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EFFECTS ON TREE SWALLOWS EXPOSED TO DIOXIN-LIKE COMPOUNDS ASSOCIATED WITH THE TITTABAWASSEE RIVER AND FLOODPLAIN NEAR MIDLAND, MICHIGAN, USA

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Abstract—Concentrations of dioxin-like compounds, primarily polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs), in soils and sediments downstream of Midland, Michigan (USA) were greater than upstream sites and prompted a site-specific hazard assessment of tree swallows breeding in the associated floodplains. Potential for adverse population-level effects from site-specific contaminant exposures were evaluated at study areas (SAs) along the Tittabawassee and Saginaw rivers downstream of Midland. The site-specific multiple lines of evidence approach to hazard assessment included endpoints for dietary- and tissue-based exposures, and population productivity measurements for tree swallows ([TS]; *Tachycineta bicolor*) measured during the 2005, 2006, and 2007 breeding seasons. Exposure to dioxin-like compounds in TS eggs were some of the greatest recorded and were similar among all upstream and downstream study sites. Conversely, concentrations in nestlings from SAs were significantly greater compared to reference areas (RAs). The pattern of relative concentrations of PCDD/DFs in eggs and nestlings at RAs was dominated by dioxin congeners, whereas at SAs it was dominated by furan congeners. No statistically significant differences were noted in exposure to PCDD/DFs or in population-level responses when compared among locations, and total clutch failures were rare. Hatching success and fledging success were weakly negatively correlated with concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) in individual eggs and nestlings, respectively. On-site concentrations of TEQs in floodplain soils were some of the greatest ever reported in the environment, and several lines of evidence indicate potential population-level effects on TS overall reproductive productivity. Environ. Toxicol. Chem. 2011;30:1354–1365. © 2011 SETAC

Keywords—*Tachycineta bicolor* Risk assessment Productivity Furans Dioxins

INTRODUCTION

Tittabawassee River sediments and floodplain soils downstream of Midland, Michigan (USA) contain polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) that are greater than upstream reference areas and the regional background level. Potential sources of the PCDD/DFs are historical production of industrial organic chemicals and on-site storage and disposal, prior to the establishment of modern waste management protocols [1]. The major chemicals of concern include 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) [2,3], which contribute to the uniqueness of the site relative to other sites that are generally contaminated with polychlorinated biphenyls (PCBs) or PCDDs. Contributions of PCDFs to the congener profile are consistent with those at sites contaminated from the use of graphite-electrodes at chloralkali plants [4,5]. Furthermore, based on chemical characteristics and best estimates of historical production data, it is likely that this unique mixture has been in place for almost a century, with most of the materials being released prior to the 1950s. The lipophilic nature and slow degradation rates of these compounds [6] when sheltered from ultraviolet solar radiation, combined with consistent inundation of the floodplain, has resulted in continued presence of PCDD/DFs in floodplain soils and sediments.

The Michigan Department of Public Health first issued fish consumption advisories in 1978 based on concentrations of PCDFs, PCDDs, and PCBs in fish collected downstream of Midland. Wild game consumption advisories were issued in 2004 based on concentrations of PCDD/DF in deer and turkey. An unpublished 2003 report from the Michigan Department of Environmental Quality concluded that elevated risk, based on dietary exposure modeling, existed for individual and population-level effects for piscivorous birds and mammals exposed to site-specific PCDD/DFs downstream of Midland.

Most toxicological responses to dioxin-like compounds are believed to be mediated through the aryl hydrocarbon receptor (AhR), and effects include carcinogenicity, immunotoxicity, and adverse effects on reproduction, development, and endocrine functions [7]. In particular, AhR-mediated compounds have been shown to decrease hatching success, adult responsiveness and immune function, and increase enzyme induction of birds [8–13]. Recent findings provide evidence of the molecular basis for variation in sensitivity among bird species to dioxin-like compounds [14,15]. Specifically, the responsiveness of birds is dependent on the sequence of amino acids in the ligand-binding domain of the AhR, which determines the binding affinity and thus occupancy on the receptor and AhR-mediated effects. If the amino acid sequence of the ligand-binding domain of the AhR is known, birds can be classified as to their relative sensitivity.

All Supplemental Data may be found in the online version of this article.
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Species at the top of the food web are generally considered to be the most likely to experience greater exposure to dioxin-like compounds [16–23]. However, higher trophic status species generally also have larger foraging ranges that can include off-site locations, potentially limiting site-specific exposures during the breeding season. An intermediate trophic status species, such as the tree swallow ([TS]; *Tachycineta bicolor*), with a completely site-specific foraging range has potential for greater exposures to site-specific contaminants than a higher trophic status species.

Tree swallows were selected to determine the extent and distribution of chemical exposure through the aquatic food chain and associated population-level risk of breeding downstream of Midland. Tree swallows eat primarily emergent aquatic invertebrates [24–26] and have been shown to have exposure links to contaminated sediments [27–32]. They readily occupy nest boxes when provided, and forage in close proximity to their nest while breeding [33,34]. In addition, tree swallows are resistant to human disturbance and have limited foraging range while nesting, so tissue concentrations are generally indicative of local exposure. This species has an almost ubiquitous distribution both locally and throughout the USA, is commonly encountered, and generally nests in close proximity to conspecific individuals [35]. These attributes alleviate concerns related to species presence on-site and obtaining the necessary numbers of active nest boxes to reach the required sample numbers per site. The use of nest boxes by TS allowed for better experimental control and eliminated time-intensive nest searching.

Several studies across North America have monitored TS for exposure to and/or effects of PCBs [27,28,30,32,36–46]. However, the exposure and potential effects of exposures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on TS has been limited to a study along the Woonasquatucket River in Rhode Island (USA) [47], and exposure and potential effects of exposures to PCDFs as co-contaminants on TS is limited to a study along the Housatonic River in New York (USA) [40].

The present study had several objectives. The first was to compare the levels of tissue- [48] and dietary-based [49] exposures of TS in several areas within the river floodplains near Midland, Michigan. Exposure was expressed as TCDD equivalents (TEQs) based on World Health Organization (WHO) TCDD equivalency factors (TEFs) for birds [7]. Concentrations of PCDD/DF-$TEQ_{WHO\text{-}Avian}$ (ng/kg wet wt) were calculated as the sum of the concentrations of individual PCDD and PCDF congeners multiplied by their TEFs. Because concentrations of PCDD/DF-$TEQ_{WHO\text{-}Avian}$ in TS eggs were similar between reference and study areas [48], both comparisons between reference and study areas in a fixed effects model and regression-based comparisons among a continuum of exposure and effects were utilized to assess risk to breeding TS based on productivity measurements (T.B. Fredricks, unpublished data). In addition, adverse effects were evaluated by calculating hazard quotients (HQs) as the quotient of concentrations of PCDD/DF-$TEQ_{WHO\text{-}Avian}$ in the diet or eggs and nestlings of TS divided by available toxicity reference values (TRVs). Several lines of evidence of potential effects [50,51] were compared to determine if differences in reproduction occurred among locations and if relationships were observed between exposure to PCDF on populations of TS breeding downstream of Midland, Michigan, where exposure to dioxin-like compounds is primarily to PCDFs. Comparisons were made between these results and similar field-based measures of exposure, productivity, and nestling growth.

METHODS

Site description

The present study was conducted on the Tittabawassee, Chippewa, and Saginaw Rivers, in the vicinity of Midland, Michigan (Fig. 1). Nest boxes were placed and all samples were collected from within the 100-year floodplain of the individual rivers. Two reference areas (RAs) were located upstream of the likely sources of PCDD/DFs [2] on the Tittabawassee (R-1) and Chippewa (R-2) Rivers (Fig. 1). Study areas (SAs) downstream of the likely 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. Study areas along the 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 S-7 site is located on a peninsula between the Tittabawassee and Saginaw Rivers just upstream of their confluence. 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 [2]. 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. The S-8 site was an exception and was only used for sediment and dietary food web sampling to establish a relationship between concentrations of PCDD and PCDF in soils and invertebrates and small mammals. 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 (T.B. Fredricks, unpublished data). 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 2005. Monitoring began one year after 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 [52], with relatively equal proportions of boxes placed in species-specific micro-habitats for each species studied.

Previous reports provide more detailed descriptions of study-specific nest monitoring and sample collection protocols [48,49]. 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. During the 2005, 2006, and 2007 breeding seasons, a total of 50 live and addled eggs and 45 nestlings were collected from individual tree swallow clutches. However, of the collected nestlings, 17 were collected from clutches in which an addled egg was also analyzed. Live eggs were collected after clutch completion, whereas addled eggs were collected after hatching. Because eggs in which concentrations of residues were quantified were collected after clutch size was noted, clutch size was not adjusted for egg sampling. However, hatching success, fledging success, and productivity measurements were calculated based on an adjusted clutch size because the fertility and hatchability of the collected egg was unknown at collection.

