Passerine Exposure to Primarily PCDFs and PCDDs in the River Floodplains Near Midland, Michigan, USA

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Abstract 
House wren (Troglodytes aedon), tree swallow (Tachycineta bicolor), and eastern bluebird (Sialia sialis) tissues collected in study areas (SAs) downstream of Midland, Michigan (USA) contained concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs) greater than in upstream reference areas (RAs) in the region. The sum of concentrations of PCDD/DFs (ΣPCDD/DFs) in eggs of house wrens and eastern bluebirds from SAs were 4- to 22-fold greater compared to those from RAs, whereas concentrations in tree swallow eggs were similar among areas. Mean concentrations of ΣPCDD/DFs and sum 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (ΣTEQsWHO-Avian), based on 1998 WHO avian toxic equivalency factors, in house wren and eastern bluebird eggs ranged from 860 (430) to 1500 (910) ng/kg wet weight (ww) and 470 (150) to 1100 (510) ng/kg ww, respectively, at the most contaminated study areas along the Tittabawassee River, whereas mean concentrations in tree swallow eggs ranged from 280 (100) to 760 (280) ng/kg ww among all locations. Concentrations of ΣPCDD/DFs in nestlings of all studied species at SAs were 3- to 50-fold greater compared to RAs. Mean house wren, tree swallow, and eastern bluebird nesting concentrations of ΣPCDD/DFs and ΣTEQsWHO-Avian ranged from 350 (140) to 610 (300) ng/kg ww, 360 (240) to 1100 (860) ng/kg ww, and 330 (100) to 1200 (690) ng/kg ww, respectively, at SAs along the Tittabawassee River. Concentrations of ΣTEQsWHO-Avian were positively correlated with ΣPCDD/DF concentrations in both eggs and nestlings of all species studied. Profiles of relative concentrations of...
Concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs) in soil and sediment in portions of the Tittabawassee and Saginaw rivers and associated floodplain downstream of Midland, Michigan (USA) are greater than the background concentration for the region (Hilscherova et al. 2003). Potential sources of the PCDD/DFs are historical production of organic chemicals and on-site storage and disposal, prior to the establishment of modern waste management protocols (Amendola and Barna 1986). The congener profile of the PCDD/DFs is dominated by 2,3,4,7,8-pentacloro dibenzofuran (PeCDF), which is consistent with the waste stream of a chloralkali plant using a graphite-electrode process (Rappe et al. 1991; Svensson et al. 1993). The lipophilic nature and slow degradation rates of these compounds (Mandal 2005), combined with consistent inundation of the floodplain, led to the continued presence of PCDD/DFs in floodplain soils and sediments.

PCDD/DFs occur in the environment as mixtures and have potential to be accumulated through the food web. Greater than background concentrations of dioxinlike compounds have been previously measured in upper trophic level organisms downstream of Midland, Michigan. The Michigan Department of Public Health first issued fish consumption advisories in 1978 based on elevated concentrations of PCDFs, PCDDs, and polychlorinated biphenyls (PCBs) in tissues of fish collected downstream of Midland. Wild game consumption advisories were issued in 2004 based on elevated concentrations in deer and turkey.

One set of toxicological responses to dioxinlike compounds is mediated through the aryl hydrocarbon receptor (AhR). These AhR-mediated responses include carcinogenicity, immunotoxicity, and adverse effects on reproduction, development, and endocrine functions (van den Berg et al. 1998). In particular, AhR-mediated compounds have been shown to decrease hatching success, adult responsiveness, and immune function and to increase CYP1A enzyme induction of birds (Hoffman et al. 1998; Nosek et al. 1992a, 1993; Powell et al. 1996, 1998; Thiel et al. 1988). Recent findings provide evidence of the molecular basis for variation in avian species sensitivity to dioxinlike compounds (Head et al. 2008; Karchner et al. 2006).

Three cavity-nesting passerine birds were selected for study to provide data for a site-specific ecological risk assessment of the Tittabawassee and Saginaw rivers and associated floodplain downstream of Midland, Michigan using the multiple-lines-of-evidence approach described by Fairbrother (2003). Prior to the initiation of research, species were selected based on their applicability and the predicted statistical power of data collected to test hypotheses associated with ecosystem health. Applicability was determined based on similarities in nesting characteristics, resistance to disturbance, foraging range and expected species density based on habitat availability, and use as a receptor in previous contamination research. These similarities allow for a more direct comparison of the parameters of interest, including differences in stressor exposure based on divergent foraging characteristics as well as differences in species stressor sensitivity.

Based on the above criteria, the tree swallow (Tachycineta bicolor), house wren (Troglodytes aedon), and eastern bluebird (Sialia sialis) were selected as study species for this research. All are obligate cavity-nesters with limited foraging range and similar site fidelity. Tree swallows are aquatic insectivores (Kuerzi 1941), primarily feeding on emergent insects (McCarty 1997; McCarty and Winkler 1999; Mengelkoch et al. 2004), and have been extensively utilized in contaminant studies (Custer et al. 2005; Echols et al. 2004; Froese et al. 1998; Neigh et al. 2006b; Shaw 1983). Eastern bluebirds and house wrens are both terrestrial insectivores (Beal 1915; Guinan and Sealy 1987) but have different habitat preferences and foraging strategies that could lead to different contaminant accumulation. Eastern bluebirds prefer open grassland habitats and feed by dropping on prey from an elevated perch, whereas house wrens primarily glean insects off foliage in brushy/forested habitats. Several studies of contaminants have used eastern bluebirds and house wrens (Burgess et al. 1999; Custer et al. 2001; Henny et al. 1977; Mayne et al. 2004; Neigh et al. 2006a, 2007).

The primary goal of the study was to characterize PCDD/DF exposure for these three passerine species representing different feeding pathways. To that end, eggs and nestlings of each species were examined for the following: (1) concentrations of ΣPCDF/DF and 2,3,7,8-tetrachlorodibenzofuran (TCDD) equivalents (TEQWHO-Avian) based on World Health Organization (WHO) 2,3,7,8-TCDD equivalency factors for birds (TEFWHO-Avian) (van den Berg et al. 1998); (2) temporal, spatial, and species-specific trends in concentrations; and (3) patterns of relative concentrations of individual congeners. Eggs were studied to account for maternal transfer of contaminants to the developing embryo, whereas concentrations of PCDD/DF in nestlings were considered to be more representative of site-specific exposures. Comparisons of congener-specific concentrations stratified by feeding pathway and site.
provided information about the sources of contaminants and species-specific exposure pathways.

