AN ENVIRONMENTAL RISK ASSESSMENT OF SEVERAL PASSERINE BIRD SPECIES EXPOSED TO ELEVATED CONCENTRATIONS OF POLYCHLORINATED DIBENZOFURANS WHILE BREEDING IN THE RIVER FLOODPLAINS DOWNSTREAM OF MIDLAND, MICHIGAN, USA

By

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ABSTRACT

AN ENVIRONMENTAL RISK ASSESSMENT OF SEVERAL PASSERINE BIRDS EXPOSED TO ELEVATED CONCENTRATIONS OF POLYCHLORINATED DIBENZOFURANS WHILE BREEDING IN THE RIVER FLOODPLAINS DOWNSTREAM OF MIDLAND, MICHIGAN, USA

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Concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) in floodplain soils and sediments are significantly greater downstream of Midland, Michigan (USA) compared to upstream areas. Moreover floodplain soils downstream of Midland have PCDD/DF concentrations 6- to 10-fold greater than those in proximal sediments and are some of the greatest on record. The majority of the contaminant mixture is composed of 2,3,7,8-tetrachlorodibenzofuran and 2,3,4,7,8-pentachlorodibenzofuran, which are likely present in the environment from the historical production, storage, and disposal of industrial organic chemicals and by-products prior to the establishment of modern waste management protocols. The lipophilic nature and slow degradation rates of the PCDD/DFs, combined with the annual inundation of the floodplain, has led to elevated concentrations of PCDD/DFs throughout the basin. In response to concerns regarding the ecological impact of these contaminants a site-specific multiple lines of evidence study was executed including dietary- and tissue-based exposures assessments and population productivity measurement. Two terrestrial [house wrens (*Troglodytes aedon*) and eastern bluebirds (*Sialia sialis*)] and one aquatic [tree swallows (*Tachycineta bicolor*)] food web-based passerine species were monitored both upstream and downstream of the putative source in order to elucidate the potential for contaminant driven adverse population-level effects. Additionally,

measured exposures were compared to toxicity reference values (TRVs), and reproductive parameters were compared to literature values. Sixty-nine, 144, and 66 nest boxes were monitored daily at two reference areas (RAs), four Tittabawassee River study areas (SAs), and two Saginaw River SAs, respectively, during the breeding seasons of 2005 through 2007. Concentrations of ΣPCDD/DF 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in both eggs and nestlings of the species studied at downstream SAs were 3- to 246-fold greater than RAs, with the exception that tree swallow eggs had similar concentrations among locations. Site-specific average $TEQ_{WHO\text{-}Avian}$ daily dose for house wrens, tree swallows, and eastern bluebirds at downstream Tittabawassee River SAs was at least 45-, 41-, and 70-fold greater than RAs, while Saginaw River SAs were intermediate. Overall reproductive parameters for the three passerine species studied were similar or greater at downstream SAs compared to upstream RAs. Of all initiated clutches 66% (n=427), 73% (n=245), and 64% (n=122) successfully fledged at least one nestling for house wrens, tree swallows, and eastern bluebirds, respectively. Dietary exposure for adult house wrens, tree swallows, and eastern bluebirds were greater than the selected TRVs however the other lines of evidence such as egg-based exposure assessments, productivity, and measures of individual health did not indicate an at risk population. The most probable cause of the apparent dichotomy among the dietary-based exposure assessment and the other lines of evidence was that the dietary-based TRVs selected are likely conservative based on the dose delivery methodology. Ongoing band return rate data will conclude in 2010 and provide additional data on species demographics including information on post-fledging survival and population recruitment.

To my families — for their unwavering support, abundant patience, and complete understanding

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Thanks to everyone who has taken part in this journey!!!

Tim

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KEY TO ABBREVIATIONS

ADD_{pot} – potential average daily dose

ARNT – AhR nuclear translocator

AhR – aryl hydrocarbon receptor

AHY – after hatch year

ASY – after second year

BW – body weight

DDT – dichloro-diphenyl-trichloroethane

DNA – deoxyribonucleic acid

DRE – dioxin-responsive element

C – concentrations

ºC – degrees centigrade

CI – confidence interval

CNC – Chippewa Nature Center

COC – chemicals of concern

d – day

DDXs – dichlorodiphenyltrichloroethane and related metabolites

DEQ – Department of Environmental Quality

dw – dry weight

EB – eastern bluebird (*Sialia sialis*)

ERA – ecological risk assessment

g – gram

h – hour

HpCB – heptachlorinated biphenyl

HpCDD – heptachlorodibenzo-*p*-dioxin

HpCDF – heptachlorodibenzofuran

HQ – hazard quotient

HW – house wren (*Troglodytes aedon*)

HxCB – hexachlorinated biphenyl

HxCDD – hexachlorodibenzo-*p*-dioxin

HxCDF – hexachlorodibenzofuran

IACUC – Michigan State University's Institutional Animal Care and Use Committee

IR – intake rate

kg – kilogram

LC50 – lethal concentration for 50% of dosed

ln – natural log

LOAEL(C) – lowest observed adverse effect level (concentration)

m – meter

MDEQ – Michigan Department of Environmental Quality

MI – Michigan

MSU-ATL – Michigan State University-Aquatic Toxicology Laboratory

ng – nanogram

NOAEL(C) – no observed adverse effect level (concentration)

OCDD – octachlorodibenzo-*p*-dioxin

OCDF – octachlorodibenzofuran

PCB – polychlorinated biphenyls

PCDD – polychlorinated dibenzo-*p*-dioxins

PCDF – polychlorinated dibenzofurans

PeCB – pentachlorinated biphenyl

PeCDD – pentachlorodibenzo-*p*-dioxin

PeCDF – pentachlorodibenzofuran

R-1 and R-2 – specific reference areas

RA – reference area

S-7 to S-9 – specific Saginaw River study areas

SA – study area

SNWR – Shiawassee National Wildlife Refuge

SR – Saginaw River

SY – second year

T-3 to T-6 – specific Tittabawassee River study areas

TCB – tetrachlorinated biphenyl

TCDD – tetrachlorodibenzo-*p*-dioxin

TCDF – tetrachlorodibenzofuran

TEF – Toxic equivalency factor

TEQ – 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent

TR – Tittabawassee River

TRB – Tittabawassee river basin

TRV – toxicity reference value

TS – tree swallow (*Tachycineta bicolor*)