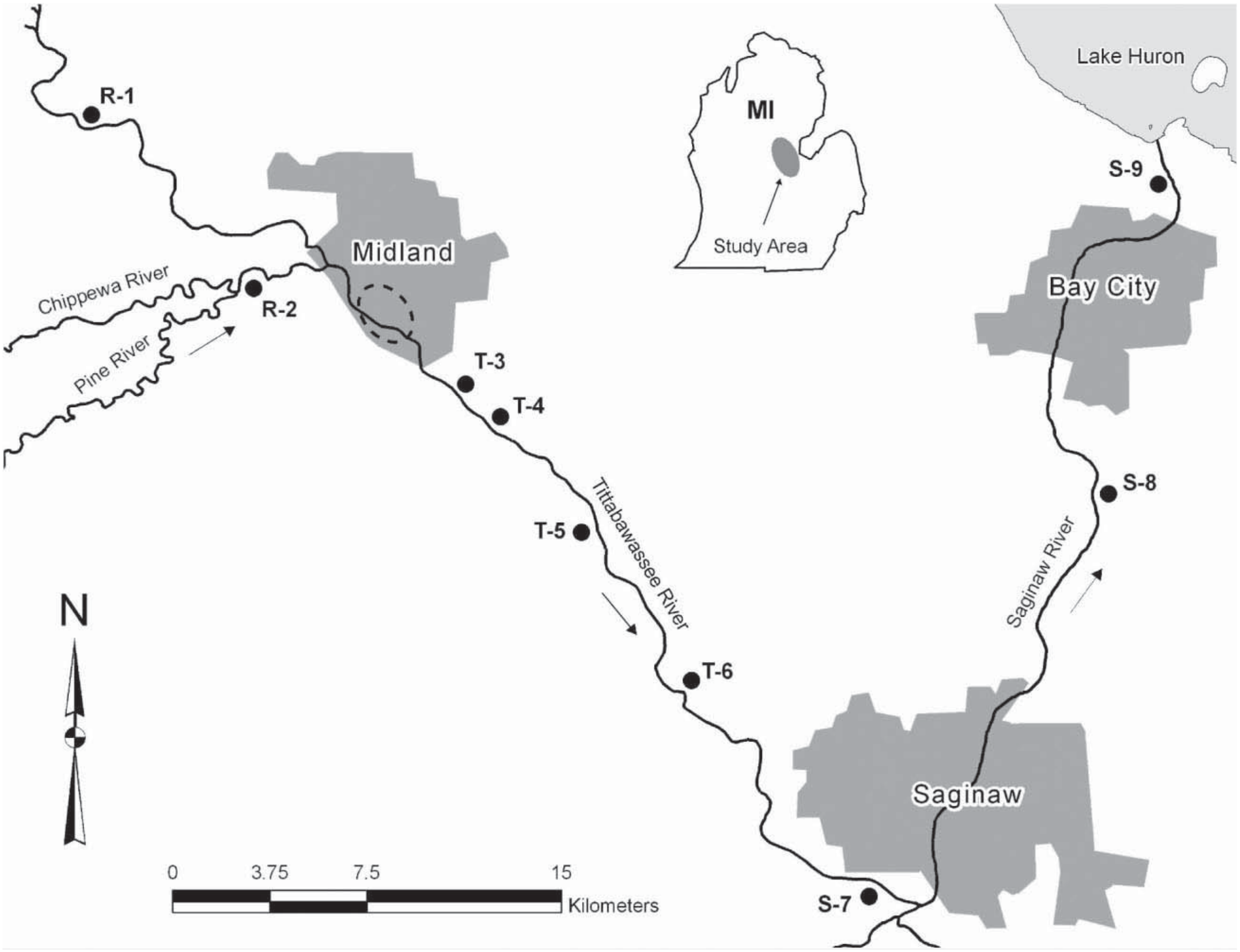

Fig. 1. Study site locations within the Chippewa, Tittabawassee, and Saginaw River floodplains, Michigan, USA. Reference Areas (R-1 to R-2), Tittabawassee River Study Areas (T-3 to T-6), and Saginaw River Study Areas (S-7 to to S-9) were monitored from 2005 to 2007. Direction of river flow is designated by arrows; suspected source of contamination is enclosed by the dashed oval.

Because the outcome that would have occurred was unknown for a sampled egg, an adjusted clutch size was defined as the clutch size excluding any eggs sampled or broken by researchers. Hatching success (number of eggs that hatch per adjusted clutch size), fledging success (number of nestlings that fledge per number of eggs that hatched), and productivity (number of nestlings that fledge per adjusted clutch size) were adjusted to account for sample collection. Measures of nesting success for all clutches were included in comparisons up to the point that they were depredated, or abandoned due to human interference, and thereafter removed from comparisons. The rates of nest occupancy and abandonment were recorded for each nesting attempt.

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 presented [49]. Briefly, 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 $30\,m \times 30\,m$ grids proximal to the river bank, one for sampling of terrestrial invertebrates and one for collection of benthic and emergent aquatic invertebrates.

Dietary items were collected as bolus samples from nestlings by using a black electrical cable-tie fitted at the base of their neck so that they could not swallow [53]. The site-specific diet was determined based on the relative proportion of the total mass represented by each invertebrate order identified in the bolus samples. In addition, 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 were estimated using the U.S. Environmental Protection Agency (U.S. EPA) *Wildlife Exposure Factors Handbook* equations for passerine birds [54]. Minimum and maximum concentrations were chosen to describe the range of possible concentrations of residues in invertebrates found on site. These are expected to include the worst-case scenario for dietary exposure. Adult dietary exposure estimates apply only to the nesting period as foraging habits and range are likely more variable outside the nesting period.

Chemical analyses

Concentrations of 17 individual 2,3,7,8-substituted PCDD/DF congeners (ΣPCDD/DFs) were quantified in all samples, whereas concentrations of the 12 non- and mono-*ortho*-substituted PCB congeners (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) and all three isomers of dichloro-diphenyl-trichloroethane (DDT) and related metabolites were measured in a subset of egg samples. Congeners were identified and quantified in accordance with U.S. EPA Method 8290/1668A with minor modifications [55]. A more detailed description of methods and the measured concentrations has been reported previously [48,49]. 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) connected to a MicroMass® high-resolution mass spectrometer (Waters). Chemical analyses included pertinent quality assurance practices, including matrix spikes, blanks, and duplicates.

In-depth species-specific site description

Concentrations of ΣPCDD/DFs and PCDD/DF-$TEQ_{WHO\text{-}Avian}$ were quantified in tree swallow eggs and nestlings collected on-site [48]. Briefly, geometric mean concentrations of PCDD/DF-$TEQs_{WHO\text{-}Avian}$ in TS eggs were similar among study locations ($F = 1.06$; $n = 50$; $p = 0.4037$). However, patterns of relative concentrations of individual congeners in eggs from more downstream SAs averaged 49 to 72% for 2,3,4,7,8-PeCDF and 13 to 27% for TCDF, as opposed to 38 to 42% for TCDD and 26 to 27% for 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin at the RAs. Concentrations of co-contaminants were measured in three TS eggs and found to be less than concentrations known to cause adverse effects. In nestlings, concentrations of PCDD/DF-$TEQs_{WHO\text{-}Avian}$ at the Tittabawassee and Saginaw River SAs were 3- to 34-fold greater than those in nestlings from RAs. The inconsistency in tree swallow egg and nestling concentrations between RAs and SAs likely indicates that, during egg laying, the expanded foraging range of tree swallows includes a proximally contaminated site with a different congener profile near the RAs.

Concentrations of ΣPCDD/DFs and PCDD/DF-$TEQ_{WHO\text{-}Avian}$ were quantified in site-specific food webs by quantifying residues in invertebrates collected from all study areas, and bolus samples collected from tree swallow nestlings at RAs and Tittabawassee River SAs [49]. Briefly, potential average daily dose (ADD_{pot}; ng/kg body wt/d) based on PCDD/DF-$TEQ_{WHO\text{-}Avian}$ concentrations in bolus-based and food web-based dietary exposure estimates were 41- and 40-fold greater at Tittabawassee River SAs than at RAs for adult TS, whereas food web-based dietary exposure estimates were 11-fold greater at Saginaw River SAs.

Reproductive parameters including nest abandonment, clutch size, egg mass, hatching success, predicted brood size, nestling growth, fledging success, predicted number of fledglings, and productivity were monitored for TS breeding in the river floodplains near Midland, Michigan (T.B. Fredricks, unpublished data). Briefly, of the initiated clutches, 73% successfully fledged at least one nestling, and only 7% were abandoned among all study sites. In general, TS exhibited greater reproductive success at Saginaw River SAs compared to Tittabawassee River SAs, whereas successes at RAs were intermediate. Specifically, clutch size, predicted brood size, and predicted number of fledglings were greater at Saginaw River SAs compared to Tittabawassee River SAs and RAs, whereas productivity at Tittabawassee River SAs was 70% compared to 80 to 81% at the other study areas. Overall hatching successes at RAs, Tittabawassee River SAs, and Saginaw River SAs were 81, 76, and 86% at RAs, respectively, and were not statistically different among areas.

