_{WHO-Avian} between nestlings and eggs divided by the nestlings' days of life [17].

Assessment of risk

The risk of potential effects on passerine health because of exposure to PCBs existing in Kalamazoo River floodplain soils was estimated by comparing concentrations of PCBs to established TRVs for eggs, nestlings, and adults of the house wren and eastern bluebird [18]. Risk was quantified as hazard quotients (HQs), which were calculated as the concentration of PCBs in the tissue divided by the TRV.

Toxicity reference values were derived from the results of studies reported in the literature. Studies were chosen for terrestrial passerine species based on several criteria. The studies judged to be the most suitable for TRV calculation used wild-life species chronically exposed over sensitive life stages, eval-

uated endpoints ecologically relevant to PCB exposure, had minimal co-contamination, and were multiyear studies.

Few studies are available for the species used in the present study except for an examination of American robin exposure and effects at the Housatonic River (NY, USA), which found no correlation between reproduction and PCB concentrations [19]. American robin eggs at the Housatonic site contained a mean PCB concentration of 84 mg/kg wet weight. From this field study, it can be concluded that the threshold for effects of PCBs on robins is greater than 84 mg/kg wet weight. Studies of European starlings (*Sturnus vulgaris*) also were considered for derivation of an appropriate TRV. European starlings exhibited a decrease in parental attentiveness and increased mortality at two PCB sites compared to a reference site when Aroclor 1254 concentrations in tissues (mean \pm SD) were 13.6 ± 2.9 mg PCB/kg wet weight in eggs, 5.9 ± 0.8 mg PCB/kg wet weight in nestlings, and 15.9 ± 5.3 mg PCB/kg wet weight in adults [20]. The study of starlings did not report reduced hatching success, and similar embryo mortality was observed at another study site contaminated with metals but with relatively low PCB concentrations. It has been suggested that adult incubation anomalies or co-contamination by dioxin may lead to embryo mortality, so these egg concentrations are an unacceptable basis for a TRV for the Kalamazoo River [20]. Effects found in adult starlings also may be affected by the fitness of nestlings, which may reduce feeding calls and, therefore, affect parental care. The starling study fulfilled several of the given criteria by taking place over several years, by measuring ecologically relevant endpoints, and by measuring concentrations at critical life stages in a terrestrial wild avian species. The confounding factors in the study by Halbrook et al. [20], and the fact that Henning et al. [19] did not observe abnormalities in reproduction at much greater concentrations, suggests that the figure reported by Henning et al. [19] is a more appropriate choice for the TRV based on the no-observed-adverse-effect level for PCBs of 84 mg/kg.

The TRV selected as a threshold for effects based on TEQ concentrations was that suggested by the U.S. Environmental Protection Agency [21] (<http://www.epa.gov/hudson/reports.htm>). That study investigated TEQ concentrations in tree swallows at the Hudson River (NY, USA) over a two-year period, was conducted on wild passerine birds, evaluated relevant ecological endpoints during critical life stages, and co-contamination was considered to be inconsequential based on the study. Tree swallows exhibited abnormal plumage, decreased hatching success, and increased abandonment during one year, but these effects were not observed the following year. The inconsistency of effects over both years suggests that the TEQ value of 13,000 ng/kg from that study should not be used as a lowest-observed-adverse-effect level but could be appropriate for a no-observed-adverse-effect level. The greatest concentration of TEQs in the year without effects was 13,000 ng/kg wet weight. No lowest-observed-adverse-effect level could be established for TEQs.

RESULTS

Gross morphology and abnormalities

No gross physiological abnormalities were observed in 180 eastern bluebird nestlings and 362 house wren nestlings, or in approximately 75 eastern bluebird adult pairs and 108 house wrens adult pairs, but some egg abnormalities were present in house wrens of the 305 eastern bluebird eggs and 607 house wren eggs evaluated. These abnormalities include desiccated

Table 1. Mean concentrations of total polychlorinated biphenyls (PCBs) and lipid content of eastern bluebirds and house wrens at the Fort Custer reference area and the Trowbridge Impoundment on the Kalamazoo River (MI, USA) Area of Concern

