SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 32, No. 6, pp. 1304–1316, 2013

Printed in the USA

MULTIPLE LINES OF EVIDENCE RISK ASSESSMENT OF AMERICAN ROBINS EXPOSED TO POLYCHLORINATED DIBENZOFURANS (PCDFS) AND POLYCHLORINATED DIBENZO-*P*-DIOXINS (PCDDS) IN THE TITTABAWASSEE RIVER FLOODPLAIN, MIDLAND, MICHIGAN, USA

DUSTIN L. TAZELAAR,*† TIMOTHY B. FREDRICKS,‡ RITA M. SESTON,‡ SARAH J. COEFIELD,‡ PATRICK W. BRADLEY,† SHAUN A. ROARK,§ DENISE P. KAY,‖ JOHN L. NEWSTED,‖ JOHN P. GIESY,‡#††‡‡ STEVEN J. BURSIAN,† and MATTHEW J. ZWIERNIK†

†Department of Animal Science, Michigan State University, East Lansing, Michigan, USA
‡Department of Zoology, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan, USA
§GEI Consultants, Denver, Colorado, USA
‖Cardno ENTRIX, Okemos, Michigan, USA
#Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
††Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong SAR, China
‡‡College of Environment, Nanjing University, Nanjing, China

(Submitted 28 July 2012; Returned for Revision 29 October 2012; Accepted 23 January 2013)

Abstract: Concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) in Tittabawassee River floodplain soils and biota downstream of Midland, Michigan, USA, are greater than regional background concentrations. From 2005 to 2008, a multiple lines of evidence approach was utilized to evaluate the potential for effects of PCDD/DFs on American robins (*Turdus migratorius*) breeding in the floodplains. A dietary-based assessment indicated there was potential for adverse effects for American robins predicted to have the greatest exposures. Conversely, a tissue-based risk assessment based on site-specific PCDD/DF concentrations in American robin eggs indicated minimal potential for adverse effects. An assessment based on reproductive endpoints indicated that measures of hatch success in study areas were significantly less than those of reference areas. However, there was no dose-response relationship between that endpoint and concentrations of PCDD/DF. Although dietary-based exposure and reproductive endpoint assessments predicted potential for adverse effects to resident American robins, the tissue-based assessment indicates minimal to no potential for adverse effects, which is reinforced by the fact the response was not dose related. It is likely that the dietary assessment is overly conservative given the inherent uncertainties of estimating dietary exposure relative to direct tissue-based assessment measures. Based on the available data, it can be concluded that exposure to PCDD/DFs in the Tittabawassee River floodplain would not likely result in adverse population-level effects to American robins. *Environ Toxicol Chem* 2013;32:1304–1316. © 2013 SETAC

Keywords: Avian Dioxin Furan Hazard quotient Reproduction

INTRODUCTION

Soils and sediments of the Tittabawassee and Saginaw River floodplains downstream of Midland, Michigan, USA, are contaminated with polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs). Concentrations of PCDDs and PCDFs (PCDD/DFs) in sediments and soils collected from the Tittabawassee River floodplain ranged from 1.0×10^2 to 5.4×10^4 ng/kg dry weight, while mean total concentrations of PCDD/DF in sediments and soils from upstream reference areas (RAs) were 10- to 20-fold less [1]. Based on the spatial distribution and profile of relative concentrations of PCDD/DF congeners in the Tittabawassee River floodplain, their presence is likely due to historical production of industrial organic chemicals and onsite storage, treatment, and disposal of associated wastes prior to the establishment of modern regulations and waste management practices [2]. In particular the pattern of PCDD/DF is indicative of a graphite electrode chlor-alkali facility [1].

A preliminary screening-level ecological risk assessment based on limited biological data concluded that there was potential for adverse effects from PCDD/DF exposure to resident wildlife [3]. Therefore, a refined ecological risk assessment and supporting field investigations of exposures and responses of receptors were conducted. Based on guidance criteria of the US Environmental Protection Agency (USEPA) [4], several species were selected to better characterize the potential for adverse effects of contamination throughout the Tittabawassee and Saginaw River floodplains [5–8]. Assessments of exposure and effects on passerine birds in terrestrial food webs have utilized a variety of species [9–11]. In the present study, the American robin (*Turdus migratorius*) was investigated as a terrestrial species with a direct relationship to floodplain soils in a site-specific, multiyear, multiple lines of evidence assessment of exposure and potential effects. This assessment was based on site-specific, empirical measures of both exposure and responses.

The American robin is the largest, most abundant and widespread North American thrush. It is easily recognizable with its very audible and unique song and defensive vocalizations. At the beginning of the breeding season, American robins construct an open cup nest from grass, soil, and earthworm castings [12], typically near short grass habitats, at heights manageable for

All Supplemental Data may be found in the online version of this article.
* Address correspondence to Dustin.Tazelaar@Cardno.com.
Published online 19 February 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2193

observation by researchers. This affords researchers an onsite species presence with sufficient numbers of nests to quantify exposure and assess populations. Furthermore, the American robin has a limited home range during the breeding season and has a diet of terrestrial plants and invertebrates, including earthworms [13,14], which results in exposure to residues in local soils.

A multiple lines of evidence approach was utilized to evaluate exposure of American robins to PCDD/DFs and potential effects, including reproductive endpoints on population parameters. The methodologies implemented minimized the uncertainties associated with predicting exposures and effects based on uncontrollable variables associated with single field-based measurement endpoints [15,16] by measuring both directly. The lines of evidence utilized in the present study included site-specific measurements of American robin exposure based on concentrations measured in diet, eggs, nestlings, and adults. To estimate the potential for adverse effects to American robins along the Tittabawassee and Saginaw River floodplains, these exposures were compared with selected toxicity reference values (TRVs). The results of these predictions were then compared with site-specific measures of population parameters, especially those related to reproduction.

MATERIALS AND METHODS

Site description

The present study was conducted throughout the Tittabawassee River system in and near Midland, Michigan, USA (Figure 1). The Tittabawassee River system receives drainage from approximately 5426 km^2 of land that is composed primarily of woodlands, agricultural lands, and urban areas. Due to episodic rain events with lesser daily variations regulated by upstream hydroelectricity generation, discharges and stage vary among seasons. The Tittabawassee River flows southeast into the Saginaw River, which flows northeast to Saginaw Bay on Lake Huron. The Saginaw River downstream of the Tittabawassee River is wider and deeper with a lesser velocity. The Saginaw River is confined by engineered banks that support shipping lanes within and onshore urban development. The Tittabawassee River has a wide natural floodplain where sediments are mobilized and deposited in the floodplain seasonally from extreme flood events and bank scouring by ice associated with spring thaw. Conversely, the Saginaw River is less susceptible to deposition within the floodplain, and as such, concentrations of PCDD/DFs in surface sediment of the Saginaw and Tittabawassee Rivers were similar, whereas concentrations in surface soil of the Tittabawassee River floodplain were greater than those of the Saginaw River [17].

Samples were collected at RAs and study areas (SAs) within the 100-year floodplains of the individual rivers. Reference areas and SAs included intermittent agricultural, forested, and short grass habitat and spanned contiguous foraging areas of between 1 and 3 km of river. Two RAs were delineated upstream of the identified sources of PCDD/DFs [1] on the Tittabawassee River (R1) and Chippewa and Pine (R2) Rivers (Figure 1). Study areas downstream of the identified sources of PCDD/DFs included

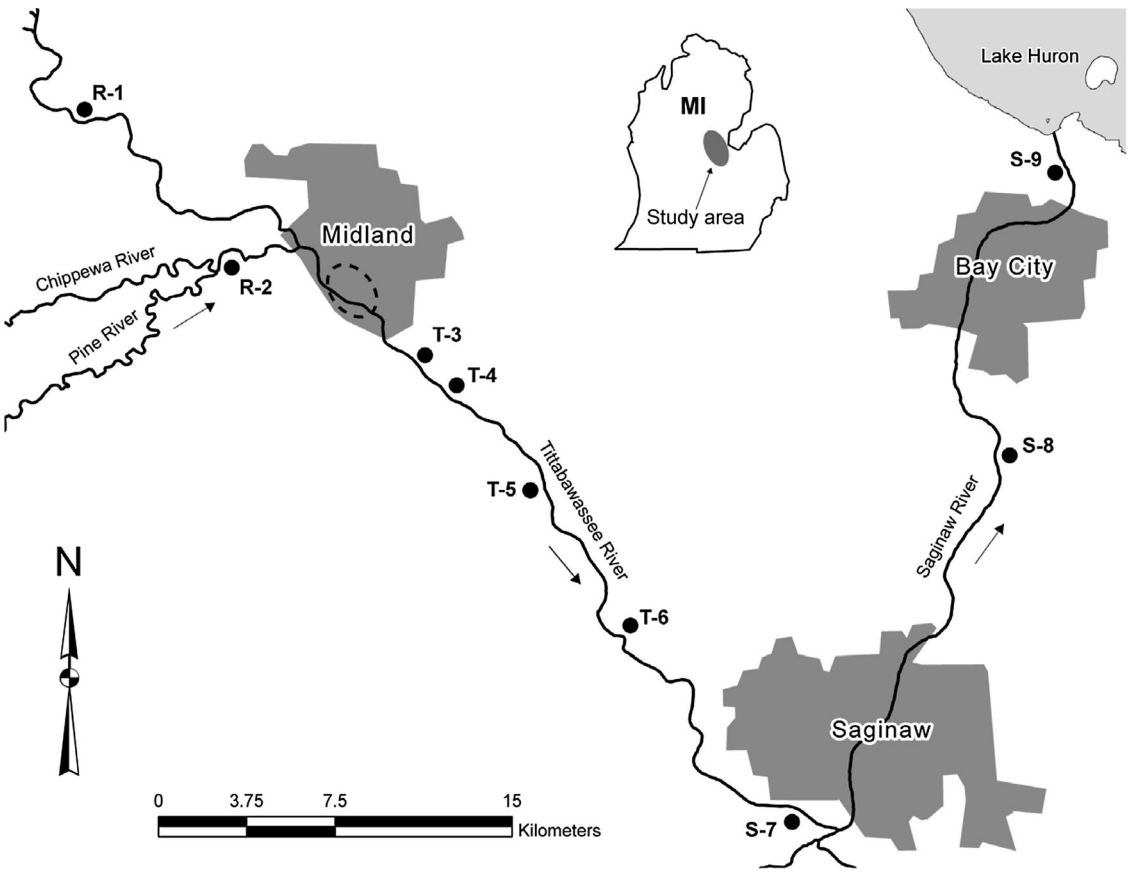

Figure 1. Study sites within the Chippewa, Pine, Tittabawassee, and Saginaw River floodplains, Michigan, USA. Reference areas (R1 and R2), Tittabawassee River study areas (SAs) T3 to T6, and Saginaw River SAs (S7 and S9) were monitored from 2005 to 2008. Only sediments and aquatic food web item collection took place at S8, with the exception of a limited number of dietary item samples. Direction of river flow is indicated with arrows; source of contamination is enclosed in a dotted oval.

approximately 72 km of free-flowing river from the upstream boundary, defined as the low-head dam within the city limits of Midland, through the confluence of the Tittabawassee and Saginaw Rivers to where the Saginaw River enters Lake Huron. The SAs along the Tittabawassee River downstream of Midland included 4 sites (T3–T6) approximately equidistant and 2 sites (S7 and S9) located at the initiation and terminus of the Saginaw River. Although S7 appears that it could be considered a Tittabawassee River site, it is located on a peninsula between the Tittabawassee and Saginaw Rivers with a majority of influence coming from the Saginaw River headwaters, including the Flint and Cass Rivers. An additional study area, S8, was included in parallel assessments for aquatic-based receptors; however, a limited number of American robin dietary items were collected from this site. The 6 SAs (T3–S9) were selected from the Tittabawassee and Saginaw Rivers based on the necessity to discern spatial trends, accessibility privileges, and worst-case receptor exposure potential based on floodplain dynamics and measured soil and sediment concentrations [1].

Monitoring of nests

Nests were monitored [18] every third day to obtain eggs and nestlings for quantification of PCDD/DFs and to make observations of reproductive endpoints. Nests were located and monitored throughout the breeding seasons from 2005 to 2008, which involved investigating suitable nest locations focusing particularly on areas where adult robins displayed defensive vocalizations. Nests located during the present study were at heights ranging from less than 1 meter (m) to greater than 17 m but typically were located at heights observable from the ground or a ladder with a bicycle mirror attached to a telescoping pole.

Reproductive endpoints examined included clutch size, hatching success, fledging success, and productivity. Hatching success was calculated in 2 ways for nests that were uninterrupted by failure and renesting. For the 1st approach, the total number of nestlings following completion of hatching was divided by the total number of eggs present prior to initiation of hatching. This approach ignored the fertility and hatchability of any egg collected and was referred to as the range-low hatching success. The 2nd approach, range-high hatching success, was adjusted for any viable egg collected and assumed that any such egg would have hatched. This was done so that concentrations of PCDD/DFs in individual clutches could be paired with outcomes of the same nest instead of collecting all of the eggs from a single nest. Analyzing the data using these 2 methods bounds the range of possible values for reproductive outcomes while allowing for the least biased measurement of concentrations of PCDD/DFs among nests. Fledging success was calculated for successful nests. The range-low fledging success is equal to the total number of juveniles fledged divided by the number of nestlings present following hatch completion, while the range-high fledging success was adjusted for any nestling collected and assumed that any such nestling would have fledged. Productivity, defined as the number of juveniles fledged following the nestling period divided by the number of eggs present prior to hatching, was represented the same way as hatching and fledging success and was presented as the range-low productivity and range-high productivity for nests observed during the egg-laying or incubation period through the nestling period. This range-low and range-high approach provides a range of values to account for tissue sampling bias [11]. Numbers of eggs in clutches were monitored; however, adjustment for collected eggs was unnecessary.