USA – United States of America

USEPA – United States Environmental Protection Agency

USFWS – United States Fish and Wildlife Service

WHO – World Health Organization

ww – wet weight

y – year

CHAPTER 1

Introduction

Timothy Brian Fredricks

Overview

Historical production of organic chemicals and on-site storage and disposal at the Dow Chemical Company Mid-Michigan Plant, prior to the establishment of modern waste management protocols, are likely sources of the polychlorinated dibenzofurans (PCDFs) and secondarily polychlorinated dibenzo-*p*-dioxins (PCDDs) in the Tittabawassee River floodplain downstream of Midland, MI, USA. The major chemicals of concern include primarily 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran which makes the site somewhat unique. Due to the persistence of these compounds, local fauna may be exposed to concentrations that could potentially affect their reproductive potential or ability to survive on-site compared to uncontaminated locations. One of the most comprehensive and impartial methods for determining if site-specific fauna are being impacted by elevated chemical concentrations is to conduct a site-specific ecological risk assessment (ERA) based on multiple lines of evidence over several years. Use of these methodologies provides information to assist in making informed decisions about the potential impact(s) of on-site exposure and aids in both the planning and evaluation of effective remedial actions.

Due to the complex nature of the ecosystem involved, the overall research focused on a range of receptor species in order to conduct a representative site-specific ERA. Representative species from different feeding guilds were selected based on their likelihood of exposure to on-site contaminants. The bioaccumulative nature of the contaminants of concern suggests that species located in the upper portions of the food web will experience the greatest exposure, thus apex predators dominated receptor selection. The American mink (*Mustela vison*) was selected as the apex mammalian predator with a primarily aquatic-based exposure pathway. The great blue heron (*Ardea herodias*) and belted kingfisher (*Ceryle alcyon*) were selected as apex avian predators from the aquatic-based exposure pathway, while the great-horned owl (*Bubo virginianus*) was selected as an apex avian terrestrial predator. Several passerine birds were selected as mid-level predators with both aquatic and terrestrial foraging strategies. Passerines are often selected as receptor species in ERAs for many reasons but mainly due to their limited foraging range and abundance as opposed to many apex predators that are more likely to be fewer in numbers and have a greater potential to forage off-site.

The range of receptors mentioned above were then assessed utilizing a multiple lines of evidence approach, which combined considerations of dietary-based and tissue-based exposures with assessments of both individual health and population level reproductive productivity in the overall assessment of risk in the Tittabawassee and Saginaw River floodplains.

The portion of the research described herein focused on contaminant exposure through the terrestrial and aquatic food webs, monitoring reproductive parameters, and assessing overall risk to passerine birds nesting in the Tittabawassee and Saginaw River floodplains by studying the aquatic foraging tree swallow (*Tachycineta bicolor*), terrestrial foraging house wren (*Troglodytes aedon*), and the primarily terrestrial and partially aquatic foraging eastern bluebird (*Sialia sialis*). In this chapter I provide: (1) a site description of the study area, (2) historical information including the production, storage and disposal of the contaminants of concern, (3) chemical descriptions for major and co-contaminants potentially found on-site, (4) a review of toxicology for the chemical of concern, (5) descriptions of the individual study species and discussion of their selection and relevance as receptor species, (6) a statement of the overall research objectives, and (7) notation of all pertinent permits, approvals, and sources of funding .

Site Description

The upstream boundary of the study area (SA) is defined by the Dow Chemical Company's Michigan operations Midland facility which began operations in 1897 and has operated continuously until the present day. The facility is currently located on approximately 1,900 acres in and around Midland, Michigan. Initial operations focused on mining brine and extracting bromine and chlorine to produce brominated and chlorinated compounds. Production of organic chemicals began in 1908 with the production of phenol and chlorobenzene and peaked in the 1980s. The use of carbon electrodes for production of chlorine-based products was common until the mid-1980s and has resulted in what was termed the "chloralkali pattern" of contaminants in the graphite sludge waste by-products (Rappe et al. 1991). Over the years, the Dow Chemical Company has produced more than 1,000 different inorganic and organic chemicals that have been used in numerous household, agricultural and industrial products. Initial waste management practices were consistent with the standards of the time, with most waste managed on site, including locations along the Tittabawassee River. Practices have evolved with the changing production and regulatory environment to include tertiary treatment with a final effluent filter and holding ponds combined with a revetment groundwater interception system that prevents groundwater contaminants from migrating to the river. Currently, effluent is strictly regulated under Clean Water Act National Pollutant Discharge and Elimination System permit (#MI0000868).

However production and discharge practices have been updated, previous research has documented elevated concentrations of PCDDs and PCDFs (PCDD/DFs), polychlorinated biphenyls (PCBs), and dichlorodiphenyltrichloroethane (DDT) and related metabolites (DDXs) in the floodplain soils and river sediments along the Tittabawassee and surrounding rivers. To focus the efforts of the site-specific Tittabawassee River ERA, chemicals of concern (COCs) were identified through the evaluation of background and local conditions both upstream and downstream of Midland, Michigan. The primary COCs identified for inclusion in the present investigation include PCDD/DFs. Concentrations of PCDD/DFs in downstream sediments and flood plain soils were 10- to 20-fold greater than those found upstream of Midland, Michigan, which correspond to sum PCDD/DF (ΣPCDD/DF) concentrations in soils and sediments ranging from 1.0×10^2 to 5.4×10^4 ng/kg, dry weight (dw) (Hilscherova et al. 2003). Contaminated rivers from industrialized areas in the eastern United States have ΣPCDD/DF concentrations that range 1.6×10^2 to 2.4×10^4 ng/kg dw with the maximum recorded concentration of ΣPCDD/DFs of 8.2×10^5 ng/kg dw (Wenning et al. 1992; Eitzer 1993; Beliveau et al. 2003). The congener profile at these sites was dominated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the Woonasquatucket River and 1,2,3,4,6,7,8,9-octachlorodizenzo-*p*-dioxin (OCDD), 1,2,3,4,6,7,8,9-octachlorodibenzofuran, 1,2,3,4,6,7,8-heptachlorodibenzofuran, and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin in the Passaic River. ΣPCDD/DF concentrations in sediment collected from the Rhine River in Western Europe ranged from 2.0×10^2 to 1.8×10^4 ng/kg dw, composed of primarily PCDFs and OCDD (Evers et al. 1988). Sediments collected from Tokyo Bay, Japan and Masan Bay, Korea contained