Toxicity reference values

Selection of appropriate toxicity reference values is an essential step in the risk assessment process. Toxicity reference values represent a concentration in food or tissues less than those for which adverse toxicological effects would be expected. Selection criteria for 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 additional uncertainties associated with the relationship between $TEQ_{WHO\text{-}Avian}$ values derived from PCB-based or PCDD/DF-based exposures [47], consideration was only given to values derived from PCDD/DF-based exposures. Literature-based no-observed-adverse-effect concentrations (NOAECs) and lowest-observed-adverse-effect concentrations (LOAECs) were used in the determination of HQs and subsequent comparison of risk. In the present study, dietary exposure- and egg exposure-based TRVs were used to evaluate the potential for adverse effects from exposure to site-specific contamination on TS.

Assessment under laboratory conditions where PCDD/DF had been fed to birds is 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-week exposure period was selected as the dietary exposure-based TRV for the present study [9]. The major limitation of the study by Nosek et al. [9] was that hens were exposed to TCDD through injections to stimulate targeted exposure levels versus a true dietary-based exposure. However, dosing exposure efficiency through injections is typically greater than that of gut transfer, thus providing a slightly conservative TRV. In addition, this study was conducted on a gallinaceous bird that is generally considered to have greater sensitivity to dioxin-like compound exposures [11,56–58], and recent findings provide evidence suggesting a molecular basis to this variation [14,15]. The diet-based TRVs were determined by converting the weekly exposure at which adverse effects on fertility and hatching success were determined (1,000 ng TCDD/kg/week) to a LOAEC for daily exposure of 140 ng TCDD/kg/d. The dosing regime was based on orders of magnitude differences, and adverse effects were not present at the next lowest dose, which was determined to be the NOAEC for dietary exposure (14 ng TCDD/kg/d) (Supplemental Data, Table S1).

Studies in which ring-necked pheasant eggs were injected with TCDD [9,10,59] were selected as the most applicable for deriving egg-based TRVs in the present study. The three studies that dosed ring-necked pheasant hens or eggs were combined to determine a geometric mean NOAEC of 710 ng/kg wet weight, and LOAEC of 7,940 ng/kg wet weight, as egg exposure-based TRVs [60]. Other egg-injection studies that dosed bobwhite quail (*Colinus virginianus*) [61] and double-crested cormorant (*Phalacrocorax auritus*) [12,58] eggs with TCDD were not selected for several reasons, including limited sample size, failure to establish a dose–response relationship, or poor hatchability of un- or vehicle-injected controls. Tree swallow field exposure studies [40,47] were also eliminated from TRV development, due to uncertainties associated with habitat characterization and the presence of co-contaminant exposure.

Hazard assessment

Overall hazard of PCDD/DFs to TS breeding in the river floodplains downstream of Midland was assessed through a multiple lines of evidence approach [50,51], which incorporated both dietary-based and tissue-based exposure estimates in addition to monitoring site-specific TS reproductive productivity. Potential effects of dietary- and egg tissue-based exposures were assessed by calculating HQs. No appropriate TRV was available for nestling tissue-based exposures, therefore HQs were not determined for this endpoint. Concentrations of PCDD/DF-TEQs$_{\text{WHO-Avian}}$ in eggs and dietary estimates expressed as the ADD$_{\text{pot}}$, were divided by egg exposure- or dietary exposure-based NOAEC or LOAEC TRVs (Supplemental Data, Table S1), respectively. Hazard quotients were determined based on the upper 95% confidence level (UCL) for geometric means at individual study locations for concentrations in eggs, as well as based on ranges at RAs, Tittabawassee River SAs, and Saginaw River SAs, for dietary exposures divided by the selected TRV, respectively. Ranges were used among study areas for dietary exposure due to limited sample sizes at most study locations. Samples of each invertebrate order from the food web sampling were composites of all individuals of an order collected per location, per sampling period. Hazard quotients for dietary exposure were calculated based on TEQs$_{\text{WHO-Avian}}$ in bolus-based dietary exposure estimates at RAs and Tittabawassee River SAs, and on food web-based dietary exposure estimates at Saginaw River SAs. Residue concentrations in bolus samples from Saginaw River SAs were not quantified. In addition to dietary- and egg-based hazard assessments, potential adverse effects on population health were concurrently monitored for ecologically relevant endpoints at site-specific downstream and upstream study areas. Clutch-specific correlations between residue concentrations and productivity endpoints were evaluated for concentration-dependent effects, and productivity endpoints were compared to relevant literature-based field studies. Incorporation of both dietary- and tissue-based assessment endpoints, in addition to monitoring productivity, has been shown to greatly reduce uncertainty in risk assessments of persistent organic pollutants [62].

Statistical analyses

Individual nesting attempts were considered the experimental unit for statistical comparisons. Comparisons among concentrations of PCDD/DF-TEQs$_{\text{WHO-Avian}}$ in eggs were made between locations [48]. 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 [49]. Prior to the use of parametric statistical procedures to compare measures of nesting success, normality was evaluated using the Shapiro–Wilks' test, and the assumption of homogeneity of variance was evaluated using Levene's test. Nest parameters that were not normally distributed were ranked prior to statistical analyses. The PROC GLM (release 9.1; SAS Institute) was used for comparisons, and when significant differences among locations were indicated, Bonferroni's *t* test was used to compare individual locations. In the present study, because no significant differences in PCDD/DF-TEQs$_{\text{WHO-Avian}}$ concentrations in eggs were observed among locations [48], a logistic regression approach was used to study the associations between concentrations of chemicals as the independent variable, and population parameters as the dependent variables. Specifically, associations between concentrations of PCDD/DF-TEQs$_{\text{WHO-Avian}}$ in eggs and nestlings, and hatching and fledging success, respectively, were modeled using PROC LOGISTIC (release 9.1; SAS Institute) [40].

Total concentrations of the 17 individual 2,3,7,8-substituted PCDD/DF congeners are reported as the sum of all congeners (ng/kg wet wt). For individual congeners for which concentrations were less than the limit of quantification, a proxy value of half the sample method detection limit was assigned. Total concentrations of 12 non- and mono-*ortho*-substituted PCB congeners are reported as the sum of these congeners (ΣDL-PCBs) for a subset of egg samples. A regression model was used to estimate total concentrations of PCBs (ΣPCBs) including the di-*ortho*-substituted congeners from ΣDL-PCBs [63]. Dichlorodiphenyl-trichloroethane (2′,4′ and 4′,4′ isomers) and dichlorodiphenyl-dichloroethylene (4′,4′) are reported as the sum of the *o,p*- and *p,p*-isomers (ΣDDT) for the same subset of egg samples as for PCBs.

Statistical analyses were performed using SAS® software (Release 9.1; SAS Institute). Because the PCDD/DF-TEQ$_{\text{WHO-Avian}}$ concentration data were mostly log-normally distributed, they were transformed using the natural log (ln) of $(x+1)$. To better understand the potential distributions of the PCDD/DF-TEQ$_{\text{WHO-Avian}}$ concentrations at each study location, a probabilistic distribution simulation approach was used to portray the distributions. The mean and standard deviation of log-transformed concentrations in eggs were used to generate a sample of 10,000 random concentrations of PCDD/DF-TEQs$_{\text{WHO-Avian}}$ concentrations in eggs based on a lognormal distribution. This probabilistic model is presented as cumulative frequency distributions based on PCDD/DF-TEQ$_{\text{WHO-Avian}}$ concentrations. Statistical significance was considered at $p < 0.05$.

RESULTS

Site-specific endpoints

A total of 245 TS clutches were initiated and monitored for productivity during the breeding seasons from 2005 to 2007. Occupancy was well distributed between sites with the exception of T-5, where few nesting attempts occurred due to box placement constraints from farming practices. Concentrations of ΣPCDD/DF were quantified in eggs ($n = 50$) and nestlings ($n = 45$) collected from individual TS nesting attempts. Samples of boluses were collected throughout the nesting season from 96 TS nesting attempts to determine site-specific foraging patterns and to determine bolus-based dietary exposure to PCDD/DFs. Previous research [64] has shown that similarly conducted bolus sampling from nestlings did not influence productivity parameters, therefore all clutches were included in comparisons of nesting success.

Hazard assessment

Because no statistically significant difference was observed between egg exposure concentrations between the study and reference areas, the data were analyzed using a type II logistic regression model. Comparisons were made for both hatching and fledging success, and concentrations of PCDD/DF-TEQs$_{\text{WHO-Avian}}$ in tree swallow eggs and nestlings, respectively, for clutches where both data endpoints were measured. Overall hatching and fledging success averages ranged from 73 to 86% and 85 to 96%, respectively, among study areas. However, the logistic regression models were significant based on the Hosmer and Lemeshow goodness-of-fit tests for both hatching ($\chi^2 = 3.7647$, $n = 39$, degrees of freedom [df] = 7, $p = 0.8064$) and fledging ($\chi^2 = 5.6599$, $n = 43$, $df = 8$, $p = 0.6853$) success (Figs. 2 and 3).