For the present study, a nest was considered successful if at least 1 juvenile American robin fledged. The Mayfield nest success index was also calculated. The Mayfield nest success index is based on the duration of observations and daily predation and survival rates [19].

Concentrations of PCDD/DFs were measured in eggs, nestlings, and adults. Eggs are accurate indicators of exposure because maternal transfer of a variety of contaminants is feasible, whereas potential adverse effects, especially of aryl hydrocarbon receptor (AhR)-mediated chemicals such as PCDD/DFs, are most likely to occur during development [20]. Addled eggs were collected opportunistically, while a single viable egg [21] was randomly collected from each nest. A maximum of 1 nestling per nesting attempt was also collected at approximately 12 d of age. The target sample sizes for tissue collections from each RA and SA were 6 eggs and 6 nestlings. Individual eggs were collected from unique nest attempts. Nestlings were also collected from unique nest attempts; however, in some instances, a nestling was collected from a nest attempt from which an egg was collected. Adults were collected with an air rifle or a small gauge/caliber firearm from known nesting areas following the breeding season but prior to migration. Adults were not identified as being associated with particular nests. Nestlings and adults were monitored for gross external morphological abnormalities during collection efforts.

Sampling of the food web

Items eaten by American robins, including invertebrates, plant matter, and soil, were collected during 2003 to 2006. The methods were previously detailed in a parallel study [8]. The present study commenced at a date later than parallel studies; however, the dietary items comprising the American robin diet had already been sampled during previous efforts. Thus, sampling of food web items preceded American robin tissue and reproductive data collection at most locations. Briefly, aerial and plant perching invertebrates were collected via sweep and/or aerial nets during the day or via Insect Vac (Bioquip Products) during the evening from a white sheet reflecting light from a metal halide lamp. Soil was collected with decontaminated shovels or trowels within the top 15 cm of soil of a 1×1 m plot with quality control samples. Vegetation and earthworms were collected from within or proximal to the aforementioned plot. A subsample of earthworms was depurated of gut contents prior to preservation and subsequent analysis. Site-specific sampling of food web items occurred at both RAs and SAs T4 and T6 in 2003. Samples were collected from the aforementioned locations and T3 to T5 in 2004, and S7 and S9 were added in 2006. To assess potential temporal variation in magnitude of exposure to contaminants through the diet, sampling occurred in mid-May, June, and September.

Quantification of contaminants

Concentrations of the 17 2,3,7,8-substituted PCDD/DF congeners were quantified in all samples, while concentrations of dioxin-like polychlorinated biphenyls (DL-PCBs) and dichloro-diphenyl-trichloroethane (DDT) and related metabolites (DDXs) were measured in a subset of eggs and nestlings. Congeners were quantified in eggs, nestlings, and adults in accordance with USEPA method 8290/1668A [22] with minor modifications as described previously [23,24]. Eggs were opened around the breadth with a chemically cleaned scalpel blade and assessed for stage of development and the presence of any abnormalities. Contents were lyophilized and stored in clean jars until analysis (I-CHEM brand). To account for any

desiccation during incubation and storage, concentrations of PCDD/DF in eggs were reported on a fresh mass basis. Adjusted fresh mass was calculated based on egg volume [25]. The mass of egg contents was determined by subtracting the mass of the eggshell at the time of processing from adjusted fresh mass. Nestling and adult whole-body samples were homogenized following removal of beaks, stomach contents, feathers and legs below the tibiotarsus. Adults were frozen with liquid nitrogen and homogenized with a Robot Coupe food processor. Nestlings were homogenized with an Osterizer blender.

Individual congeners and compounds were identified and quantified by use of high-resolution gas chromatography/high-resolution mass spectroscopy (HRGC/HRMS) via a Hewlett-Packard 6890 GC (Agilent Technologies) connected to a MicroMass high-resolution mass spectrometer (Waters Corporation) conducted at AsureQuality Ltd, Lower Hutt, New Zealand. Sample processing blanks, matrix spikes, matrix spike duplicates, blind check samples, and unspiked sample replicates were included during chemical analysis as quality control samples. Evaluation of recovery and relative percent difference for matrix spike and spike duplicate samples and unspiked replicate samples were within $\pm$ 30% at a rate of greater than 95% acceptability.

Concentrations of PCDD/DF were expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) equivalents (ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$). Concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ were calculated as the sum of the products of the concentrations of each of the 17 PCDD/DF congeners multiplied by the appropriate 2,3,7,8-TCDD equivalency factors ($TEF_{WHO\text{-}Avian}$) as specified for birds by the World Health Organization (WHO) [26] and concentrations expressed as ng ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$/kg on a wet weight basis for biota and on a dry weight basis for soil.

Calculation of potential average daily dose

Site-specific (R1 and R2, T3–T6, S7 and S9) potential average daily doses ($ADDs_{pot}$) were calculated. The ADD_{pot}, expressed as ng ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$/kg body mass/d, was calculated by use of the wildlife dose equation for dietary exposures, equations 4 through 8, of the USEPA *Wildlife Exposure Factors Handbook* (WEFH) [27]. Rate of intake of food was calculated by use of the USEPA WEFH equations 3 and 4 with a body mass of 77 g. This approach assumed that all foraging was done within the study area.

Relative proportions of items consumed in the diet were estimated from the literature [13,14]. This composition included 25% Coleoptera (beetles), 25% Lepidoptera (mostly moths), 18% Oligochaeta (earthworms), 7% plant, and 25% miscellaneous Arthropoda. Invertebrates comprising the miscellaneous Arthropoda included Orthoptera (mostly grasshoppers), Hemiptera (largely shield bugs or stink bugs), Homoptera (particularly leafhoppers), and Arachnida (spiders). Concentrations of PCDD/DFs were greater in invertebrates than in plants. Thus, proportions of dietary items used to calculate exposure via the diet were adjusted to be appropriate during the breeding season when more invertebrates are consumed relative to plants. The strategy applied assured that $ADDs_{pot}$ were nearer the greater end of the exposure distributions that resulted in an exposure assessment that is protective of the population.

Toxicity reference values

Potential for adverse effects was evaluated by comparing concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in the diet or eggs with available TRVs. Toxicity reference values are quantitative measures of toxicity used to estimate risk utilizing the hazard quotient (HQ) method where the estimate of exposure is compared with a threshold concentration for effect. Several factors were considered during selection of TRVs, including appropriateness of receptor species, chemical compound, presence of a dose-response relationship, and quantification of ecologically relevant endpoints associated with sensitive life stages. In an effort to minimize uncertainties associated with derivations of total ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ [28], consideration was given only to TRVs derived from exposure to PCDD/DF. No-observed-adverse-effect concentrations (NOAECs) and lowest-observed-adverse-effect concentrations (LOAECs) were used in the determination of HQs and subsequent assessment of risk. In the present study, TRVs based on concentrations in the diet or in eggs were used to evaluate the potential adverse effects of site-specific exposure to ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$.

The dietary ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ TRV selected for the present study was established based on a study in which adult hen ring-necked pheasants (*Phasianus colchicus*) were exposed via intraperitoneal injections of 2,3,7,8-TCDD for a 10-wk exposure period [29]. Toxicity reference values based on concentrations in diet were determined by converting the weekly exposure from which adverse effects on fertility and hatching success were determined (1000 ng 2,3,7,8-TCDD/kg/wk) to a LOAEC for daily exposure of 140 ng 2,3,7,8-TCDD/kg/d. The dosing regime was based on orders of magnitude differences, and adverse effects were not present at the next lesser dose, 14 ng TCDD/kg/d, which was determined to be the NOAEC for dietary exposure.

The TRV selected for comparison to concentrations of PCDD/DF (ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$) in American robin eggs was based on an egg-injection study that involved dosing eastern bluebird (*Sialia sialis*) eggs with 2,3,7,8-TCDD [30]. Field-collected eastern bluebird eggs collected from the wild were injected with concentrations of 2,3,7,8-TCDD in 10-fold increments ranging from 1 to 100 000 ng/kg wet weight prior to replacement to their original clutches and subsequent incubation by unexposed adults. Hatching success was significantly adversely affected at doses greater than 10 000 ng 2,3,7,8-TCDD/kg wet weight (LOAEC), whereas endpoints associated with eggs exposed to less than 1000 ng 2,3,7,8-TCDD/kg wet weight (NOAEC) were not significantly different than those of vehicle-injected controls. Additionally, the key measurement endpoint of the study was hatching success, an ecologically relevant endpoint, for which a dose-response relationship had been observed. The minimal taxonomic distance between the species further strengthened the applicability of the bluebird egg TRV to the eggs of the American robin. Closely related species are expected to exhibit similar sensitivity to dioxin-like compounds [29], and both bluebirds and American robins are of the family Turdidae. Not unexpectedly, both have the same genetic sequence in the ligand-binding domain (LBD) of the AhR that appears to dictate species sensitivity to dioxin-like compounds further confirming their direct comparability (S.W. Kennedy, Environment Canada, National Wildlife Research Centre, Ottawa, ON, Canada, personal communication).

Several studies in which eggs were injected with 2,3,7,8-TCDD were also considered for the American robin egg TRV, including studies with ring-necked pheasant [31] and double-crested cormorant (*Phalacrocorax auritus*) [32]. However, based on the criteria adopted for selection of a TRV, including species relatedness, ecologically relevant endpoints, a clear

dose-response relationship, valid control groups, and power to discern effects, none was as robust as the study of the eastern bluebird [30].

Assessment of risk

Overall, the risk of PCDD/DFs to American robins was assessed utilizing a multiple lines of evidence approach that incorporated estimates of exposure based on concentrations in both the diet and in eggs as well as quantification of site-specific productivity measurement endpoints [15]. Potential effects of exposure based on concentrations of PCDD/DFs in the diet and in eggs were assessed by calculating HQs. Concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ (ng/kg wet wt) in eggs and estimates of dietary exposure ADD_{pot}, expressed as ng ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$/kg body weight/d, were divided by TRVs based on either concentrations in eggs or diet and based on either the NOAEC or LOAEC, respectively. Hazard quotients were determined based on the upper 95% confidence level for arithmetic means of concentrations in eggs at individual study locations and based on 95th and 50th centiles of dietary exposures. Arithmetic means were presented rather than geometric means because arithmetic means were greater and provided a more conservative basis from which inferences could be drawn. Incorporation of both dietary- and tissue-based assessments of exposure has been shown to reduce uncertainty in risk assessments of persistent organic pollutants (POPs) [33].

In addition to the point estimates of risk, semiprobabilistic estimates of risk were determined by comparing probability distributions of expected cumulative percent frequencies of exposure based on concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in eggs of American robin and ADD_{pot} based on ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in the diet to appropriate TRVs. Predicted probabilistic distributions were generated by use of a Monte Carlo approach in SAS software (Release 9.1 and 9.3; SAS Institute) for the egg-based risk assessment and a resampling approach in R software (R 2.10.0; http://www.R-project.org/) for the dietary-based risk assessment.

Statistical analyses

Mean concentrations of the 17 individual 2,3,7,8-substituted PCDD/DF congeners are reported as the arithmetic mean expressed as ng/kg wet weight for American robin eggs, nestlings, adults, and dietary items and as ng/kg dry weight for soils. Mean concentrations of co-contaminants in American robin eggs and nestlings are also reported as the arithmetic mean expressed as ng/kg wet weight. Individual congeners for which concentrations were less than the limit of quantification (LOQ) had a proxy value of one-half the LOQ assigned.

Statistical analyses were performed using SAS and R software. The experimental unit for reproductive measurements was individual nest attempt. Eggs, nestlings, or adults were considered individual experimental units. To assess fixed effects spatially while treating year as a random variable, PROC GLIMMIX was utilized. In some instances, data were transformed to satisfy the assumption of normality. Fledging success data were arcsine square root transformed. Square root transformations were used for hatching success and range-high productivity data. Egg and nestling compound concentration data were log transformed. Type III tests for fixed effects were used to identify significance in differences among areas. Least squares means tests were used to identify significance in differences between areas. A Wilcoxon 2-sample test was used to detect significant differences for data for a single year. Differences were considered to be statistically significant at $p < 0.05$. A regression assessment was performed by use of PROC REG in SAS software to assess potential relationships between hatching success and ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs.

To describe the ADD_{pot} as accurately as possible using the measured concentrations in dietary items, a resampling approach using R software was used to estimate 50th and 95th centiles and maximum ADD_{pot}. This method was similar to assessments of American mink (*Mustela vison*) and great horned owl (*Bubo virginianus*) exposure to PCDD/DFs in the Tittabawassee River floodplain [5,7].

RESULTS

Site-specific endpoints

A total of 240 American robin nests among all sites were monitored for opportunities to collect samples for quantification of residues and to make observations on reproductive endpoints during the 4 breeding seasons including 2005 through 2008. Concentrations of PCDD/DFs were quantified in 84 eggs and 53 nestlings collected from the aforementioned nesting attempts. To avoid pseudoreplication issues, the egg with the greatest concentration of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ was selected for use in assessments in instances where data were available for more than 1 egg from a single clutch. This was done to provide a greater central tendency for the exposure calculation so as to result in a more conservative risk assessment. The number of eggs from each site, in which the concentration of ΣPCDD/DF was determined, ranged from 4 to 18 ($n = 10$ at R1, $n = 9$ at R2, $n = 7$ at T3, $n = 8$ at T4, $n = 10$ at T5, $n = 18$ at T6, $n = 8$ at S7, and $n = 4$ at S9). Nestlings that were less or greater than approximately 12 d posthatch were deemed not comparable to the majority of nestlings collected (45 nestlings), thus 8 of the nestlings sampled/salvaged were excluded. The number of nestlings analyzed for ΣPCDD/DF quantification from each site ranged from 2 to 7 ($n = 6$ at R1, $n = 6$ at R2, $n = 6$ at T3, $n = 6$ at T4, $n = 6$ at T5, $n = 7$ at T6, $n = 6$ at S7, and $n = 2$ at S9). Following the breeding season, 12 adults ($n = 6$ at R1, $n = 3$ at T3, and $n = 3$ at T6) were collected for quantification of PCDD/DFs. Concentrations of ΣPCDD/DFs were measured in 160 composite samples of individual dietary items collected from RAs and SAs during the breeding seasons from 2003 through 2006. These concentrations were used along with estimates of relative proportions of each component of the diet to determine the daily intake of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$. Co-contaminants were quantified in 5 eggs from RAs, 23 eggs from Tittabawassee River SAs, and 11 eggs from Saginaw River SAs (Supplemental Data, Table S1). Co-contaminants were also quantified in a subset of nestlings ($n = 1$ in RAs, $n = 5$ in Tittabawassee River SAs, and $n = 5$ in Saginaw River SAs). Adult American robins and dietary items were not analyzed for co-contaminants.

Concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in eggs, nestlings, and adults

Concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs were significantly different among RAs and SAs ($p < 0.0001$). Results of least squares means tests indicated significant differences between concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs from RAs and Tittabawassee River SAs ($p < 0.0001$) and those from RAs and Saginaw River SAs ($p = 0.0012$). Mean concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in eggs from the Tittabawassee River SAs were 8- to 79-fold greater than those from RAs

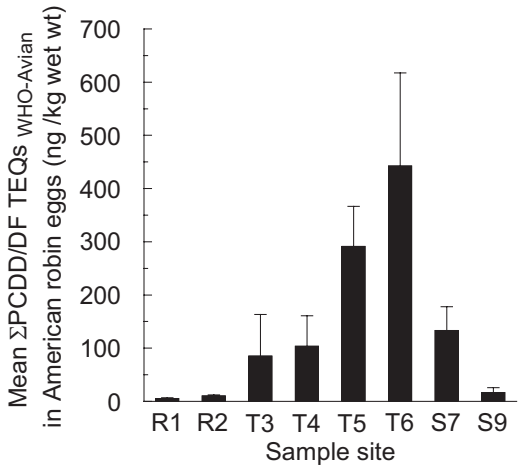

Figure 2. Mean concentrations of polychlorinated dibenzofuran and polychlorinated dibenzo-*p*-dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$) in American robin eggs collected during 2005 to 2008 from the river floodplains near Midland, Michigan, USA. Error bars indicate 95% upper confidence level. R1 and R2 = reference sites; T3 to T6 = Tittabawassee River study sites; S7 and S9 = Saginaw River study sites.

(Figure 2), while concentrations in eggs from the Saginaw River SAs were 2- to 24-fold greater than those of eggs collected from RAs. Mean concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in eggs from Tittabawassee River SAs were as much as 28-fold greater than those of eggs collected in Saginaw River SAs. The maximum concentration of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ observed in eggs was 1700 ng/kg wet weight in an egg from T6.

Profiles of relative concentrations of PCDD/DF congeners in American robin eggs were dominated by PCDD congeners at RAs, while downstream SAs were dominated by PCDF congeners (Supplemental Data, Figure S1). The dominant congener in SAs was 2,3,4,7,8-PeCDF, which contributed means of 35% and 24% to ΣPCDD/DFs concentrations at Tittabawassee River SAs and Saginaw River SAs, respectively. The dominant congener in RA eggs was octachlorodibenzo-*p*-dioxin (OCDD), which contributed a mean of 27% to ΣPCDD/DF concentrations. The dominant congener contribution to the mean ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ concentration in eggs in RAs was 2,3,7,8-TCDD (36%) and 2,3,4,7,8-PeCDF in Tittabawassee River SAs (84%) and Saginaw River SAs (71%).

Concentration differences of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ in eggs were statistically significant among RAs and SAs ($p = 0.0498$). Results of least squares means tests indicated significant differences between RAs and Saginaw River SAs ($p = 0.0173$). Mean concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ in eggs were not statistically significantly different between RAs and Tittabawassee River SAs or between Tittabawassee River SAs and Saginaw River SAs. Mean concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ were 8- and 3-fold greater at Saginaw River SAs than RAs and Tittabawassee River SAs, respectively. The greatest concentration of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ was observed in an egg collected from S9 (11 ng/kg wet weight), in which PCB-77 contributed approximately 42% of the ΣDL-PCB $TEQ_{WHO\text{-}Avian}$.

Concentrations of ΣDL-PCB in eggs were not significant among RAs and SAs ($p = 0.2775$). Mean concentrations of ΣDL-PCB at Saginaw River SAs were 8-fold greater than RAs and 2-fold greater than Tittabawassee River SAs.

Concentrations of ΣDDX in eggs were not statistically significantly different among RAs and SAs, although the *p* value ($p = 0.0596$) was near the α value. Results of least squares means tests indicated significant differences between Tittabawassee River SAs and Saginaw River SAs ($p = 0.0249$). Mean concentrations of ΣDDX were 2-fold greater at Tittabawassee River SAs than RAs and Saginaw River study areas.

Concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin nestlings (Supplemental Data, Figure S2) were statistically significantly different among RAs and SAs ($p < 0.0001$). Results of least squares means tests indicated significant differences between RAs and Tittabawassee River SAs ($p < 0.0001$) and between RAs and Saginaw River SAs ($p = 0.0074$). Mean concentrations were as much as 21-fold greater in Tittabawassee River SAs. Mean concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in nestlings were 4- to 120-fold greater in Tittabawassee River SAs than RAs, while mean concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in nestlings from Saginaw River SAs were 2- to 34-fold greater than nestlings collected from RAs. The maximum concentration of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in nestlings of 710 ng/kg wet weight was observed at T5.

Profiles of relative concentrations of PCDD/DF congeners in nestlings resembled those in eggs and were comprised primarily of PCDD congeners in nestlings collected in RAs, while profiles in nestlings from SAs were dominated by PCDF congeners (Supplemental Data, Figure S3). The dominant congener in SAs was 2,3,4,7,8-PeCDF, which contributed means of 30% and 28% to ΣPCDD/DF concentrations at Tittabawassee River SAs and Saginaw River SAs, respectively. The dominant congener in RAs was OCDD, which contributed a mean of 37% to ΣPCDD/DF concentrations. The dominant congener contribution to the mean of the ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ concentration in nestlings in RAs was 2,3,7,8-TCDD (33%) and 2,3,4,7,8-PeCDF in Tittabawassee River SAs (79%) and Saginaw River SAs (72%).

Co-contaminants in nestlings were not significantly different among RAs, Tittabawassee River SAs, and Saginaw River SAs for concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ ($p = 0.6263$), ΣDL-PCBs ($p = 0.6727$), and ΣDDXs ($p = 0.1181$). Mean concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ were 12- and 4-fold greater at Saginaw River SAs than RAs and Tittabawassee River SAs, respectively. The greatest concentration of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ was from a nestling collected from S9 (4.2 ng/kg wet weight), in which PCB-81 contributed approximately 54% to the concentration of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$. Mean ΣDL-PCB concentrations at Saginaw River SAs were 18-fold greater than RAs and 4-fold greater than Tittabawassee River study areas. Mean concentrations of ΣDDX were 10-fold greater at Tittabawassee River SAs than RAs and 5-fold greater than Saginaw River study areas.

Concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in adult American robins (Supplemental Data, Figure S4) from the Tittabawassee River SAs were significantly greater (49-fold) than those from RAs ($p = 0.0039$). Profiles of relative concentrations of ΣPCDD/DFs in adults resembled those in eggs and nestlings. Profiles in adults from RA were primarily comprised of PCDD congeners (27% OCDD), while profiles in adults from SA were dominated by 2,3,4,7,8-PeCDF (Supplemental Data, Figure S5). Mean 2,3,4,7,8-PeCDF congener contribution was approximately 42% to ΣPCDD/DF in Tittabawassee River SAs. The maximum concentration of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in adult American robins was 270 ng/kg wet weight from T6.

Dietary exposure

Concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in co-located soils, terrestrial plants, and invertebrates were generally greater in dietary items from SAs than those from RAs (Table 1). Mean

Table 1. Median (95th centile) concentrations of polychlorinated dibenzofuran and polychlorinated dibenzo-*p*-dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$) (ng/kg wet wt) in American robin dietary items collected during 2003 to 2006 from the river floodplains near Midland, Michigan, USA

	R1 and R2		T3 to T6		S7 and S9	
	n	Median (95th centile)	*n*	Median (95th centile)	*n*	Median (95th centile)
Beetles	9	3.3 (14)	17	460 (1600)	6	100 (240)
Moths and butterflies	7	0.95 (1.5)	6	66 (95)	5	16 (34)
Miscellaneous[a]	13	1.2 (3.1)	15	24 (280)	7	3.5 (34)
Earthworms	6	1.4 (2.3)	12	220 (440)	4	120 (450)
Plants	9	0.57 (1.4)	18	4.6 (13)	12	0.58 (1.8)
Soil	11	5.7 (17)	23	3400 (11000)	8	1200 (6300)

[a]Comprised of Orthoptera (mostly grasshoppers), Hemiptera (largely shield bugs or stink bugs), Homoptera (particularly leafhoppers), and Arachnida (spiders).
R1 and R2 = reference sites; T3 to T6 = Tittabawassee River study sites; S7 and S9 = Saginaw River study sites.

concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in dietary items were as much as 160-fold greater in invertebrates from Tittabawassee River SAs than those from RAs, whereas concentrations from Saginaw River SAs were intermediate. Mean concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ were least in terrestrial plants. The maximum concentration of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ was 1900 ng/kg wet weight in Coleoptera collected from T4.

Profiles of relative concentrations of ΣPCDD/DFs in the 4 primary dietary items (plants, Coleoptera, Lepidoptera, and Oligochaeta) varied in mean proportions of PCDDs to PCDFs among areas (Supplemental Data, Figures S6–S9). Profiles of relative concentrations of congeners of PCDD/DF in the dietary items were dominated by octachlorodibenzo-*p*-dioxin (OCDD), which accounted for 57% to 74%, 35% to 53% and 25% to 52% of mean concentrations of ΣPCDD/DF in dietary items from RAs, Tittabawassee River SAs, and Saginaw River SAs, respectively. In general, profiles of relative concentrations of ΣPCDD/DF in dietary items consisted of a greater percentage of PCDF congeners in SAs than in RAs. Mean contributions percentages of PCDF congeners to ΣPCDD/DF ranged from 14% to 24% in RAs, 38% to 56% in Tittabawassee River SAs, and 36% to 58% in Saginaw River SAs.

Potential average daily dose

Potential average daily doses (ADD_{pot}) of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$, expressed as ng/kg body weight/d for adult American robins, were greater in SAs than RAs. The median ADD_{pot} was 130- and 50-fold greater at Tittabawassee River SAs and Saginaw River SAs, respectively, than in RAs. The 95th centile $ADDs_{pot}$ values were 120- to 40-fold greater at Tittabawassee River SAs and Saginaw River SAs, respectively, than at RAs. The maximum ADD_{pot} of 610 ng/kg body weight/d was observed at Tittabawassee River SAs (Table 2).

Table 2. Polychlorinated dibenzofuran and polychlorinated dibenzo-*p*-dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$) potential average daily doses (ADD_{pot}; ng/kg body wt/d) for adult American robins breeding in the floodplains near Midland, Michigan, USA, during 2005 to 2008

	R1 and R2	T3 to T6	S7 and S9
Median	1.4	190	68
95th centile	3.6	420	140
Maximum	4.4	610	210

R1 and R2 = reference sites; T3 to T6 = Tittabawassee River study sites; S7 and S9 = Saginaw River study sites.

Reproductive success

Reproductive endpoints were monitored in 68 RA nests, 133 Tittabawassee River SA nests, and 39 Saginaw River SA nests. There were 215 American robin nests for which the outcome was determined. At least 1 nestling fledged (successful nest) at 42%, 50%, and 37% of nests in RAs, Tittabawassee River SAs, and Saginaw River SAs, respectively (Table 3). The number of nests monitored per site varied, with as few as 15 nests located at S9 and as many as 64 nests located at T6 over the duration of the study.

Mayfield estimates of daily mortality rates were significantly different among RAs (743.5 exposure days), Tittabawassee River SAs (1284 exposure days), and Saginaw River SAs (359 exposure days) (chi-square test; $p < 0.0001$). Daily mortality rates were 0.048 nest losses/exposure day in RAs, 0.044 nest losses/exposure day in Tittabawassee River SAs, and 0.053 nest losses/exposure day in Saginaw River SAs. Daily mortality rates were significantly different between RAs and Tittabawassee River SAs ($p < 0.001$), between RAs and Saginaw River SAs ($p = 0.0008$) and between Tittabawassee River SAs and Saginaw River SAs ($p < 0.001$).

Nests that were preyed upon comprised the majority of nests that were not successful. Criteria for depredation included the loss of all eggs or nestlings prior to a date at which nestlings would have been expected to fledge; the presence of damaged eggs, such as resulting from an avian predator puncturing the shell; or evidence of preyed upon nestlings or adults, such as lacerations on the carcass of dead birds or piles of feathers below the nest. No predators were observed in the act of depredating American robin adults, nestlings, or eggs during the study; however, potential predators observed on site included American mink, squirrels (Scuridae), raccoons (*Procyon lotor*), and avian species such as blue jays (*Cyanocitta cristata*) and crows (*Corvus brachyrhynchos*).