	Fort Custer			Trowbridge		
	<i>n</i>	% Lipid	PCB (mg/kg)	<i>n</i>	% Lipid	PCB (mg/kg)
Eastern bluebird						
Egg						
2001	2	6.0 (2.4) ^a	0.25 (0.077)	2	8.2 (3.1)	11 (2.9)
2002	7	8.4 (2.7)	0.13 (0.076)	3	15 (15)	11 (3.9) ^b
2003	5	8.7 (3.1)	0.19 (0.13)	2	5.9 (0.40)	1.8 (0.32) ^b
Total	14	8.2 (2.8)	0.17 (0.10)	7	11 (10)	8.3 (5.1) ^c
Nestling						
2001	6	5.7 (0.84)	0.011 (0.003)	1	4.8 (NA)	0.41 (NA)
2002	5	5.0 (0.42)	0.010 (0.004)	3	4.6 (1.2)	1.4 (2.1) ^b
2003	6	4.0 (1.2)	0.013 (0.009)	2	5.0 (1.3)	1.8 (0.83) ^b
Total	17	4.9 (1.1)	0.011 (0.006)	6	4.8 (0.96)	1.3 (1.4) ^d
House wren						
Egg						
2001	8	19 (17)	0.14 (0.15)	6	9.3 (5.4)	4.5 (2.0) ^b
2002	6	7.1 (4.0)	0.10 (0.053)	8	11 (6.2)	6.8 (5.6) ^b
Total	14	14 (14)	0.12 (0.12)	14	10 (5.7)	5.8 (4.4) ^d
Nestling						
2001	7	4.5 (1.8)	0.029 (0.024)	5	5.1 (1.4)	0.8 (0.79) ^b
2002	6	6.3 (2.6)	0.010 (0.005)	11	6.9 (2.1)	0.71 (0.62) ^b
2003	NA ^e	NA	NA	1	2.4 (NA)	1.1 (NA)
Total	13	5.3 (2.3)	0.020 (0.020)	17	6.1 (2.2)	0.77 (0.64) ^f
Adult						
2002	5	5.5 (1.1)	0.087 (0.029)	6	5.2 (1.3)	3.6 (2.3) ^b
2003	3	5.3 (0.86)	0.045 (0.022)	3	5.6 (2.1)	2.5 (1.6) ^b
Total	8	5.4 (0.93)	0.072 (0.033)	9	5.4 (1.5)	3.2 (2.1) ^d

^a Values in parentheses represent the SD.

^b Mean concentration was significantly greater than at the Fort Custer reference site (Student's *t* test or Kruskal-Wallis; $p < 0.05$).

^c Mean concentration was significantly greater than at the Fort Custer reference site, but interactions between location and year exist two-way analysis of variance [ANOVA]; location, $p = 0.000$; year, $p = 0.030$; location \times year, $p = 0.020$; $n = 21$).

^d Mean concentration was significantly greater than at the Fort Custer reference site (two-way ANOVA; $p < 0.000$).

^e NA = not available.

^f Mean concentration was significantly greater than at the Fort Custer reference site, but year was a significant cofactor (two-way ANOVA; location, $p = 0.000$; year, $p = 0.029$; location \times year, $p = 0.662$; $n = 30$).

contents, irregular shape, and abnormal texture. A proportion of house wren nests contained eggs with shells that appeared to be thinned (4% of all nests, 12% of TB nests), but the thickness of the shells was not measured.

Total PCB concentrations in tissue

Polychlorinated biphenyl concentrations in eggs, nestlings, and adults (house wrens only) from TB were significantly greater than those at the reference location (FC; two-way ANOVA: $p < 0.001$) for both eastern bluebirds and house wrens (Table 1). Year was a significant cofactor for eastern bluebird eggs (two-way ANOVA: year, $p = 0.030$, $n = 21$) and house wren nestlings (two-way ANOVA: year, $p = 0.029$, $n = 30$), and the interaction between location and year was significant for eastern bluebird eggs (two-way ANOVA: location \times year, $p = 0.020$, $n = 21$). For all tissues analyzed, PCB concentrations differed by 39- to 118-fold. The trend in concentration differences between sites was the same when concentrations were lipid-normalized. In general, eastern bluebirds at TB had the greatest concentrations of PCBs in eggs and nestlings (Table 1), but the greatest tissue concentration in the study was

measured in a house wren egg from TB in 2002 (PCB concentration, 26 mg/kg wet wt).

