ABSTRACT

A site-specific multiple lines of evidence risk assessment was conducted for house wrens (Troglodytes aedon) and eastern bluebirds (Sialia sialis) along the Tittabawassee River downstream of Midland, Michigan, where concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs) in floodplain soils and sediments are greater compared to upstream areas and some of the greatest anywhere in the world. Lines of evidence supporting the population-level assessment endpoints included site-specific dietary- and tissue-based exposure assessments and population productivity measurements during breeding seasons 2005–2007. While a hazard assessment based on site-specific diets suggested that populations residing in the downstream floodplain had the potential to be affected, concentrations in eggs compared to appropriate toxicity reference values (TRVs) did not predict a potential for population-level effects. There were no significant effects on reproductive success of either species. The most probable cause of the apparent difference between the dietary- and tissue-based exposure assessments was that the dietary-based TRVs were overly conservative based on intraperitoneal injections in the ring-necked pheasant. Agreement between the risk assessment based
on concentrations of PCDFs and PCDDs in eggs and reproductive performance in both species supports the conclusion of a small potential for population-level effects at this site.

**Key Words:** house wren, eastern bluebird, reproductive success, dioxins, furans, hazard quotient.

**INTRODUCTION**

Polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-\(p\)-dioxins (PCDDs) present in the floodplain soils and sediments of the riverine systems downstream of Midland, Michigan (Hilscherova et al. 2003) are likely associated with the historical production of industrial organic chemicals and on-site storage and disposal of by-products, prior to the establishment of modern waste management protocols (Amendola and Barna 1986). The Tittabawassee River flows through Midland Michigan and is one of three rivers that unite to become the Saginaw River. The Saginaw River is a larger slower moving river that is less prone to rapid changes in stage, with a wider channel and more urban surroundings than the Tittabawassee River. It is generally contained within its banks, limiting the interaction with the floodplain soils that occur on the Tittabawassee River. Total concentrations of PCDD/DFs (\(\Sigma_1\)PCDD/DFs) collected from floodplain soils and sediments along the Tittabawassee River ranged from 1.0 \(\times\) 10^2 to 5.4 \(\times\) 10^4 ng/kg dw, while mean \(\Sigma_1\)PCDD/PCDF concentrations in soils and sediments in the reference area (RA) upstream of Midland were 10- to 20-fold less (Hilscherova et al. 2003). Floodplain soils of the Tittabawassee River downstream of the putative sources have concentrations of \(\Sigma_1\)PCDD/PCDF, which are 6- to 10-fold greater than the proximal river sediment, while the floodplain soil to sediment relationship is opposite for the Saginaw River. In contrast to the Tittabawassee River, the floodplain soils along downstream reaches of the Saginaw River have approximately 10-fold lesser \(\Sigma_1\)PCDD/DF concentrations than river sediments (Kannan et al. 2008).

The primary objective of this study was to evaluate the potential for adverse effects on house wrens and eastern bluebirds breeding in the river floodplains downstream of Midland, Michigan using a multiple lines of evidence approach (USEPA 1998a; Fairbrother 2003). Extensive site-specific measures of exposure included concentrations of PCDD/DFs in eggs and nestlings, as well as in the diet that was studied by measuring concentrations in invertebrates and bolus samples collected from the site. Both site- and species-specific dietary compositions were determined from bolus samples. Sufficient masses of site-specific invertebrates were collected so that concentrations of PCDD/DFs could be measured and used in the calculation of weighted average dietary exposure concentrations. In addition, 3 years of population-level reproductive endpoints (e.g., clutch size, hatching success, hatchling growth, and fledging success) were measured on a site-specific basis.

Receptor species selection is an essential step in the risk assessment process. The nature of contamination within the Tittabawassee and Saginaw rivers is variable and receptor species were selected to account for these differences. While tree swallows...
(Tachycineta bicolor) have proven to be a sufficient study species for many contaminated sites, their aquatic-based diet (McCarty 1997; McCarty and Winkler 1999; Mengelkoch et al. 2004) would not account for the greater $\sum$PCDD/DF concentrations in the floodplain soils along the Tittabawassee River. Therefore, the current study focused on the terrestrial-based assessment of risk to house wrens (Troglodytes aedon) and eastern bluebirds (Sialia sialis). Site-specific assessments of exposures and related effects for a variety of terrestrial passerine species have been conducted (Ankley et al. 1993; Bishop et al. 1995; Custer et al. 2001; Henning et al. 2003; Arenal et al. 2004; van den Steen et al. 2006, 2007), but more commonly, tree swallows have been selected as target species in assessments of risk in aquatic-based studies (Shaw 1983; DeWeese et al. 1985; Beaver 1992; Ankley et al. 1993; Bishop et al. 1995; Froese et al. 1998; Custer et al. 1998; Secord et al. 1999; Custer et al. 2000; Harris and Elliott 2000; Custer et al. 2002, 2003; Echols et al. 2004; Custer et al. 2005; Smits et al. 2005; Neigh et al. 2006b; Spears et al. 2008). However, house wrens and eastern bluebirds have been effectively used as receptors at terrestrially contaminated study sites (Thiel et al. 1988; Burgess et al. 1999; Custer et al. 2001; Mayne et al. 2004; Neigh et al. 2006a).

Based on multiple desirable characteristics, house wrens and eastern bluebirds were selected to determine the extent and distribution of exposure to $\sum$PCDD/DFs through the terrestrial food chain and associated risk downstream of Midland. Eastern bluebirds primarily forage by dropping onto prey from an elevated perch in upland habitats with sparse ground cover (e.g., old fields and pastures). House wrens primarily glean insects from shrub foliage along field edges and upland forested habitats. Subtle differences in foraging characteristics and dietary composition (Fredricks et al. 2011a) between these two species enables the comparison of two distinct terrestrial feeding guilds. In addition, these two species have an almost ubiquitous distribution both locally and throughout the United States, are relatively common, and are often multi-brooded per season. Both are obligate cavity nesters and readily occupy a provided nest box that allows for better experimental control and eliminates time-intensive nest searching. Additionally, house wrens and eastern bluebirds are resistant to disturbance and have limited foraging range while nesting, so egg and nestling tissue residue concentrations are generally indicative of local exposure.

Potential for adverse effects was evaluated by comparing concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents (TEQWHO-Avian) based on World Health Organization (WHO) TCDD equivalency factors for birds (TEFWHO-Avian) (van den Berg et al. 1998) in the diet and tissues of house wrens and eastern bluebirds to available toxicity reference values (TRVs). Predicted hazard quotients based on TRVs were compared to site-specific measures of population condition (Fredricks et al. 2011b) to evaluate potential differences between lines of evidence. Additionally, comparisons were made between these results and similar field-based measures of exposure and productivity. The hazard assessment combined with site-specific multiple lines of evidence for two species over three reproductive field seasons strengthens confidence, minimizes uncertainty, and broadens the applicability of risk assessment outcomes.
METHODS

Site Description

The study was conducted on the Tittabawassee, Chippewa, and Saginaw rivers, in the vicinity of Midland, Michigan (Figure 1). The site-specific hydrology of the Tittabawassee River combined with the lipophilic nature and slow degradation rates of dioxin-like compounds (Mandal 2005) resulted in the presence of historical contamination (ATS 2007, 2009) in both the aquatic and terrestrial food webs downstream of Midland. The Tittabawassee River system receives drainage from approximately 5426 km² of land, composed primarily of woodlands, agricultural lands, and urban areas. Water levels fluctuate naturally throughout the year. Increased flow due to spring thaw combined with the breakup of ice sheets along the river creates conditions that favor bank scouring and mobilization of sediments and floodplain soils. Annual floods suspend particulates that are deposited within the Tittabawassee River floodplain soils downstream of Midland, Michigan. Two reference areas were located upstream of the putative sources of PCDD/DFs (Hilscherova et al. 2003)

Figure 1. Study site locations within the Chippewa, Tittabawassee, and Saginaw river floodplains, Michigan, USA. Reference Areas (R-1 to R-2), Tittabawassee River Study Areas (T-3 to T-6), and Saginaw River Study Areas (S-7 to to S-9) were monitored from 2004–2007. Direction of river flow is designated by arrows; suspected source of contamination is enclosed by the dashed oval.
Risk Assessment of Passerines Exposed to PCDFs

on the Tittabawassee (R-1) and Chippewa (R-2) rivers (Figure 1). Study areas (SAs) downstream of the putative sources of PCDD/DFs include approximately 72 km of free-flowing river from the upstream boundary defined as the low-head dam near Midland, Michigan, through the confluence of the Tittabawassee and Saginaw rivers to where the Saginaw River enters Saginaw Bay in Lake Huron. The SAs along Tittabawassee River downstream of Midland included four sites (T-3 to T-6) approximately equally spaced, and three sites (S-7 to S-9) located at the initiation, median, and terminus of the Saginaw River. The seven SAs (T-3 to S-9) were selected for the Tittabawassee and Saginaw rivers, respectively, based on the necessity to discern spatial trends, ability to gain access privileges, and maximal receptor exposure potential based on floodplain width and measured soil and sediment concentrations (Hilscherova et al. 2003). Three hundred and 52 nest boxes were placed and all samples were collected from within the 100-year floodplain of the individual rivers. Nest box trails at each study site contained between 30 and 60 nest boxes and spanned a continuous foraging area of between 1 and 3 km of river. S-8 was an exception and was only used for sediment and dietary food web sampling. No studies of birds were conducted at this location.