Statistical significance was variable among reproductive endpoints, both among and between RAs and SAs (Table 4). In contrast to the Mayfield approach to estimating daily mortality, the following reproductive endpoint data do not include data influenced by depredation. Range-low and range-high fledging success and range-low productivity were not statistically different among or between areas. Although range-low hatching success was not statistically significantly different among RAs, Tittabawassee River SAs and Saginaw River SAs, it was significantly different between RAs and Saginaw River SAs ($p = 0.0365$). Range-high hatching success was not statistically significantly different among areas ($p = 0.0600$). Similarly, range-high hatching success was not statistically significantly different between RAs and Tittabawassee River SAs

Table 3. Nest outcomes for American robins breeding in the floodplains near Midland, Michigan, USA, during 2005 to 2008

	R1 and R2	T3 to T6	S7 and S9[a]
	Outcome %[b] (n)	Outcome % (n)	Outcome % (n)
2005			
Hatched[c]	63% (8)	75% (12)	–
Fledged[d]	56% (9)	58% (12)	–
Predated	44% (9)	25% (12)	–
Abandoned	0% (0)	8% (12)	–
Other	0% (0)	8% (12)	–
Unknown	0% (0)	0% (0)	–
Failed[e]	0% (0)	0% (0)	–
2006			
Hatched	100% (2)	58% (12)	80% (10)
Fledged	0% (1)	29% (17)	40% (10)
Predated	100% (1)	59% (16)	60% (10)
Abandoned	0% (1)	6% (16)	0% (10)
Other	0% (1)	0% (16)	0% (10)
Unknown	0% (1)	0% (16)	0% (10)
Failed	0% (1)	0% (16)	0% (10)
2007			
Hatched	44% (32)	63% (43)	44% (9)
Fledged	35% (31)	44% (43)	33% (9)
Predated	58% (31)	56% (43)	66% (9)
Abandoned	6% (31)	0% (43)	0% (9)
Other	0% (31)	0% (43)	0% (9)
Unknown	0% (31)	0% (43)	0% (9)
Failed	0% (31)	0% (43)	0% (9)
2008			
Hatched	64% (22)	67% (51)	64% (11)
Fledged	45% (22)	60% (50)	36% (11)
Predated	50% (22)	38% (50)	64% (11)
Abandoned	0% (22)	0% (50)	0% (11)
Other	5% (22)[f]	2% (50)[g]	0% (11)
Unknown	0% (22)	0% (50)	0% (11)
Failed	0% (22)	0% (50)	0% (11)
Overall			
Hatched	54% (64)	65% (118)	62% (30)
Fledged	42% (63)	50% (122)	37% (30)
Predated	53% (63)	46% (121)	63% (30)
Abandoned	3% (64)	2% (121)	0% (30)
Other	2% (64)[g]	3% (121)[g]	0% (30)
Unknown	0% (64)	0% (121)	0% (30)
Failed	0% (64)	0% (121)	0% (30)

[a]S7 and S9 were monitored 2006–2008.
[b]Percentage of *n*.
[c]At least 1 egg in clutch hatched.
[d]At least 1 nestling from brood fledged.
[e]Each egg failed to hatch.
[f]Human disturbance–related failure.
[g]Weather-related failure.
R1 and R2 = reference sites; T3 to T6 = Tittabawassee River study sites; S7 and S9 = Saginaw River study sites.

($p = 0.0601$). However, these p values were only slightly greater than the α value. Range-high hatching success was significantly different between RAs and Saginaw River SAs ($p = 0.0306$). Finally, although differences in range-high productivity were not statistically significant among areas, the p value was only slightly greater than the α value between RAs and Saginaw River SAs ($p = 0.0597$).

Effects assessment

Estimated ADD_{pot} values for SAs were generally greater than TRVs based on dietary exposure. Both the 50th and 95th centiles and the maximum ADD_{pot} for Tittabawassee River SAs and Saginaw River SAs were greater than both the dietary-based LOAEC and NOAEC, with the exception of the Saginaw River SA 50th centile ADD_{pot}, which was less than the LOAEC. Based on the NOAEC and LOAEC, the estimated 95th centile HQs at Tittabawassee River SAs and Saginaw River SAs were > 1.0. The RA ADD_{pot} was less than each dietary-based TRV (Figure 3).

The predicted probabilistic distributions of expected cumulative probabilities based on concentrations of ADD_{pot} calculated from site-specific food web–based dietary exposures for adult American robins were compared with selected TRVs. The predicted probabilities of the ADD_{pot} exceeding the NOAEC at Tittabawassee River SAs and Saginaw River SAs were approximately 99% and 98%, respectively, while the probability for the RA was $< 1\%$ (Figure 4). Predicted probabilities of the ADD_{pot} exceeding the LOAEC at Tittabawassee River SAs and Saginaw River SAs, were approximately 75% and 6%, respectively, while that of the RA was $< 1\%$.

The 95% upper confidence level concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs were 9.2, 370, and 130 ng/kg wet weight at RAs, Tittabawassee River SAs, and Saginaw River SAs, respectively, with all calculated HQs being < 1.0 for all sites (Figure 5). Concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ were not included in the effects assessment because a comprehensive assessment of concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ was beyond the scope of the present study. However, for eggs that were analyzed for co-contaminants, concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ contributed minimally to the total concentrations of $TEQs_{WHO\text{-}Avian}$ (4% in RAs, $< 1\%$ in Tittabawassee River SAs and 9% in Saginaw River SAs), and ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ concentrations were substantially less than a recognized TEQ-based NOAEL TRV [34].

Predicted probabilistic distributions of expected cumulative percent frequencies based on concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs were compared with several TRVs (Figure 6). Approximately 5% of the Tittabawassee River SA predicted concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs exceeded the NOAEC, while approximately $< 1\%$ in the RAs and Saginaw River SAs exceeded the NOAEC. Approximately $< 1\%$ of the predicted concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs of the RAs and SAs exceeded the LOAEC.

DISCUSSION

Species selection

American robins were a suitable receptor species to characterize exposure and potential effects via the soil-to-plant and invertebrate food web during the present study. Both eggs and nestlings were of sufficient individual mass to meet analytical detection limits. Widespread distribution and sufficient breeding and foraging habitat in RAs and SAs allowed for assessment of reproductive performance of the population. Furthermore, the use of floodplain soils in American robin nest construction may lead to a greater exposure potential than that of cavity nesting terrestrial species. However, unlike cavity-nesting terrestrial species, such as eastern bluebirds and house wrens (*Troglodytes aedon*) [8], American robins were not isolated to artificial nest boxes or platforms. Therefore, more effort was required to locate nests.

Multiple lines of evidence

American robins residing within the Tittabawassee River floodplain were exposed to dioxin-like compounds. However, the comprehensive site-specific data, when employed in a multiple lines of evidence approach, did not identify with any certainty the potential for, or site-measured, contaminant-related

Table 4. Productivity measurements for American robins breeding in the floodplains near Midland, MI, during 2005–2008

	All nesting attempts					
	R1 and R2		T3 to T6		S7 and S9[a]	
	n	Mean (SE)[b]	*n*	Mean (SE)	*n*	Mean (SE)
Clutch Size	30	3.2 (0.3)	42	3.2 (0.3)	12	3.5 (0.5)
Range-low hatching success	24	88%^{A} (3.5%)	42	75%^{AB} (3.6%)	10	68%^{B} (7.2%)
Range-high hatching success[c]	24	94%^{A} (2.7%)	42	81%^{AB} (3.6%)	10	74%^{B} (8.4%)
Range-low fledging success	24	75% (6.1%)	60	80% (3.4%)	10	79% (6.0%)
Range-high fledging success[d]	24	93% (3.6%)	60	97% (1.4%)	10	100% (0.0%)
Range-low productivity[e]	18	70% (6.9%)	33	61% (5.2%)	6	50% (6.5%)
Range-high productivity[f]	18	84% (4.7%)	33	72% (4.1%)	6	63% (11%)

[a]S7 and S9 were monitored during 2006 to 2008.
[b]Means with differing uppercase letters were statistically significantly different ($p < 0.05$).
[c]Range-high hatching success includes any eggs removed for contaminant analyses as successfully hatched eggs.
[d]Range-high fledging success includes nestlings collected for contaminant analyses if remainder of clutch was successful.
[e]Productivity is defined as the number of nestlings fledged per eggs laid.
[f]Range-high productivity considers eggs removed for contaminant analyses as fledglings.
R1 and R2 = reference sites; T3 to T6 = Tittabawassee River study sites; S7 and S9 = Saginaw River study sites; SE = standard error.

individual- or population-level adverse effects. Establishing whether a site-relevant contaminant exposure has the potential to adversely affect individuals is a key component in ascertaining causation when differences in individual or population health are noted in field-measured parameters. Two different methods of exposure and effects assessments (dietary- and egg-based) indicated contrasting potential for adverse effects to American robins in the SA.

Exposure and assessment based on concentrations in the diet

The risk assessment based on estimated $ADDs_{pot}$ and applicable TRVs indicated there was potential for adverse effects for American robins in Tittabawassee River SAs and most likely for American robins in Saginaw River SAs, whereas no potential for adverse effects was indicated in RAs. The expected threshold for effects should be observed at concentrations between the LOAEC and NOAEC. The HQ based on the LOAEC for the 50th centile was < 1.0; however, potential for adverse effects was indicated because the HQ based on the LOAEC for the 95th centile and both HQs based on the 50th and 95th centile of exposure relative to the NOAEC were >1.0 for dietary items in Saginaw River SAs (Figure 3). Although the potential for adverse effects is indicated for American robin foraging in Saginaw River SAs, this line of evidence has the greatest amount of associated uncertainty.

In comparison, the estimated 50th centile for the $ADDs_{pot}$ at the SA calculated from data collected during the present study were similar to concentrations of $\Sigma PCDD/DF\ TEQs_{WHO\text{-}Avian}$ in

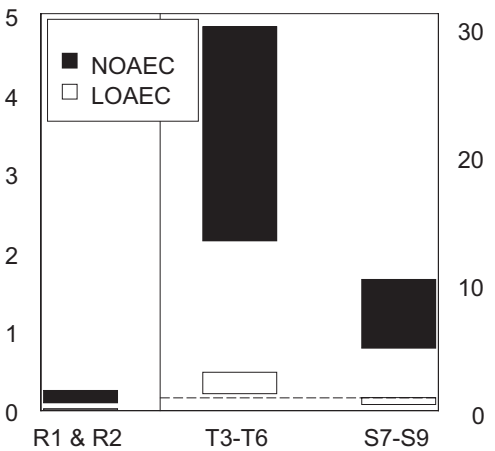

Figure 3. Hazard quotients (HQs) for the effects of potential polychlorinated dibenzofuran and polychlorinated dibenzo-*p*-dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($\Sigma PCDD/DF\ TEQ_{WHO\text{-}Avian}$) average daily dose ($ADD_{pot}$) from site-specific food web-based dietary exposure for adult American robins during 2003 to 2006 from the river floodplains near Midland, Michigan, based on the no-observable-adverse-effect concentration (NOAEC) and the lowest-observable-adverse-effect concentration (LOAEC). Hazard quotients based on measured concentration ranges are presented; left y-axis for reference areas (R1 and R2); right y-axis for Tittabawassee River study areas (T3–T6), and Saginaw River study areas (S7 and S9); lower end of bars bound by 50th centile HQ value and upper end bound by 95th centile HQ value; dashed horizontal reference line of right y-axis indicates HQ value of 1.

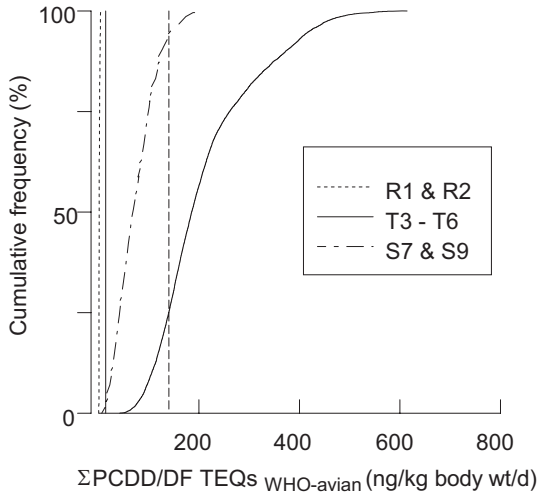

Figure 4. Modeled probabilistic distribution of expected cumulative percent frequencies for potential average 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($\Sigma PCDD/DF\ TEQ_{WHO\text{-}Avian}$) daily dose ($ADD_{pot}$; ng/kg body wt/d) calculated from site-specific food web-based dietary exposure for adult American robins breeding during 2003 to 2006 within the floodplains near Midland, Michigan, USA. Ten thousand replications per site; reference areas, R1 and R2, indicated by a dotted line; Tittabawassee River study areas, T3 to T6, indicated by a solid line; Saginaw River study areas, S7 and S9, indicated by a dotted-dashed line; y-axis offset to show R1 and R2; no-observable-adverse-effect concentration (NOAEC) indicated by a vertical solid bar; lowest-observable-adverse-effect concentration (LOAEC) indicated by a vertical dashed bar; toxicity reference values (TRVs) derived from Nosek et al. [29].