Dichlorodiphenyltrichloroethane metabolites were detected in all egg samples from a random subset analyzed for DDT concentrations. Concentrations of *p,p'*-dichlorodiphenyldichloroethylene (DDE) comprised 95 to 98% of the total sum of all DDT metabolites in eastern bluebirds and house wrens. Total DDT is defined as the sum of all isomers measured. Concentrations in eastern bluebirds were sevenfold greater than those in house wrens at FC and three-fold greater than those in house wrens at TB. Concentrations of total DDT (mean \pm SD) contained in eastern bluebird eggs were 2.1 ± 1.1 mg/kg wet weight at TB and 1.5 ± 1.8 mg/kg wet weight at FC, but differences between sites were not statistically significant (Student's *t* test: $p = 0.317$). House wren eggs at TB contained DDT at a concentration of 0.66 ± 0.87 mg/kg wet weight, and eggs at FC contained DDT at a concentration of 0.20 ± 0.21 mg/kg wet weight. Again, however, differences between locations were not statistically significant (Student's *t* test: $p = 0.135$). The only statistically significant difference was for concentrations of *p,p'*-dichlorodiphenyldichloroethane in eggs

Table 2. Mean concentrations of dioxin equivalents (TEQs) and relative potency in eastern bluebird and house wren tissues sampled from the Fort Custer reference area and the former Trowbridge Impoundment area (both, MI, USA) contaminated with polychlorinated biphenyls (PCBs)

	Fort Custer			Trowbridge		
	<i>n</i>	TEQ (ng/kg)	Relative potency (ng TEQ/kg PCB)	<i>n</i>	TEQ (ng/kg)	Relative potency (ng TEQ/kg PCB)
Eastern bluebird						
Egg						
2001	2	10 (10) ^a	37 (29)	2	70 (4.8)	7.4 (0.63)
2002	NA ^b	NA	NA	1	220 (NA)	25 (NA)
2003	1	2.2 (NA)	47 (NA)	2	15 (10)	8.5 (3.3)
Total	3	7.6 (8.6)	40 (21)	5	77 (82) ^c	11 (8.0)
Nestling						
2001	6	1.5 (1.1)	130 (99.0)	1	3.2 (NA)	7.8 (NA)
2002	5	1.3 (0.34)	150 (71)	3	7.3 (4.9) ^c	25 (18) ^d
2003	6	1.2 (0.20)	120 (45)	2	6.3 (4.4) ^c	3.3 (0.91)
Total	17	1.3 (0.64)	130 (71)	6	6.3 (4.0) ^c	15 (16)
House wren						
Egg						
2001	4	8.0 (4.7)	300 (280)	5	160 (54) ^c	40 (16) ^d
2002	4	9.2 (3.6)	150 (130)	6	530 (380) ^c	62 (20)
Total	8	8.6 (3.9)	220 (220)	11	360 (330) ^c	52 (21) ^f
Nestling						
2001	7	1.7 (1.2)	74 (53)	5	73 (63) ^c	94 (35) ^g
2002	6	1.1 (0.43)	130 (66)	11	61 (43) ^c	97 (42) ^d
2003	NA	NA	NA	1	41 (NA)	38 (NA)
Total	13	1.4 (0.96)	100 (64)	17	63 (47) ^f	93 (40) ^d
Adult						
2002	5	5.2 (2.6)	66 (43)	6	120 (40) ^c	43 (30)
2003	3	10 (8.7)	260 (180)	3	91 (91) ^c	47 (38)
Total	8	7.1 (5.7)	140 (140)	9	110 (57) ^f	45 (30) ^h

^a Values in parentheses represent the SD.

^b NA = not available.

^c Mean concentration was significantly greater than at the Fort Custer reference site (Student's *t* test; *p* < 0.05).

^d Mean relative potency was significantly lesser than at the Fort Custer reference site (Student's *t* test; *p* < 0.05).