Nest Box Monitoring

Standard passerine nest boxes with wire mesh predator guards around the entrance hole and mounted to a greased metal post were used to facilitate monitoring of nesting activity and collection of samples (Fredricks et al. 2010). Nest boxes were placed at individual study sites R-1 to T-6 in 2004, and two additional sites (S-7 and S-9) were added in year 2005. Monitoring began one year subsequent to placement of nest boxes and continued through 2007 at all sites. Individual nest boxes were placed at study sites to maximize occupancy of several passerine species (Horn et al. 1996) with relatively equal proportions of boxes placed in species-specific microhabitats for each species studied.

Previous reports provide more detailed descriptions of study-specific nest monitoring and sample collection protocols used in the current study (Fredricks et al. 2010,c). In general, boxes were monitored twice a week for occupancy beginning in early April. Boxes were monitored daily after clutch initiation through incubation and subsequently near the expected hatch or fledge day for each species. Masses of eggs were determined on the date laid, and masses of nestlings were measured four times over the brood rearing period. Eggs for use in residue quantification were collected after clutch completion and prior to the fifth day of incubation. Therefore, clutch size was not adjusted for egg sampling (i.e., clutch size equals total eggs produced). However, measures of hatching success, fledging success, and productivity were calculated based on an adjusted clutch size (i.e., total eggs produced minus eggs removed) since the fertility and hatchability of the collected egg was unknown at collection. Additionally, brood size and number of fledglings were predicted based on the adjusted hatching success and productivity, respectively. A maximum of one nestling per nesting attempt was collected from randomly selected boxes for residue quantification, 10-d post-hatch for house wrens or 14-d post-hatch for eastern bluebirds. Since fully developed nestlings were collected just prior to fledge, it was assumed that any nestlings collected would have successfully fledged.
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provided the remaining portion of the nesting attempt was successful. Therefore, fledging success and productivity were not adjusted for number of sampled nestlings (i.e., based on total number of fully developed nestlings).

House wren and eastern bluebird nestlings and adults were banded with U.S. Fish and Wildlife Service aluminum leg bands throughout the study. Adults were actively trapped by researchers at the nest box during each nesting attempt. During routine handling nestlings and adults were monitored for gross external morphological abnormalities.

**Dietary Exposure**

Detailed site descriptions and protocols for collecting and handling samples of representative invertebrate orders collected on-site and dietary bolus samples collected from nestlings have been previously described (Fredricks et al. 2011a). Concentrations of PCDD/DF in diets of house wrens and eastern bluebirds were estimated by two methods: (1) food web–based diet: multiplying study-specific dietary compositions for major (>1% by mass) prey items by respective area-specific TEQWHO-Avian concentrations in associated prey items for each study species, and (2) bolus-based diet; area-specific average, minimum, and maximum concentrations from actual bolus samples collected from nestlings of each species studied.

In support of the food web–based analysis, site-specific collections of invertebrates were made during 2003 at R-1, R-2, T-4 and T-6, 2004 at R-1, R-2 and T-3 to T-6, and 2006 at S-7 to S-9 at multiple times throughout the breeding season. Each site included two 30 m × 30 m grids proximal to the river bank, one for sampling of terrestrial invertebrates and one for collection of benthic and emergent aquatic invertebrates. Sites in the SA were selected based on maximizing the potential for collecting food items with the greatest contaminant concentrations for a given nest box trail given the available soil and sediment data. Sampling methods were designed to target aquatic emergent insects, benthic invertebrates, and terrestrial invertebrates in order to collect the necessary biomass for residues analyses and to obtain a representative sample of available dietary items at each site. Invertebrates were categorized taxonomically to the order level for each life stage collected during each sampling period per site. Samples were then homogenized and stored at –20°C until extraction.

In support of the bolus-based direct measurement of ingestion, bolus samples from nestling house wrens and eastern bluebirds were collected by use of a black electrical cable-tie fitted at the base of their neck (Mellott and Woods 1993). Samples were collected from nestlings between the ages of 3- and 9-d post-hatch for house wrens and 4- and 12-d post-hatch for eastern bluebirds. Bolus samples were collected from nestlings approximately 1 h after ligature application. Nests were not sampled on consecutive days. Invertebrates in each bolus sample were classified to order and the total number and mass of each order was recorded for each sample. The site-specific diet for both species was determined based on the relative proportion of the total mass represented by each invertebrate order identified in the bolus samples. Additionally, bolus samples were recombined for residue analyses based on clutch from which each sample was collected and combined with other proximally and temporally located boxes to obtain the necessary biomass for residue quantification.
Dietary exposures of adults and nestlings were estimated using the U.S. Environmental Protection Agency (USEPA) *Wildlife Exposure Factors Handbook* (WEFH) equations for passerine birds (USEPA 1993). USEPA WEFH Equation 3–4 was used to calculate food intake rate based on site-, species-, and age-specific body masses. Potential average daily dose (ADD$_{pot}$; ng TEQ$_{WHO-Avian}$/kg body weight/d) was calculated using Equation 4–3 (USEPA 1993) assuming that 100% of the foraging range for each species was within the associated study area. Concentrations of PCDD/DF in diets of house wrens and eastern bluebirds were estimated by use of web-based diet by multiplying study-specific dietary compositions for major (>1% by mass) prey items by respective area-specific (R-1 to R-2; T-3 to T-6; S-7 to S-9) average, minimum, and maximum concentrations of TEQ$_{WHO-Avian}$ in associated prey items for each study species. Dietary concentrations in food items were estimated for bolus-based diet by area-specific average, minimum, and maximum concentrations from actual bolus samples collected from nestlings of each species studied. Minimum and maximum concentrations were chosen to describe the range of possible invertebrate concentrations found on site, which the authors expected to include the worst-case scenario for dietary exposure. Dietary exposure estimates apply only to the nesting period for both adults and nestlings because foraging habits and range are likely more variable outside the nesting period.

Quantification of PCDD/DF

Concentrations of all of the 17 2,3,7,8-substituted PCDD/DF congeners are reported for all samples whereas concentrations of polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDT) and related metabolites are reported for a subset of eggs. Congeners were quantified in accordance with USEPA Method 8290/1668A with minor modifications (USEPA 1998b). A more detailed description of methods and the measured concentrations have been previously reported (Fredricks *et al.* 2011a, 2010). Briefly, samples were homogenized with anhydrous sodium sulfate, spiked with known amounts of $^{13}$C-labeled analytes (as internal standards), and Soxhlet extracted. Ten percent of the extract was removed for lipid content determination. Sample purification included the following: treatment with concentrated sulfuric acid, silica gel, sulfuric acid silica gel, acidic alumina and carbon column chromatography. Components were analyzed using high-resolution gas chromatography/high-resolution mass spectroscopy, a Hewlett-Packard 6890 GC (Agilent Technologies, Wilmington, DE) connected to a MicroMass® high-resolution mass spectrometer (Waters Corporation, Milford, MA). Chemical analyses included pertinent quality assurance practices, including matrix spikes, blanks, and duplicates.