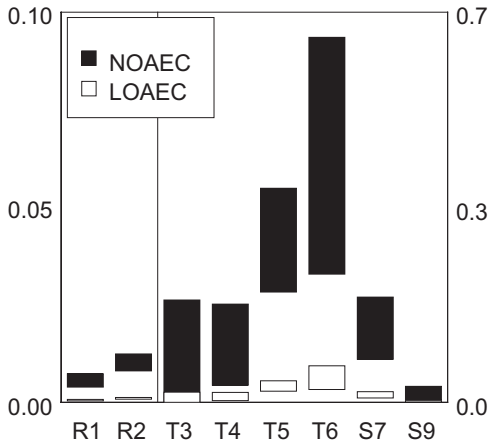

Figure 5. Hazard quotients (HQs) for the effects of polychlorinated dibenzofuran and polychlorinated dibenzo-*p*-dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$) for American robin eggs collected during 2005 to 2008 from the river floodplains near Midland, Michigan, based on the no-observable-adverse-effect concentration (NOAEC) and the lowest-observable-adverse-effect concentration (LOAEC). Hazard quotients based on 95% lower and upper confidence limits derived from arithmetic mean concentrations are presented; left y-axis for reference areas (R1 and R2); right y-axis for Tittabawassee River study areas (T3–T6) and Saginaw River study areas (S7 and S9); lower end of bars bound by 95% LCL HQ value and upper end bound by 95% upper confidence level HQ value.

pooled dietary samples from a tree swallow study where reduced hatching success was observed. Stomach contents of tree swallow nestlings exposed to dioxin-like compounds ranged from 72 to 230 ng ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$/kg from the Woonasquatucket River in Rhode Island, USA [28]. This corresponded to reduced hatching success for populations of tree swallows. Although this approach suggests exposure of tree

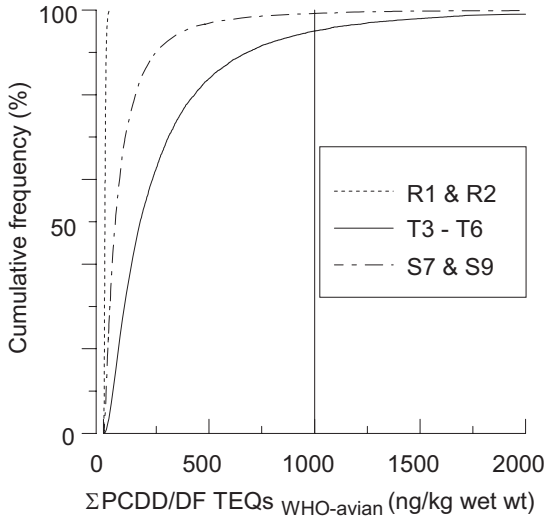

Figure 6. Modeled probabilistic distribution of expected cumulative percent frequencies for American robin egg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$) concentrations ng/kg wet weight in site-specific eggs collected from the river floodplains near Midland, Michigan, in 2005 to 2008. Ten thousand replications per site; reference areas, R1 and R2, indicated by a dotted line; Tittabawassee River study areas (SAs), T3 to T6, indicated by a solid line; Saginaw River SAs, S7 and S9, indicated by a dotted-dashed line; y-axis offset to show R1 and R2; no observable adverse effect concentration (NOAEC) indicated by a vertical solid bar; lowest observable adverse effect concentration (LOAEC) (not indicated) is 10 000 ng ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$/kg wet weight; toxicity reference values (TRVs) derived from Thiel et al. [30].

swallows along the Woonasquatucket River was 6 to 18 times greater than concentrations associated with the threshold for effects that were selected for that study, the dietary samples were pooled by reference or study area and subsequently not directly comparable to the estimated ADD_{pot} approach of the present study. Also, the profiles of congeners in dietary items were dissimilar between the studies. During the study of the Woonasquatucket River, dietary items were dominated by 2,3,7,8-TCDD; 1,2,3,4,6,7,8-HpCDD; and OCDD. The SAs of the present study, however, were dominated by 2,3,7,8-TCDF; OCDD; and to a lesser extent OCDF, further reducing study comparability.

Assessing exposure by use of the ADD_{pot} is generally less certain than directly measuring concentrations in tissues of adults, nestlings, or eggs. Application of the ADD_{pot} is useful if it is not possible to make measurements of concentrations in eggs or nestlings and information is available for potential dietary items or these concentrations can be predicted from measurements in soils. In applying the ADD_{pot} approach, it is assumed that composition of the diet based on information in the literature is appropriate. Furthermore, the relative composition of the diet selected for calculation of the ADD_{pot} in the present study is likely conservative because the proportion of earthworms was greater than that suggested in the literature from which the composition was derived. The proportion of earthworms in the diet was likely underestimated in those studies [13,14] because the frequencies were based on analyses of stomach contents that might misrepresent the contribution by Oligochaeta to the diet because they are soft-bodied and more readily digestible relative to more chitinous invertebrates. The ADD_{pot} approach also assumes that the normalized ingestion rate is appropriate and that American robins limit their foraging to the floodplain. Furthermore, the estimated ADD_{pot} is what was available, not necessarily what is bioavailable for uptake. Another uncertainty in applying TRVs based on concentrations in the diet is that correction by relative potencies among congeners by applying $TEFs_{WHO\text{-}Avian}$ does not correct for differences in rates of assimilation or biotransformation and clearance that would affect the internal dose resulting from a dietary dose, particularly in this instance where the selected comparison TRV is based on an intraperitoneal study rather than true dietary exposure. For these reasons, we consider measurements of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in eggs to be a more accurate estimate of exposure, especially to the more sensitive life stage.

Assessment of exposure based on concentrations in eggs, nestlings, and adult American robins

Overall, concentrations of PCDD/DFs in American robin eggs, nestlings, adults, and dietary items were greater in SAs than in the RAs. The dominant furan congener, however, differed between dietary items and tissues of American robins. Profiles of relative concentrations of congeners in dietary items were dominated by 2,3,7,8-TCDF, while the profiles in American robin tissues were dominated by 2,3,4,7,8-PeCDF. This could be the result of TCDF being metabolised and PeCDF being retained by American robins [5,35]. This difference was also observed for other receptor species in parallel studies [5,7,8,23,36].

In contrast to the dietary-based assessment, an assessment based on concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ measured in eggs indicated there was minimal potential for adverse effects of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ on American robins upstream and downstream of Midland because HQs based on both the LOAEC and NOAEC were < 1.0 (Figure 5). The predicted frequency

distributions of concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in eggs suggest approximately 5% of the eggs in Tittabawassee River SAs exceed the NOAEC, while < 1% of Saginaw River SAs exceed the same threshold value (Figure 6). The same distributions indicate that < 1% of eggs from the Tittabawassee River SA and Saginaw River SA exceed the LOAEC. Assuming that the actual threshold for effects occurs between the NOAEC and the LOAEC, based on relevant egg-based TRVs and 95% upper confidence level exposures in eggs, adverse effects on American robins would not be expected for either the RAs or SAs.

Results from comparable studies of other species of birds suggest a minimal potential for effects to American robins in RAs and SAs in the vicinity of Midland, Michigan, or other areas. The results of the study of tree swallows on the Woonasquatucket River indicated that an estimated lethal concentration for 50% (LCE50) of tree swallow eggs exposed to dioxin-like compounds was 1700 ng TCDD/kg wet weight based on hatching [28]. This LCE50 value is more than 6-fold greater than the mean concentration of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs from the most contaminated SAs of the Tittabawassee River floodplain and thus suggests minimal potential for adverse effects. However, this does not suggest concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ similar to those in eggs from the Tittabawassee River would not occur at an ecologically relevant point on the lethal concentration estimate curve derived for tree swallows along the Woonasquatucket River.

Measures of individual and population condition

Of the individual and population health parameters quantified as indicators of effects observed in the field, hatching was deemed the most sensitive and robust. Other ecologically relevant endpoints were measured as metrics of individual health, such as morphology of embryos, nestlings, and adults, while fledging and productivity were quantified to understand overall population health. There were no morphological deformities in either observed and/or collected nestlings or adults. There were no deformities observed in embryos during egg homogenization processing. The TRVs selected with the greatest certainty are based on hatching because these allowed for a direct comparison of the same measurement endpoint between the field and controlled laboratory studies. Although each quantified endpoint is relevant to the overall individual and population condition assessment, hatching success should be considered of significant importance due to the aforementioned criteria.

There were significant differences between RA and SA range-low hatching success and RA and SA range-high hatching success. However, these differences did not appear to be related to PCDD/DF exposure. A key factor for establishing causation is the presence of a dose-response relationship. For the present study the range-low and range-high hatching success for the Tittabawassee River SAs were intermediate, while the American robin tissue ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ concentrations were greatest. Conversely, range-low hatching success and range-high hatching success were least in Saginaw River SAs, where ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ concentrations were intermediate. This suggests that differences in hatching success are not a direct result of exposure to PCDD/DFs.

Similarly, the Mayfield daily mortality rates between RAs and SAs differed significantly, and again the differences were not related to dose of PCDD/DFs. The greatest daily mortality rate was observed where concentrations of PCDD/DF in tissues were intermediate and least where concentrations in tissues were greatest. Where daily mortality rate was greatest (Saginaw River SAs), no nests were abandoned. Depredation accounted for the loss of each failed nest in Saginaw River SAs. Although depredation accounted for the majority of nest failure in RAs and Tittabawassee River SAs, each area experienced minimal nest failure due to abandonment (2 nests per area).

Hatching success observed for American robins nesting within the Tittabawassee River floodplain were comparable to values reported as species norms. Range-high hatching success and range-low hatching success were greater than or similar to that of American robins from a study in which hatching success (60–69%) was unaffected by exposure to DDT [37]. Range-high hatching success and range-low hatching success were also within the range of measured hatching success (45–100%) for 99 avian species from a genetic similarity and hatching success study [38]. Additionally, both range-high and range-low hatching successes in the present study were greater than the hatching success reported for the reference areas in a study of American robin breeding and nesting behavior [39]. The proportions of successful nests of RAs and all SAs during the present study were also greater than those (25 and 21–24%) reported for American robin in the literature [11,40].

Regression assessment

As mentioned previously, hatching successes of American robins observed in the present study were not related to concentrations of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs. A significantly lesser hatching success and greater ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs were observed in SAs relative to RAs. A regression assessment indicated no apparent relationship between range-low ($r^2 < 0.01$, $n = 30$) or range-high hatching success ($r^2 < 0.01$, $n = 30$) and ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ in American robin eggs for nesting attempts with both data points quantified.

Analysis of uncertainty of the assessment

The greatest uncertainty regarding the results of the present study, like other risk assessments, was related to the selection of TRVs because the chosen TRVs could have a significant influence on the subsequent assessment of hazard and associated risk. Recently, methods have been suggested [41] for deriving TRVs through compilation of data from multiple studies for a single species, or multiple species where applicable, from which dose-response curves may be generated to isolate appropriate effective doses (EDs) to use as TRVs rather than the more conventional NOAEC and LOAEC approach of HQ quantification. Although we recognize the validity of the ED approach, sufficient data were not available to generate EDs for the present study.

The greatest proportion of research investigating effects of dioxin-like compounds on avian species has been conducted on the domestic chicken (*Gallus domesticus*) and has overwhelmingly acknowledged that the chicken is the most sensitive species to PCDD/DF [42–44]. Although this research has resulted in reliable ED data relative to applicable endpoints, there is now a data set robust enough to conclude that the selection of chicken-derived EDs as TRVs will most likely result in overly conservative estimates of hazard. TRVs were selected from studies based on species relatedness, including genetic congruence of the ligand-binding domain of the AhR construct to that of the American robin. Estimates of TRVs based on concentrations in eggs were based on a field study of the eastern bluebird, which like the American robin is a member of the

family Turdidae [30]. Toxicity reference values were derived from intraperitoneal injections of TCDD in hen ring-necked pheasants [29]. The major limitation of the present study stems from the differences in contaminant absorption, distribution, metabolism, and excretion from intraperitoneal injections rather than through ingestion in a true dietary dosing study.

Additional confidence in selection of TRVs is available from recent research investigating the differences between species-specific sensitivities to dioxin-like compounds, which suggests that sensitivities are determined by differences in sequences of amino acids of the AhR LBD among species [45]. Based on these AhR LBD results, the American robin was classified as a species with moderate sensitivity to dioxin-like compounds. The eastern bluebird has an AhR LBD that is identical to the American robin, whereas the ring-necked pheasant is only 1 substitution different but responds similarly to exposure to dioxin-like compounds. Thus, we concluded that the most scientifically defensible TRVs for this American robin risk assessment were the individual studies selected. The sequence of amino acids in the LBD was deemed to be more predictive of sensitivity to dioxin-like compounds than was taxonomic classification of feeding guild.

Uncertainty within the dietary-based TRV further suggests that the assessment based on measured concentrations in eggs is more reliable. One uncertainty in applying dietary TRVs is that correction by relative potencies among congeners by applying $TEFs_{WHO\text{-}Avian}$ does not correct for differences in rates of assimilation or biotransformation and clearance that would affect the internal dose resulting from a dietary dose.

CONCLUSIONS

Assessments of risk associated with concentrations of PCDD/DFs in the diet of American robins downstream of Midland indicated that there was potential for adverse effects. However, in contrast, neither tissue-based exposures based on concentrations of PCDD/DF in eggs nor assessment of individual health were indicative of the potential for adverse effects. Subsequent assessment of reproductive endpoints revealed significant differences between hatching success of American robin populations in RAs and SAs in the floodplains near Midland. Although significant differences were observed in hatching success among RAs and SAs, hatching successes were greater where exposure was greatest and lesser where exposure was intermediate. Thus, the effect did not appear to be dose-related. Moreover, all of the measurement parameters associated with individuals and populations for American robins exposed to dioxin-like compounds in the present study were similar to or greater than those reported in the literature for unexposed American robin populations. Possible explanations for the disagreement between the tissue- and dietary-based exposure assessments include the possibility that the tissue-based TRVs were too liberal as the doses utilized, which established the NOAEC and LOAEC, may not have accurately characterized true threshold values for potential effects or the dietary-based TRVs may have been overly conservative based on intraperitoneal injections in the ring-necked pheasant instead of true dietary absorption. Uncertainties within the estimate of ADD_{pot} values including dietary composition and time spent onsite may also explain this disparity. A comprehensive assessment of co-contaminants was beyond the scope of the present study; however, a subset of the samples indicated concentrations of co-contaminants were generally not significantly different, with the exception of concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ in eggs. However, concentrations of ΣDL-PCB $TEQ_{WHO\text{-}Avian}$ were less than an established TRV. Furthermore, concentrations of DL-PCB $TEQ_{WHO\text{-}Avian}$ contributed minimally to the total concentrations of $TEQs_{WHO\text{-}Avian}$. It is feasible that significant differences in hatching success can be explained by differences in habitat quality, but habitat quality measures were beyond the scope of this assessment.