^e Mean concentration was significantly greater than at the Fort Custer reference site, but year was a significant cofactor (two-way analysis of variance [ANOVA]; location, *p* = 0.000; year, *p* = 0.020; location × year, *p* = 0.101; *n* = 19).

^f Mean concentration was significantly greater than at the Fort Custer reference site (two-way ANOVA; *p* < 0.000).

^g Mean relative potency was significantly greater than at the Fort Custer reference site (Student's *t* test; *p* < 0.05).

^h Mean relative potency was significantly lesser than at the Fort Custer reference site (two-way ANOVA; *p* < 0.000).

of house wrens between sites (Student's *t* test: *p* = 0.021), but sample sizes were small (*n* = 10). Addled eggs of eastern bluebirds at FC contained 2.6-fold greater concentrations of lipid-normalized total DDT than fresh eggs contained, but addled eggs of eastern bluebirds at TB had lipid-normalized total DDT concentrations 82% those in fresh eggs from TB. Addled eggs of house wrens had lipid-normalized concentrations 7.4- and 5.7-fold greater than those in fresh eggs at FC (*n* = 5) and TB (*n* = 5), respectively. Samples sizes were insufficient to perform statistical tests comparing addled and fresh eggs.

Dioxin equivalents (TEQ_{WHO-Avian})

For all tissue matrices in both species, TEQ_{WHO-Avian} concentrations were significantly greater at TB than at FC (Student's *t* test or two-way ANOVA: *p* < 0.001) (Table 2). In the tissues of eastern bluebirds, TEQ_{WHO-Avian} concentrations were 5- to 10-fold greater at TB than at FC, whereas TEQ_{WHO-Avian} concentrations in the tissues of house wrens were 15- to 47-

fold greater at TB compared to FC. An addled egg of a house wren contained the greatest TEQ_{WHO-Avian} concentration for all tissues in the study (1,800 ng/kg wet wt).

The relative contributions of non-*ortho*-substituted and mono-*ortho*-substituted congeners to total TEQ_{WHO-Avian} were evaluated at each site. Congener 169 was not detected in 67 to 69% of all samples for eastern bluebirds and house wrens, and in the samples in which PCB 169 was detected, it represented less than 1% of the total TEQ_{WHO-Avian} concentration. House wren eggs, nestlings, and adults from TB were the only matrix in which PCBs 77, 81, and 126 were all detected in the majority of samples. Congeners 77, 81, and 126 comprised the greatest proportion of the total TEQ_{WHO-Avian} concentration for all species (Fig. 2). In house wrens at FC, PCB 126 comprised the greatest portion of the total TEQ_{WHO-Avian} concentration, but at TB, PCB 77 made the greatest contribution to the TEQ_{WHO-Avian} concentration. Contributions of every congener to the total TEQ_{WHO-Avian} concentration were significantly

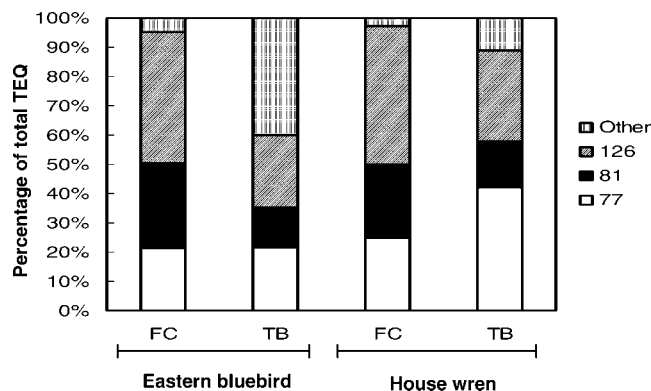


Fig. 2. Contribution of individual non-*ortho*-substituted and mono-*ortho*-substituted congeners to dioxin equivalents (TEQs) in the tissues of passerine species at the Fort Custer State Recreation Area (FC) and the Trowbridge Impoundment (TB), Michigan, USA.

different between FC and TB for house wrens (Student's *t* test: $p < 0.05$). In eastern bluebirds, PCBs 81 and 126 comprised significantly different proportions of the TEQ_{WHO-Avian} concentration between sites (Student's *t* test: $p = 0.001$), but PCB 77 was similar between locations (Student's *t* test: $p > 0.05$). Congener 118 was frequently detected (83–100% of samples from both locations and all matrices and species).