The portion of the research described here focused on tissue-based exposure analyses. Results for the dietary-based exposure (Fredricks et al. 2009a) and nest productivity (Fredricks et al. 2009c) are reported elsewhere. Incorporation of all three lines of evidence into an ecological risk assessment will eventually lead to informed decisions about the potential impact(s) of on-site exposure and will aid in both the planning and evaluation of effective remedial actions.

**Methods**

**Site Description**

The research was conducted on the Tittabawassee, Chippewa, and Saginaw rivers, in the vicinity of Midland, Michigan (Fig. 1). The reference areas (RAs) were located upstream of the putative sources of PCDD/DF (Hilscherova et al. 2003) on the Tittabawassee (R-1) and Chippewa (R-2) rivers (Fig. 1). The area downstream of the putative PCDD/DF sources, defined as the study area (SA), includes ~72 km of the Tittabawassee and Saginaw rivers. The SA stretched from the upstream boundary, defined as the low-head dam near Midland, Michigan, to where the Saginaw River enters Saginaw Bay. Throughout the SA, the Tittabawassee River is free-flowing to the confluence with the Saginaw River and eventually Saginaw Bay. The SA consisted of two areas: the Tittabawassee River study areas, which included four locations (T-3 to T-6), and the Saginaw River study areas, which included two locations (S-7 and S-9). S-7 is located on a peninsula between the Tittabawassee and Saginaw rivers just upstream of their confluence. The six SAs were selected based on availability of landowner access to the sites and expected high-end exposure based on a previous study that measured soil and sediment concentrations (Hilscherova et al. 2003).

**Nest Boxes**

Nest boxes were used to facilitate monitoring of nesting activity and collection of samples. Standard passerine nest boxes (cedar; ~12 cm × 12 cm × 20 cm with a 3.5-cm hole) were fitted with a wire mesh predator guard around the entrance, mounted on 2.13-m metal T-posts covered in lubricating grease (to deter predator access), and placed at individual study locations R-1 to T-6 in 2004. Two additional sites (S-7 and S-9) were added in 2005. Nest boxes were placed in appropriate habitats to accommodate and target all three species (Horn et al. 1996; Parren 1991) and, when possible, to prevent competition among species (Prescott 1982). Nests were monitored from mid-April through the end of the breeding season beginning in 2005 and for the following 2 years (2006 and 2007). Procedures
generally followed those used by McCarty and Secord (1999).

Tissue Collection

Both eggs and nestlings were collected for quantification of PCDD/DFs. Nest boxes were randomly selected from the active nest boxes at a given location for either live egg or nestling collections but not both. Fresh egg mass was determined on the date laid. Abandoned and addled eggs were collected for possible quantification of PCDD/DF congeners and determination of degree of development after no activity for 7 days (3–4 days posthatch for addled eggs) or by the presence of new nesting material. Addled eggs were defined as those that failed to hatch 3–4 days posthatch of the remainder of the clutch. Eggs were individually stored wrapped in chemically cleaned foil in a chemically clean glass jar (I-CHEM, Rockwood, TN) at ambient temperature in the field and refrigerated at 4°C. In the laboratory, collected eggs were opened around the girth with a chemically cleaned scalpel blade and assessed for the laboratory, collected eggs were opened around the girth with a chemically cleaned scalpel blade and assessed for the presence of any abnormalities (Giesy et al. 1994; Larson et al. 1996). Nestlings (one per box) were collected at 10 days posthatch for house wrens or 14 days posthatch for eastern bluebirds and tree swallows and then euthanized via cervical dislocation. Nestlings were stored in similar glass jars on wet ice in the field and at −20°C until analyses. In the laboratory, collected nestlings were homogenized without feathers, bill, legs, and gizzard and crop contents with a chemically cleaned stainless-steel Omni-mixer® (Omni International, Marietta, GA). The homogenates were stored at −20°C until extraction.

During the 2005–2007 breeding seasons, a total of 49, 50, and 35 live and addled eggs were collected from unique house wren, tree swallow, and eastern bluebird clutches, respectively. An additional 9 eggs from 4 house wren clutches, 10 eggs from 4 tree swallow clutches, and 13 eggs from 5 eastern bluebird clutches were collected for within-clutch variability monitoring. During the same sampling period, 48, 45, and 30 nestlings were collected from unique house wren, tree swallow, and eastern bluebird clutches, respectively. However, of the collected nestlings, 10, 17, and 10 nestlings were collected from clutches in which an addled egg was also analyzed for house wrens, tree swallows, and eastern bluebirds, respectively.

Identification and Quantification of PCDD/DF Congeners

Concentrations of seventeen 2,3,7,8-substituted PCDD/DF congeners were measured in all samples, whereas concentrations of 12 non- and mono-ortho-substituted PCBs and dichloro-diphenyl-trichloroethane and related metabolites (DDXs) were determined in a subset of these samples. Eggs were lyophilized and stored at −20°C until extraction. Egg content mass was calculated by subtracting the egg shell mass at dissection from the total fresh egg mass measured on the day laid. Masses were calculated for quantification purposes and to account for any desiccation of the eggs during incubation and storage. Because actual fresh egg masses were determined on the date laid, it was not necessary to adjust for moisture loss, as has been suggested by previous research (Adrian and Stevens 1979; Heinz et al. 2009; Peakall and Gilman 1979; Stickel et al. 1973).