Predicted probabilistic distributions of expected cumulative percent frequencies of PCDD/DF-$TEQ_{WHO\text{-}Avian}$ concentrations in eggs were compared to selected TRVs. Predicted distributions of PCDD/DF-$TEQ_{WHO\text{-}Avian}$ concentrations in TS eggs were greater than the NOAEC (710 ng/kg wet wt) [60] for all sites other than S-9 (Fig. 4). Reference-1 and T-6 had 7.4% and 17% of the predicted distribution greater than the NOAEC, respectively, whereas less than 4% of the distributions at other locations were greater than the NOAEC. Based on the predicted distributions at all study sites, less than 1% of the cumulative exposure frequency was greater than the LOAEC (7,940 ng/kg wet wt) [51].

Upper 95% confidence level (geometric mean) concentrations of PCDD/DF-$TEQs_{WHO\text{-}Avian}$ in TS eggs among all study sites were not greater than the species-specific egg-based LOAEC or NOAEC TRVs. Resulting HQs based on LOAECs were less than 1.0 among all study sites. The greatest HQ determined was less than 1.0 based on NOAEC at T-6 (Supplemental Data, Fig. S1).

Dietary exposures based on minimum measured concentrations of PCDD/DF-$TEQs_{WHO\text{-}Avian}$ at Tittabawassee and Saginaw River SAs were greater than the diet-based NOAEC TRV, regardless of whether food web- or bolus-based estimates of dietary exposure were used at Tittabawassee River SAs. Dietary exposure-based estimates of HQs based on maximum measured concentrations of PCDD/DF-$TEQs_{WHO\text{-}Avian}$ at Tittabawassee River SAs were greater than the LOAEC TRV, whereas Saginaw River SAs HQs were less than 1.0 (Fig. 5). Both food web- and bolus-based estimates of dietary exposure were less than dietary-based LOAEC and NOAEC TRVs at RAs.

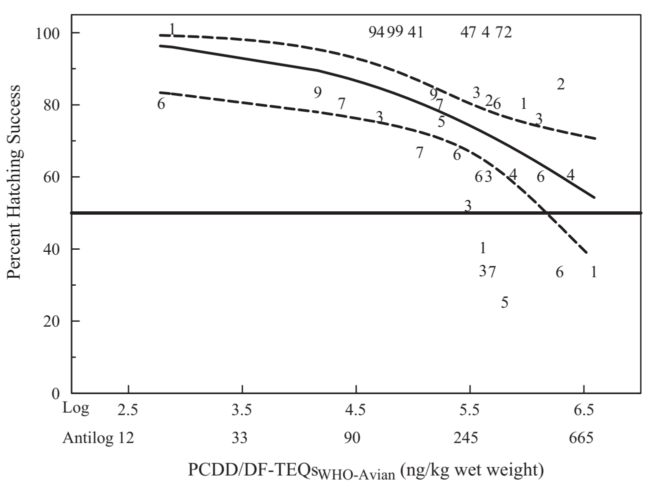

Fig. 2. Logistic regression of percentage hatching success of tree swallows (TS) and natural log of the sum polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxin (PCDD) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) based on World Health Organization (WHO) TCDD equivalency factors for birds [10] (PCDD/DF-$TEQs_{WHO\text{-}Avian}$) in tree swallow eggs collected in the Tittabawassee River floodplain near Midland, Michigan (USA) from 2005 to 2007. Numbers indicate sampling site of samples collected from nests with associated productivity measurements ($n = 39$; 1 = R-1; 2 = R-2; 3 = T-3; 4 = T-4; 5 = T-5; 6 = T-6; 7 = S-7; 9 = S-9); the 95% upper and lower confidence intervals are the dotted lines; the predicted values are plotted with the solid line; the 50% level for fledging success is indicated by the horizontal bar.

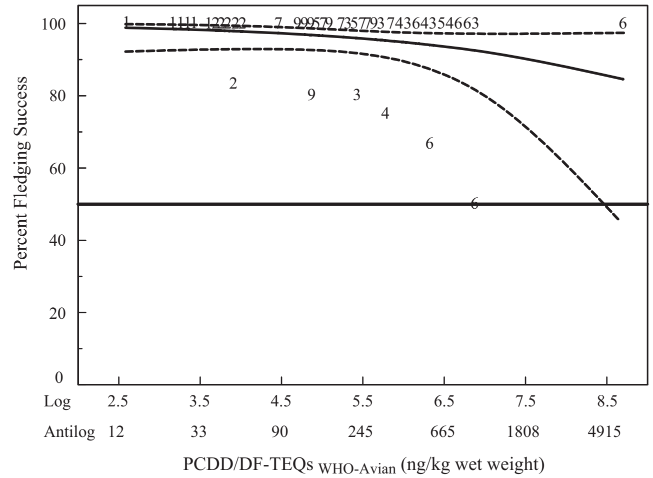

Fig. 3. Logistic regression of percentage fledging success and natural log of the sum polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxin (PCDD) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) based on World Health Organization (WHO) TCDD equivalency factors for birds [10] (PCDD/DF-$TEQs_{WHO\text{-}Avian}$) in tree swallow (TS) nestlings collected in the Tittabawassee River floodplain near Midland, Michigan (USA) from 2005 to 2007. Numbers indicate sampling site of samples collected from nests with associated productivity measurements (1 = R-1; 2 = R-2; 3 = T-3; 4 = T-4; 5 = T-5; 6 = T-6; 7 = S-7; 9 = S-9); the 95% upper and lower confidence intervals are the dotted lines; the predicted values are plotted with the solid line; the 50% level for fledging success is indicated by the horizontal bar.

DISCUSSION

The TS is a relevant study species for the assessment of risk in aquatic environments due to ease of study, sufficient occupancy rates, sufficient sample masses, and availability of prior research for comparisons. A possible limitation raised for their continued widespread use as a receptor species has been

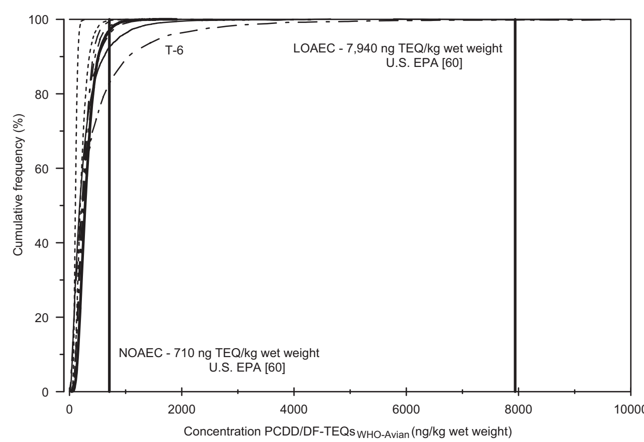

Fig. 4. Simulated probabilistic distribution of expected cumulative percent frequencies for tree swallow egg sum polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxin (PCDD) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) based on World Health Organization (WHO) TCDD equivalency factors for birds [10] (PCDD/DF-$TEQs_{WHO\text{-}Avian}$ ng/kg wet weight) for site-specific tree swallow eggs collected in the river floodplains around Midland, Michigan (USA) from 2005 to 2007. Ten thousand replications 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; no-observed-adverse-effect concentration (NOAEC) and lowest-observed-adverse-effect concentration (LOAEC) indicated by vertical bars. U.S. EPA = U.S. Environmental Protection Agency [60].

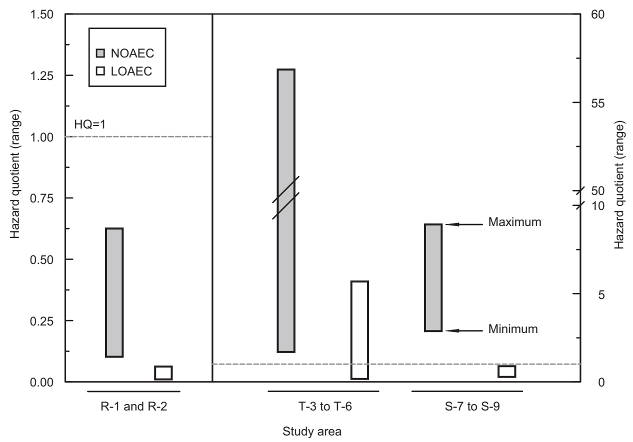

Fig. 5. Hazard quotients (HQ) for the effects of potential sum polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxin (PCDD) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) based on World Health Organization TCDD equivalency factors for birds [10] (PCDD/DF-TEQs$_{\text{WHO-Avian}}$) daily dietary dose calculated from site-specific bolus-based (R-1 to T-6) and food web-based (S-7 to S-9) dietary exposure for adult tree swallows collected from 2005 to 2007 in the river floodplains near Midland, Michigan (USA), based on the no-observable-adverse-effect concentration (NOAEC) and the lowest-observable-adverse-effect concentration (LOAEC). The HQs based on measured concentration ranges are presented; left *y* axis for reference areas (R-1 and R-2); right *y* axis (broken from 10–50) 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 because no bolus samples were collected from those sites.

concern over the sensitivity of the species to dioxin-like compounds [40,44,47,65–67]. By combining multiple lines of evidence for TS exposed to dioxin-like compounds near Midland, Michigan, a balanced assessment of aquatic-based risk was possible.