Biomagnification

Ratios of concentrations between life stages of avian species for lipid-normalized and wet-weight total PCBs and TEQs_{WHO-Avian} were less than 1.0 for house wren eggs to nestlings and greater than 1.0 for house wren nestlings to adults at FC and TB. Eastern bluebird egg to nestling ratios (< 1.0) were less than those in house wrens (Table 3). Ratios of biomagnification between egg and nestling life stages for eastern bluebirds and house wrens were greater at FC than at TB when comparing TEQs_{WHO-Avian}.

Daily accumulation rates were calculated based on total PCBs and TEQs_{WHO-Avian}. Total PCB daily accumulation rates on a wet-weight basis for house wren nests were 0.0068 $\mu\text{g}/\text{d}$ at FC during 2001 and 0.32 and 0.40 $\mu\text{g}/\text{d}$ at TB during 2001 and 2002, respectively. Daily accumulation rates based on TEQs_{WHO-Avian} on a wet-weight basis were 1.9 pg/d at FC during 2001 and 26 and 16 pg/d at TB during 2001 and 2002, respectively. Accumulation rates on a wet-weight basis for eastern bluebirds at FC were 0.0053 and 0.054 $\mu\text{g}/\text{d}$ for 2002 and 2003, respectively. Total TEQ_{WHO-Avian} accumulation rates on a wet-weight basis for eastern bluebirds were 2.1 and 12 pg/d at FC during 2002 and 2003, respectively.

Relative potency (ng TEQ/kg PCB) of PCB mixtures in

samples were calculated by dividing the TEQ_{WHO-Avian} concentration by the total PCB concentration for each sample (Table 3). The resulting relative potency is an indicator of the overall effect of environmental weathering and differential bioaccumulation on the toxicity of the PCB mixture [22]. Relative potencies generally were greater and more variable at FC than at TB for both species, because concentrations of PCBs generally were low and TEQ_{WHO-Avian} concentrations were driven by the detection limit of the analytical method. Ratios of relative potencies among different sample types reflect changes in the potency of the PCB mixture. At TB, the potency ratio from egg to nestling was 1.3 and 1.8 for eastern bluebird and house wren, respectively, and the potency ratio at FC for the same life stages of these species was 3.3 and 0.45, respectively. Ratios for nestling to adult were 0.48 at TB and 1.4 at FC for house wrens.

DISCUSSION

DDT concentrations

Little is known about the differences in species sensitivity between other birds and eastern bluebirds or house wrens. A DDE concentration of 2.0 mg/kg in eggs has been suggested as a threshold for effects in bald eagles (*Haliaeetus leucocephalus*) [23]. In another passerine bird, the tree swallow, total DDT residue levels were 1.3 mg/kg in the attended eggs and 2.8 mg/kg in unattended eggs [24]. Although the value associated with unattended eggs may represent a threshold for effects on behavior, the likely DDE threshold for effects on reproduction of passerine birds is near 8 or 9 mg/kg [24]. No eggs of either species at the Kalamazoo River contained DDE concentrations greater than the threshold for reproductive effects, and mean concentrations were fourfold less than this same threshold. Concentrations of DDE and DDT in the eggs of eastern bluebirds were near the threshold for effects on behavior, but the eggs of house wrens had total DDT concentrations threefold less than that threshold. The co-contaminant, DDT, exists at many locations, including the Kalamazoo River Area of Concern, which makes it difficult to attribute abnormal reproduction or behavior to the presence of one contaminant versus another. At both Kalamazoo River locations, DDT is detected, but the presence of DDT is not expected to be causing reproductive impairment in the species examined.