Based on the evidence, we were not able to conclude that American robins foraging and breeding within the Tittabawassee River floodplain are at risk to experience adverse population-level effects as a result of their exposure to PCDD/DFs. Although the dietary-based assessment as well as noted differences in hatching success suggested both the potential for and presence of adverse effects, the remaining lines of evidence either conflicted with or weakened this interpretation. The directly measured tissue-based exposure assessment did not identify the potential for adverse effects. Furthermore, measures of effects on both individuals and populations including clutch size, fledging success, and productivity were not different between exposed and reference areas, and like hatching success, were not different from values reported as normal in the literature. Estimates of survival calculated by use of the Mayfield method indicated that measures of reproductive fitness were greatest in the Tittabawassee River SAs, which consistently had the greatest contamination. Moreover, hatching success, which was generally lesser in the contaminated areas, could not be directly linked to contaminant exposure, as a key criteria for establishing stressor causation is the identification of a dose response. When measured concentrations of residues in eggs were compared to hatching success by the 8 individual study sites, the response was not dose related.

Animal use

All procedures that included the use of animals were conducted following standard operating procedures approved by Michigan State University's Institutional Animal Care and Use Committee. All required agency permits and approvals are archived at the Michigan State University Wildlife Toxicology Laboratory (MSU-WTL).

SUPPLEMENTAL DATA

Table S1.
Figures S1–S9. (253 KB PDF).

Acknowledgment—The authors would like to thank the staff and students of the Michigan State University (MSU) Wildlife Toxicology Laboratory team and the researchers of Cardno ENTRIX, Okemos, Michigan for their dedicated efforts. The authors recognize J. Dastyck and S. Kahl of the US Fish and Wildlife Service – Shiawassee National Wildlife Refuge for their assistance and refuge access, T. Lenon of Chippewa Nature Center for his assistance and nature center access, and the more than 50 cooperating parks and landowners for granting property access. The authors recognize the statistical analysis efforts of P. Reeb and W. Wang of the MSU Statistical Consulting Center. Funding was provided via unrestricted grants from The Dow Chemical Company, Midland, Michigan to M. Zwiernik and J. Giesy of Michigan State University. J. Giesy was supported by the Canada Research Chair Program.

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Supplemental Information

MULTIPLE LINES OF EVIDENCE RISK ASSESSMENT OF AMERICAN ROBINS EXPOSED TO POLYCHLORINATED DIBENZOFURANS (PCDFS) AND POLYCHLORINATED DIBENZO-*P*-DIOXINS (PCDDS) IN THE TITTABAWASSEE RIVER FLOODPLAIN, MIDLAND, MICHIGAN, USA

Dustin L Tazelaar, Timothy B Fredricks, Rita M Seston, Sarah J Coefield, Patrick W Bradley, Shaun A Roark, Denise P Kay, John L Newsted, John P Giesy, Steven J Bursian and Matthew J Zwiernik

Supplemental figure 1. Mean congener percent contributions in American robin eggs collected during 2005-2008 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA.

Supplemental figure 2. Mean concentrations of $\sum$PCDD/DF $TEQs_{WHO\text{-}Avian}$ in American robin nestlings collected during 2005-2008 from the river floodplains near Midland, Michigan, USA.

Supplemental figure 3. Mean congener percent contributions in nestling American robins collected during 2005-2008 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA.

Supplemental figure 4. Mean concentrations of $\sum$PCDD/DF $TEQs_{WHO\text{-}Avian}$ in American robin adults collected during 2005-2008 from the river floodplains near Midland, Michigan, USA.

Supplemental figure 5. Mean congener percent contributions in adult American robins collected during 2007 from the Chippewa and Tittabawassee river floodplains, Midland, Michigan, USA.

Supplemental figure 6. Mean congener percent contributions in terrestrial plants collected during 2003-2006 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA.

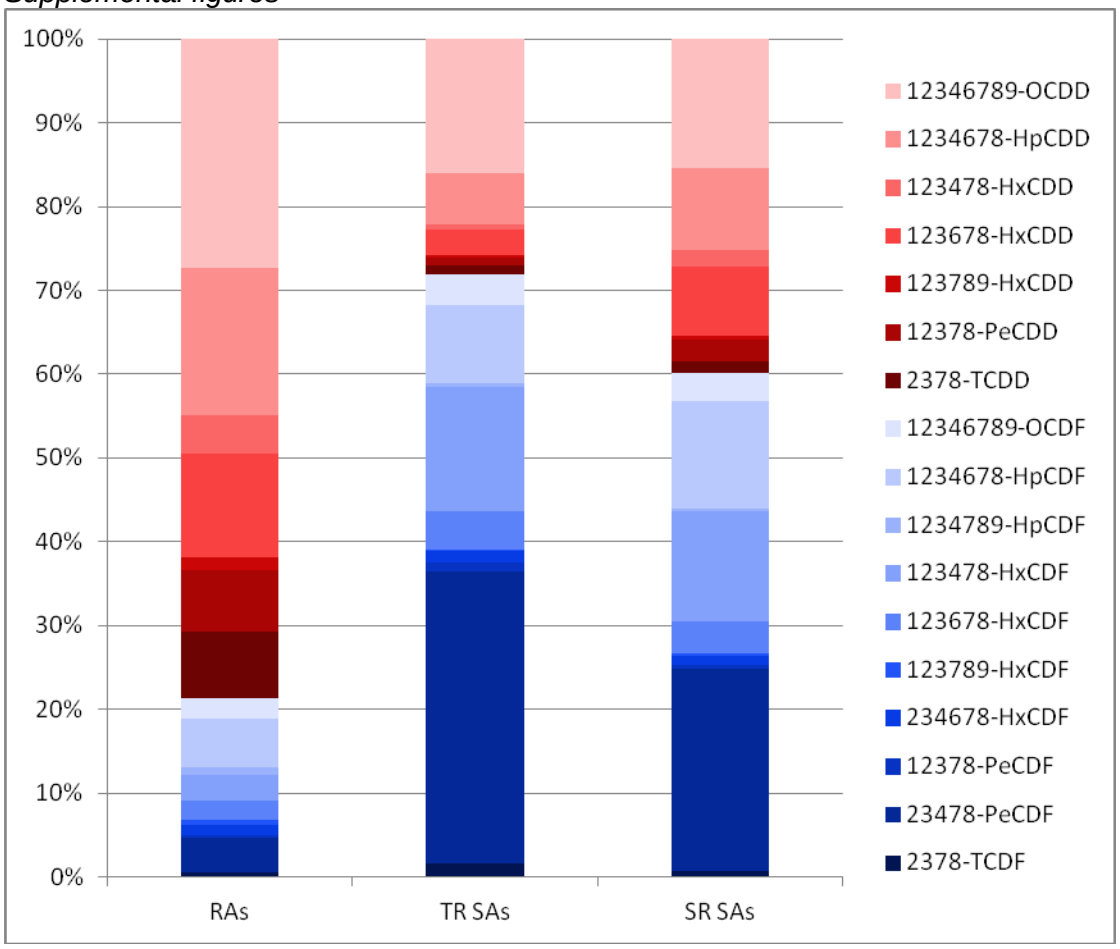
Supplemental figure 7. Mean congener percent contributions in terrestrial Coleoptera collected during 2003-2006 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA.

Supplemental figure 8. Mean congener percent contributions in terrestrial Lepidoptera collected during 2003-2006 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA.

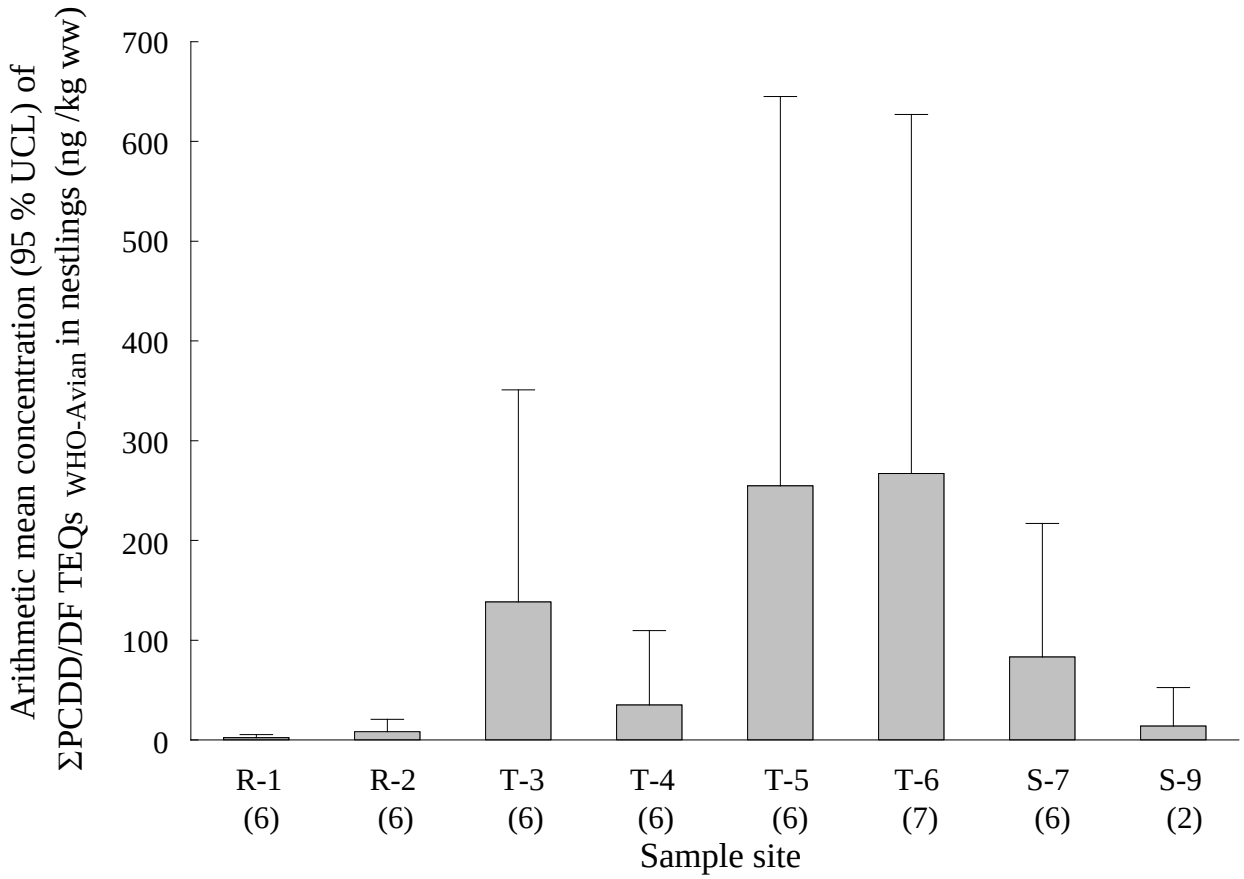
Supplemental figure 9. Mean congener percent contributions in depurated terrestrial Oligochaeta collected during 2003-2006 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA.

Supplemental Table 1. Concentrations of co-contaminants in American robin tissues collected during 2005 to 2008 from the river floodplains near Midland, Michigan, USA.