Toxicity reference values

Despite the widespread use of TS as a receptor at contaminated sites, its utility is still limited by the paucity of controlled studies on the effects of dioxin-like compounds from which to determine thresholds for effects. Recent research has determined potential molecular differences in sensitivities of birds to dioxin-like compounds [14,15]. Specifically, the difference between species-specific sensitivities to dioxin-like compounds is potentially tied to amino acid substitution differences in the AhR ligand-binding domain between species (S.W. Kennedy, unpublished data). Based on these findings, the TS was classified as a species with moderate sensitivity to dioxin-like compounds, which is the same classification given to the American robin (*Turdus migratorius*), eastern bluebird (*Sialia sialis*), house wren (*Troglodytes aedon*), and house sparrow (*Passer domesticus*), and is similar to the ring-necked pheasant. After considering a number of criteria, the TRVs used herein were based on studies of the ring-necked pheasant [9,10,59], and were less conservative compared to TRVs based on chicken exposures [11,56,57,68–70]. For the dietary exposure-based HQs, the TRVs based on intraperitoneal injections of TCDD in hen ring-necked pheasants [9] would be expected to overestimate the effects thresholds for tree swallows. This is because intraperitoneal injections are not a true dietary dose and do not take into account exposure reductions due to sequestration, metabolism, excretion, as well as reduced bioavailability of contaminants bound to dietary biota [17,71–75]. However, instead of relying solely on the HQ values derived by use of this TRV, the results of the hazard assessment based on this TRV were interpreted in the context of the results of other field studies of the effects of AhR-active compounds on the TS.

Assessment of hazard

Assessing the potential for adverse effects by use of the HQ approach, based on the most appropriate TRVs available, can provide information on the likelihood of site-specific effects. For all study locations, hazard quotients were less than 1.0 based on reported 95% UCLs for concentrations of PCDD/DF-TEQ$_{\text{WHO-Avian}}$ in TS eggs. However, TRVs based on a study in which TCDD was injected into eggs of eastern bluebirds [13] were less conservative than the pheasant study (NOAEC, 1,000 ng TCDD/kg wet weight; LOAEC, 10,000 ng TCDD/kg wet wt) and, if used, would result in HQs that range from 0.14 to 0.7 for all study locations. Hazard quotients greater than 1.0 indicate that exposures exceed the threshold for adverse effects, which suggests that the potential exists for effects to occur. In general, due to the relatively conservative nature of the values used for exposure and TRVs, population-level effects are not expected at HQ values less than 10. Compared to the predicted distributions of concentrations of PCDD/DF-TEQ$_{\text{WHO-Avian}}$ in eggs at these sites, the percent of the frequency distribution greater than the NOAEL was as great as 17% (Fig. 4). However, less than 1% of the frequency distribution for concentrations of PCDD/DF-TEQ$_{\text{WHO-Avian}}$ in TS eggs was greater than the LOAEC among all study locations. Based on three samples that were screened for ΣDL-PCB TEQs$_{\text{WHO-Avian}}$ in the present study, it was determined that the addition of ΣDL-PCB TEQs$_{\text{WHO-Avian}}$ to the PCDD/DF-TEQs$_{\text{WHO-Avian}}$ would not change the outcome of the egg-based HQ assessment. Despite the uncertainties associated with co-contamination by PCBs on-site and based on the conservatively selected egg-based TRVs and 95% UCL exposures, minimal potential for effects on individual TS exists among study locations based on the egg-based HQ approach.

Hazard quotient values, based on concentrations of PCDD/DF-TEQ$_{\text{WHO-Avian}}$ in bolus samples from nestling TS, were greater than or equal to 1.0 at Tittabawassee and Saginaw River SAs based on the minimum value of PCDD/DF-TEQ$_{\text{WHO-Avian}}$ concentrations and the NOAEC. Food web-based dietary exposure HQs were similar to bolus-based HQs at Tittabawassee River SAs [49]. Bolus-based dietary exposures were selected because they represented actual invertebrates collected on-site by TS, and included the greatest potential exposure estimates due to a greater range of values. Because bolus-based exposures were not available for Saginaw River SAs, food web-based dietary exposures were used to determine HQs, which were only slightly less than those at Tittabawassee River SAs based on food web-based exposures.

Dietary exposures measured in tree swallow nestlings exposed primarily to TCDD on the Woonasquatucket River in Massachusetts ranged from 0.87 to 6.6 and from 72 to 230 ng TEQ/kg wet weight at unexposed and exposed sites, respectively [47]. If these data are converted to a daily dietary dose, based on site- and species-specific ingestion rates calculated from data collected in the present study, tree swallow exposure at the contaminated sites along the Woonasquatucket River would range from 61 to 190 ng TEQ/kg body weight/d. In that study, hatching success was less at exposed sites; while beyond the scope of their conclusions, it is likely that adult dietary exposure prior to breeding was similar to measured nestling exposures. Maximum measured daily intake based on bolus concentrations of PCDD/DF-TEQs$_{\text{WHO-Avian}}$ in the present study was 800 ng/kg body weight/d, which is fourfold greater than the maximum dietary sample collected along the Woonasquatucket River. Therefore, similar effects on hatching success could be expected for the dietary exposures measured at the Tittabawassee and Saginaw River SAs (Supplemental Data, Table S2). However, despite greater dietary exposure, lesser concentrations of PCDD/DF-TEQs$_{WHO\text{-}Avian}$ in eggs in the present study indicate potential differences in metabolism and sequestration of these site-specific PCDD/DF congeners. In addition, some uncertainty exists in the dietary exposure estimates along the Tittabawassee River because dietary-based samples were not screened for co-contaminants, of which PCBs would be of greatest concern considering egg-based residues analyses. Based on the range of exposures to PCDD/DF-TEQs$_{WHO\text{-}Avian}$ for TS at SAs downstream of Midland, and available dietary-based TRVs, the potential exists for population-level effects on hatching success. However, one possibility for the discrepancy between HQ based on estimates of dietary exposure, and those based on concentrations in the eggs and nestlings, is that the dietary-based HQs are likely more conservative due to the TRV based on intraperitoneal injections that likely resulted in an overestimation of both the NOAEC and LOAEC [9], as opposed to more realistic exposure routes such as dietary gavages or spiked diets.

To compare across the continuum of site-specific exposures, logistic regression rates of hatching and fledging success were developed as a function of concentrations of PCDD/DF-TEQs$_{WHO\text{-}Avian}$ in eggs and nestlings, respectively. Interpretation of the regression of hatching success and egg concentrations (Fig. 2) shows that a potential field-based threshold of 50% effects occurs at a concentration of approximately 750 ng PCDD/DF-TEQs$_{WHO\text{-}Avian}$/kg. This estimate is consistent with the NOAEL of 710 ng PCDD/DF-TEQ/kg wet weight in eggs [51], which was used in the present study. A similar shaped regression of hatching success and egg concentrations was determined on the Woonasquatucket River for tree swallows exposed to primarily TCDD [47]. These similar findings on two independent rivers further supports the potential for effects on hatching success for tree swallows exposed to PCDD/DFs. The regression for fledging success (Fig. 3) shows less effect with 20% reduction in fledging success at a concentration of approximately 5.0×10^3 ng PCDD/DF-TEQs$_{WHO\text{-}Avian}$/kg, wet weight in nestlings. However, based on the trend indicated by the six samples with fledging success ranging from 50 to 80% running parallel to the predicted curve, the possibility exists that a more sensitive portion of the population could be affected at lesser concentrations (Fig. 3). The potential field-based threshold for a 50% effect on fledging success for these individuals was approximately 1.0×10^3 ng PCDD/DF-TEQs$_{WHO\text{-}Avian}$/kg wet weight. The finding of a potential negative association between fledging success and nestling exposure to PCDD/DFs along the Tittabawassee River is novel, and further supports the potential site-specific effects along the Tittabawassee River.

Multiple lines of evidence and population-level effects

Various means can be used to assess the potential effects of residues on wildlife populations [76]. We have applied several of these in this assessment. Predicted effects on productivity based on concentrations of PCDD/DF-TEQs$_{WHO\text{-}Avian}$ in eggs and nestlings, or in the diet, were compared with nesting success observed for TS in a site-specific multiple lines of evidence assessment of hazard [50,64,77–79]. Sampling a single egg per nest was selected for this assessment, based on previous studies [38,80,81] that indicated that laying order does not affect concentrations, and that exposure is better estimated than from extrapolation from abiotic matrices and can be accounted for in subsequent nesting success calculations. Exposure and productivity were directly measured to minimize uncertainties associated with predicting the potential for adverse effects based solely on concentrations in abiotic matrices [62,82]. Endpoints related to reproductive performance of TS were quantified.