The PCDD/DFs, PCBs, and DDXs were quantified in accordance with EPA Method 8290/1668A with minor modifications (USEPA 1998). Briefly, samples were homogenized with anhydrous sodium sulfate and Soxhlet extracted in hexane:dichloromethane (1:1) for 18 h. Before extraction, known amounts of 13C-labeled analytes were added to the sample as internal standards. The extraction solvent was exchanged to hexane and the extract was concentrated to 10 mL. Ten percent of this extract was removed for lipid content determination. Extracts were initially purified by treatment with concentrated sulfuric acid. The extract was then passed through a silica gel column containing silica gel and sulfuric acid silica gel and eluted with hexane. The extract was then separated into two fractions by elution through acidic alumina: Fraction one contained most PCBs and pesticide compounds and fraction two contained PCDD/DFs and coplanar PCBs. Fraction two of the alumina column was then passed through a carbon gel column containing silica gel and sulfuric acid silica gel and eluted with hexane. The first carbon fraction, eluted with various solvent mixtures, was combined with the fraction one eluate from the acidic alumina column and retained for PCBs and DDXs analyses. The PCBs and DDXs extract was split, and separate analyses were performed using high resolution gas chromatography/high resolution mass spectroscopy (HRGC/HRMS) under the guidance of EPA method 1668, revision A. The second fraction, eluted with toluene, contained the PCDD/DFs and PCBs (IUPAC Nos. 77, 81, 126, and 169). Components were analyzed using HRGC/HRMS, a Hewlett-Packard 6890 GC (Agilent Technologies, Wilmington, DE) connected to a MicroMass® high-resolution mass spectrometer (Waters Corporation, Milford, MA). PCDF, PCDD, PCB, and DDX congeners were separated on a DB-5 capillary column (Agilent Technologies, Wilmington, DE) coated at 0.25 μm (60 m × 0.25 mm inner diameter). The mass spectrometer was operated at an electron-impact ionization (EI) energy of 60 eV and an ion current of 600 μA. Congeners were identified and quantified by use of single-ion monitoring (SIM) at the two most intensive ions at the molecular ion cluster. Concentrations of certain PCDD/DF
congeners, particularly 2,3,7,8-TCDD and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) congeners, were confirmed by using a DB-225 (60 m × 0.25 mm inner diameter, 0.25 µm film thickness) column (Agilent Technologies, Wilmington, DE). Losses of congeners during extraction and cleanup were corrected based on recoveries of 13C-labeled analytes as outlined in EPA Method 8290/1668A. Quality control samples generated during chemical analyses included laboratory method blanks, sample processing blanks (equipment rinsate and atmospheric), matrix spike and matrix spike duplicate pairs, unspiked sample replicates, and blind check samples. Results of method and field blank analyses indicated no systematic laboratory contamination issues. Evaluation of the percent recovery and relative percent difference data for the matrix spike and matrix spike duplicate samples and unspiked replicate samples were within ±30% at a rate of greater than 95% acceptability.

Statistical Analyses

Total concentrations of the 17 2,3,7,8-substituted PCDD/DF congeners are reported as the sum of all congeners [ng/kg wet weight (ww)]. Individual congeners that were less than the limit of quantification were assigned a value of half the sample method detection limit. Total concentrations of 12 non- and mono-ortho-substituted PCB congeners are reported as the sum of these congeners (ΣPCBs; ng/kg ww). Concentrations of TEQWHO-Avian (ng/kg ww) were calculated for both PCDD/DFs and PCBs by summing the product of the concentration of each congener, multiplied by its avian TEF WHO-Avian (van den Berg et al. 1998). Additionally, dichloro-diphenyl-trichloroethane (2’,4’ and 4’,4’ isomers) and dichloro-diphenyl-dichloroethylene (4’,4’) are reported as the sum of the o,p and p,p isomers (ΣDDXs; µg/kg ww) for the same subset of samples as for PCBs. Geometric means and 95% confidence intervals are presented.

Sample sizes reported for both eggs and nestlings were collected from individual nest boxes from unique nesting attempts. However, some clutches had analytical data reported for both an addled egg and nestling, which is similar to previous research (Custer et al. 2003, 2005). Multiple eggs sampled from the same nesting attempt only were used for investigating clutch variability trends, with the exception that a single egg was randomly selected and included in the between-site comparisons. Comparisons of site-specific differences between live and addled eggs were made using the same egg dataset as for among sites comparisons. Correlations between concentrations of ΣPCDD/DFs and TEQWHO-Avian in eggs and lay order, clutch initiation dates, and date collected were made by species and only included the eggs used for between-site comparisons. No statistical comparisons were made for analytical results for cocontaminants or among multiple eggs sampled from the same nesting attempt for clutch variability trends.

Statistical analyses were performed using SAS® software (Release 9.1; SAS Institute Inc., Cary, NC, USA). Prior to the use of parametric statistical procedures, normality was evaluated using the Shapiro–Wilks test and the assumption of homogeneity of variance was evaluated using Levene’s test. Values that were not normally distributed were transformed using the natural log (ln) of (x + 1). Concentrations in eggs and nestlings were initially tested for overall effects, including the following class variables: YEAR, SPECIES, SAMPLE, and SITE without interaction terms for concentrations of both ΣPCDD/DFs and TEQ WHO-Avian. Subsequent one-way comparisons were made for all YEARs combined and separated by SPECIES and SAMPLE testing for differences in concentrations of ΣPCDD/DFs and TEQ WHO-Avian among SITES. Due to sample size limitations, one-way comparisons between live and addled eggs were made by species for only sites R-1 and R-2, T-3 to T-6, and S-7 and S-9. PROC TTEST was used to compare individual locations. PROC TTEST was used to compare between only two groups. The associations between concentrations of both ΣPCDD/DFs and TEQ WHO-Avian and order in which eggs were laid (relative position of egg within laying sequence) and date egg laid (Julian date) were evaluated individually by Pearson’s correlation coefficients. No statistical comparisons were made between multiple eggs collected from the same clutch (within-clutch variability) or for samples screened for potential cocontaminants (PCBs and DDXs). Differences were considered to be statistically significant at p < 0.05.

Results

ΣPCDD/DF, ΣPCB, and ΣDDX Concentrations

Concentrations of ΣPCDD/DFs in neither eggs nor nestlings were different among years, but concentrations of ΣPCDD/DFs did vary between eggs and nestlings as well as among species and locations. Because there was no difference in concentrations of ΣPCDD/DFs among years (F = 2.46, p = 0.0877), comparisons were made by species (F = 9.53, p = 0.0001) and sample type (F = 54.19, p < 0.0001) when comparing among sampling locations (F = 44.52, p < 0.0001).

Concentrations of ΣPCDD/DF in eggs of both house wrens and eastern bluebirds were significantly different among sampling locations, whereas concentrations of
PCDD/DF in eggs of tree swallows were not (Table 1). Mean concentrations of PCDD/DFs in house wren and eastern bluebird eggs were 10- to 19-fold and 4- to 16-fold greater at Tittabawassee River SAs than RAs, respectively, whereas house wren eggs at Saginaw River SAs tended to be intermediate between the two and comparisons at this location were not possible for eastern bluebird eggs due to a limited sample size. Maximum concentration of PCDD/DF in eggs of house wrens, tree swallows, and eastern bluebirds were 7200 ng/kg at T-4, 2000 ng/kg at R-1, and 2400 ng/kg at T-6, respectively.