Toxicity Reference Value Selection

Selection of appropriate toxicity reference values (TRVs) is an essential step in the risk assessment process. TRVs represent a concentration in food or tissues that is equal to or less than the threshold associated with adverse toxicological effects. Selection criteria for studies reporting potential TRVs involved consideration of several factors including: chemical compound, measurement endpoints associated with sensitive life-stages (development and reproduction), limited risk of co-contaminants.
causing an effect, measurement endpoints associated with ecologically relevant responses, evidence of a dose–response relationship, and use of a closely related or wildlife species. In an effort to minimize uncertainties associated with the relationship between TEQ_{WHO-Avian} values derived from PCB-based or PCDD/DF-based exposures (Custer et al. 2005), only values derived from PCDD/DF-based exposures were considered. Literature-based no observed adverse effect concentrations (NOAECs) and lowest observed adverse effect concentrations (LOAECs) were used in the determination of hazard quotients (HQs) and subsequent assessment of risk. In this study, TRVs based on concentrations in the diet and eggs were used to evaluate the potential adverse effects of site-specific contamination on two primarily terrestrial foraging passerines.

Laboratory-based dosing studies incorporating PCDD/DF dietary exposure–based effects assessments are lacking for passerines and limited in general for avian species. A study that dosed adult hen ring-necked pheasants (Phasianus colchicus) with intraperitoneal injections of TCDD for a 10 wk exposure period was selected as the dietary exposure–based TRV for this study (Nosek et al. 1992a). The major limitation of this study was that hens were exposed to TCDD via injections versus the diet. However, dosing exposure efficiency through injections should be greater than that of gut transfer thus providing a more conservative TRV. Although this study was not conducted on a passerine species, galliforms are generally considered to have greater sensitivity to dioxin-like compound exposures (Brunström and Reutergardh 1986; Brunström 1988; Powell et al. 1996, 1997a). In addition, recent evidence on the molecular basis for variation in sensitivities to dioxin-like compounds among avian species (Karchner et al. 2006; Head et al. 2008) suggests that the ring-necked pheasant exhibits a sensitivity that is equivalent to the passerines studied (SW Kennedy personal communication) but more tolerant than the domestic chicken. The diet-based TRVs were determined by converting the weekly exposure at which adverse effects on fertility and hatching success were determined (1000 ng TCDD/kg/wk) to a LOAEC for daily exposure of 140 ng TCDD/kg/d (Table 1). The dosing regime was

| Table 1. | Toxicity reference values (TRVs) for total TEQ_{WHO-Avian}^a concentrations selected for comparison to terrestrial passerines exposed to PCDD/DFs in the river systems downstream of Midland, Michigan, during 2005–2007. |
| Species | NOAEC | LOAEC | Reference |
|----------|--------|--------|-----------|
| House wren Diet. exposure–based^b | 14 | 140 | Nosek et al. 1992a |
| Egg exposure–based^c | 710 | 7,940 | USEPA 2003^d |
| Eastern bluebird Diet. exposure–based^b | 14 | 140 | Nosek et al. 1992a |
| Egg exposure–based^c | 1,000 | 10,000 | Thiel et al. 1988 |

^aTEQ_{WHO-Avian} were calculated based on the 1998 avian WHO TEF values.

^bng/kg/d ww.

^cng/kg ww.

^dcalculated from studies by Nosek et al. 1992a,b and Nosek et al. 1993.
based on orders of magnitude differences and adverse effects were not observed at
the next lowest dose, which was determined to be the NOAEC for dietary exposure
(14 ng TCDD/kg/d).

A study in which eastern bluebird eggs were injected with TCDD (Thiel et al. 1988) was selected to determine an egg tissue residue–based TRV for eastern blue-
birds in the current study. Field-collected eastern bluebird eggs were dosed with
concentrations of TCDD that ranged from 1 to 100,000 ng/kg wet weight (ww; in
10-fold increments), and then returned to their clutch and incubated by unexposed
adults. Hatching success was significantly affected at exposures greater than 10,000
ng/kg ww (LOAEC), while exposures less than 1000 ng/kg ww (NOAEC) resulted
in effects that were similar to those of the vehicle-injected controls. Despite having
only 7 to 13 eggs per dosage group, this study was selected as the eastern bluebird
egg exposure–based TRV due to species-specific applicability. Overall good hatching
success in treatment groups, presence of a dose–response relationship, and effects
were measured in an ecologically relevant endpoint.

A more conservative egg exposure–based TRV was selected for house wrens be-
because differences in species-specific sensitivity between eastern bluebirds and house
wrens was unknown. When the results of three studies (Nosek et al. 1992a,b, 1993)
that dosed ring-necked pheasant hens or eggs were combined as the geometric
mean, the NOAEC was 710 ng/kg ww while the LOAEC was 7940 ng/kg ww as egg
exposure–based TRVs for house wrens (USEPA 2003).

Additional egg-injection studies that were evaluated but not selected for deriv-
ing TRVs included studies of bobwhite quail (Colinus virginianus) (McMurry and
Dickerson 2001) and double-crested cormorant (Phalacrocorax auritus) (Powell et al.
1997b, 1998) studies. Reasons for not selecting them included limited sample size,
failure to establish a dose–response relationship, and/or poor hatchability of non-
or vehicle-injected controls.

Hazard Characterization Methods

Overall hazard of PCDD/DFs to house wrens and eastern bluebirds breeding
in the river floodplains downstream of Midland was assessed with several lines of
evidence (USEPA 1998a; Fairbrother 2003) that incorporated both dietary- and egg
tissue–based exposure estimates in addition to measures of site-specific reproduc-
tive success. Potential effects of dietary- and tissue-based exposures were assessed
by calculating ranges of hazard quotients (HQ) for each species. Concentrations
of \( \Sigma_{1} \) PCDD/DF TEQs \( \text{WHO-Avian} \) (ng/kg ww) in eggs and dietary estimates [potential
average daily dose (ADD \( \text{pot} \), ng/kg/d)] were divided by egg exposure– or dietary
exposure–based NOAEC or LOAEC TRVs (Table 1), respectively.

Hazard quotients for egg exposures were determined based on the upper 95%
confidence level (UCL) of the geometric mean egg tissue residue concentrations at
each study location. Hazard quotients for dietary exposures were based on ranges
of concentrations at RAs, Tittabawassee River SAs, and Saginaw River SAs divided
by the selected TRV, respectively. Ranges were used for dietary exposure estimates
due to limited sample sizes at most study locations. Furthermore, samples of inver-
tebrates from the food web were composites of all individuals of an order collected
per location per sampling period, which provide an accurate estimate of the central
tendency of the concentration estimates, but limit the information about variability within each order at a location. HQs for dietary exposure were calculated based on TEQ$_{\text{WHO-Avian}}$ in bolus-based dietary exposure estimates at reference and Tittabawassee River SAs, and on food web–based dietary exposure estimates at Saginaw River SAs. Concentrations of residues were not measured in bolus samples from Saginaw River SAs. In addition to dietary- and egg-based hazard assessments, potential adverse effects on population health were concurrently evaluated for ecologically relevant endpoints at site-specific downstream and upstream study areas, and compared to relevant literature-based field studies. Incorporation of both dietary- and tissue-based assessment endpoints has been shown to greatly reduce uncertainty in risk assessments of persistent organic pollutants (Leonards et al. 2008).

Statistical Analyses

Each individual nesting attempt was considered the experimental unit for statistical comparisons (i.e., if an individual nested multiple times on site each attempt was considered independent). Egg-based exposure comparisons were made between sampling locations (Fredricks et al. 2010). Samples from individual locations were combined by study area for comparisons of bolus- and food web–based dietary concentrations due to limited biomass collected at each location (Fredricks et al. 2011a). Detailed descriptions of productivity measures and associated statistical analyses have been provided previously (Fredricks et al. 2011b).

Total concentrations of the 17 individual 2,3,7,8-substituted PCDD/DF congeners are reported as the sum of all congeners (ng/kg ww). For individual congeners that were less than the limit of quantification a proxy value of half the sample method detection limit was assigned. Concentrations of TEQ$_{\text{WHO-Avian}}$ (ng/kg ww) were calculated for PCDD/DFs by summing the product of the concentration of each congener, multiplied by its avian TEF$_{\text{WHO-Avian}}$ (van den Berg et al. 1998). Total concentrations of twelve non- and mono-ortho-substituted PCB congeners are reported as the sum of these congeners (ΣPCBs) for a subset of egg samples. Also, concentrations of 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 (DDT metabolites) for the same subset of samples as for PCBs.