ΣPCDD/DF concentrations that ranged from $3.2x10^3$ to $2.0x10^5$ ng/kg dw (Sakurai et al. 2000) and $1.2x10^2$ to $1.7x10^4$ ng/kg dw (Kannan et al. 2007), respectively. OCDD and more chlorinated PCDF congeners were dominant in the sediment samples from these locations. Concentrations of PCDD/DFs in sediments and floodplain soils collected near Midland, Michigan, along the Tittabawassee and associated rivers are similar to or greater than other global industrialized areas and well above the nominal EPA recommended cleanup level of 1.0 to 20 ng/kg dw.

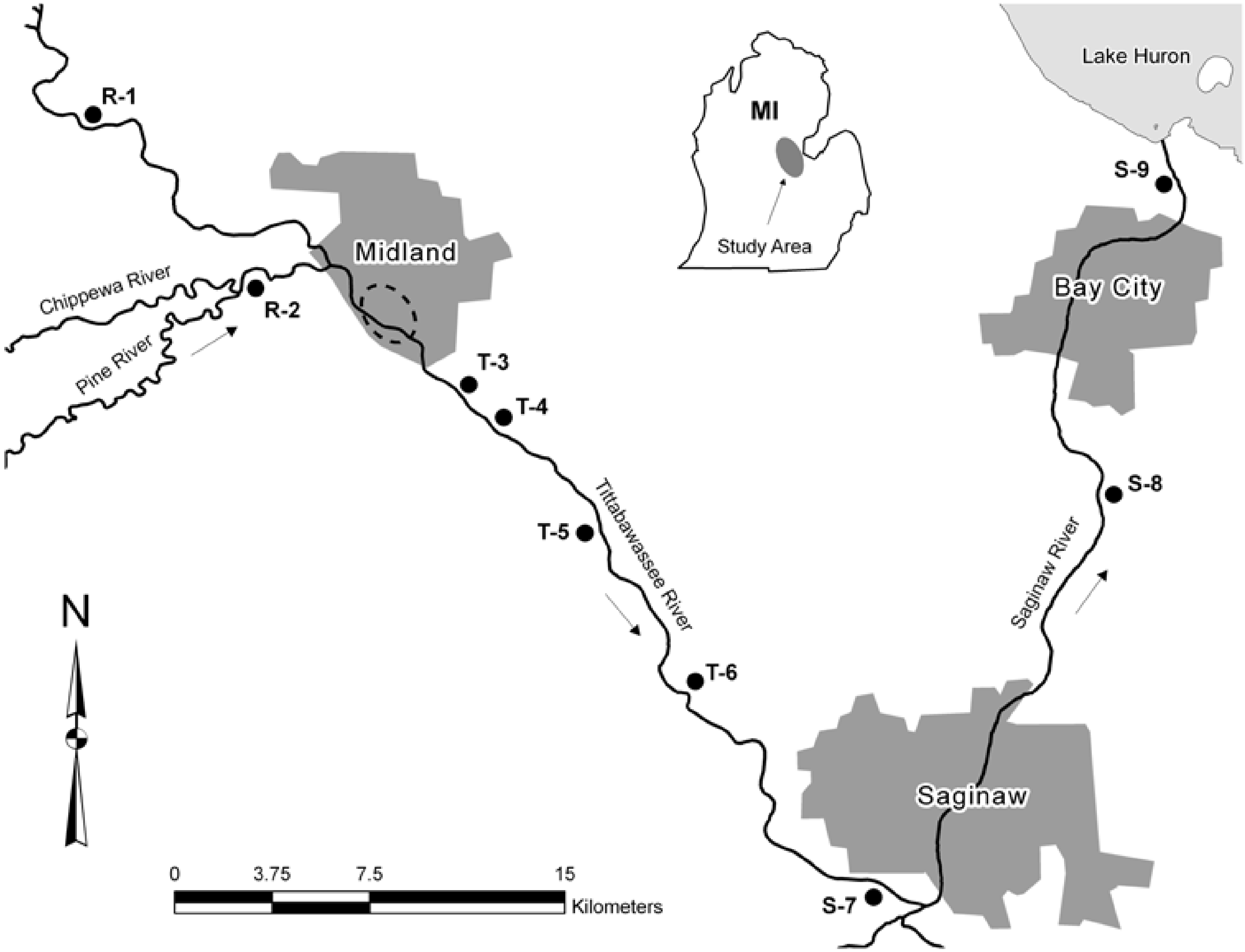
PCBs and DDXs were identified as secondary COCs due to previous research on the Saginaw River and from screening level assessments on the Tittabawassee River and associated tributaries. Concentrations of the sum of the PCB congeners (ΣPCB) in the Saginaw River and Saginaw Bay downstream of the Tittabawassee River have been measured in relatively great concentrations (Ludwig et al. 1993; Summer et al. 1996; Giesy et al. 1997; Froese et al. 1998). More recently, soil and sediment collected from the Saginaw River and Bay, which are downstream of the Tittabawassee River, were determined to have elevated concentrations of primarily PCBs (greater than 1,000 μg/kg dw) but also many PCDD/DFs were detected (Kannan et al. 2008). However, ΣPCB concentrations were not detected or were less than 150 μg ΣPCBs /kg dw in sediments from the Tittabawassee River upstream of the junction with the Saginaw River (Michigan DEQ 2002; Hilscherova et al. 2003).