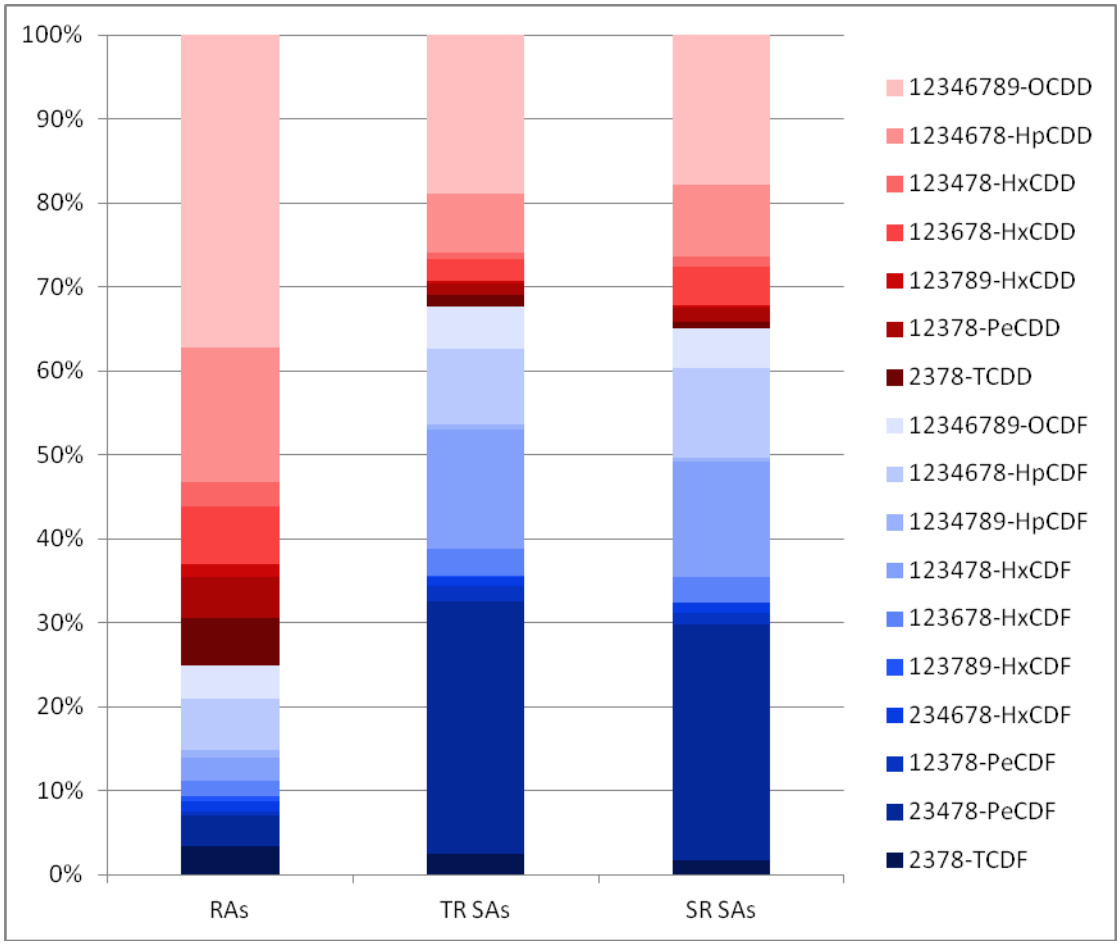
Supplemental figures



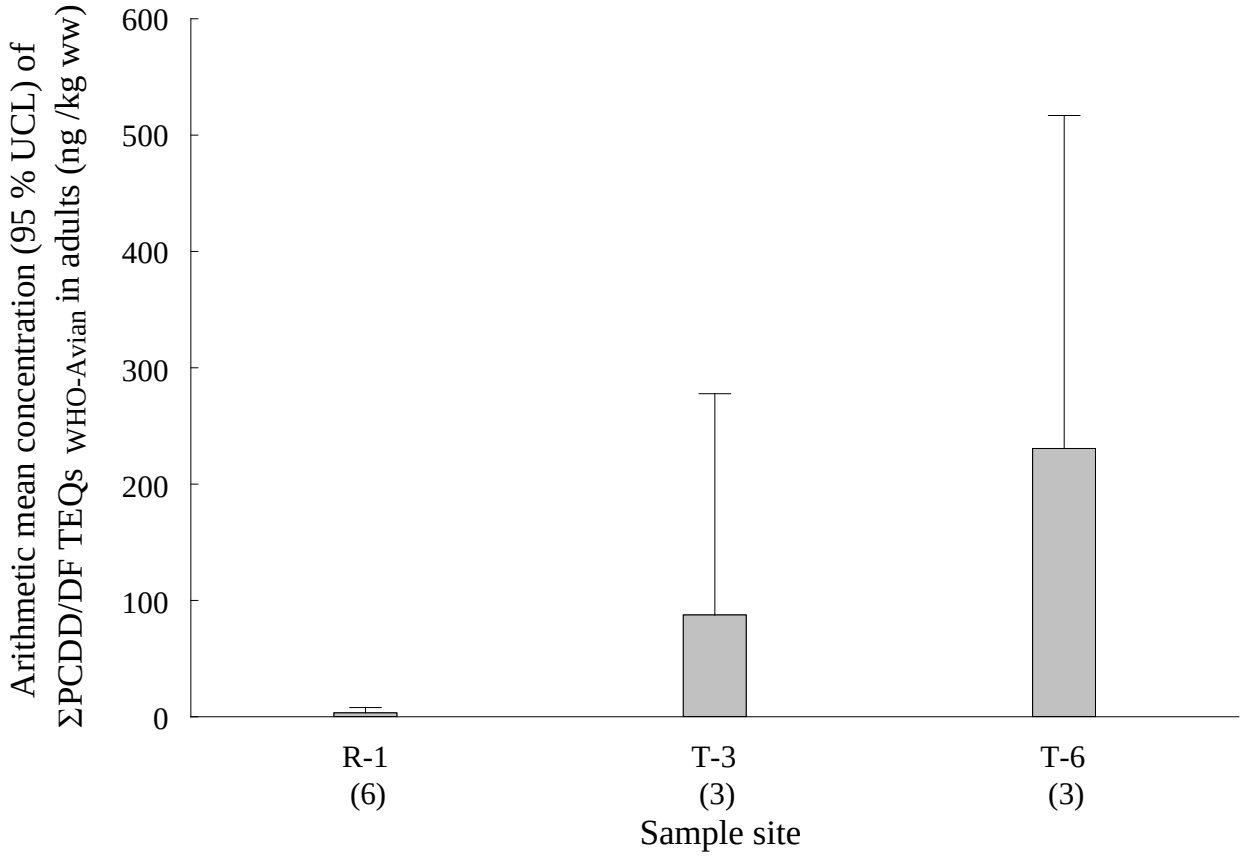
Supplemental figure 1. Mean congener percent contributions in American robin eggs collected during 2005-2008 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA. R-1 to R-2 includes reference areas, T-2 to T-6 includes Tittabawassee River study areas and S-7 and S-9 includes Saginaw River study areas. Congeners include octachlorodibenzo-*p*-dioxin (OCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), tetrachlorodibenzo-*p*-dioxin (TCDD), octachlorodibenzofuran (OCDF), heptachlorodibenzofuran (HpCDF), hexachlorodibenzofuran (HxCDF), pentachlorodibenzofuran (PeCDF) and tetrachlorodibenzofuran (TCDF).



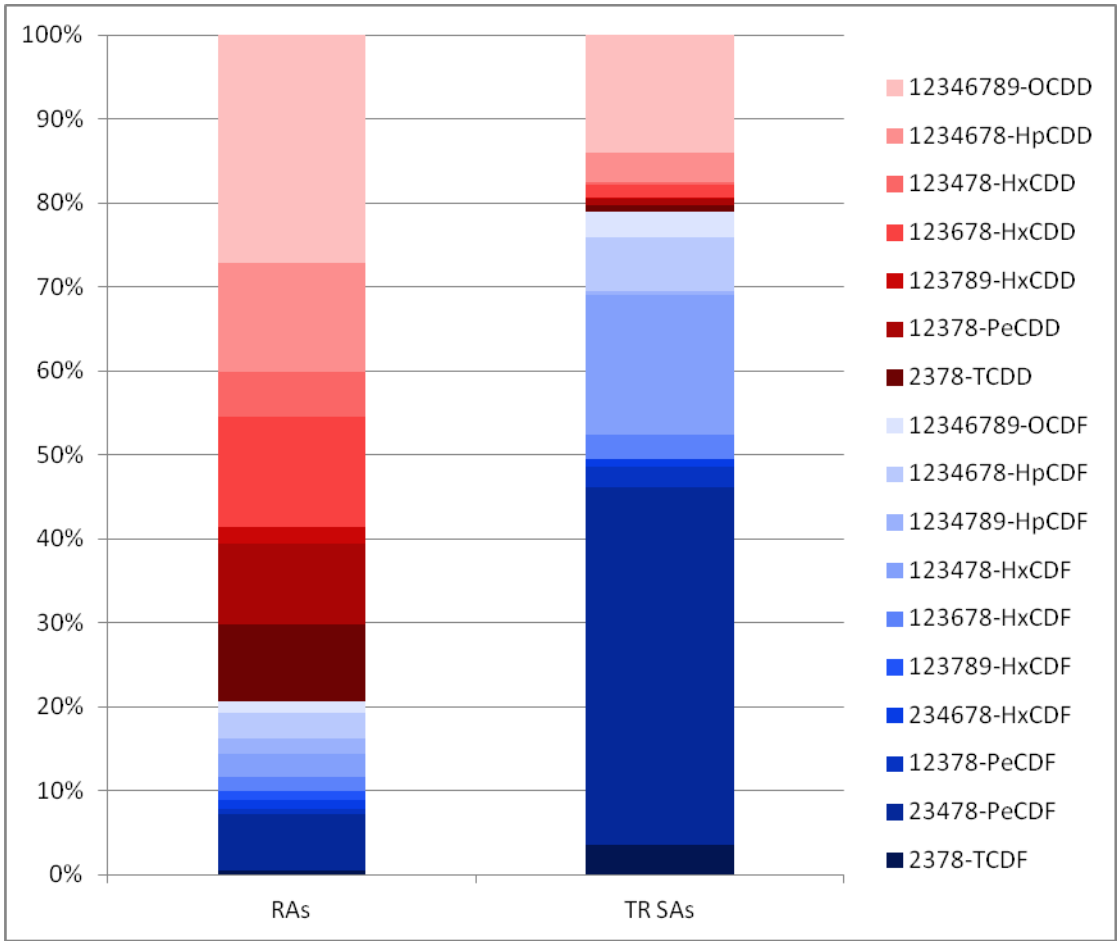
Supplemental figure 2. Mean concentrations of $\sum$PCDD/DF $TEQs_{WHO\text{-}Avian}$ in American robin nestlings collected during 2005-2008 from the river floodplains near Midland, Michigan, USA. Error bars indicate the 95% upper confidence level; Reference areas (R-1 and R-2); Tittabawassee River study areas (T-3 to T-6); and Saginaw River study areas (S-7 and S-9). Samples sizes are indicated in parentheses below the sample sites.



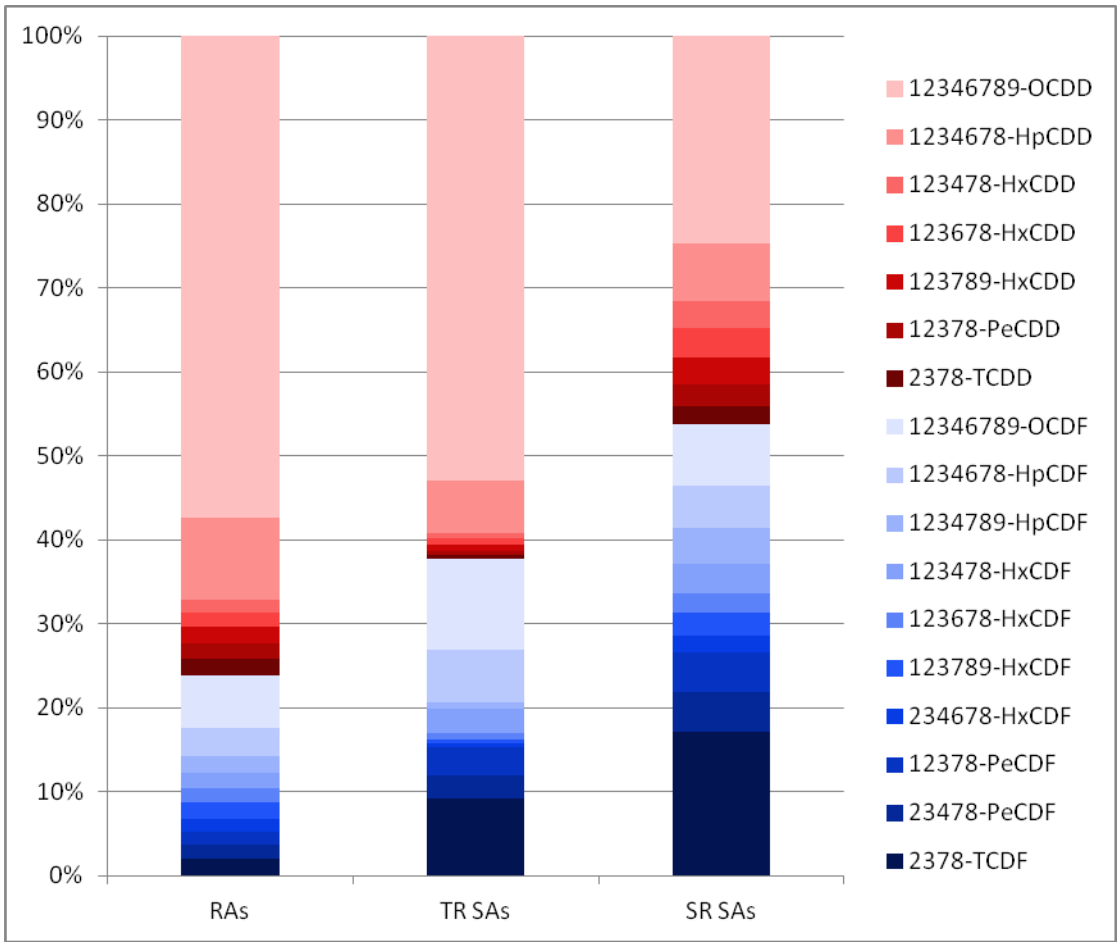
Supplemental figure 3. Mean congener percent contributions in nestling American robins collected during 2005-2008 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA. R-1 to R-2 includes reference areas, T-2 to T-6 includes Tittabawassee River study areas and S-7 and S-9 includes Saginaw River study areas. Congeners include octachlorodibenzo-*p*-dioxin (OCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), tetrachlorodibenzo-*p*-dioxin (TCDD), octachlorodibenzofuran (OCDF), heptachlorodibenzofuran (HpCDF), hexachlorodibenzofuran (HxCDF), pentachlorodibenzofuran (PeCDF) and tetrachlorodibenzofuran (TCDF).