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. For concentration data that were not normally distributed, the data were transformed using the natural log (ln) of $(x + 1)$. To better understand the potential distributions of the TEQ$_{\text{WHO-Avian}}$ egg concentrations at each study location a probabilistic modeling approach was used to portray the distributions. Probabilistic models were developed as cumulative frequency distributions based on ΣPCDD/DF TEQ$_{\text{WHO-Avian}}$ concentrations in eggs. The mean and standard deviation of transformed egg values were used to generate a sample of 10,000 random egg values based on a lognormal distribution. The association between concentrations of ΣPCDD/DF TEQ$_{\text{WHO-Avian}}$ and hatching success by species was evaluated with Pearson’s correlation coefficients for nesting attempts in which both data were collected. Statistical significance was considered at $p < .05$.
RESULTS

Site-Specific Endpoints

Among all study sites, 427 house wren clutches and 122 eastern bluebird clutches were initiated and monitored for productivity during the breeding seasons from 2005 to 2007. Both species nested at all sites with the exception that no eastern bluebird clutches were initiated at S-9. Additionally, concentrations of \( \Sigma_{1} \text{PCDD/DF} \) were quantified in eggs and nestlings collected from individual house wren (49 and 48, respectively) and eastern bluebird (35 and 30, respectively) nesting attempts. Samples of boluses were collected throughout the nesting season from 135 house wren and 51 eastern bluebird nesting attempts to determine site-specific foraging patterns and to determine bolus-based dietary exposure to PCDD/DFs.

Tissue residues

Concentrations of PCDD/DFs and TEQ_{WHO-Avian} are reported for eggs and nestlings of house wrens and eastern bluebirds collected on-site (Fredricks et al. 2010). Geometric mean concentrations of TEQ_{WHO-Avian} in eggs of house wrens and eastern bluebirds from Tittabawassee River SAs were 5- to 91-fold greater.
Figure 3. Geometric mean concentrations of $\Sigma$ PCDD/DF TEQ$_{\text{WHO-Avian}}$ in eastern bluebird eggs collected during 2005–2007 from the river floodplains near Midland, Michigan. Error bars show the 95% upper confidence level (UCL); Reference areas (R-1 and R-2); Tittabawassee River study areas (T-3 to T-6); and Saginaw River study areas (S-7 and S-9); sample size is indicated in parentheses under the sample site; range presented for S-7 where $n = 2$.

than those from RAs (Figures 2 and 3), while concentrations in eggs collected from the Saginaw River SAs were intermediate. Patterns of relative concentrations of congeners in eggs from more downstream SAs were dominated primarily by 2,3,4,7,8-pentadibenzofuran (2,3,4,7,8-PeCDF) and to a lesser extent 2,3,7,8-tetrachlorodibenzofuran (TCDF) opposed to primarily dioxin congeners at RAs. Maximum concentration of TEQ$_{\text{WHO-Avian}}$ in eggs of house wrens and eastern bluebirds were 2300 ng/kg at T-3 and 1000 ng/kg at T-6, respectively. Co-contaminants in eggs, including DDT and metabolites, and PCBs, were not significantly greater than established regional background concentrations for the two species. In addition, concentrations of $\Sigma$ PCDD/DFs in nestlings of both species at SAs were 8- to 50-fold greater than those in nestlings from RAs (Fredricks et al. 2010). Maximum concentration of TEQ$_{\text{WHO-Avian}}$ in nestlings of house wrens and eastern bluebirds occurred at T-6 and were 1200 ng/kg and 1400 ng/kg, respectively. The relative potency of the exposure mixture was reasonably consistent and associated concentrations of TEQ$_{\text{WHO-Avian}}$ were positively correlated with concentrations of $\Sigma$ PCDD/DFs in both eggs and nestlings of all studied species (Fredricks et al. 2010). Nestling-based
Table 2. Potential average (range) $\text{TEQ}_{\text{WHO-Avian}}$ daily dose (ADD$_\text{pot}$; ng/kg body weight/d) calculated from site-specific bolus-based and food web–based dietary exposure for adult house wrens and eastern bluebirds breeding during 2004–2006 within the river floodplains near Midland, Michigan.

|                      | R-1 and R-2$^b$ | T-3 to T-6 | S-7 and S-9 |
|----------------------|----------------|------------|-------------|
| **House wren**       |                |            |             |
| Bolus                | 1.1 (0.73–1.7)$^{c,d}$ | 150 (38–430) | —$^e$ |
| Food web             | 1.5 (0.54–3.0) | 68 (13–140) | 16 (5.9–34) |
| **Eastern bluebird** |                |            |             |
| Bolus                | 0.88 (0.44–1.9) | 110 (13–450) | — |
| Food web             | 1.1 (0.47–2.2) | 77 (24–180) | 41 (6.2–110) |

$^a$TEQ$_{\text{WHO-Avian}}$ were calculated based on the 1998 avian WHO TEF values.
$^b$R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area.
$^c$Values were rounded and represent only two significant figures.
$^d$Food ingestion rate was calculated from equations in The Wildlife Exposure Factors Handbook (USEPA 1993).
$^e$Residue analyses were not conducted on bolus collected invertebrates at S-7 and S-9.

Congener profiles were similar to egg-based profiles for both species studied and among study areas (Fredricks et al. 2010).

Dietary exposures

Dietary exposures were greater in the SA than the RA. When concentrations of TEQ$_{\text{WHO-Avian}}$ quantified in site-specific and bolus samples collected from both house wren and eastern bluebird nestlings (Fredricks et al. 2011a) were used to calculate site-specific dietary composition based on mass of individual invertebrate orders to the overall dietary mass from bolus samples the potential average daily dose (ADD$_\text{pot}$; ng TEQ$_{\text{WHO-Avian}}$/kg body weight/d) for house wrens was 136-fold greater at the Tittabawassee River SAs compared to RAs. Bolus-based ADD$_\text{pot}$ estimates were intermediate at Saginaw River SAs (Table 2). ADD$_\text{pot}$ for bluebirds based on TEQ$_{\text{WHO-Avian}}$ concentrations were 125-fold greater at Tittabawassee River SAs compared to RAs, while ADD$_\text{pot}$ were intermediate at the Saginaw River SAs (Table 2).

Productivity

Reproductive parameters including clutch size, egg mass, hatching success, predicted brood size, nestling growth, fledging success, predicted number of fledglings, and productivity for house wrens and eastern bluebirds breeding in the river floodplains were similar or greater at downstream SAs compared to upstream RAs among all study years (Fredricks et al. 2011b). Of all initiated clutches, 66% and 64% successfully fledged at least one nestling for house wrens and eastern bluebirds, respectively. Although there were several differences, house wren fledging success was greater at RAs (86%) compared to Saginaw River SAs (73%), while Tittabawassee River SAs (82%) were intermediate. However predicted brood size was greater at...
Saginaw River SAs (5.1 nestlings/brood) compared to Tittabawassee River SAs (4.5 nestlings/brood), while RAs (5.0 nestlings/brood) were intermediate. Since adult females were captured and uniquely identified during nesting attempts it was possible to determine overall nesting success per female for the duration of the study. Total numbers of nestlings fledged per female from 2005 to 2007 were similar among study areas and averaged (range) 5.2 (0–25) and 5.4 (0–13) for house wrens and eastern bluebirds, respectively. Nestling growth rate constants and mass gained per day were similar among study areas for both species studied (Fredricks et al. 2011b).

Additional information pertaining to post-fledge survival and recruitment of recently fledged nestlings might offer additional insight into population health and sustainability. However, due to the relatively short duration of this portion of the study and inherently small recruitment and site fidelity of yearling passerines (Summers-Smith 1956; Adams et al. 2001; Robinson et al. 2007; Wells et al. 2007; Rush and Stutchbury 2008; Fredricks et al. 2011b) a comprehensive band monitoring data set of extended duration (2005 to 2009) for the birds described is ongoing.

**Correlation Assessment**

Hatching success was not correlated with concentrations of $\Sigma$PCDD/DF TEQ$_{\text{WHO-Avian}}$ in either house wren or eastern bluebird eggs for clutches with both data points measured. House wren eggs from RAs had lesser TEQ$_{\text{WHO-Avian}}$ but similar hatching success compared to downstream SAs, which resulted in a slightly negative correlation coefficient ($R = -0.14526$, $p = 0.3587$, $n = 42$; Figure 4) that was not significant. Overall mean hatching success for eastern bluebirds at RAs (70%) was not significantly less than Tittabawassee River SAs (84%), however the trend resulted in a significant positive correlation with TEQ$_{\text{WHO-Avian}}$ concentrations ($R = 0.47213$, $p = 0.0198$, $n = 24$; Figure 5).