From approximately 1936 until the early 1970s, Michigan Chemical/Velsicol Corporation produced various brominated and chlorinated chemicals which were released into the Pine River, an upstream tributary of the Chippewa River and both rivers are upstream tributaries of the Tittabawassee River. The presence of relatively great concentrations of DDT and DDXs in Pine River sediments (Michigan DEQ 2000) may pose risks to wildlife in the Tittabawassee River floodplain and therefore were evaluated in passerine egg samples. Despite the addition of several secondary COCs, PCDD/DFs in the Tittabawassee and Saginaw River floodplains remain the primary concern due to their elevated concentrations in respect to the predicted toxic thresholds.

The Tittabawassee River study area includes sediments and floodplain soils for approximately 37 kilometers of the Tittabawassee River downstream of Midland, MI (Figure 1.1). Specifically, the Tittabawassee River SA extended from the upstream boundary of the Dow Chemical Company (low-head dam present) to the confluence of the Tittabawassee and Shiawassee rivers downstream of Greenpoint Island, as defined in the Hazardous Waste Management Facility Operating License which was issued by Michigan Department of Environmental Quality. The Saginaw River SA was defined in 2006 and starts near the terminus of the Tittabawassee River SA and extends down the Saginaw River to the mouth of Saginaw Bay and Lake Huron. Reference areas (RAs) include the Tittabawassee River north of Midland to the Sanford dam, and the Chippewa and Pine rivers which are tributaries of the Tittabawassee River north and west of the downstream study areas.

Two reference areas (R-1 and R-2) were selected to serve as background or control sites based on location, and access was granted to place and monitor nest boxes. R-1 (N43 39; W84 22) was located approximately 15 km upstream of the downstream study areas on two privately owned properties along the Tittabawassee River near the Sanford dam. These properties were approximately 1.5 km apart and contain a mix of open-managed (mowed once a year) prairie habitat, unmanaged prairie with some

Figure 1.1. 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 and S-9) were monitored from 2005–2007. Direction of river flow is designated by arrows; suspected source of contamination is enclosed the dashed oval.


R-1
R-2
Chippewa River
Pine River
Midland
T-3
T-4
T-5
T-6
Tittabawassee River
MI
Study Area
Lake Huron
S-9
Bay City
S-8
Saginaw River
Saginaw
S-7
N
0
3.75
7.5
15
Kilometers

progressively invasive autumn olive (*Elaeagnus umbellate*), and forested habitat. R-2 (N43 36; W84 17) was located approximately 5 km upstream of the Tittabawassee River SAs on Chippewa Nature Center (CNC) owned property. The CNC is a private nature center that originated in the late 1960s as a place to help people enjoy and learn about the environment. The property was adjacent to both the Pine and Chippewa rivers and includes a mix of both prairie and forested habitats.

Four Tittabawassee River SAs (T-3 to T-6) were selected downstream of the RAs and located within the Tittabawassee River floodplain. The Tittabawassee River SA locations were selected based on the same conditions used to establish the RAs. T-3 (N43 33; W84 10) and T-4 (N43 32; W84 09) were located approximately 5.0 and 6.5 km downstream of Midland, respectively, on property of the Dow Chemical Company, on the east side of the Tittabawassee River. Both properties contained a mix of floodplain forest and farm fields planted annually in corn (2004), winter wheat (2005), corn (2006), and soybeans (2007). Farming practices involved minimal soil disruption, no pesticide application, and minimal herbicide applications usually done prior to harvest and after the avian breeding season. Placement of nest boxes at this location was not constrained due to farming practices (i.e. some boxes were placed in farm fields to accommodate all species' habitat requirements). T-5 (N43 30; W84 07) was located approximately 12 km downstream of Midland on the west side of the Tittabawassee River and included both Freeland Township property and several privately owned properties. This site contained primarily floodplain forest with several large agricultural fields (primarily planted in corn, soybeans, or sugar beets) and a small patch of prairie habitat. Box placement at this location was largely constrained to the forest edges due to farming practices with only a few boxes placed in the available prairie habitat. T-6 (N43 27; W84 04) was located approximately 20 km downstream of Midland on the east side of the Tittabawassee River and included both Saginaw Township property and a privately owned parcel. This site contained primarily floodplain forest and prairie-buffered agricultural land (primarily soybean and corn), and some city park (mowed landscape). It was possible to place nest boxes in the prairie habitat and along forest edges to accommodate all species' habitat requirements.

Three Saginaw River study areas (S-7 to S-9) were established in 2005 based on requests for information further downstream. S-7 (N43 23; W84 00) was located approximately 32 km downstream of Midland on the south side of the Tittabawassee River just prior to the confluence with the Shiawassee River on Shiawassee National Wildlife Refuge property. This site contained both floodplain forest and open prairie habitats that are gradually being encroached upon by some secondary successional plant species. S-8 (N43 30; W83 52) was located approximately 43 km downstream of Midland on the south side of the Saginaw River adjacent to Veteran's Memorial Park. S-8 was only used for sediment and dietary food web sampling, and no studies of birds were conducted at this location. S-9 (N43 38; W83 51) was located approximately 70 km downstream of Midland on the west side of the Saginaw River near its entry into Saginaw Bay. This site was dominated by inlets from the Saginaw River lined with phragmites (*Phragmites australis*) combined with managed (mowed) grass areas with little floodplain forest habitat.