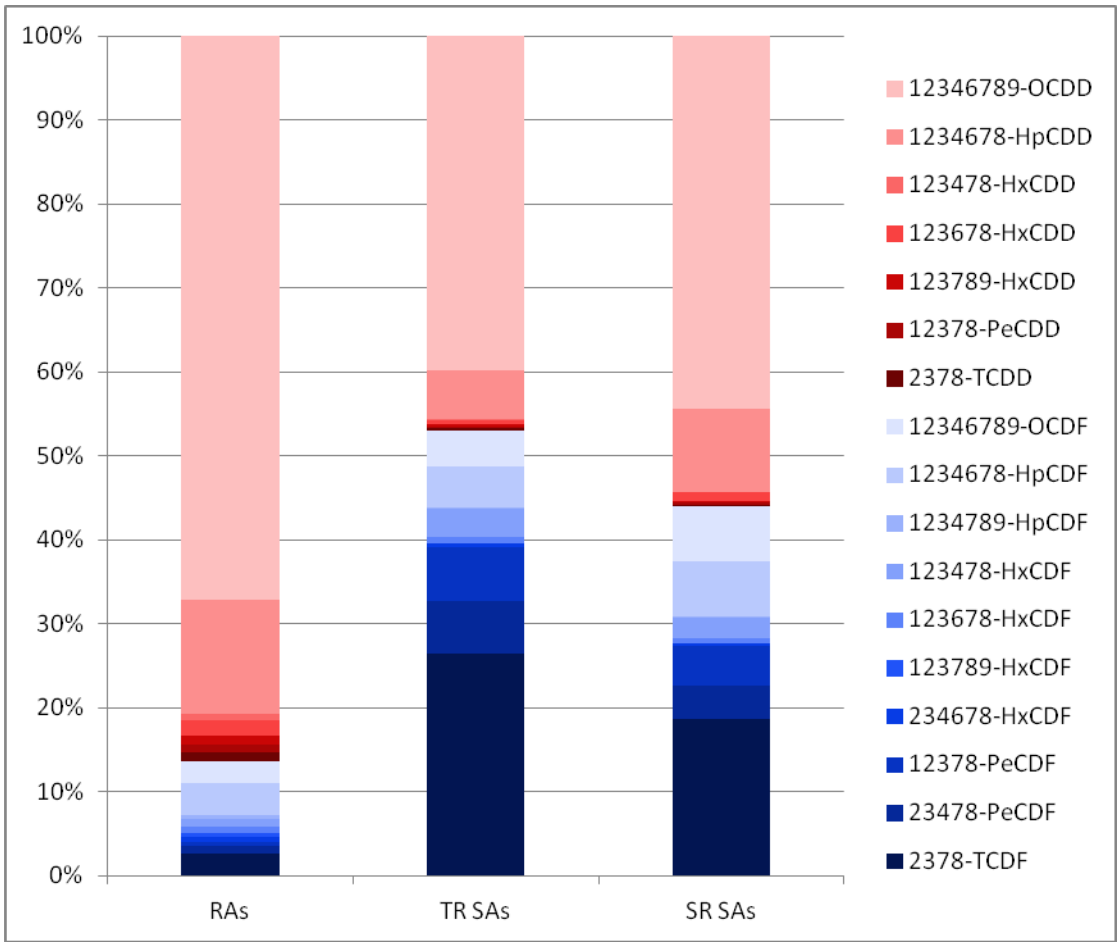
Supplemental figure 4. Mean concentrations of $\sum$PCDD/DF $TEQs_{WHO\text{-}Avian}$ in American robin adults collected during 2005-2008 from the river floodplains near Midland, Michigan, USA. Error bars indicate the 95% upper confidence level; Reference areas (R-1 and R-2); and Tittabawassee River study areas (T-3 to T-6). Samples sizes are indicated in parentheses below the sample sites.



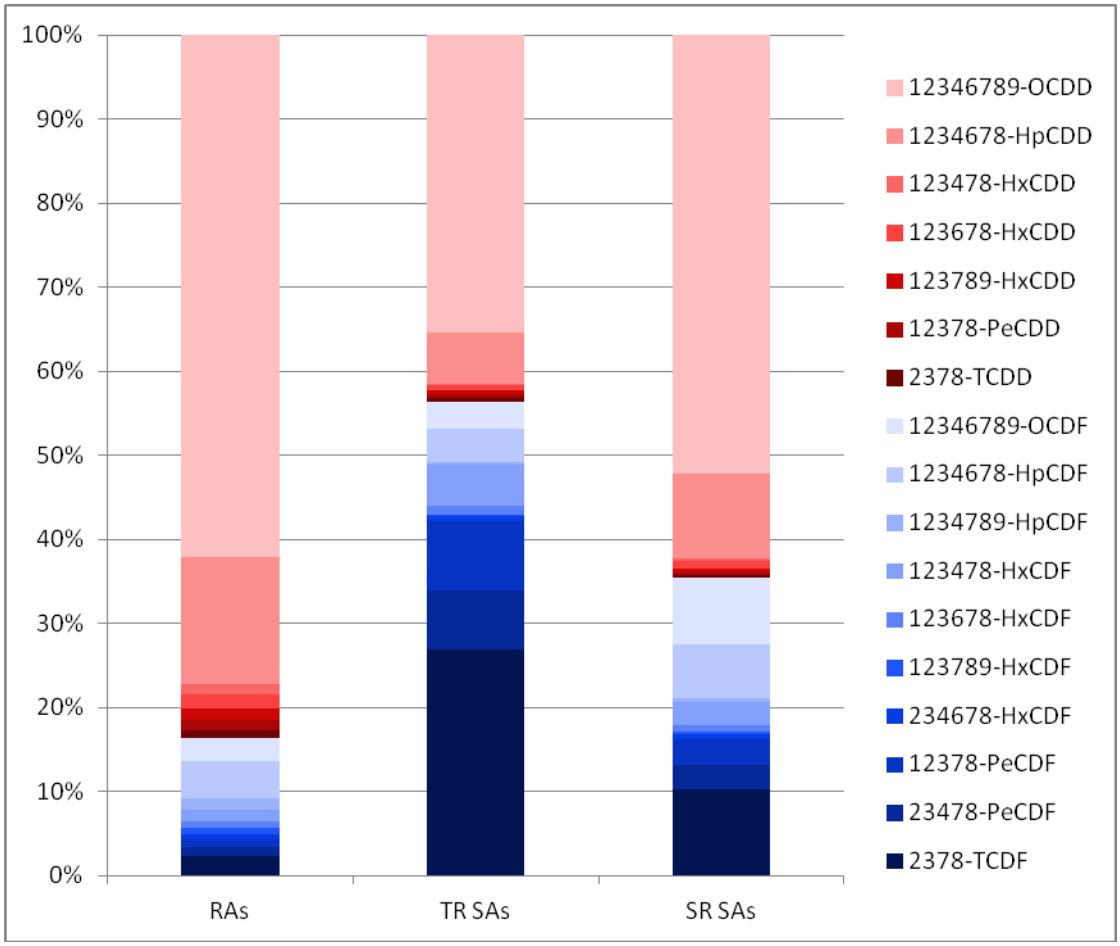
Supplemental figure 5. Mean congener percent contributions in adult American robins collected during 2007 from the Chippewa and Tittabawassee river floodplains, Midland, Michigan, USA. R-1 to R-2 includes reference areas and T-3 to T-6 includes Tittabawassee River study areas. Congeners include octachlorodibenzo-*p*-dioxin (OCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), tetrachlorodibenzo-*p*-dioxin (TCDD), octachlorodibenzofuran (OCDF), heptachlorodibenzofuran (HpCDF), hexachlorodibenzofuran (HxCDF), pentachlorodibenzofuran (PeCDF) and tetrachlorodibenzofuran (TCDF).



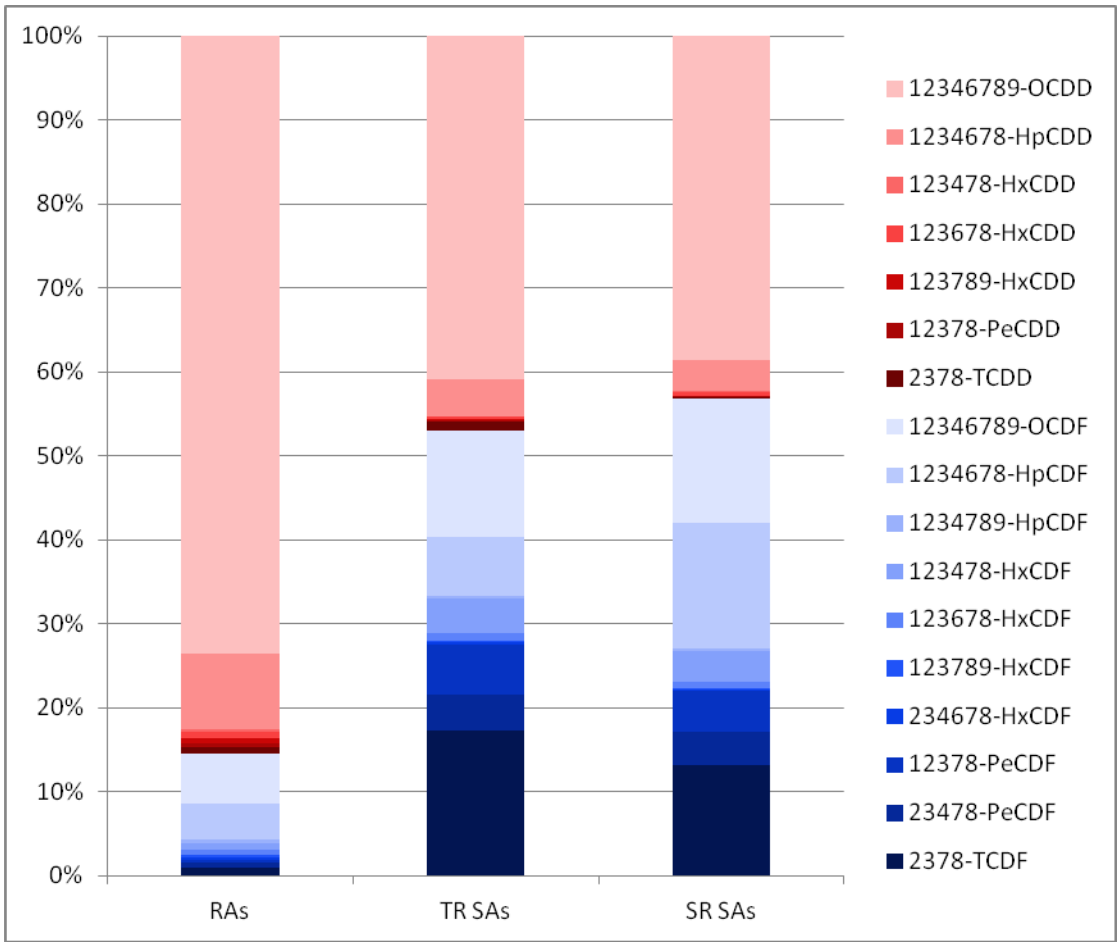
Supplemental figure 6. Mean congener percent contributions in terrestrial plants collected during 2003-2006 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA. R-1 to R-2 includes reference areas, T-3 to T-6 includes Tittabawassee River study areas and S-7 and S-9 includes Saginaw River study areas. Congeners include octachlorodibenzo-*p*-dioxin (OCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), tetrachlorodibenzo-*p*-dioxin (TCDD), octachlorodibenzofuran (OCDF), heptachlorodibenzofuran (HpCDF), hexachlorodibenzofuran (HxCDF), pentachlorodibenzofuran (PeCDF) and tetrachlorodibenzofuran (TCDF).



Supplemental figure 7. Mean congener percent contributions in terrestrial Coleoptera collected during 2003-2006 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA. R-1 to R-2 includes reference areas, T-3 to T-6 includes Tittabawassee River study areas and S-7 and S-9 includes Saginaw River study areas. Congeners include octachlorodibenzo-*p*-dioxin (OCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), tetrachlorodibenzo-*p*-dioxin (TCDD), octachlorodibenzofuran (OCDF), heptachlorodibenzofuran (HpCDF), hexachlorodibenzofuran (HxCDF), pentachlorodibenzofuran (PeCDF) and tetrachlorodibenzofuran (TCDF).



Supplemental figure 8. Mean congener percent contributions in terrestrial Lepidoptera collected during 2003-2006 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA. R-1 to R-2 includes reference areas, T-3 to T-6 includes Tittabawassee River study areas and S-7 and S-9 includes Saginaw River study areas. Congeners include octachlorodibenzo-*p*-dioxin (OCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), tetrachlorodibenzo-*p*-dioxin (TCDD), octachlorodibenzofuran (OCDF), heptachlorodibenzofuran (HpCDF), hexachlorodibenzofuran (HxCDF), pentachlorodibenzofuran (PeCDF) and tetrachlorodibenzofuran (TCDF).



Supplemental figure 9. Mean congener percent contributions in depurated terrestrial Oligochaeta collected during 2003-2006 from the Chippewa, Tittabawassee and Saginaw river floodplains, Midland, Michigan, USA. R-1 to R-2 includes reference areas, T-3 to T-6 includes Tittabawassee River study areas and S-7 and S-9 includes Saginaw River study areas. Congeners include octachlorodibenzo-*p*-dioxin (OCDD), heptachlorodibenzo-*p*-dioxin (HpCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), pentachlorodibenzo-*p*-dioxin (PeCDD), tetrachlorodibenzo-*p*-dioxin (TCDD), octachlorodibenzofuran (OCDF), heptachlorodibenzofuran (HpCDF), hexachlorodibenzofuran (HxCDF), pentachlorodibenzofuran (PeCDF) and tetrachlorodibenzofuran (TCDF).

Supplemental tables

Supplemental Table 1. Concentrations of co-contaminants in American robin tissues collected during 2005 to 2008 from the river floodplains near Midland, Michigan, USA.

	R-1 and R-2			T-3 to T-6			S-7 and S-9[a]		
	n	Mean[b]	Range	*n*	Mean	Range	*n*	Mean	Range
Egg									
ΣDL-TEQ$_{PCB\text{-}WHO\text{-}Avian}$	5	0.34^{A}	0.12-0.49	23	0.81^{AB}	0.19-3.3	11	2.7^{B}	0.28-11
ΣDL-PCB	5	8.1×10^{2}	4.4×10^{2}-1.4×10^{3}	23	2.5×10^{3}	4.4×10^{2}-1.8×10^{4}	11	6.2×10^{3}	6.2×10^{2}-2.7×10^{4}
ΣDDX	5	91^{AB}	38-2.3×10^{2}	23	1.6×10^{2A}	30-5.8×10^{2}	11	65^{B}	21-1.9×10^{2}
Nestling									
ΣDL-TEQ$_{PCB\text{-}WHO\text{-}Avian}$	1	0.13	n/a	5	0.53	0.18-1.0	5	1.6	0.21-4.2
ΣDL-PCB	1	2.4×10^{2}	n/a	5	1.3×10^{3}	2.4×10^{2}-3.5×10^{3}	5	4.5×10^{3}	3.2×10^{2}-1.4×10^{4}
ΣDDX	1	9.5	n/a	5	95	37-1.8×10^{2}	5	21	7.5-49

[a] S-7 and S-9 were monitored during 2006-2008

[b] Means with differing uppercase letters were statistically significantly different ($p < 0.05$)

Units are µg/kg for ΣDDX and ng/kg for ΣDL-PCB and ΣDL-TEQ$_{PCB\text{-}WHO\text{-}Avian}$