Concentrations of PCDD/DF in nestlings of house wrens, tree swallows, and eastern bluebirds occurred at T-6 and were 1700 ng/kg, 7300 ng/kg, and 2100 ng/kg, respectively. Concentrations of PCDD/DF in live and addled eggs of tree swallows were significantly different at the RAs (t = 3.08, p = 0.0095) but not at SAs. PCDD/DF concentrations in live and addled eggs were not significantly different for house wrens or eastern bluebirds. Tree swallow addled egg PCDD/DF concentrations were twofold greater than live eggs collected at RAs (Fig. 2).

Concentrations of PCDD/DF in nestlings of house wrens, tree swallows, and eastern bluebirds were individually correlated with collection day in nestlings of house wrens, tree swallows, and eastern bluebirds at RAs (t = -0.70022, p = 0.0112, n = 12; R = 0.70403, p = 0.0106, n = 12; R = 0.68281, p = 0.0144, n = 12; respectively), whereas all correlations

### Table 1
Total concentrations of furans and dioxins (ΣPCDD/DF) and TEQs WHO-Avian in eggs of house wrens, tree swallows and eastern bluebirds collected during 2005–2007 from the Chippewa River, Tittabawassee River, and Saginaw River floodplains, Midland, Michigan, USA

|                      | Reference area | Study area |
|----------------------|----------------|------------|
|                      | R-1            | R-2        | T-3  | T-4  | T-5  | T-6  | S-7  | S-9  |
| House wren           |                |            |      |      |      |      |      |      |
| ΣPCDD/DF             | 73 (6) A       | 82 (6) A   | 1400 (9) C | 990 (7) C | 860 (6) BC | 1500 (6) C | 480 (6) BC | 200 (3) AB |
|                      | (28–190)       | (53–130)   | (810–2500) | (390–2500) | (510–1400) | (860–2600) | (220–1000) | (33–1200) |
| TEQs WHO-Avian       | 10 (6) A       | 25 (6) AB  | 860 (9) D | 360 (7) D | 430 (6) D | 910 (6) D | 240 (6) CD | 79 (3) BC |
|                      | (4.8–21)       | (14–44)    | (420–1700) | (190–650) | (220–820) | (460–1800) | (100–580) | (12–470) |
| Tree swallow         |                |            |      |      |      |      |      |      |
| ΣPCDD/DF             | 660 (7) A      | 760 (7) A  | 470 (8) A | 540 (6) A | 2(2)    | 380 (7) A | 400 (7) A | 280 (6) A |
|                      | (340–1300)     | (440–1300) | (300–740) | (350–850) | (460–490) | (170–850) | (280–570) | (220–360) |
| TEQs WHO-Avian       | 180 (7) A      | 280 (7) A  | 220 (8) A | 240 (6) A | 2(2)    | 220 (7) A | 190 (7) A | 100 (6) A |
|                      | (73–420)       | (180–430)  | (140–360) | (130–460) | (190–330) | (73–700) | (120–300) | (78–140) |
| Eastern bluebird     |                |            |      |      |      |      |      |      |
| ΣPCDD/DF             | 51 (6) A       | 130 (6) AB | 470 (6) BC | 620 (6) C | 770 (3) C | 1100 (6) C | 2(2)    | N/A |
|                      | (20–130)       | (84–220)   | (200–1100) | (430–890) | (570–1000) | (620–2000) | (110–240) |      |
| TEQs WHO-Avian       | 10 (6) A       | 30 (6) A   | 150 (6) B | 210 (6) B | 390 (3) B | 510 (6) B | 2(2)    | N/A |
|                      | (4.2–22)       | (16–57)    | (59–370) | (160–280) | (210–710) | (260–1000) | (63–92) |  

### Note
- Values (ng/kg ww) were rounded and represent only two significant figures; they are given as the geometric mean with the sample size given in parentheses (n) over the 95% confidence interval
- Eggs include both live and addled eggs
- Means identified with the same letter are not significantly different among locations (across) at the p = 0.05 level using the Bonferroni means separation test
- TEQs WHO-Avian were calculated based on the 1998 avian WHO TEF values
- Range reported for sites with only two samples. Sites were not included in the between location statistical comparisons
- N/A = no samples collected from this location
were not significant at downstream SAs. For the clutches analyzed, the within-clutch variability of concentrations of \( \text{RPCDD/DF} \) in eggs varied by only 10–38% across species and sites (Table 3).

Concentrations of \( \text{RPCBs} \) in eggs were greatest for tree swallows, intermediate for house wrens, and least for eastern bluebirds (Table 4). Concentrations of \( \Sigma DDXs \) were greatest in tree swallow eggs and were primarily composed of 4,4'-dichloro-diphenyl-dichloroethylene (Table 4).

TCDD Equivalents (TEQWHO-Avian)

The TEQWHO-Avian concentrations in eggs or nestlings were not different among years, but TEQWHO-Avian concentrations did vary between eggs and nestlings as well as among species and locations. Because there was no statistically significant difference in TEQWHO-Avian concentrations among years \( (F = 2.48, p = 0.0855) \) data were pooled across years and comparisons were made by species \( (F = 26.18, p < 0.0001) \) and sample type \( (F = 34.02, p < 0.0001) \) when comparing among sampling locations \( (F = 66.62, p < 0.0001) \).

The TEQWHO-Avian concentrations in eggs of both house wrens and eastern bluebirds were significantly different among sampling locations, whereas TEQWHO-Avian concentrations in eggs of tree swallows were not (Table 1). Mean TEQWHO-Avian concentrations in house wren and eastern bluebird eggs were 15- to 91-fold and 5- to 46-fold greater at Tittabawassee River SAs than RAs, respectively, whereas concentrations in house wren eggs at Saginaw River SAs tended to be intermediate between the two and comparisons at this location were not possible for eastern bluebird eggs due to a limited sample size. Maximum TEQWHO-Avian concentrations in eggs of house wrens, tree swallows, and eastern bluebirds were 2300 ng/kg at T-3, 730 ng/kg at R-1, and 1000 ng/kg at T-6, respectively.