Contaminant Descriptions

PCDD/DFs and PCBs are chemically classified as a group of halogenated aromatic hydrocarbons. The 75 possible PCDD congeners consist of two benzene rings joined by two oxygen atom bridges with varying numbers and positions of chlorine atom substitutions. The 135 PCDFs are structurally very similar, and consist of two benzene rings fused to one central furan ring with varying numbers and positions of chlorine atom substitutions. PCB congeners consist of two benzene rings connected by a single C–C bond with varying numbers and positions of chlorine atom substitutions. Of the 209 possible PCB congeners, 12 are either mono-*ortho*- or non-*ortho*-substituted and are structurally and conformationally similar to PCDD/DFs. Those PCDD/DF congeners with chlorine atoms substituted for hydrogen atoms at the 2,3,7,8-positions exhibit the greatest toxicity, and are thus of the greatest interest (7 PCDD and 10 PCDF congeners). TCDD is thought to be the most potent congener and thus most widely studied of these compounds. The structures of TCDD and related compounds are shown in Figure 1.2. This suite of 17 PCDD/DF and 12 PCB congeners will be referred to as dioxin-like compounds throughout the remainder of this document.

Despite their structural relatedness, each PCDD/DF and PCB congener has unique physical-chemical properties that affect its fate, transport, bioavailability, and toxicity (Eisler 1986; Eisler and Belisle 1996). To help investigate and quantify the complex mixtures of compounds commonly present in the environment, toxic equivalency factors (TEFs) specific to mammals, fish, and birds were established at an expert meeting organized by the World Health Organization (WHO) (van den Berg et al. 1998). The TEF approach is based on a number of assumptions, including dioxin-like compounds

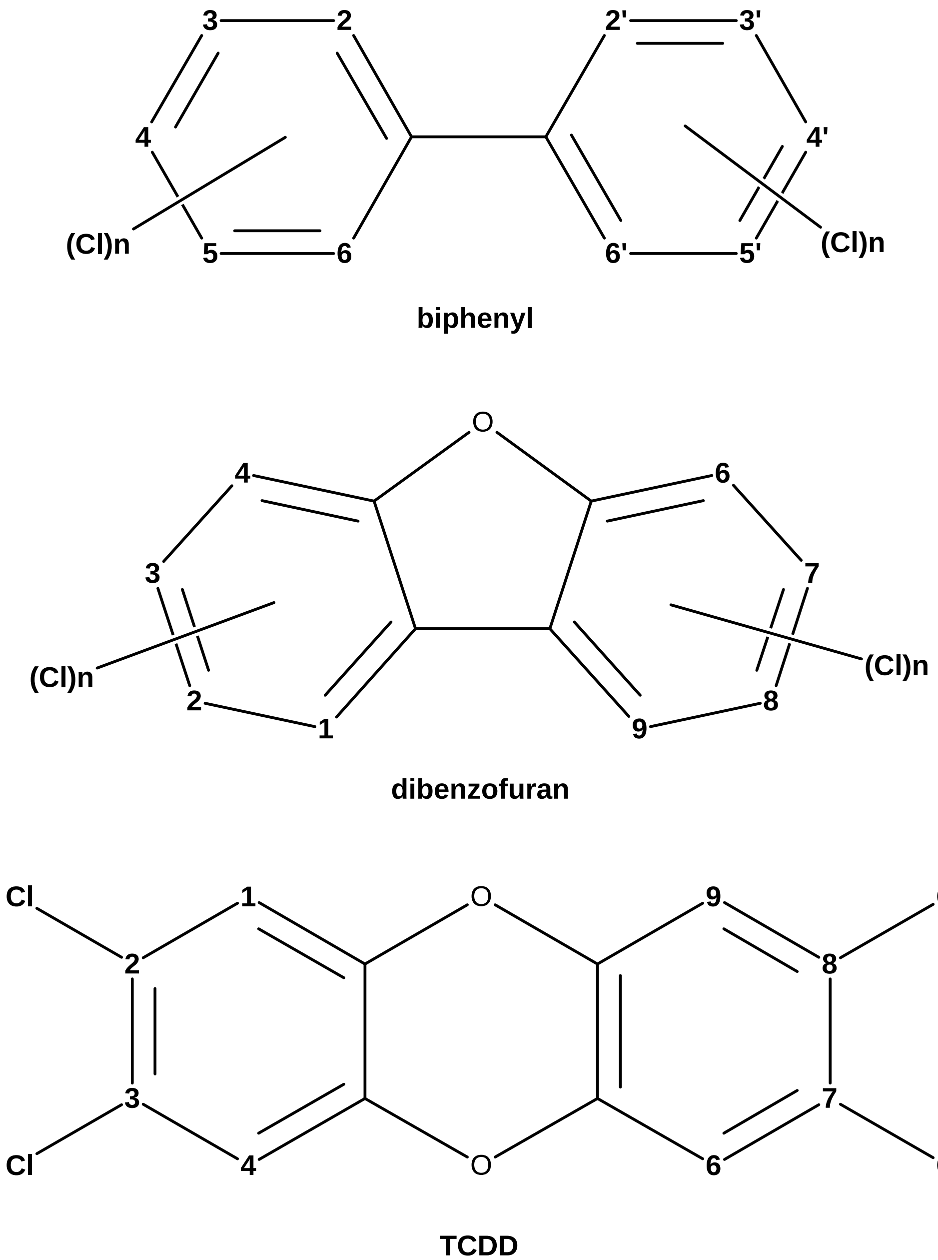


Figure 1.2. Chemical structure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and general dibenzofuran and biphenyl rings with potential halogenation sites numbered.

exhibit their toxicity through the same mechanism of action and the toxicity of a mixture of these compounds, once scaled to TCDD, is predicted to be additive. Specific TEF values were developed to express the relative scaled toxicity of the 17 2,3,7,8-substituted PCDD/DF and 12 mono-*ortho*- or non-*ortho*-substituted PCB congeners to TCDD (Table 1.1). Concentrations of each congener in a mixture can be converted to TCDD equivalents (TEQs) by multiplying the concentration of the individual congener with its specific TEF. The user can then sum the TEQs to arrive at a predicted toxic potential for the mixture in terms of a single congener, TCDD. It must be noted that the TEF concept cannot be used for calculation of bioaccumulation factors since specific congeners bioaccumulate to different degrees across trophic levels (van den Berg et al. 1998; Russell et al. 1999; Stephens et al. 1995; Wan et al. 2005).