Several ways can be used to estimate exposure. Concentrations in the diet or in the tissues of the wildlife can be compared to TRVs based on both laboratory and field studies. This is best suited to situations where only one or a small set of contaminants exists, especially when the primary contaminants cause effects through the same or similar mechanisms of action. Estimating exposure through the diet is uncertain due to differences in assimilation and/or metabolism or excretion, or even sequestration among PCDD/CF congeners. Basically, due to this problem, it is impossible to translocate TEQ from the food into the interior of the predator. Although the relative potencies of individual congeners are corrected by use of the WHO TEFs, they do not correct for differences in chemical–physical properties and assimilation. For this reason, estimating exposure by measuring concentrations of PCDD/DF congeners in eggs and/or nestlings, and then calculating the TEQ and relating them to TRVs, is a more accurate approach. Both of these measures of exposure need to be related to some threshold for effect before the potential for effect can be inferred. As we have seen, uncertainties exist in both estimating exposure and response.

Another method to assess effects is to directly measure ecologically relevant responses along a continuum of exposures, estimated either in tissues or diet. This sort of site-specific effects study, while obviating the needs for TRVs and free of the uncertainties of estimating exposure, is limited by edaphic effects and exposure to multiple stressors and chemicals. However, in addressing effects of all of these factors at a site, no better way exists than to directly measure sensitive, ecologically relevant, population-level parameters at the location of interest. In this case, two basic experimental designs can be applied. These include use of either a fixed effects (type I) model in which values from the site of interest are compared to a reference location, or a regression (type II) model, in which associations between the observed outcomes and concentrations of individual or groups of potential causative agents are compared. The fixed effects model makes the assumption that habitats being compared are identical except for the effects of a single stressor; thus, the target and reference sites are assigned without error. In the type II model, both the dependent and independent variables are allowed to vary along a continuum, and are assumed to be measured with error. Both approaches have advantages and disadvantages, and both are appropriate to address particular questions. The former approach, in which the results from a study area are compared to a reference location, is limited by the fact that it is impossible to find two habitats that are identical except for a single stressor. However, this approach does have the advantage of being able to assess the potential effects of all of the stressors at a site, relative to an adjacent reference location that is deemed to be sufficiently similar based on habitat suitability indices. The latter approach is analogous to epidemiological approaches used with cohorts of humans. This approach has the advantage of not needing to have an identical reference habitat, and for that reason has been suggested as the better alternative. However, the type II model has limitations as well. For example, there is the issue of covarying chemical residues, and a situation where the gradient of exposure magnitude is limited or clustered as a few greater and a few lesser values, which results in a two-point regression and a potential overestimate of the association. Because this is a continuing complication of risk assessment methodologies, in the present study, we have compared the two approaches to determine the similarity of results in a multiple lines of evidence approach. Specifically, the effects of comparing the results from a target area to an imperfect reference area on the conclusions drawn were investigated.

The threshold for effects, estimated as the 20% effect on hatching success, developed from the regression between concentrations of PCDD/DF-TEQs$_{WHO\text{-}Avian}$ in TS eggs and hatching success was estimated to be 200 ng PCDD/DF-TEQs$_{WHO\text{-}Avian}$/kg wet weight. Due to the presence of DL-PCB congeners, this value is likely underestimated by approximately 35%. Therefore, the threshold concentration was estimated to be 270 ng PCDD/DF-TEQs$_{WHO\text{-}Avian}$/kg wet weight. Concentrations of PCBs were measured in only a few subsamples, because initial information on the site indicated that concentrations of PCDD/DF were so great [2]. The concentrations of PCBs, expressed as ΣDL-PCB TEQs$_{WHO\text{-}Avian}$ were less than those estimated to cause adverse effects in TS [30]. In addition, a similar relationship between hatching success and concentrations of TCDD in eggs was reported for tree swallows breeding along the Woonasquatucket River in Rhode Island [47], which lends further support to this line of evidence.

Concentrations of PCDD/DF-TEQs$_{WHO\text{-}Avian}$ in the diets of TS on the Tittabawassee River were similar to, or greater than, those of TS on the Woonasquatucket River [47]. Therefore, comparisons were made with the estimated threshold for a 50% reduction in hatching based on concentrations in the egg that was reported in that study to be 1,700 ng TCDD/kg wet weight. The threshold measured on the Woonasquatucket River was greater than twice the threshold for effect estimated during the present study (~750 ng PCDD/DF-TEQs$_{WHO\text{-}Avian}$/kg wet wt). This difference in threshold could potentially be from exposures to different combinations of site-specific dioxin-like compounds not being fully accounted for under the current TEF approach [7]. Using the threshold for a decrease in hatching success from the present study, and comparing it to the predicted distribution of PCDD/DF-TEQs$_{WHO\text{-}Avian}$ for TS among all study sites, a 50% reduction in hatching success would have been expected for approximately 4% of the population, which is consistent with the HQ based on concentrations in eggs. However, based on the relationship between hatching success and concentrations in eggs, a potentially population-level relevant 20% decrease in hatching success would be expected for almost 52% of the population at sites near Midland, Michigan. Overall, hatching success for TS at Tittabawassee River SAs (76%) or Saginaw River SAs (86%) were not significantly less than at RAs (81%) (T.B. Fredricks, unpublished data) based on type I analyses, but hatching success was negatively correlated with ΣPCDD/DF TEQ$_{WHO\text{-}Avian}$ concentrations in eggs for individual clutches (Fig. 2). The comparison was limited by the presence of clutches that had relatively great concentrations of contaminants but still had good hatching success, which may again indicate a small subpopulation of birds that is less sensitive to the effects of dioxin-like compounds. Another possibility is that the sensitivity to dioxin-like compounds is normally this variable within a given species. Reduced hatching at sites along the Woonasquatucket River was associated with total clutch losses as opposed to a reduction in hatching success [47], whereas decreased hatching success along the Housatonic River, which is primarily contaminated with PCBs, was variable and only significant during some years [40]. Complete loss of clutches did occur in TS breeding near Midland, Michigan, but was limited to 2% of all clutches that were incubated. Average hatching success was similar among all study areas near Midland, Michigan and only slightly less than at other study sites in North America [27,83–85].

In summary, multiple lines of evidence support the conclusion that reproductive effects occurred on tree swallows, particularly near Midland, Michigan, associated with exposure to dioxin-like compounds. We found that concentrations of PCDD/DFs in eggs and diets of TS were comparable to other populations exposed to dioxin-like compounds [40,47]; HQ values were greater than one at SAs downstream of Midland; and a negative correlation was observed between concentrations PCDD/DF-TEQs$_{WHO\text{-}Avian}$ in TS eggs and nestlings, and hatching success and fledging success, respectively. The relationship between fledging success and exposure in nestling tissues is novel and highlights the continued importance of high-quality field studies that relate exposure to field-level effects. Despite these findings, it is important to note that overall productivity through fledging was similar among all study sites, which is an important estimate of the number of offspring produced. Tree swallows on the Woonasquatucket River [47] contained TEQ$_{WHO\text{-}Avian}$ that were contributed primarily from TCDD (>89% of total TEQ$_{WHO\text{-}Avian}$), whereas for the Housatonic River [40] exposures were primarily from PCBs (86% of total TEQ$_{WHO\text{-}Avian}$), as compared to the present study where exposure of TS was primarily from TCDF and secondarily from 2,3,4,7,8-PeCDF and PCBs. Potential differences in the distribution and metabolism of specific congeners by birds [72,74,86], or differences in species-specific sensitivities to dioxin-like compounds [14,15], could account for the potential differences between some literature-based thresholds and the effects observed in the present study.

SUPPLEMENTAL DATA

Table S1. Toxicity reference values for total TEQs$_{WHO\text{-}Avian}$ concentrations selected for comparison to tree swallows exposed to PCDD/DFs in the river systems downstream of Midland, Michigan, USA during 2005–2007.

Fig. S1. Hazard quotients for the effects of PCDD/DF-TEQs$_{WHO\text{-}Avian}$ for tree swallow eggs collected in 2005–2007 in the river floodplains near Midland, Michigan, USA based on the NOAEC and the LOAEC.

Table S2. Potential average (range) TEQ$_{WHO\text{-}Avian}$ daily dose calculated from site-specific bolus-based and food web-based dietary exposure for adult tree swallows breeding during 2004–2006 within the river floodplains near Midland, Michigan, USA.