Concentrations of TEQWHO-Avian in nestlings of house wrens, tree swallows, and eastern bluebirds were significantly different among sampling locations (Table 2). Mean TEQWHO-Avian concentrations in house wren and eastern bluebird eggs were 15- to 91-fold and 5- to 46-fold greater at Tittabawassee River SAs than RAs, respectively, whereas concentrations in house wren eggs at Saginaw River SAs tended to be intermediate between the two and comparisons at this area were not possible for eastern bluebird eggs due to a limited sample size. Maximum TEQWHO-Avian concentrations in eggs of house wrens, tree swallows, and eastern bluebirds were 2300 ng/kg at T-3, 730 ng/kg at R-1, and 1000 ng/kg at T-6, respectively.

**Table 2** Total concentrations of furans and dioxins (\( \Sigma PCDD/DF \)) and TEQWHO-Avian in nestlings\(^a\) of house wrens, tree swallows, and eastern bluebirds collected during 2005–2007 from the Chippewa River, Tittabawassee River, and Saginaw River floodplains, Midland, Michigan (USA)

| Reference area | Study area |
|----------------|------------|
|                | R-1        | R-2        |
|                | T-3        | T-4        | T-5        | T-6        | S-7        | S-9        |
| House wren     |            |            |            |            |            |            |
| \( \Sigma PCDD/DF \) | 14 (6) A\(^b\) | 24 (6) AB   | 610 (7) D  | 350 (7) CD | 420 (6) CD | 530 (6) CD | 180 (6) C  | 55 (4) B   |
|                 | (9.2–21)   | (19–31)    | (330–1100)| (230–540) | (230–760) | (270–1000)| (83–410)   | (33–90)    |
| TEQWHO-Avian   | 3.4 (6) A  | 6.5 (6) AB  | 290 (7) D | 140 (7) CD | 210 (6) CD | 300 (6) D | 78 (6) C   | 18 (4) B   |
|                 | (1.7–6)    | (5.7–7.5)  | (140–580)| (110–180) | (93–460) | (140–660) | (34–180)   | (14–22)    |
| Tree swallow    |            |            |            |            |            |            |            |
| \( \Sigma PCDD/DF \) | 64 (6) A   | 110 (6) AB  | 460 (6) CD| 460 (6) CD| 360 (3) C | 1100 (6) D| 270 (6) BC | 250 (6) BC |
|                 | (32–130)   | (81–140)   | (320–670)| (320–660)| (74–1700)| (390–3000)| (180–410) | (190–350)  |
| TEQWHO-Avian   | 25 (6) A   | 47 (6) A    | 340 (6) BC| 320 (6) BC| 240 (3) B | 860 (6) C | 190 (6) B  | 150 (6) B  |
|                 | (16–36)    | (42–52)    | (230–500)| (210–500) | (54–1100)| (310–2400)| (120–320) | (100–210)  |
| Eastern bluebird|            |            |            |            |            |            |
| \( \Sigma PCDD/DF \) | 24 (6) A   | 41 (6) A    | 520 (6) BC| 330 (5) B | N/A\(^d\) | 1200 (5) C| (2)        | N/A        |
|                 | (12–48)    | (22–75)    | (260–1000)| (180–590) | (150–152) | (310–2400)| (120–320) | (100–210)  |
| TEQWHO-Avian   | 2.8 (6) A  | 7.6 (6) A   | 190 (6) B | 100 (5) B | N/A        | 690 (5) C | (2)        | N/A        |
|                 | (2–3.7)    | (4.9–11)   | (65–570) | (50–210)  |            | (300–1600)| (49–70)   |            |

Note: Values (ng/kg ww) were rounded and represent only two significant figures; they are given as the geometric mean with the sample size given in parentheses (n) over the 95% confidence interval

\(^a\) House wren nestlings were collected on day 10 and tree swallow and eastern bluebird nestlings were collected on day 14 (hatch = day 0)

\(^b\) Means identified with the same letter are not significantly different among locations (across) at the \( p = 0.05 \) level using the Bonferroni means separation test

\(^c\) TEQWHO-Avian were calculated based on the 1998 avian WHO TEF values

\(^d\) N/A = no samples collected from this location

\(^e\) Range reported for sites with only two samples. Sites were not included in the between location statistical comparisons
bluebirds occurred at T-6 and were 1200 ng/kg, 6000 ng/kg, and 1400 ng/kg, respectively.

Concentrations of TEQWHO-Avian in live and addled eggs exhibited the same trends as concentrations of ΣPCDD/DF (Fig. 2) for all studied species. TEQWHO-Avian concentrations in addled tree swallow eggs were greater than in live eggs at RAs ($t = 3.52, p = 0.0042$). Concentrations of TEQWHO-Avian in tree swallow eggs were correlated with egg lay order ($R = 0.60047, p = 0.0232, n = 14$) at the RAs but not at the SAs. TEQWHO-Avian concentrations in eggs of house wrens and eastern bluebirds were not correlated with egg lay order. Concentrations of TEQWHO-Avian in eggs and nestlings of all species were not correlated with date laid or collection day, respectively, across all areas. For the clutches analyzed, the within-clutch variability of concentrations of TEQWHO-Avian in eggs varied by only 7–42% across species and sites (Table 3). Concentrations of ΣPCB TEQWHO-Avian in tree swallow eggs comprised from 23 to 47% of the ΣTEQWHO-Avian, whereas in house wren and eastern bluebird eggs ΣPCB TEQWHO-Avian concentrations only comprised <1–8% (Table 4).

Congener Patterns

Relative proportions of PCDD/DF concentrations contributed by individual congeners varied between the eggs and nestlings as well as among species and sampling areas. Congener profiles were characterized by principle component analysis (PCA) by relative orderings of PCDD/DF concentrations normalized to the ΣPCDD/DF concentration. The PCA model that included two principle components (PC1 and PC2) explained 85% of the total variance. All samples collected in RAs had negative greatest eigenvectors for both PC1 and PC2, whereas tree swallow samples were separated by positive vectors for PC1 (loading score of 0.89 for 2,3,7,8-TCDF). House wren and eastern bluebird had positive vectors for PC2 (loading score of 0.84 for 2,3,4,7,8-PeCDF) and negative vectors for PC1 (Fig. 3).