Mixtures of halogenated compounds are ubiquitous in the environment largely as a result of industrialization. Historically, many of these compounds were products of natural combustion and geological processes that resulted in trace quantities being produced. However, essentially all substantial quantities of PCDD/DFs in the environment were undesired and unidentified products of various anthropogenic activities (Czuczwa et al. 1984; Schecter et al. 1988). Sources of PCDD/DFs in the environment are varied and include byproducts of various types of incineration and combustion (Czuczwa and Hites 1984; Rappe and Kjeller 1987), through the use of elemental chlorine in the bleaching process at pulp and paper mills (Swanson et al. 1988; Bright et al. 1999), and through the production of chlorine and chlorinated compounds (Hutzinger et al. 1985; Rappe et al. 1991; Svensson et al. 1993). PCDD/DFs and related hydrocarbons are persistent and lipophilic (Mandal 2005), and have a great potential to

Table 1.1. Avian toxic equivalency factors (TEFs) from the World Health Organization (WHO) for the 17 2,3,7,8-chlorine substituted PCDD/DF congeners and 12 PCB congeners.

PCDD/DFs[a]	TEF[b]	PCBs[c]	TEF[b]
Polychlorinated dibenzo-p-dioxins		*Non-ortho-substituted*	
2,3,7,8-TCDD	1	3,3',4,4'-TCB (77)	0.05
1,2,3,7,8-PeCDD	1	3,4,4',5-TCB (81)	0.1
1,2,3,4,7,8-HxCDD	0.05	3,3,',4,4',5-PeCB (126)	0.1
1,2,3,6,7,8-HxCDD	0.01	3,3',4,4',5,5'-HxCB (169)	0.001
1,2,3,7,8,9-HxCDD	0.1		
1,2,3,4,6,7,8-HpCDD	<0.001		
1,2,3,4,6,7,8,9-OCDD	0.0001		
Polychlorinated dibenzofurans		*Mono-ortho-substituted*	
2,3,7,8-TCDF	1	2,3,3',4,4'-PeCB (105)	0.0001
1,2,3,7,8-PeCDF	0.1	2,3,4,4',5-PeCB (114)	0.0001
2,3,4,7,8-PeCDF	1	2,3',4,4',5-PeCB (118)	0.00001
1,2,3,4,7,8-HxCDF	0.1	2',3,4,4',5-PeCB (123)	0.00001
1,2,3,6,7,8-HxCDF	0.1	2,3,3',4,4',5-HxCB (156)	0.0001
1,2,3,7,8,9-HxCDF	0.1	2,3,3',4,4',5'-HxCB(157)	0.0001
2,3,4,6,7,8-HxCDF	0.1	2,3',4,4',5,5'-HxCB (167)	0.00001
1,2,3,4,6,7,8-HpCDF	0.01	2,3,3',4,4',5,5'-HpCB (189)	0.00001
1,2,3,4,7,8,9-HpCDF	0.01		
1,2,3,4,6,7,8,9-OCDF	0.0001		

[a] TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran

[b] van den Berg et al. 1998

[c] TCB = tetrachlorinated biphenyl; PeCB = pentachlorinated biphenyl; HxCB = hexachlorinated biphenyl; HpCB = heptachlorinated biphenyl

accumulate through the food web from contaminated sediments and soils (Custer et al. 1998; Russell et al. 1999; Blankenship et al. 2005; Kay et al. 2005; Smits et al. 2005; Wan et al. 2005; Maul et al. 2006), which makes improper waste storage and the disruption of contaminated sites important sources of these compounds into the broader environment. Previous research determined that the predominant congeners found downstream of Midland on the Tittabawassee River and later the Saginaw River are PCDFs and OCDD, which suggest that the PCDD/DFs originated from the production of chlorine by the graphitic electrode process (Rappe et al. 1991).

Relevant Toxicological Research

TCDD is usually considered to be one of the most toxic compounds known (Eisler 1986) and, as such, has been extensively studied. The mechanism of action for dioxin-like compounds is widely believed to be dependent on the activation of the aryl hydrocarbon receptor (AhR) that is a ligand-activated cytosolic transcription factor (Safe 1986). TCDD is known to bind with high affinity to the AhR (Poland and Knutson 1982), while other dioxin-like compounds have varying degrees of binding affinity. Activation of this receptor-mediated pathway can result in a diverse array of effects, including biochemical changes such as enzyme induction, developmental deformities, reproductive failure, hepato-toxicity, immuno-toxicity, carcinogenicity, wasting syndrome, and eventually death (Poland and Knutson 1982; van den Berg et al. 1998). The AhR resides in the cytoplasm associated with several co-chaperones (Hsp90 and XAP2), where this AhR complex binds with a ligand (e.g. dioxin-like compounds), the chaperones dissociate and the AhR translocates into the nucleus. Once inside the nucleus, AhR dimerizes with the AhR nuclear translocator (ARNT). This heterodimer complex is able to bind with specific DNA response elements, known as dioxin-responsive elements (DREs) to alter gene expression and can lead to an increase in the transcription of certain genes, such as CYP1A1 and CYP1A2 (mammalian) and CYP1A4 and CYP1A5 (avian). However, the relationship between the induction or suppression of these genes and associated toxicity of the ligands responsible for them is not completely understood (Schmidt and Bradfield 1996).