**Hazard Assessment**

When predicted probabilistic distributions of expected cumulative percent frequencies based on concentrations of $\Sigma$PCDD/DF TEQ$_{\text{WHO-Avian}}$ in eggs of house wren and eastern bluebirds were compared to selected TRVs, the predicted distributions of concentrations in house wren eggs were greater than the NOAEC (710 ng/kg ww; (USEPA 2003)) for all sites other than RAs and S-9 (Figure 6). Sites T-3 and T-6 had 58% and 65% of the predicted distribution greater than the NOAEC, while S-9, T-4, and T-5 had 10%, 15%, and 21% of the frequency distribution greater than the NOAEC, respectively. Based on the predicted distributions at all study sites, less than 1% of the cumulative frequency of exposure concentrations was greater than the LOAEC (7940 ng/kg ww; (USEPA 2003)). Predicted distributions of concentrations of TEQ$_{\text{WHO-Avian}}$ in eastern bluebird eggs were greater than the NOAEC (1000 ng/kg ww; (Thiel et al. 1988)) at the Tittabawassee River SAs, while those at RAs and the Saginaw River SAs were not (Figure 7). Sites T-3 and T-6 had 1% and 15% of the predicted distribution greater than the NOAEC, while no study sites were greater than the LOAEC (10,000 ng/kg ww; (Thiel et al. 1988)).
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Figure 4. Correlation plot of percent hatching success and $\Sigma$PCDD/DF TEQs WHO-Avian in house wren eggs for nesting attempts with data collected for both variables from the river floodplains near Midland, Michigan during 2005–2007. R- and p-values and sample size indicated; 1 = R-1; 2 = R-2; 3 = T-3; 4 = T-4; 5 = T-5; 6 = T-6; 7 = S-7; 9 = S-9.

Hazard quotients (HQs) calculated as the upper 95% confidence level (UCL; geometric mean) concentrations of $\Sigma$PCDD/DF TEQs WHO-Avian in house wren and eastern bluebird eggs divided by the species-specific egg-based LOAEC TRVs were less than one among all study sites. Tittabawassee River SAs T-6, T-3, and T-5 had HQs greater than one for house wren eggs based on the 95% UCL and NOAEC, but at all other sites HQs were less than 1.0 (Figure 8). Hazard quotients for eastern bluebird eggs based on the 95% UCL and NOAEC TRV were less than one for all sites except T-6 at which it was approximately one (Figure 9).

Bolus-based HQs based on either the NOAEC or LOAEC at Tittabawassee River SAs were greater than 1.0 for house wrens and eastern bluebirds (Figure 10). Food web–based dietary exposure HQs at Saginaw River SAs for both house wrens and eastern bluebirds were greater than 1.0 (Figure 10). Diet-based on maximum measured $\Sigma$PCDD/DF TEQs WHO-Avian concentrations at the Tittabawassee and Saginaw River SAs were greater than the diet-based NOAEC TRV for both species studied whether food web– or bolus-based estimates of dietary exposure were used at Tittabawassee River SAs. Dietary exposure–based estimates of minimum measured concentrations for both house wrens and eastern bluebirds at Tittabawassee River SAs were greater

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Figure 5. Correlation plot of percent hatching success and $\sum$PCDD/DF TEQs WHO-Avian in eastern bluebird eggs for nesting attempts with data collected for both variables from the river floodplains near Midland, Michigan during 2005–2007. $R$- and $p$-values and sample size indicated; $1 = R-1$; $2 = R-2$; $3 = T-3$; $4 = T-4$; $5 = T-5$; $6 = T-6$; $7 = S-7$.

than the LOAEC TRV, while Saginaw River SAs were less. Both food web– and bolus-based estimates of dietary exposure were less than associated LOAEC and NOAEC TRVs at RAs.

DISCUSSION

Risk Characterization

Assessing the potential for adverse effects by use of a HQ approach that is based on the most appropriate TRVs available for the species studied can provide information on the presence of site-specific effects. HQs greater than 1.0 are indicative of exposures that exceed the threshold for adverse effects and suggest there is the potential for adverse effects to occur. Compared to the predicted distributions of concentrations of TEQs WHO-Avian in eggs at these sites, the percent of the frequency distribution greater than the NOAEL ranged from 21 to 65% for house wrens and was 15% for eastern bluebirds (Figures 6 and 7). However, less than 1% of the frequency distribution for concentrations of TEQs WHO-Avian in house wren eggs was greater than the LOAEC, and 0% of the predicted distribution was greater for eastern bluebirds. The actual effect threshold for individuals is likely between the established no- and
Figure 6. Modeled probabilistic distribution of expected cumulative percent frequencies for house wren egg TEQ_{WHO-Avian} concentrations ng/kg ww in site-specific eggs collected from the river floodplains near Midland, Michigan in 2005–2007. 10,000 simulations per site; R-1 and R-2 indicated by solid lines; T-3 to T-6 indicated by dash-dot-dash lines; S-7 and S-9 indicated by dotted lines; Y-axis offset to show R-1 and R-2; NOAEC and LOAEC indicated by vertical bars.

lowest-effect TRV values. Based on conservatively selected, egg-based TRVs (likely based on a species with greater sensitivity) and 95% UCL exposures the potential for effects on individual house wrens at Tittabawassee River SAs is minimal, and effects on eastern bluebirds are not expected.

Hazard quotient values based on concentrations of TEQ_{WHO-Avian} in food bolus for both house wrens and eastern bluebirds had similar trends and were greater than or equal to 1.0 at Tittabawassee River SAs based on the minimum value of TEQ_{WHO-Avian} concentrations and NOAEC. Dietary exposures of house wrens and eastern bluebirds on-site were similar to dietary exposures measured in tree swallow nestlings exposed to primarily TCDD on the Woonasquatucket River in Massachusetts that ranged from 0.87 to 6.6 and from 72 to 230 ng TEQ/kg ww at unexposed and exposed sites, respectively (Custer et al. 2005). House wren and eastern bluebird exposure at Tittabawassee River SAs would range from 66 to 209 and from 57 to 179 ng TEQ/kg BW/d, respectively, when converted to a daily dietary dose based on site- and species-specific ingestion rates calculated from data collected in the current study. In the Woonasquatucket River study on tree swallows, hatching success was negatively impacted at exposed sites, and although beyond the scope of
Figure 7. Modeled probabilistic distribution of expected cumulative percent frequencies for eastern bluebird egg $\text{TEQ}_{\text{WHO-Avian}}$ concentrations ng/kg ww in site-specific eggs collected from the river floodplains near Midland, Michigan in 2005–2007. 10,000 simulations per site; R-1 and R-2 indicated by solid lines; T-3 to T-6 indicated by dash-dot-dash lines; S-7 indicated by a dotted line; Y-axis offset to show R-1 and R-2; NOAEC indicated by a vertical bar; LOAEC (not indicated) is 10,000 ng TEQs/kg ww (Thiel et al. 1988).

their conclusions it is likely that adult dietary exposure prior to breeding was similar to nestling exposures. Therefore, similar effects on hatching success could be predicted at the comparable exposures measured at the Tittabawassee River SAs (Table 2).

Multiple Lines of Evidence and Population-Level Effects

Predicted effects on productivity based on tissue- and dietary-based exposure estimates were compared with measured productivity of the terrestrial passerines studied to provide a site-specific multiple lines of evidence assessment of potential for adverse effects (Menzie et al. 1996; Fairbrother 2003; Hull and Swanson 2006; Neigh et al. 2006a; Barnthouse et al. 2009). To minimize potential uncertainties associated with predicting the potential for adverse effects based solely on concentrations in abiotic matrices, in this study both the exposure to PCDD/DF and reproductive performance expressed as productivity were directly measured (Chapman et al. 2002; Leonards et al. 2008). Uncertainties were also minimized due to the robust
Figure 8. Hazard quotients (HQ) for the effects of $\Sigma$PCDD/DF TEQ$_{\text{WHO-Avian}}$ for house wren eggs collected in 2005–2007 from the river floodplains near Midland, Michigan, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). 95% confidence intervals (LCL/UCL) based on the geometric mean concentrations are presented; Left y-axis for reference areas (R-1 and R-2); Right y-axis for Tittabawassee River study areas (T-3 to T-6) and Saginaw River study areas (S-7 and S-9).