For all three species, dioxins dominated the congener profile at RAs and furans dominated at the SAs. For example, at RAs, tree swallow mean egg PCDD/DF concentration congener profiles were dominated by 79% dioxin congeners compared to SAs that only had 44% (Fig. 4). For all species studied, congener profiles of eggs and nestlings at Saginaw River SAs were similar to those at Tittabawassee River SAs (supplemental information in Fig. 6). Mean PCDD/DF congener profiles of egg and nesting house wrens and eastern bluebirds were dominated by a combination of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF at Tittabawassee River SAs (Fig. 4). An even larger proportion of the total was 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF for tree swallows at the SAs. Mean nesting PCDD/DF concentration congener profiles for all species at Tittabawassee River SAs were
composed of between 69 and 84% furan congeners, with 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF making up 35–60% of the total. The majority of congeners were above the detection limit in over 50% of samples with the exception of the dioxinlike congeners 1,2,3,7,8,9-hexachlorodibenzofuran, 1,2,3,4,7, 8,9-heptachlorodibenzofuran, 1,2,3,4, 6,7,8,9-octachlorodibenzofuran (supplemental information: Tables 5–10).

Table 3 Within-clutch variability of total concentrations of furans and dioxins (ΣPCDD/DF) and TEQs WHO-Avian in eggs of house wrens, tree swallows, and eastern bluebirds collected during 2005–2007 from the Chippewa River and Tittabawassee River floodplains, Midland, Michigan (USA)

| Clutch initiation b | Site c | E1 d,e | E2 | E3 | E4 | E5 | Percent difference f |
|--------------------|--------|--------|----|----|----|----|----------------------|
| House wren         |        |        |    |    |    |    |                      |
| Clutch 1 21 Jun 06 | R-1    | 240 (LE) 51 (2) | 150 (AE) 32 (5) | 200 (AE) 30 (7) | 37 | 41 |
| Clutch 2 10 May 06 | R-1    | 77 (LE) 8.4 (1) | 73 (AE) 8.2 (3) | 60 (AE) 9.6 (5) | 21 | 15 |
| Clutch 3 03 Jun 07 | T-4    | 1400 (AE) 630 (1) | 1600 (AE) 480 (3) | 1700 (AE) 780 (4) | 19 | 39 |
| Clutch 4 24 May 06 | T-5    | 330 (AE) 140 (1) | 320 (AE) 140 (3) | 240 (AE) 100 (4) | 27 | 28 |
| Tree swallow       |        |        |    |    |    |    |                      |
| Clutch 1 09 May 06 | R-1    | 430 (LE) 120 (1) | 370 (LE) 110 (2) | 330 (LE) 110 (3) | 23 | 7 |
| Clutch 2 30 May 05 | R-2    | 510 (LE) 270 (1) | 550 (AE) 290 (2) | 660 (AE) 340 (3) | 740 (AE) 370 (4) | 370 (5) | 34 |
| Clutch 3 08 May 06 | T-3    | 690 (AE) 210 (1) | 670 (LE) 210 (2) | 560 (AE) 190 (3) | 210 (1) 190 (3) | 27 |
| Clutch 4 19 May 06 | T-6    | 1000 (AE) 690 (2) | 1500 (AE) 1100 (3) | 1700 (AE) 1200 (4) | 38 | 42 |
| Eastern bluebird   |        |        |    |    |    |    |                      |
| Clutch 1 02 Jun 06 | R-1    | 24 (AE) 6.8 (1) | 25 (AE) 6.4 (2) | 24 (AE) 5.9 (3) | 27 (AE) 6.4 (4) | 14 |
| Clutch 2 30 May 07 | R-2    | 120 (AE) 28 (1) | 99 (AE) 27 (3) | 87 (AE) 25 (5) | 25 | 9 |
| Clutch 3 14 May 06 | T-3    | 380 (LE) 170 (1) | 390 (LE) 170 (2) | 360 (LE) 170 (3) | 350 (LE) 160 (4) | 180 (5) | 29 |
| Clutch 4 27 Apr 07 | T-3    | 210 (AE) 100 (1) | 300 (AE) 170 (4) | 280 (AE) 160 (4) | 10 | 9 |
| Clutch 5 01 Aug 05 | T-6    | 1400 (AE) 1000 (2) | 1600 (AE) 1200 (2) | 1400 (AE) 1000 (3) | 11 | 11 |

Note: ΣPCDD/DF (ng/kg ww) with egg type given in parentheses over TEQ WHO-Avian with egg number laid given in parentheses

a TEQ WHO-Avian were calculated based on the 1998 avian WHO TEF values

b Clutch initiation is the day the first egg was discovered

c R-1 to R-2 are reference areas and T-3 to T-6 are Tittabawassee River study areas
d Values were rounded and represent only two significant figures
e E1–E5 indicate individual eggs analyzed per clutch

f Percent difference is calculated as the maximum value minus the minimum divided by the maximum times 100 for each clutch (prior to rounding)

g Egg type: LE = live egg and AE = addled egg

h Egg #: Numbered in order as laid starting with 1. If two eggs have the same number, they were both found new, so lay order is unknown

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Discussion

ΣPCDD/DF, ΣPCB, and ΣDDX Concentrations

Concentrations of ΣPCDD/DF in all passerine tissues collected except tree swallow eggs were greatest at Tittabawassee River SAs, whereas those from Saginaw River SAs had intermediate concentrations and those from RAs had the least. ΣPCDD/DF concentrations in tree swallow nestlings collected in the same areas mirror the site-specific sediment trends, as expected. These results are similar to those for ΣPCB concentrations in tree swallow eggs collected from an upstream reference site along the Champlain Canal that were similar to those collected from downstream sites with known contamination along the Hudson River, New York (USA) (Secord et al. 1999). In that study, concentrations in nestlings were less at reference areas, which is similar to the findings in the current study. One possible explanation for these observations is that migrating swallows follow aquatic systems (Butler 1988) and could accumulate PCDD/DFs in route.