Total PCBs

Limited data regarding passerine birds exposed to PCBs through terrestrial diets are available for comparison with the results of the present study. Tissue concentrations were similar between species at FC and other species at other relatively

Table 3. Ratios of accumulation between life stages of the eastern bluebird and house wren based on the mean lipid-normalized and wet-weight^a total polychlorinated biphenyls (PCBs) and dioxin equivalents (TEQs) in eggs and live-sampled nestlings at the Fort Custer State Recreation Area (FC) and the Trowbridge Impoundment (TB; both MI, USA)

	Accumulation ratio (Total PCBs)				Accumulation ratio (TEQs)			
	Eastern bluebird		House wren		Eastern bluebird		House wren	
	FC	TB	FC	TB	FC	TB	FC	TB
Egg to nestling	0.11 (0.060)	0.20 (0.62)	0.79 (0.23)	0.22 (0.17)	0.31 (0.13)	0.0094 (0.48)	0.50 (0.25)	0.34 (0.24)
Nestling to adult	NA ^b	NA	3.2 (3.4)	4.4 (4.2)	NA	NA	4.3 (4.8)	1.9 (1.7)
Adult to egg	NA	NA	0.40 (1.3)	1.0 (1.4)	NA	NA	0.47 (0.82)	1.6 (2.5)

^a Wet-weight accumulation ratios are given in parentheses.

^b NA = not available.

Table 4. Bioaccumulation factors for wet-weight concentrations of total polychlorinated biphenyls (PCBs) and dioxin equivalents (TEQs) from the diet (average potential daily dose) [10] to bird tissues at the Fort Custer State recreation area (FC) and the Trowbridge Impoundment (TB; both MI, USA)

	Bioaccumulation factors (total PCB)				Bioaccumulation factors (TEQ)			
	Eastern bluebird		House wren		Eastern bluebird		House wren	
	FC	TB	FC	TB	FC	TB	FC	TB
Diet to egg	10	16	6.4	45	2.8	1.1	8.9	13
Diet to nestling	0.64	2.5	0.91	5.5	0.48	0.09	0.56	2.0
Diet to adult	NA ^a	NA	3.3	23	NA	NA	2.8	3.5

^a NA = not available.

uncontaminated sites in the region [19,25]. Total concentrations of PCBs in house wren and eastern bluebird eggs and nestlings at TB were less than concentrations contained in American robin eggs and nestlings at the Housatonic River [19], but concentrations of PCBs at TB were greater than concentrations in red-winged blackbird (*Agelaius phoeniceus*) eggs at locations in the Great Lakes–St. Lawrence River Basin (ON, Canada) with the exception of one site [25]. Although few comparisons to other studies can be made for these specific species, the fact remains that total PCB concentrations were significantly greater at TB than at FC in all matrices examined. Concentrations in the tissues corresponded to greater concentrations of total PCBs in the soil at TB compared to the soil at FC [26], which suggests that concentrations in the tissues of the species examined reflected local contamination in the soil.

Tissue concentrations, expressed on a wet-weight basis, generally were greater in eastern bluebirds than in house wrens. When concentrations in all matrices were lipid-normalized, concentrations in house wrens and eastern bluebirds were more similar, but eastern bluebirds still contained greater mean total concentrations of PCBs. Differences in concentrations of PCBs between house wrens and eastern bluebirds likely were indicative of on-site dietary exposure. For eggs and nestlings at TB, eastern bluebirds contained total PCB concentrations 1.7-fold greater than those of house wrens. When site-specific average potential daily doses were calculated for TB, exposure in the diet of eastern bluebirds was found to be 3.7-fold greater than that of house wrens [10]. At FC, the present study measured greater daily accumulation rates for the nestlings of house wrens compared to eastern bluebirds, which suggests that they obtain greater concentrations from the diet at FC. Indeed, dietary exposure for house wrens was greater than that for eastern bluebirds at FC. Further study is needed, but general trends in concentration tissue gradients, described as daily accumulation rates, appear to reflect daily dietary exposure.

Dioxin equivalent (TEQ) concentrations

One measure of the toxicity of PCBs is based on toxic equivalency factors, which compare the toxicity of individual PCB congeners to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [14]. The greatest TEQ_{WHO-Avian} values were consistently measured in adults and eggs of house wrens from TB. This was opposite of what was measured for total PCB concentrations for which eastern bluebirds contained consistently greater concentrations in their tissues than house wrens. Mono-*ortho*-substituted and non-*ortho*-substituted (coplanar) congeners, which comprise TEQ_{WHO-Avian} concentrations, apparently accumulate between life stages to a greater degree in house wrens than in eastern bluebirds. House wrens had TEQ_{WHO-Avian} concentrations in