Animal Use. (79.5 KB DOC)

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REFERENCES

1. U.S. Environmental Protection Agency. 1986. Michigan dioxin studies. Dow Chemical wastewater characterization study: Tittabawassee River sediments and native fish. EPA-905/4-88-003. Region V, Westlake, OH.
2. Hilscherova K, Kannan K, Nakata H, Hanari N, Yamashita N, Bradley PW, McCabe JM, Taylor AB, Giesy JP. 2003. Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. *Environ Sci Technol* 37:468–474.
3. Kannan K, Yun S, Ostaszewski A, McCabe J, Mackenzie-Taylor D, Taylor A. 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.
4. Rappe C, Kjeller LO, Kulp SE, Dewit C, Hasselsten I, Palm O. 1991. Levels, profile and pattern of PCDDs and PCDFs in samples related to the production and use of chlorine. *Chemosphere* 23:1629–1636.
5. Svensson BG, Barregard L, Sallsten G, Nilsson A, Hansson M, Rappe C. 1993. Exposure to polychlorinated dioxins (PCDD) and dibenzofurans (PCDF) from graphite-electrodes in a chloralkali plant. *Chemosphere* 27:259–262.
6. Mandal PK. 2005. Dioxin: A review of its environmental effects and its aryl hydrocarbon receptor biology. *J Comp Physiol B* 175:221–230.
7. van den Berg M, Birnbaum L, Bosveld ATC, Brunström B, Cook P, Freeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen R, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrank D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106:775–792.
8. Hoffman DJ, Melancon PN, Klein JD, Eisemann JD, Spann JW. 1998. Comparative developmental toxicity of planar polychlorinated biphenyl congeners in chickens, American kestrels and common terns. *Environ Toxicol Chem* 17:747–757.
9. Nosek JA, Craven SR, Sullivan JR, Hurley SS, Peterson RE. 1992. Toxicity and reproductive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in ring-necked pheasant hens. *J Toxicol Environ Health* 35:187–198.
10. Nosek JA, Sullivan JR, Craven SR, Gendron-Fitzpatrick A, Peterson RE. 1993. Embryotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the ring-necked pheasant. *Environ Toxicol Chem* 12:1215–1222.
11. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Giesy JP, Stromborg KL, Bursian SJ. 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* 31:404–409.
12. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Kelly ME, Stromborg KL, Melancon MJ, Fitzgerald SD, Bursian SJ. 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* 17:2035–2040.
13. Thiel DA, Martin SG, Duncan JW, Lemke MJ, Lance WR, Peterson RE. 1988. Evaluation of the effects of dioxin-contaminated sludges on wild birds. *Proceedings*, Technical Association of Pulp and Paper Environmental Conference, Charleston, SC, USA, April 18–20, pp 145–148.
14. Head JA, Hahn ME, Kennedy SW. 2008. Key amino acids in the aryl hydrocarbon receptor predict dioxin sensitivity in avian species. *Environ Sci Technol* 42:7535–7541.
15. Karchner SI, Franks DG, Kennedy SW, Hahn ME. 2006. The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA* 103:6252–6257.
16. Baker S, Sepúlveda MS. 2009. An evaluation of the effects of persistent environmental contaminants on the reproductive success of great blue herons (*Ardea herodias*) in Indiana. *Ecotoxicology* 18:271–280.
17. Drouillard KG, Fernie KJ, Smits JE, Bortolotti GR, Bird DM, Norstrom RJ. 2001. Bioaccumulation and toxicokinetics of 42 polychlorinated biphenyl congeners in American kestrels (*Falco sparverius*). *Environ Toxicol Chem* 20:2514–2522.
18. Elliott KH, Cesh LS, Dooley JA, Letcher RJ, Elliott JE. 2009. PCBs and DDE, but not PBDEs, increase with trophic level and marine input in nestling bald eagles. *Sci Total Environ* 407:3867–3875.
19. Harris ML, Elliott JE, Butler RW, Wilson LK. 2003. Reproductive success and chlorinated hydrocarbon contamination of resident great blue herons (*Ardea herodias*) from coastal British Columbia, Canada, 1977 to 2000. *Environ Pollut* 121:207–227.
20. Naito W, Murata M. 2007. Evaluation of population-level ecological risks of dioxin-like polychlorinated biphenyl exposure to fish-eating birds in Tokyo Bay and its vicinity. *Integr Environ Assess Manag* 3:68–78.
21. Straub C, Maul J, Halbrook R, Spears B, Lydy M. 2007. Trophic transfer of polychlorinated biphenyls in great blue heron (*Ardea herodias*) at Crab Orchard National Wildlife Refuge, Illinois, United States. *Arch Environ Contam Toxicol* 52:572–579.
22. Strause KD, Zwiernik MJ, Im SH, Bradley PW, Moseley PP, Kay DP, Park CS, Jones PD, Blankenship AL, Newsted JL, Giesy JP. 2007. Risk assessment of great horned owls (*Bubo virginianus*) exposed to polychlorinated biphenyls and DDT along the Kalamazoo River, Michigan, USA. *Environ Toxicol Chem* 26:1386–1398.
23. Zwiernik MJ, Bursian S, Aylward LL, Kay DP, Moore J, Rowlands C, Woodburn K, Shotwell M, Khim JS, Giesy JP, Budinsky RA. 2008. Toxicokinetics of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF in mink (*Mustela vison*) at ecologically relevant exposures. *Toxicol Sci* 105:33–43.
24. McCarty JP. 1997. Aquatic community characteristics influence the foraging patterns of tree swallows. *Condor* 99:210–213.
25. McCarty JP, Winkler DW. 1999. Foraging ecology and diet selectivity of tree swallows feeding nestlings. *Condor* 101:246–254.
26. Mengelkoch JM, Niemi GJ, Regal RR. 2004. Diet of the nestling tree swallow. *Condor* 106:423–429.
27. Custer CM, Custer TW, Allen PD, Stromborg KL, Melancon MJ. 1998. Reproduction and environmental contamination in tree swallows nesting in the Fox River drainage and Green Bay, Wisconsin, USA. *Environ Toxicol Chem* 17:1786–1798.
28. Echols KR, Tillitt DE, Nichols JW, Secord AL, McCarty JP. 2004. Accumulation of PCB congeners in nestling tree swallows (*Tachycineta bicolor*) on the Hudson River, New York. *Environ Sci Technol* 38:6240–6246.
29. Maul JD, Belden JB, Schwab BA, Whiles MR, Spears B, Farris JL, Lydy MJ. 2006. Bioaccumulation and trophic transfer of polychlorinated biphenyls by aquatic and terrestrial insects to tree swallows (*Tachycineta bicolor*). *Environ Toxicol Chem* 25:1017–1025.
30. Neigh AM, Zwiernik MJ, Bradley PW, Kay DP, Park CS, Jones PD, Newsted JL, Blankenship AL, Giesy JP. 2006. Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund site, Michigan, USA. *Environ Toxicol Chem* 25:428–437.
31. Papp Z, Bortolotti GR, Sebastian M, Smits JEG. 2007. PCB congener profiles in nestling tree swallows and their insect prey. *Arch Environ Contam Toxicol* 52:257–263.
32. Smits JEG, Bortolotti GR, Sebastian M, Ciborowski JJH. 2005. Spatial, temporal, and dietary determinants of organic contaminants in nestling tree swallows in Point Pelee National Park, Ontario, Canada. *Environ Toxicol Chem* 24:3159–3165.
33. Dunn PO, Hannon SJ. 1992. Effects of food abundance and male parental care on reproductive success and monogamy in tree swallows. *Auk* 109:488–499.
34. Quinney TE, Ankney CD. 1985. Prey size selection by tree swallows. *Auk* 102:245–250.
35. Muldal A, Gibbs HL, Robertson RJ. 1985. Preferred nest spacing of an obligate cavity-nesting bird, the tree swallow. *Condor* 87:356–363.
36. Ankley GT, Niemi GJ, Lodge KB, Harris HJ, Beaver DL, Tillitt DE, Schwartz TR, Giesy JP, Jones PD, Hagley C. 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 Contam Toxicol* 24:332–344.
37. Bishop CA, Koster MD, Chek AA, Hussell DJT, Jock K. 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.
38. Custer CM, Custer TW, Coffey M. 2000. Organochlorine chemicals in tree swallows nesting in pool 15 of the upper Mississippi River. *Bull Environ Contam Toxicol* 64:341–346.
39. Custer TW, Custer CM, Hines RK. 2002. Dioxins and congener-specific polychlorinated biphenyls in three avian species from the Wisconsin River, Wisconsin. *Environ Pollut* 119:323–332.
40. Custer CM, Custer TW, Dummer PM, Munney KL. 2003. Exposure and effects of chemical contaminants on tree swallows nesting along the Housatonic River, Berkshire county, Massachusetts, USA, 1998–2000. *Environ Toxicol Chem* 22:1605–1621.