The toxicity of dioxin-like compounds, particularly TCDD, has been well established in birds. Several studies have investigated domestic chicken (*Gallus gallus*) egg hatching success after injection with TCDD and have calculated 50% lethal dose (LD50) values that range between $1.5x10^2$ and $3.0x10^2$ ng/kg, wet weight (ww) (Powell et al. 1996; Henshel et al. 1997; Blankenship et al. 2003). Ring-necked pheasant (*Phasianus colchicus*) egg survival after injection with TCDD varied by injection type. For albumin or yolk injection, the LD50 was $1.4x10^3$ and $2.2x10^3$ ng/kg ww egg, respectively (Nosek et al. 1993). Developmental abnormalities observed after *in ovo* exposure to TCDD and other dioxin-like compounds in various avian species include edemas of the head and neck, liver damage, and skeletal and beak deformities (Powell et al. 1996; Hoffman et al. 1998; Blankenship et al. 2003), however similar abnormalities were not always present (Nosek et al. 1993). Comparative egg injection studies have demonstrated large difference in species sensitivity to the toxic effects of dioxin-like compounds. Chickens have been shown to be up to 250-fold more sensitive than turkeys (*Meleagris gallopavo*), ring-necked pheasants, mallards (*Anas platyrhynchos*), goldeneyes (*Bucephala clangula*), domestic geese (*Anser anser*), herring gulls (*Larus argentatus*), and black-headed gulls

(*Larus ridibundus*) (Brunström and Reutergardh 1986; Brunström and Lund 1988; Brunström 1988).

In addition to egg injection studies, the toxic effects of dioxin-like compounds have been observed in field studies of avian species. Beginning in the 1960s, researchers noticed population declines in colonial fish-eating birds of the Great Lakes which were largely attributed to exposure to high levels of PCBs (Gilbertson et al. 1991). For instance, Ludwig *et al*. (1996) monitored the incidence of embryonic deformities and death rates in double-crested cormorants (*Phalacrocorax auritus*) and Caspian terns (*Hydroprogne caspia*) nesting in colonies in the Great Lakes exposed to primarily PCBs but correlations to other dioxin-like compounds (based on bioassays for TEQs) were also presented. Subsequently, a related study in a colony of double-crested cormorants exposed to PCBs reported decreased hatching success and increased incidence of nestling deformities in contaminated colonies compared to a reference colony (Larson et al. 1996). Exposure to a mixture of PCDD/DFs and PCBs in Foster's tern (*Sterna forsteri*) eggs resulted in impaired reproductive success (Kubiak et al. 1989). Additionally, the concentrations of TCDD in eggs appeared to be related to the severity of reproductive failure observed in colonies of herring gulls around the Great Lakes, however a casual relationship could not be established (Gilbertson 1983). The symptoms observed were consistent with those of chick-edema disease in domestic chickens exposed to dioxin-like compounds, and in wildlife was termed the Great Lakes embryo, mortality, edema, and deformities syndrome (GLEMEDS) (Gilbertson et al. 1991).

Toxicological studies focused on passerine species were originally less widespread but are becoming more common. Beyond the currently selected study species, exposure to PCBs has been documented for several passerine species including red-winged blackbirds (*Agelaius phoeniceus*; Ankley et al. 1993; Bishop et al. 1995), American robins (*Turdus migratorius*; Henning et al. 2003), European starlings (*Sturnus vulgaris*; Arenal et al. 2004; van den Steen et al. 2007), barn swallows (*Hirundo rustica*; Custer et al. 2006), and great tits (*Parus major*; van den Steen et al. 2006). The great tit is widely studied in Europe with a great deal of information known about most aspects of its life history, while the other species do not receive nearly as much research attention.

Studies monitoring the exposure and/or effects of dioxin-like compounds in tree swallows are rather common with relatively few toxicological studies investigating house wrens and eastern bluebirds. Tree swallow exposure to PCBs has been documented on several sites throughout North America including various locations in Alberta (Shaw 1983), Gilpin County, Colorado (DeWeese et al. 1985), Saginaw River, Michigan (Beaver 1992), Green Bay, Wisconsin (Ankley et at. 1993), St. Lawrence River basin, Ontario (Bishop et al. 1995), Lower Fox River, Wisconsin (Custer et al. 1998), Saginaw Bay, Michigan (Froese et al. 1998), Hudson River, New York (Secord et al. 1999; Echols et al. 2004), Thompson River, British Columbia (Harris and Elliot 2000), Mississippi River, Iowa (Custer et al. 2000), Wisconsin River, Wisconsin (Custer et al. 2002), Housatonic River, New York (Custer et al. 2003), Point Pelee National Park, Ontario (Smits et al. 2005), Kalamazoo River, Michigan (Neigh et al. 2006), and Crab Orchard National Wildlife Refuge, Illinois (Spears et al. 2008). House wren and eastern bluebird tissue- and dietary-based exposures to PCBs along the Kalamazoo River in Michigan were investigated (Neigh et al. 2006). Limited numbers of other studies on house wren

(Custer et al. 2001) and eastern bluebird (Burgess et al. 1999; Mayne et al. 2004) exposure to contaminants have been conducted.

Several studies have investigated exposure and effects of TCDD on eastern bluebirds and tree swallows. Eastern bluebird exposures to TCDD on plots treated with dioxin-contaminated sludge from a paper mill and an associated field based egg injection study were conducted in Wisconsin (Thiel et al. 1988). The study had three primary conclusions: (1) based on the egg injection study, the lowest observed adverse effect level (LOAEL) was 1.0×10^4 ng TCDD/kg ww egg, (2) based on the treated plots study, concentrations in eggs and invertebrates were 6.6×10^0 to 1.1×10^1 and 1.6×10^0 to 6.7×10^0 ng TCDD/kg ww, respectively, and (3) based on the treated plots study, there were no adverse effects on reproduction or growth. Tree swallows exposed to primarily TCDD on the Woonasquatucket River in Rhode Island had decreased hatching success compared to less exposed sites (Custer et al. 2005). At the Woonasquatucket River study site a 50% reduction in hatching success was estimated at approximately 1.7×10^3 ng/kg ww, and is one of the few field studies to document a reduced hatching success from exposure to dioxins.