eggs 5.7-fold greater than those in eastern bluebird eggs, and house wren nestlings contained TEQ_{WHO-Avian} concentrations 10-fold greater than those in eastern bluebird nestlings. Bioaccumulation factors from the diet to tissues (calculated from data reported by Neigh et al. [10]) also suggest that accumulation of both TEQ_{WHO-Avian} and total PCBs were greater in house wrens than in eastern bluebirds, especially at TB (Table 4). Unlike the relationship between total PCB concentrations and dietary exposure, TEQ_{WHO-Avian} for dietary exposures do not correspond with tissue concentrations or accumulation rates. Eastern bluebirds were found to have greater accumulation rates and dietary exposure to TEQ_{WHO-Avian} compared to house wrens at both TB and FC [10].

For both species, PCBs 81 and 126 contributed more to the total TEQs at FC than at TB. However, these congeners were detected in less than 30% of the samples from FC, so the detection limit of each congener contributed greatly to the analyses, being that half the detection limit was used to calculate contributions in the remaining samples. At TB, PCB 77 was the greatest contributor in most matrices. The contribution of PCB 77 to total TEQ_{WHO-Avian} increased from eggs to nestlings of house wrens, but it decreased from eggs to nestlings of eastern bluebirds. Congener 126 followed an opposite trend by contributing more to the total TEQ_{WHO-Avian} between eggs and nestlings of eastern bluebirds and less between eggs and nestlings of house wrens. Adult concentrations did not follow any discernible trend. Adults likely are exposed to a variety of contamination sources over a lifetime, whereas patterns in nestlings more closely reflect local sources of contamination [25,27].

Biomagnification

Ratios of accumulation were similar for terrestrial species and tree swallows from the Kalamazoo River [3]. Ratios ranging from approximately 2.0 to 5.0 between nestlings and adults suggest that birds are accumulating PCBs from their diet, but ratios less than 1.0 between eggs and nestlings suggest that the concentration or the rate of consumption is not great enough in nestlings to overcome growth dilution. Tree swallows from the Great Lakes–St. Lawrence River Basin also followed a similar trend, with eggs having greater concentrations than nestlings [25]. The greater accumulation ratio at FC compared to TB between eggs and nestlings for eastern bluebirds and house wrens may be a result of variation around the limits of detection for chemical analysis because of the small sample mass of many eggs.

Daily accumulation rates were less in house wrens at the Kalamazoo River than in other bird species at locations contaminated with PCBs. House wrens at TB had accumulation rates more than 10-fold less than the accumulation rates for

tree swallows at the same location [3] but from 10- to 240-fold less than those at locations on the Housatonic River [28]. Likewise, dietary exposure was estimated to be six- to eight-fold greater for concentrations of PCBs in the diet of the tree swallow compared to that of the house wren [10]. House wrens and eastern bluebirds also had accumulation rates that were less than those in black-crowned night heron (*Nycticorax nycticorax*) and Forster's tern (*Sterna forsteri*) from Green Bay (WI, USA) [17,29].

Relative potency and potency ratios calculated for birds were intended to describe the increase or decrease in toxicity of the PCB mixture at the study locations. Potency ratios greater than 1.0 were observed for most comparisons, which suggests that the mixture of congeners in the food chain become more potent at increasingly greater trophic levels through the bioaccumulation of aryl hydrocarbon receptor-active congeners [30]. Relative potencies at FC were influenced by several samples with low concentrations of PCBs or concentrations of $TEQ_{S_{WHO-Avian}}$ driven by the detection limit of the analytical method. These scenarios led to greater relative potencies in FC samples compared to TB samples, but the ratio of the relative potencies between trophic levels at each location (potency ratio) was similar. Ubiquitous congeners, such as PCBs 118 and 156, appear to bioaccumulate regardless of local sources of contamination, which contributes to the overall $TEQ_{S_{WHO-Avian}}$ concentrations at the reference location. The presence of these ubiquitous congeners in the diet adds to the increased potency of the internal mixture as nestlings and adults feed. Congener 126, which has the greatest toxic equivalency factors for PCBs in avian species [11], contributes the greatest relative percentage to the total $TEQ_{S_{WHO-Avian}}$ concentrations in samples from FC. The contribution of this congener also increases with trophic level, which suggests that PCB 126 is not easily metabolized and bioaccumulates through the food web. These factors led to the increase in relative potency between trophic levels at FC. At TB, PCBs and 126 contribute large portions to the total $TEQ_{S_{WHO-Avian}}$ because of the presence of these congeners in the original mixture released at the site [1].