41. DeWeese LR, Cohen RR, Stafford CJ. 1985. Organochlorine residues and eggshell measurements for tree swallows *Tachycineta bicolor* in Colorado. *Bull Environ Contam Toxicol* 35:767–775.
42. Froese KL, Verbrugge DA, Ankley GT, Niemi GJ, Larsen CP, Giesy JP. 1998. Bioaccumulation of polychlorinated biphenyls from sediments to aquatic insects and tree swallow eggs and nestlings in Saginaw Bay, Michigan, USA. *Environ Toxicol Chem* 17:484–492.
43. Harris ML, 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 Pollut* 110:307–320.
44. Secord AL, McCarty JP, Echols KR, Meadows JC, Gale RW, Tillitt DE, McCarty JP, Echols KR, Meadows JC, Gale RW, Tillitt DE. 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* 18:2519–2525.
45. Shaw GG. 1983. Organochlorine pesticide and PCB residues in eggs and nestlings of tree swallows, *Tachycineta bicolor*, in Central Alberta. *Can Field– Nat* 98:258–260.
46. Spears BL, Brown MW, Hester CM. 2008. Evaluation of polychlorinated biphenyl remediation at a Superfund site using tree swallows (*Tachycineta bicolor*) as indicators. *Environ Toxicol Chem* 27:2512–2520.
47. Custer CM, Custer TW, Rosiu CJ, Melancon MJ, Bickham JW, Matson CW. 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.
48. Fredricks TB, Zwiernik MJ, Seston RM, Coefield SJ, Plautz SC, Tazelaar DL, Shotwell MS, Bradley PW, Kay DP, Giesy JP. 2010. Passerine exposure to primarily PCDFs and PCDDs in the river floodplains near Midland, Michigan, USA. *Arch Environ Contam Toxicol* 58:1048–1064.
49. Fredricks TB, Giesy JP, Coefield SJ, Seston RM, Haswell MM, Tazelaar DL, Bradley PW, Moore JN, Roark SA, Zwiernik MJ. 2011. Dietary exposure of three passerine species to PCDD/DFs from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. *Environ Monit Assess* 172:91–112.
50. Fairbrother A. 2003. Lines of evidence in wildlife risk assessments. *Human and Ecological Risk Assessment* 9:1475–1491.
51. U.S. Environmental Protection Agency. 1998. Guidelines for ecological risk assessment. EPA/630/R-95/002F. Washington, DC.
52. Horn DJ, Benninger-Truax M, Ulaszewski DW. 1996. The influence of habitat characteristics on nest box selection of eastern bluebirds (*Sialia sialis*) and four competitors. *Ohio J Sci* 96:57–59.
53. Mellott RS, Woods PE. 1993. An improved ligature technique for dietary sampling in nestling birds. *J Field Ornithol* 64:205–210.
54. U.S. Environmental Protection Agency. 1993. *Wildlife Exposure Factors Handbook*, Vols I, II, III. EPA/60/R-93/187B. Washington, DC.
55. U.S. Environmental Protection Agency. 1998. 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.
56. Brunström B, 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 Pollut A* 42:37–45.
57. Brunström B. 1988. Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3′,4,4′-tetrachlorobiphenyl. *Poult Sci* 67:52–57.
58. Powell DC, Aulerich RJ, Meadows JC, Tillitt DE, Powell JF, Restum JC, Stromborg KL, Giesy JP, Bursian SJ. 1997. 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 auritus*) eggs. *Environ Toxicol Chem* 16:1450–1455.
59. Nosek JA, Craven SR, Sullivan JR, Olson JR, Peterson RE. 1992. Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in ring-necked pheasant hens, chicks, and eggs. *J Toxicol Environ Health* 35:153–164.
60. U.S. Environmental Protection Agency. 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. Washington, DC.
61. McMurry CS, Dickerson RL. 2001. Effects of binary mixtures of six xenobiotics on hormone concentrations and morphometric endpoints of northern bobwhite quail (*Colinus virginianus*). *Chemosphere* 43:829–837.
62. Leonards PE, van Hattum B, Leslie H. 2008. Assessing the risks of persistent organic pollutants to top predators: a review of approaches. *Integr Environ Assess Manag* 4:386–398.
63. Bhavsar SP, Fletcher R, Hayton A, Reiner EJ, Jackson DA. 2007. Composition of dioxin-like PCBs in fish: An approach for risk assessment. *Environ Sci Technol* 41:3096–3102.
64. Neigh AM, Zwiernik MJ, Blankenship AL, Bradley PW, Kay DP, MacCarroll MA, Park CS, Jones PD, Millsap SD, Newsted JW, Giesy JP. 2006. 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. *Human Ecol Risk Assess* 12:924–946.
65. McCarty J. 2001. Use of tree swallows in studies of environmental stress. *Rev Toxicol* 4:61–104.
66. McCarty JP, Secord AL. 1999. Reproductive ecology of tree swallows (*Tachycineta bicolor*) with high levels of polychlorinated biphenyl contamination. *Environ Toxicol Chem* 18:1433–1439.
67. Neigh AM, Zwiernik MJ, MacCarroll MA, Newsted JL, Blankenship AL, Jones PD, Kay DP, Giesy JP. 2006. Productivity of tree swallows (*Tachycineta bicolor*) exposed to PCBs at the Kalamazoo River Superfund site. *J Toxicol Environ Health A* 69:395–415.
68. Blankenship AL, Hilscherova K, Nie M, Coady KK, Villalobos SA, Kannan K, Powell DC, Bursian SJ, Giesy JP. 2003. Mechanisms of TCDD-induced abnormalities and embryo lethality in white leghorn chickens. *Comp Biochem Physiol C* 136:47–62.
69. Brunström B, Halldin K. 1998. EROD induction by environmental contaminants in avian embryo livers. *Comp Biochem Physiol C* 121:213–219.
70. Henshel DS, Hehn B, Wagey R, Vo M, Steeves JD. 1997. The relative sensitivity of chicken embryos to yolk- or air-cell-injected 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Toxicol Chem* 16:725–732.
71. Braune BM, Norstrom RJ. 1989. Dynamics of organochlorine compounds in herring-gulls—III: Tissue distribution and bioaccumulation in Lake Ontario gulls. *Environ Toxicol Chem* 8:957–968.
72. Elliott JE, Norstrom RJ, Lorenzen A, Hart LE, Philibert H, Kennedy SW, Stegeman JJ, Bellward GD, Cheng KM. 1996. Biological effects of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in bald eagle (*Haliaeetus leucocephalus*) chicks. *Environ Toxicol Chem* 15:782–793.
73. Kubota A, Iwata H, Tanabe S, Yoneda K, Tobata S. 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* 25:1007–1016.
74. Norstrom RJ, Risebrough RW, Cartwright DJ. 1976. Elimination of chlorinated dibenzofurans associated with polychlorinated biphenyls fed to mallards (*Anas platyrhynchos*). *Toxicol Appl Pharmacol* 37:217–228.
75. Wan Y, Hu J, An W, Zhang Z, An L, Hattori T, Itoh M, Masunaga S. 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* 40:1462–1468.
76. Newman MC. 2010. *Fundamentals of Ecotoxicology*, 3rd ed. CRC, Boca Raton, FL, USA.
77. Barnthouse LW, Glaser D, DeSantis L. 2009. Polychlorinated biphenyls and Hudson River white perch: Implications for population-level ecological risk assessment and risk management. *Integr Environ Assess Manag* 5:435–444.
78. Hull RN, Swanson S. 2006. Sequential analysis of lines of evidence—An advanced weight-of-evidence approach for ecological risk assessment. *Integr Environ Assess Manag* 2:302–311.
79. Menzie C, Henning MH, Cura J, Finkelstein K, Gentile J, Maughan J, Mitchell D, Petron S, Potocki B, Svirsky S, Tyler P. 1996. Report of the Massachusetts weight-of-evidence workgroup: A weight-of-evidence approach for evaluating ecological risks. *Human and Ecological Risk Assessment* 2:277–304.
80. Custer CM, Gray BR, Custer TW. 2010. Effects of egg order on organic and inorganic element concentrations and egg characteristics in tree swallows, *Tachycineta bicolor*. *Environ Toxicol Chem* 29:909–921.
81. van den Steen E, Dauwe T, Covaci A, Jaspers VLB, Pinxten R, Eens M. 2006. Within- and among-clutch variation of organohalogenated contaminants in eggs of great tits (*Parus major*). *Environ Pollut* 144:355–359.
82. Chapman PM, Ho KT, Munns WR, Solomon K, Weinstein MP. 2002. Issues in sediment toxicity and ecological risk assessment. *Mar Pollut Bull* 44:271–278.

83. Chapman LB. 1955. Studies of a tree swallow colony (third paper). *Bird Banding* 26:45–70.
84. Elliott JE, Martin PA, Arnold TW, Sinclair PH. 1994. Organochlorines and reproductive success of birds in orchard and non-orchard areas of central British Columbia, Canada, 1990–91. *Arch Environ Contam Toxicol* 26:435–443.
85. Smits JE, Wayland ME, Miller MJ, Liber K, Trudeau S. 2000. Reproductive, immune, and physiological end points in tree swallows on reclaimed oil sands mine sites. *Environ Toxicol Chem* 19:2951–2960.
86. Norstrom RJ, Clark TP, Jeffrey DA, Won HT, Gilman AP. 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* 5:41–48.