Sample sizes obtained for measurement endpoints for both house wrens and eastern bluebirds at sites studied near Midland, Michigan.

Since dietary exposures to $\Sigma$PCDD/DF TEQ$_{\text{WHO-Avian}}$ on the Tittabawassee River were similar to those based on tree swallows on the Woonasquatucket River (Custer et al. 2005) and due to the lack of field studies on house wrens and eastern bluebirds exposed to PCDD/DFs, comparisons were made with the threshold for effects on hatching success reported as 1700 ng TCDD/kg ww in eggs. The threshold for a decrease in hatching success based on the predicted distribution of TEQ$_{\text{WHO-Avian}}$ for house wrens at T-3 and T-5 would have been exceeded for approximately 20–25% of the population, while for eastern bluebirds at T-6 less than 5% would have been affected (Figures 5 and 6). However, statistical comparison of group means for hatching success of house wrens from Tittabawassee River SAs (77%) was not significantly less than that at RAs (81%) (Fredricks et al. 2011b), and was not correlated with concentrations in eggs for individual clutches (Figure 3). Although statistical power to discern differences between measures of productivity for eastern bluebirds on-site were possibly limited by occupancy, reproductive parameters among study
Figure 9. Hazard quotients (HQ) for the effects of $\sum$PCDD/DF TEQ$^{\text{WHO-Avian}}$ for eastern bluebird eggs collected in 2005–2007 from the river floodplains near Midland, Michigan, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). 95% confidence intervals (LCL/UCL) based on the geometric mean concentrations are presented; range presented for S-7 where $n = 2$; Left y-axis for reference areas (R-1 and R-2); Right y-axis for Tittabawassee River study areas (T-3 to T-6) and Saginaw River study areas (S-7 and S-9).

areas (Fredricks et al. 2011b) were similar to those reported for uncontaminated sites (Pinkowski 1979; Bauldry et al. 1995).

Despite dietary- and tissue-based exposures for both house wrens and eastern bluebirds that were comparable to tree swallows exposed to primarily TCDD at similarly contaminated sites (Custer et al. 2005) and elevated HQs at study areas downstream of Midland, overall productivity through fledging was unaffected. For the Woonasquatucket River, TEQ$^{\text{WHO-Avian}}$ exposures were primarily from TCDD (Custer et al. 2005) as compared to primarily 2,3,4,7,8-PeCDF and TCDF in terrestrial passerines tissue- and dietary-based exposures in the current study. Potential differences in the distribution and metabolism of specific congeners by birds (Norstrom et al. 1976, 1986; Elliott et al. 1996) or differences in species-specific sensitivities to dioxin-like compounds (Karchner et al. 2006; Head et al. 2008) could also account for potential differences between some literature-based thresholds and the lack of effects observed.
Figure 10. Hazard quotients (HQ) for the effects of potential $\Sigma$PCDD/DF TEQ$_{\text{WHO-Avian}}$ daily dietary dose calculated from site-specific bolus-based (R1 to T-6) and food web–based (S-7 to S-9) dietary exposure for adult house wren and eastern bluebird collected in 2005–2007 from the river floodplains near Midland, Michigan, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). HQs based on measured concentration ranges are presented; Left y-axis for reference areas (R-1 and R-2); Right y-axis for Tittabawassee River study areas (T-3 to T-6) and Saginaw River study areas (S-7 to S-9); food web–based dietary exposure is presented for S-7 to S-9 since no bolus samples were collected from those sites.

Species Selection

Overall, house wrens and eastern bluebirds were shown to be well suited to evaluate terrestrial-based contaminant exposures. The general abundance, wide distributions, and lenient habitat requirements of house wrens permitted collection of more than adequate measures of reproductive success and population health measurements. Challenges for house wren use included small nestling mass (10-d nestlings averaged approximately 10 g) and egg mass (averaged approximately 1.4 g) that may result in the need to pool samples to meet analytical detection limit requirements depending on the site and the analyte. Related dietary sampling of boluses for house wrens can also be limited by collection masses due to smaller invertebrates being fed to nestlings. Alternatively, eastern bluebirds nestlings and eggs are larger (14-d nestlings averaged approximately 28 g and eggs averaged...
approximately 3.1 g) as are dietary items, but populations are smaller and habitat requirements are more stringent. Therefore, adequate sample masses are available but often reproductive success and population health measures can be limited by low box occupancy. Additionally, species-specific diet and foraging habitat selections were reflected in egg, nestling, and dietary contaminant concentrations among these two terrestrial passerines, which reiterates the importance of receptor selection in the RA process. By combining multiple lines of evidence for these two passerine species, a balanced assessment of risk for the site of terrestrial-based contamination near Midland, Michigan, was possible.

Uncertainty Assessment

Uncertainties in this risk assessment to passerines included: availability of appropriate studies to determine dietary- and egg-based TRVs, potential inter-species sensitivity differences, and potential variability in dietary exposures based on order-level analyses. Alternatively, this study was able to collect ample data over three breeding seasons on site-specific reproductive parameters, dietary composition, and dietary- and tissue-based exposures for two terrestrial species to increase the confidence in the assessment despite these uncertainties. Through the incorporation of this extensive site-specific database over multiple measurement endpoints this assessment was able to overcome some of the greatest limitations faced by traditional point estimate based hazard assessments.

For most assessments the greatest limiting factor for developing accurate assessments of risk for birds exposed to dioxin-like compounds is a lack of comprehensive studies designed to determine thresholds for effects in ecologically-relevant species. Recent advancements in TRV selection and calculations involving the combination of multiple suitable studies into a dose–response curve (Allard et al. 2010) although appropriate were not feasible with the limited number of acceptable studies.

The domestic chicken (Gallus domesticus) is considered to be the most sensitive bird species to the effects of dioxin-like compounds (Brunström and Reutergardh 1986; Brunström 1988; Powell et al. 1996; Henshel et al. 1997; Brunström and Halldin 1998; Blankenship et al. 2003). Considering a number of data usability criteria, the TRVs used herein were based on studies of the ring-necked pheasant and eastern bluebird rather than the more conservative chicken effects data. Thus, despite limited sample sizes, potential confounding factors based on field-incubated eggs, the lack of a true dose–response relationship, and potential congener-specific differences, the TRVs based on eastern bluebird egg injections (Thiel et al. 1988) are the best available for eastern bluebird egg exposure and hatching success due to species-similarity considerations. For dietary exposure–based TRVs the intraperitoneal injections of TCDD in hen ring-necked pheasants (Nosek et al. 1992a) likely overestimates effects thresholds for the passerine species studied here. A major limitation of this TRV is that the exposure route is not a true dietary dose, which does not take into account sequestration, metabolism, excretion, and bioavailability of the contaminants when bound to dietary items (Norstrom et al. 1976; Braune and Norstrom 1989; Elliott et al. 1996; Drouillard et al. 2001; Kubota et al. 2006; Wan et al. 2006).
CONCLUSIONS

The hazard assessment based on estimated dietary exposures suggested that both populations residing in the downstream floodplain would be negatively affected. However, when concentrations of PCDD/DF in eggs were compared to appropriate TRVs, a low probability of population-level effects was predicted. This prediction is consistent with the reproductive success of the breeding populations as measured with no effects observed. The most probable cause of the apparent dichotomy between the dietary- and tissue-based exposure assessments was that the dietary-based TRVs selected were overly conservative based on the use of intraperitoneal injection dosing in those ring-necked pheasant studies. However, agreement between the two strongest lines of evidence, predicted and measured, for both species provides convincing evidence that supports the conclusion of a low potential for population-level effects at this site. The results of this study indicate that unless appropriate measures of both exposure and response are used in the assessment of hazard, the potential for adverse effects can be overestimated. The results of our study also indicated when appropriate estimates of exposure and response are used that an accurate prediction of measured responses under field conditions can be made.

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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-WTL.