Maternal contaminant deposition from body burdens

Table 4 Concentrations of selected co-contaminants in eggs of house wrens, tree swallows, and eastern bluebirds collected during 2005–2007 from the Chippewa and Tittabawassee River floodplains, Midland, Michigan (USA)

| Sitea | Egg typethe | Egg # | ΣPCBs TEQsdf | ΣPCDD/DFs TEQs | ΣPCBs f | 2′,4′-DDT | 4′,4′-DDE | 4′,4′-DDE |
|-------|-------------|-------|--------------|----------------|----------|------------|----------|----------|
| House wren | | | | | | | | | |
| Sample 1 R-1 | LE | 1 | 12 | 620 | 1.3E-2 | 6.4E-5 | 6.6E-2 | 8.9E-4 | 6.7E-2 |
| Sample 2 R-2 | LE | 1 | 62 | 110 | 2.1E-2 | 1.1E-3 | 1.3E-1 | 4.2E-3 | 1.4E-1 |
| Tree swallow | | | | | | | | | |
| Sample 1 T-3 | LE | 1 | 62 | 110 | 2.1E-2 | 1.1E-3 | 1.3E-1 | 4.2E-3 | 1.4E-1 |
| Sample 2 T-4 | LE | 1 | 62 | 110 | 2.1E-2 | 1.1E-3 | 1.3E-1 | 4.2E-3 | 1.4E-1 |
| Sample 3 T-6 | LE | 1 | 62 | 110 | 2.1E-2 | 1.1E-3 | 1.3E-1 | 4.2E-3 | 1.4E-1 |
| Eastern bluebird | | | | | | | | | |
| Sample 1 R-1 | LE | 1 | 0.57 | 6.8 | 1.3E-3 | 2.8E-5 | 1.0E-2 | 2.3E-4 | 1.0E-2 |
| Sample 2 R-2 | LE | 1 | 0.73 | 170 | 2.6E-2 | 9.0E-6 | 6.8E-2 | 3.9E-3 | 7.1E-2 |
| Sample 3 R-3 | LE | 1 | 0.95 | 540 | 6.1E-3 | 2.0E-5 | 1.6E-1 | 3.3E-3 | 1.6E-1 |
| Sample 4 T-6 | LE | 2 | 2.1 | 1000 | 6.2E-3 | 2.5E-5 | 7.7E-2 | 8.3E-4 | 7.8E-2 |

Note: Values of TEQsWHO-Avian are presented in ng/kg ww and PCBs and DDXs are presented in mg/kg ww. ΣDDXs = sum of dichloro-diphenyl-trichloroethane (2′,4′ and 4′,4′ DDT isomers) and dichloro-diphenyl-dichloroethylene (4′,4′-DDE)

a R-1 to R-2 are reference areas and T-3 to T-6 are Tittabawassee River study areas
b Egg type: LE = live egg and AE = addled egg
c Egg #: Numbered in order as laid starting with 1. R-1 to R-2 are reference areas and T-3 to T-6 are Tittabawassee River study areas
d Values were rounded and represent only two significant figures
e TEQsWHO-Avian were calculated based on the 1998 avian WHO TEF values
f ΣPCBs included only the 12 non- and mono-ortho-substituted congeners
g Each sample is an individual egg from unique clutches
h E = 10^9
might provide another explanation. Maternal transfer of contaminants to eggs has been shown to vary depending on overwintering areas in black-crowned night-herons (Henny and Blus 1986). Thus, adult female tree swallows could be exposed to concentrations of $\Sigma$PCDD/DF along migration or on wintering grounds universally, but previous research demonstrated that ring-necked pheasant hens ($Phasianus colchicus$) were only able to translocate *1% of their cumulative dosage amount to each egg (Nosek et al. 1992b). Because the congener profiles in tree swallow eggs were different between RAs and SAs, the most plausible possibility is that, prior to breeding, foraging ranges of swallows at upstream RAs temporarily include a proximal contaminated site. This explanation seems most reasonable because adult tree swallows arrive at breeding areas to defend breeding territories several weeks prior to clutch initiation (Stutchbury and Robertson 1987) and have slightly larger foraging ranges than during brood rearing (Quinney and Ankney 1985). Additionally, most passerines are considered income-breeders (meaning that the majority of resources for egg production are acquired through the daily diet during egg development), and this further confirms the hypothesis that tree swallow females are likely traveling to a proximal contaminated site during egg production at RAs (Langin et al. 2006; Nager 2006).

The $\Sigma$PCDD/DF concentrations in live and addled eggs were similar for all areas, with the exception of tree swallow eggs in the RAs. Addled eggs (arithmetic mean: 5.2%; $n = 63$) had significantly lower ($t = -2.67$, $p = 0.0085$) percent lipids compared to live eggs (6.1%; $n = 71$); however, this small difference can be attributed to partial embryo development in addled eggs. A greater percent lipids in addled eggs would have been expected if differences between fresh and addled eggs were due to desiccation. Comparisons of $\Sigma$PCDD/DF concentrations
in live and addled eggs could provide insight into exposure concentrations at which eggs lose viability. However, metabolism of these compounds by the developing embryo can result in differences that are an artifact of embryo survival rather than fecundity. Recent egg injection studies have noted significant embryo metabolism of one of the major site-related PCDF congeners (M. J. Zwiernik, personal communication). Based on comparisons of congener specific adult biomagnification factors in herring gulls (*Larus argentatus*), TCDF was determined to be rapidly metabolized as opposed to 2,3,4,7,8-PeCDF, for which metabolism was determined to be variable and possibly linked to species-specific differences in distribution or metabolism (Braune and Norstrom 1989). Previous research on mallards (*Anas platyrhynchos*; Norstrom et al. 1976) and bald eagles (*Haliaeetus leucocephalus*; Elliott et al. 1996) have discussed similar trends in metabolism for PCDF congeners. Furthermore, concentrations of ΣPCBs in live and addled eggs were not different for tree swallows exposed to PCBs in the Kalamazoo River floodplain, Michigan (USA) (Neigh et al. 2006b). In addition, the concentration of ΣPCDD/DF in eggs of tree swallows at RAs in both live and addled eggs from the current study were below a predicted threshold of effects (Custer et al. 2005).

Concentrations of ΣPCDD/DF and the lay or collection day for eggs of all species at RAs and nestlings of tree swallows at Saginaw River SAs were significantly correlated. Examining the data further revealed that the marginal correlations, with coefficients of determination ($r^2$) ranging from 0.32 to 0.49, were spurious and not indicative of true temporal trends in the concentrations. It was hypothesized that eggs laid or nestlings collected later in the nesting season would have stable or lesser concentrations at RAs and greater concentrations at SAs corresponding to extended site-specific exposure. The eggs of house wrens at RAs had a negative correlation, as expected, but it was influenced by small sample size late in the season. Additionally, if the correlations were valid, similar correlations could have been expected for concentrations of TEQ$_{\text{WHO-Avian}}$, but none were observed.