Assessment of risk based on multiple lines of evidence

Multiple lines of evidence were used to assess the risk of PCB exposure to terrestrial birds inhabiting the former impoundments at the Kalamazoo River Superfund site. Exposure assessments of PCB concentrations in eggs, nestlings, and adults are described here. A number of reproductive parameters and dietary exposures, calculated as daily dietary dose, also were assessed for both eastern bluebirds and house wrens [10,12]. Potential adverse effects based on exposure data were determined by calculating HQs derived from literature-based TRVs. All HQs at FC for total PCBs and $TEQ_{S_{WHO-Avian}}$ were less than 0.002 for all tissues of house wrens and eastern bluebirds. Similarly, HQs at TB based on the mean and upper 95% confidence limit were less than 0.16 for all species and tissue types. In addition, no individual tissue concentration of PCBs exceeded any of the threshold values for effects.

To assist in the evaluation of risk, dietary exposures, which have been described in detail elsewhere [10], were examined concurrently as another way of estimating exposure and subsequent risk potential [18]. Because little toxicity data were available for terrestrial passerine birds, dietary exposure studies reported risk based on a range of HQ values. Concurrent studies suggest little risk to terrestrial passerines exposed to

PCBs in the diet [10]. Given this, the only exposure calculations resulting in HQs greater than 1.0 were $TEQ_{S_{WHO-Avian}}$ in the diet of eastern bluebirds and house wrens in the exposed area (TB) based on the most conservative TRVs. Hazard quotient values, based on more appropriate TRVs calculated from field measurements of PCBs in wild passerine birds [21], were less than the threshold for effects based on the mean dietary exposure [10]. It is expected that actual risk lies somewhere within the range of calculated HQs, but it likely lies closer to the hazard quotient based on the field measurements of wild passerine birds. The presence of PCBs in tissues at concentrations less than a threshold for effects suggests that the HQ estimate based on dietary exposure of wild passerine birds likely is a better estimate of risk.

The final line of evidence involving reproductive health suggested some reproductive impairment that likely was attributable to other factors [12]. Depressed reproduction in house wrens and eastern bluebirds was observed in some, but not all, nesting attempts and in some, but not all, parameters evaluated. Sample sizes were small, especially for eastern bluebirds, and a 40% decrease in reproductive success at TB during 2001 could be attributed to a single female. Conversely, fledging success, which is the most important measure of reproductive success, was significantly greater in the PCB-contaminated area for house wrens. Similar field studies found no significant effect on reproductive performance of passerine birds at concentrations found in birds of the Kalamazoo River, or at even greater concentrations [28,31]. Likewise, studies of reproductive success conducted at the Kalamazoo River did not find statistically significant decreases in reproductive health of tree swallows at the contaminated TB location relative to the FC reference location [13]. Another study examined dioxin exposure to eastern bluebird eggs, and no effects were observed at toxic equivalent concentrations greater than those contained in bluebird eggs at the Kalamazoo River [32]. Without observing consistency across all nesting attempts and indices, and because of the contribution of a few adults to the reproductive measurements at TB, it cannot be concluded that the observed responses were caused by exposure to PCBs but, rather, were caused by other exogenous factors, such as co-contaminants, habitat suitability, and prey availability.

The results of the lines of evidence for exposure and effects in the present study suggest that concentrations of PCBs to which house wrens and eastern bluebirds are exposed are less than the threshold for effects. Few studies have directly investigated the sensitivity of these species to PCBs, so uncertainties exist regarding the applicability of TRVs derived from other species relative to the susceptibility of the terrestrial species in the present study to the effects of PCBs. Based on the lines of evidence, there does not appear to be substantial risk of adverse effects for terrestrial passerine birds exposed to PCBs originating from the floodplain soils of the Kalamazoo River.

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