REFERENCES

Adams AAY, Skagen SK, and Adams RD. 2001. Movements and survival of lark bunting fledglings. Condor 103:643–7
Allard P, Fairbrother A, Hope BK, et al. 2010. Recommendations for the development and application of wildlife toxicity reference values. Integ Environ Assess Manag 6:28–37
Amendola GA and Barna DR. 1986. Dow Chemical Wastewater Characterization Study: Tittabawassee River Sediments and Native Fish. EPA-905/4–88-003:1–118, Westlake, Ohio, USA
Ankley GT, Niemi GJ, Lodge KB, et al. 1993. Uptake of planar polychlorinated biphenyls and 2,3,7,8-substituted polychlorinated dibenzofurans and dibenzo-p-dioxins by birds nesting in the lower Fox River and Green Bay, Wisconsin, USA. Arch Environ Contamin Toxicol 24:332–44
Arenal CA, Halbrook RS, and Woodruff M. 2004. European starling (Sturnus vulgaris): Avian model and monitor of polychlorinated biphenyl contamination at a Superfund site in southern Illinois, USA. Environ Toxicol Chem 23:93–104
ATS. 2007. Remedial Investigation Work Plan, Tittabawassee River and Floodplain Soils, Midland, Michigan, December 2006; revised September 2007, Ann Arbor, Michigan, USA
ATS. 2009. Final GeoMorph® Site Characterization Report, Tittabawassee River and Floodplain Soils, Volume II of VI—Evaluation of Constituents of Interest, Supplemental Information, Midland, Michigan, June 2009, Ann Arbor, Michigan, USA
Barnthouse LW, Glaser D, and DeSantis L. 2009. Polychlorinated biphenyls and Hudson River white perch: Implications for population-level ecological risk assessment and risk management. Int Environ Assess Manag 5:435–44
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Bauldry VM, Muschitz DM, Radunzel LA, et al. 1995. A 27-year study of eastern bluebirds in Wisconsin: productivity, juvenile return rates and dispersal outside the study area. N Am Bird Band 20:111–9

Beaver DL. 1992. Analysis of Tree Swallow Reproduction and Growth and Maturation of Nestlings in the Saginaw Bay Area. Final Report submitted to Natural Resources Research Institute, Duluth, Minnesota, USA

Bishop CA, Koster MD, Chek AA, et al. 1995. Chlorinated hydrocarbons and mercury in sediments, red-winged blackbirds (Agelaius phoeniceus) and tree swallows (Tachycineta bicolor) from wetlands in the Great Lakes-St. Lawrence River basin. Environ Toxicol Chem 14:491–501

Blankenship AL, Hilscherova K, Nie M, et al. 2003. Mechanisms of TCDD-induced abnormalities and embryo lethality in white leghorn chickens. Comp Biochem Physiol C-Toxicol & Pharmacol 136:47–62

Braune BM and Norstrom RJ. 1989. Dynamics of organochlorine compounds in herring-gulls—3. Tissue distribution and bioaccumulation in Lake-Ontario gulls. Environ Toxicol Chem 8:957–68

Brunström B. 1998. Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3′,4,4′-tetrachlorobiphenyl. Pltry Sci 67:52–7

Brunström B and Halldin K. 1998. EROD induction by environmental contaminants in avian embryo livers. Comp Biochem Physiol C–Toxicol & Pharmacol 121:213–9

Brunström B and Reutergardh L. 1986. Differences in sensitivity of some avian species to the embryotoxicity of a PCB, 3,3′,4,4′-tetrachlorobiphenyl, injected into the eggs. Environ Poll Ser A-Ecol Bio 42:37–45

Burgess NM, Hunt KA, Bishop CA, et al. 1999. Cholinesterase inhibition in tree swallows (Tachycineta bicolor) and eastern bluebirds (Sialia sialis) exposed to organophosphorus insecticides in apple orchards in Ontario, Canada. Environ Sci Technol 18:708–16

Chapman PM, Ho KT, Munns WR, et al. 2002. Issues in sediment toxicity and ecological risk assessment. Marine Poll Bull 44:271–8

Custer TW, Custer CM, and Coffey M. 2000. Organochlorine chemicals in tree swallows nesting in pool 15 of the upper Mississippi River. Bull Environ Contam and Toxicol 64:341–6

Custer CM, Custer TW, Dummer PM, et al. 2003. Exposure and effects of chemical contaminants on tree swallows nesting along the Housatonic River, Berkshire county, Massachusetts, USA, 1998–2000. Environ Toxicol Chem 22:1605–21

Custer CM, Custer TW, Rosiu CJ, et al. 2005. Exposure and effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in tree swallows (Tachycineta bicolor) nesting along the Woonasquatucket River, Rhode Island, USA. Environ Toxicol Chem 24:93–109

Custer TW, Custer CM, Dickerson K, et al. 2001. Polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, trace elements, and monooxygenase activity in birds nesting on the North Platte River, Casper, Wyoming, USA. Environ Toxicol Chem 20:624–31

Custer TW, Custer CM, and Hines RK. 2002. Dioxins and congener-specific polychlorinated biphenyls in three avian species from the Wisconsin River, Wisconsin. Environ Poll 119:323–32

DeWeese LR, Cohen RR, and Stafford CJ. 1985. Organochlorine residues and eggshell measurements for tree swallows Tachycineta bicolor in Colorado. Bull Environ Contam Toxicol 35:767–75

Drouillard KG, Fernie KJ, Smits JE, et al. 2001. Bioaccumulation and toxicokinetics of 42 polychlorinated biphenyl congeners in American kestrels (Falco sparverius). Environ Toxicol Chem 20:2514–22

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Echols KR, Tillitt DE, Nichols JW, et al. 2004. Accumulation of PCB congeners in nestling tree swallows (Tachycineta bicolor) on the Hudson River, New York. Environ Sci Technol 38:6240–6

Elliott JE, Norstrom RJ, Lorenzen A, et al. 1996. Biological effects of polychlorinated dibenzo-\(p\)-dioxins, dibenzofurans, and biphenyls in bald eagle (Haliaeetus leucocephalus) chicks. Environ Toxicol Chem 15:782–93

Fairbrother A. 2003. Lines of evidence in wildlife risk assessments. Hum Ecol Risk Assess 9:1475–91

Fredricks TB, Zwiernik MJ, Seston RM, et al. 2011b. Reproductive success of house wrens, tree swallows, and eastern bluebirds exposed to elevated concentrations of PCDFs in a river system downstream of Midland, Michigan, USA. Environ Sci Tech

Fredricks TB, Giesy JP, Coefield SJ, et al. 2011a. Dietary exposure of three passerine species to PCDD/DFs from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Environ Mon Assess 172:91–112

Fredricks TB, Zwiernik MJ, Seston RM, et al. 2010. Passerine exposure to primarily PCDFs and PCDDs in the river floodplains near Midland, Michigan, USA. Arch Environ Contam Toxicol 58:1048–64

Froese KL, Verbrugge DA, Ankley GT, et al. 1998. Bioaccumulation of polychlorinated biphenyls from sediments to aquatic insects and tree swallow eggs and nestlings in Saginaw Bay, Michigan, USA. Environ Toxicol Chem 17:484–92

Harris ML and Elliott JE. 2000. Reproductive success and chlorinated hydrocarbon contamination in tree swallows (Tachycineta bicolor) nesting along rivers receiving pulp and paper mill effluent discharges. Environ Poll 110:307–20

Head JA, Hahn ME, and Kennedy SW. 2008. Key amino acids in the aryl hydrocarbon receptor predict dioxin sensitivity in avian species. Environ Sci Technol 42:7535–41

Henning MH, Robinson SK, McKay KJ, et al. 2003. Productivity of American robins exposed to polychlorinated biphenyls, Housatonic River, Massachusetts, USA. Environ Toxicol Chem 22:2783–8

Henshel DS, Hehn B, Wagey R, et al. 1997. The relative sensitivity of chicken embryos to yolk- or air-cell-injected 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin. Environ Toxicol Chem 16:725–32

Hilscherova K, Kannan K, Nakata H, et al. 2003. Polychlorinated dibenzo-\(p\)-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. Environ Sci Technol 37:468–74

Horn DJ, Benninger-Truax M, and Ulaszewski DW. 1996. The influence of habitat characteristics on nest box selection of eastern bluebirds (Sialia sialis) and four competitors. OH J Sci 96:57–9

Hull RN and Swanson S. 2006. Sequential analysis of lines of evidence—an advanced weight-of-evidence approach for ecological risk assessment. Integ Environ Assess Manag 2:302–11