Concentrations of ΣPCDD/DF in multiple eggs from the same nesting attempt were measured for all three species to investigate possible concentration-dependent differences in laying order or absolute concentrations. Conflicting research exists both confirming (Custer et al. 1990; Pan et al. 2008; van den Steen et al. 2006) and rebutting...
(Reynolds et al. 2004) the idea that eggs from the same clutch have similar concentrations. First, middle, or ultimate eggs of two passerine species had nearly equal likelihood of containing the maximum concentration of DDE from a given clutch (Reynolds et al. 2004). Similarly, concentrations of ΣPCDD/DF in eggs from house wrens, tree swallows, and eastern bluebirds from this study had within-clutch variability that would suggest that no relationship exists between residue concentrations and order in which eggs were laid. The results of this study are consistent with the conclusion made by Reynolds et al. (2004), that spatial distribution of contaminants on-site and daily feeding patterns likely affect concentrations of contaminants in eggs more than lay order.

In contrast with other primarily PCB contaminated study sites (Arenal et al. 2004; Custer et al. 2002, 2003, 2006; Neigh et al. 2006a, b; Secord et al. 1999), concentrations of PCBs on the Tittabawassee River were similar to the reference areas or at “background” for all species. Similarly, concentrations of ΣDDXs in eggs of all three study species were again similar to other reference or non-point-source impacted sites across the United States (Custer et al. 2000, 2002, 2005; Harris and Elliott 2000; Neigh et al. 2006a, b).

TCDD Equivalents (TEQ<sub>WHO-Avian</sub>)

The TEQ<sub>WHO-Avian</sub> concentrations in eggs and nestlings of house wrens, tree swallows, and eastern bluebirds were greater at downstream SAs, and, like ΣPCDD/DF concentrations, they were greatest at the T-6 location. One possible explanation for consistently greatest values at the T-6 location involves the natural hydrology of the Tittabawassee River. When at flood stage, the river flows across the large bends near T-6 instead of following the normal river channel (Fig. 1). The water loses momentum and energy quickly and deposits large amounts of sediment over those areas, creating a “sink” location for sediment-bound contaminants.

Based on site-specific contamination and a gradient of exposures among locations, correlations were expected between concentrations of ΣPCDD/DF and TEQ<sub>WHO-Avian</sub>, TEQ<sub>WHO-Avian</sub>, and ΣPCDD/DF concentrations for eggs and nestlings were positively correlated for all study species (Fig. 5). This was due to the consistent prevalence of three congeners with high TEF<sub>WHO-Avian</sub> values at SAs. Three PCDD/DF congeners (2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, and 1,2,3,7,8-pentachlorodibenzo-p-dioxin) have TEF<sub>WHO-Avian</sub> (van den Berg et al. 1998) values equivalent to TCDD. Combined with TCDD, these four congeners make up between 85 and 95% of the TEQ<sub>WHO-Avian</sub> concentrations for both eggs and nestlings at the Tittabawassee River and Saginaw River SAs. Individual congener correlations for the concentrations of 17 PCDD/DFs and TEQ<sub>WHO-Avian</sub> for eggs and nestlings by species were all highly correlated (unpublished data). Strong positive correlations indicate a site-specific contaminant gradient among samples across study areas.

An egg collected from a house wren nest at T-4 contained the greatest measured concentration of ΣPCDD/DF from this study (7200 ng/kg). The primary constituent was octachlorodibenzo-p-dioxin and the concentration of TEQ was only 350 ng/kg TEQ<sub>WHO-Avian</sub>, which was made up of congeners that represented only 5% of the ΣPCDD/DF concentration. The egg was collected in mid-May 2005, was the third egg laid in the clutch, and the adult female was never recaptured again on-site. For comparison, at RAs, egg and nestling mean percent ΣPCDD/DF of TEQ<sub>WHO-Avian</sub> concentrations ranged from 23 to 33% and from 16.5 to 42.2%, respectively, whereas at SAs, egg and nestling means ranged from 40 to 55% and from 39 to 73%, respectively. Due to this discrepancy, this egg was removed from correlations between TEQ<sub>WHO-Avian</sub> and
Conclusions

Overall, egg and nestling exposures for house wrens, tree swallows, and eastern bluebirds were greater downstream of Midland than upstream, and the downstream congener pattern was dominated by furan congeners, rather than PCDDs, which were dominant upstream. Eggs of tree swallows at RAs had \( \Sigma \text{PCDD/DF} \) and TEQ\text{WHO-Avian} concentrations that were similar to SAs, albeit primarily based on PCDD congeners, compared to the PCDF congeners associated with eggs collected from SAs. Despite anomalies associated with tree swallow egg concentrations at RAs, nestling concentrations of both \( \Sigma \text{PCDD/DF} \) and TEQ\text{WHO-Avian} in all species studied were less at RAs compared to SAs. We stress the importance of site-specific tissue exposure monitoring and, due to the potentially different sources to each, the necessity of both egg and nestling samples. To our knowledge, this is the first site-specific study of passerines exposed to elevated concentrations of mixtures dominated by furan congeners. Cocontaminants, including DDXs and PCBs, were generally at background levels for all three species studied based on egg data, with the exception of \( \Sigma \text{PCB TEQ}_{\text{WHO-Avian}} \) in tree swallows. However, because only a small subset of tree swallow eggs was analyzed for PCBs, there is some uncertainty associated with this conclusion. Overall, based on egg and nestling tissue concentrations, passerine birds breeding in the Tittabawassee River floodplain downstream of Midland, Michigan have significant exposure to \( \Sigma \text{PCDD/DFs} \). Subsequent articles will discuss implications of these results by incorporating data from dietary exposure (Fredricks et al. 2009a) and productivity (Fredricks et al. 2009c) into terrestrial-based (Fredricks et al. 2009b) and aquatic-based (Fredricks et al. 2009d) risk assessments of passerines nesting near Midland, Michigan.

Animal Use

All aspects of the study that involved the use of animals were conducted in the most humane way possible. To achieve that objective, all aspects of the study design were performed following standard operating procedures (Protocol for Monitoring and Collection of Box-Nesting Passerine Birds 03/04-045-00; Field studies in support of Tittabawassee River Ecological Risk Assessment 03/04-042-00) approved by Michigan State University’s Institutional Animal Care and Use Committee (IACUC). All of the necessary state and federal approvals and permits (Michigan Department of Natural Resources Scientific Collection Permit SC1252, US Fish and Wildlife Migratory Bird Scientific Collection Permit MB102552-1, and subpermitted under US Department of the Interior Federal Banding Permit 22926) are on file at MSU-ATL.
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