Kannan K, Yun S, Ostaszewski A, et al. 2008. Dioxin-like toxicity in the Saginaw River watershed: polychlorinated dibenzo-\(p\)-dioxins, dibenzofurans, and biphenyls in sediments and floodplain soils from the Saginaw and Shiawassee rivers and Saginaw Bay, Michigan, USA. Arch Environ Contam Toxicol 54:9–19

Karchner SI, Franks DG, Kennedy SW, et al. 2006. The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor. PNAS 103:6252–7

Kubota A, Iwata H, Tanabe S, et al. 2006. Congener-specific toxicokinetics of polychlorinated dibenzo-\(p\)-dioxins, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls in black-eared kites (Milvus migrans): Cytochrome P450A-dependent hepatic sequestration. Environ Toxicol Chem 25:1007–16

Leonards PE, van Hattem B, and Leslie H. 2008. Assessing the risks of persistent organic pollutants to top predators: A review of approaches. Integ Environ Assess Manag 4:386–98
Risk Assessment of Passerines Exposed to PCDFs

Mandal PK. 2005. Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. J Comp Physiol B-Biochem Syst Environ Physiol 175:221–30

Mayne GJ, Martin PA, Bishop CA, et al. 2004. Stress and immune response of nestling tree swallows (Tachycineta bicolor) and eastern bluebirds (Sialia sialis) exposed to nonpersistent pesticides and p,p'-dichlorodiphenyldichloroethylene in apple orchards of southern Ontario, Canada. Environ Toxicol Chem 23:2930–40

McCarty JP. 1997. Aquatic community characteristics influence the foraging patterns of tree swallows. Condor 99:210–3

McCarty JP and Winkler DW. 1999. Foraging ecology and diet selectivity of tree swallows feeding nestlings. Condor 101:246–54

McMurry CS and Dickerson RL. 2001. Effects of binary mixtures of six xenobiotics on hormone concentrations and morphometric endpoints of northern bobwhite quail (Colinus virginianus). Chemosphere 43:829–37

Mellott RS and Woods PE. 1993. An improved ligature technique for dietary sampling in nestling birds. J Fld Ornith 64:205–10

Mengkelkoch JM, Niemi GJ, and Regal RR. 2004. Diet of the nestling tree swallow. Condor 106:423–9

Menzie C, Henning MH, Cura J, et al. 1996. Report of the Massachusetts weight-of-evidence workgroup: A weight-of-evidence approach for evaluating ecological risks. Hum Ecol Rsk Assess 2:277–304

Neigh AM, Zwiernik MJ, Blankenship AL, et al. 2006a. Exposure and multiple lines of evidence assessment of risk for PCBs found in the diets of passerine birds at the Kalamazoo River Superfund site, Michigan. Hmn Ecol Rsk Assess 12:924–46

Neigh AM, Zwiernik MJ, Bradley PW, et al. 2006b. Tree swallow (Tachycineta bicolor) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund Site, Michigan, USA. Environ Toxicol Chem 25:428–37

Norstrom RJ, Clark TP, Jeffrey DA, et al. 1986. Dynamics of organochlorine compounds in herring-gulls (Larus argentatus). 1. Distribution and clearance of [C-14] DDE in free-living herring-gulls (Larus argentatus). Environ Toxicol Chem 5:41–8

Norstrom RJ, Risebrough RW, and Cartwright DJ. 1976. Elimination of chlorinated dibenzofurans associated with polychlorinated biphenyls fed to mallards (Anas platyrhynchos). Toxicol Appl Pharmacol 37:217–28

Nosek JA, Craven SR, Sullivan JR, et al. 1992a. Toxicity and reproductive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in ring-necked pheasant hens. J Toxicol Environ Hlth 35:187–98

Nosek JA, Craven SR, Sullivan JR, et al. 1992b. Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin in ring-necked pheasant hens, chicks, and eggs. J Toxicol Environ Hlth 35:153–64

Nosek JA, Sullivan JR, Craven SR, et al. 1993. Embryotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the ring-necked pheasant. Environ Toxicol Chem 12:1215–22

Pinkowski BC. 1979. Annual productivity and its measurement in a multi-brooded passerine, the eastern bluebird. Auk 96:562–72

Powell DC, Aulerich RJ, Meadows JC, et al. 1996. Effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) injected into the yolks of chicken (Gallus domesticus) eggs prior to incubation. Arch Environ Contam Toxicol 31:404–9

Powell DC, Aulerich RJ, Meadows JC, et al. 1998. Effects of 3,3’,4,4’-5-pentachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin injected into the yolks of double-crested cormorant (Phalacrocorax auritus) eggs prior to incubation. Environ Toxicol Chem 17:2035–40

Powell DC, Aulerich RJ, Meadows JC, et al. 1997a. Effects of 3,3’,4,4’-5-pentachlorobiphenyl (PCB 126), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), or an extract derived from
field-collected cormorant eggs injected into double-crested cormorant (Phalacrocorax auri-tus) eggs. Environ Toxicol Chem 16:1450–5
Powell DC, Aulerich RJ, Meadows JC, et al. 1997b. Organochlorine contaminants in double-crested cormorants from Green Bay, Wisconsin. 2. Effects of an extract derived from cormorant eggs on the chicken embryo. Arch Environ Contam Toxicol 32:316–22
Robinson RA, Baillie SR, and Crick HQP. 2007. Weather-dependent survival: Implications of climate change for passerine population processes. Ibis 149:357–64
Rush SA and Stutchbury BJM. 2008. Survival of fledgling hooded warblers (Wilsonia citrina) in small and large forest fragments. Auk 125:183–91
Secord AL, McCarty JP, Echols KR, et al. 1999. Polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in tree swallows from the upper Hudson River, New York State, USA. Environ Toxicol Chem 18:2519–25
Shaw GG. 1983. Organochlorine pesticide and PCB residues in eggs and nestlings of tree swallows, Tachycineta bicolor, in Central Alberta. Can Fld Nat 98:258–60
Smits JEG, Bortolotti GR, Sebastian M, et al. 2005. Spatial, temporal, and dietary determinants of organic contaminants in nestling tree swallows in Point Pelee National Park, Ontario, Canada. Environ Toxicol Chem 24:3159–65
Spears BL, Brown MW, and Hester CM. 2008. Evaluation of polychlorinated biphenyl remediation at a superfund site using tree swallows (Tachycineta bicolor) as indicators. Environ Toxicol Chem 27:2512–20
Summers-Smith D. 1956. Mortality of the house sparrow. Brd Stdy 3:265–70
Thiel DA, Martin SG, Duncan JW, et al. 1988. Evaluation of the effects of dioxin-contaminated sludges on wild birds. In Proceedings 1988 Technical Association of Pulp and Paper Environmental Conference, Charleston, SC, USA, April 18–20, 1988:145–148
USEPA (US Environmental Protection Agency). 1993. Wildlife Exposure Factors Handbook Volumes I, II, and III. EPA/60/R-93/187B. Office of Research and Development, Washington, DC, USA
USEPA. 1998a. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F. Washington, DC, USA
USEPA. 1998b. Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS). Revision 1. Method 8290A. SW-846. Washington, DC, USA
USEPA. 2003. Analyses of Laboratory and Field Studies of Reproductive Toxicity in Birds Exposed to Dioxin-Like Compounds for Use in Ecological Risk Assessment. EPA/600/R-03/114F. National Center for Environmental Assessment, Offices of Research and Development, Cincinnati, OH, USA
Van Den Berg M, Birnbaum L, Bosveld ATC, et al. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Hlth Persp 106:775–92
Van Den Steen E, Dauwe T, Covaci A, et al. 2006. Within- and among-clutch variation of organohalogenated contaminants in eggs of great tits (Parus major). Environ Poll 144:355–9
Van Den Steen E, Covaci A, Jaspers VLB, et al. 2007. Experimental evaluation of the usefulness of feathers as a non-destructive biomonitor for polychlorinated biphenyls (PCBs) using silastic implants as a novel method of exposure. Environ Internat 33:257–64
Wan Y, Hu J, An W, et al. 2006. Congener-specific tissue distribution and hepatic sequestration of PCDD/Fs in wild herring gulls from Bohai Bay, North China: Comparison to coplanar PCBs. Environ Sci Technol 40:1462–8
Wells KMS, Ryan MR, Millsbaugh JJ, et al. 2007. Survival of postfledging grassland birds in Missouri. Condor 109:781–94