When comparing the current study to previous research, it is helpful to use calculated $TEQ_{WHO\text{-}Avian}$ values since most studies report exposures based on a mixture of compounds. In doing so, it is important to keep in mind that the TEQ scheme, though helpful in summing the toxic potential of the different PCDD/DF and PCB congeners, does not account for all uncertainties, so comparisons between studies reporting TEQs must be done with caution. Not all researchers monitored or screened for possible co-contaminants present on site, may have reported different or incomplete congener lists, or even used different TEF values to arrive at their end values (Safe 1990; Ahlborg et al. 1992; Rappe 1994; Bosveld et al. 1995; Kennedy et al. 1996; van den Berg et al. 2006). These restrictions have been taken into account when interpreting results throughout this dissertation.

Selection of Receptor Species

Possibly the most essential step for an ERA to be effective is the selection of receptor species. This is especially true from the monetary and time-management perspectives due to the great amounts of both money and time necessary to complete extensive site-specific field studies. The predicted intensity of exposure to COCs is the primary selection tool for a species to serve as a receptor in a site-specific ERA (EPA 1994). The lipophilic nature and slow degradation rates of these compounds (Mandal 2005), combined with consistent inundation of the floodplain, led to the continued presence of PCDD/DFs in floodplain soils and sediments. However uptake of PCDD/DFs from contaminated soil into plant tissue is very limited (Hulster and Marschner 1993; Welsch-Pausch et al. 1995), other organisms are exposed through the incidental ingestion of contaminated particulate matter and the consumption of prey items that have intimate contact with the sediment or floodplain soil. In general, dioxin-like compounds are resistant to biological degradation, specifically congeners without two adjacent unoccupied halogenation sites, which make these dioxin-like compounds likely to bioaccumulate through the food web. Species that are at the top of the food web are generally considered the most likely to experience greater exposure to dioxin-like compounds. However, high trophic status species also often have larger foraging ranges that can include off-site locations, thus potentially limit site-specific exposures. An intermediate trophic status species with a completely site-specific foraging range can potentially have greater exposures to site-specific contaminants than higher trophic status species.

Prior to the initiation of research, species were selected based on their applicability and the predicted statistical power of data collected to test hypotheses associated with ecosystem health. Applicability was determined based on similarities in nesting characteristics, resistance to disturbance, foraging range and expected species density based on habitat availability, and use as a receptor in previous contamination research. These similarities allow for a more direct comparison of the parameters of interest including differences in stressor exposure based on divergent foraging characteristics as well as differences in species stressor sensitivity.

Species-specific sensitivities to dioxin-like compounds are another important factor to consider during the selection of a receptor species. Due to both financial and logistical limitations it is rarely feasible to study representative species present at a site, let alone all species, so only those that are sensitive to the COCs should be considered as receptors. Despite the fact that terrestrial and aquatic invertebrates are in direct contact with and ingest relatively great quantities of soils and sediments, they lack an AhR-mediated pathway, and are thus not sensitive to PCDD/DFs. However, invertebrates do accumulate relatively great quantities of dioxin-like compounds and can be a direct vector of contaminants from the soil and sediments to upper trophic species (Blankenship et al. 2005; Kay et al. 2005). In mammals, toxicity of exposure to PCDD/DFs varies significantly among species, and has been widely studied (reviews: Eisler 1986; Safe

1986; van den Berg et al. 1998; van den Berg et al. 2006). Laboratory studies have shown birds, particularly during the embryonic stage, are particularly sensitive to the effects of dioxin-like compounds (Barron et al. 1995). However, great differences in species-specific susceptibility to these toxic effects have been observed in birds, with the domestic chicken being 10- to 100- fold more sensitive to these effects than other species (Brunström 1988; Kennedy et al. 1996; Hoffman et al. 1998). The observed differences in sensitivity to dioxin-like compounds among birds may be attributable to varying concentrations of the AhR in certain tissues, differences in degradation potential of certain dioxin-like compounds, or species-specific differences in the AhR construct and associated ligand binding affinity (Hahn 1998). Recent research has investigated the molecular characterization of the AhR and potential receptor-specific binding affinity differences between avian species that exhibit different sensitivities to AhR-active compounds (Karchner et al. 2006; Head et al. 2008). Developing species-specific relative sensitivities to dioxin-like compounds will hopefully further refine the selection of appropriate receptor species so that selected species could be considered protective of other on-site species that are classified as less-sensitive but similarly exposed.

Based on the above criteria, tree swallows, house wrens, and eastern bluebirds were selected as study species for this dissertation. All are obligate cavity nesters with limited foraging range and similar site fidelity. Tree swallows are aquatic insectivores (Kuerzi 1941) primarily feeding on emergent insects (McCarty 1997; McCarty and Winkler 1999; Mengelkoch et al. 2004), and have been utilized extensively in contaminant studies. Eastern bluebirds and house wrens are both terrestrial insectivores (Beal 1915; Guinan and Sealy 1987), but have different habitat preferences and foraging strategies that could lead to different contaminant accumulation. Eastern bluebirds prefer open grassland habitats and feed by dropping on prey from an elevated perch, while house wrens primarily glean insects off foliage in brushy/forested habitats. Several studies of contaminants have used eastern bluebirds and house wrens.

Research Objectives

The overall goal of this dissertation is to better understand the site-specific risk to passerine species breeding in the Tittabawassee River floodplain downstream of Midland, Michigan, USA. To meet this goal, exposure and associated effects were monitored over several breeding seasons for house wrens, tree swallows, and eastern bluebirds breeding on-site. More specifically, study objectives included first quantifying contaminant exposure through both dietary-based and tissue-based evaluations as well as measurements of individual and population health, and then comparing site-specific exposures and health measures to literature based effects levels or to species norms for reproductive measures. Additionally, passerine data collected downstream of Midland was compared to that collected from upstream reference locations to help explain site-specific ecological responses and provide insight on causality. However, since the upstream reference areas are not in fact controls, but just appropriately selected field sites, the lack of adverse effects means that the contaminants are not causing effects, while the presence of adverse effects means something is causing effects. The multiple lines of evidence approach provides a framework that allows the researcher to converge upon a likely suspect to the cause of adverse effects (Fairbrother, 2003). Using a multiple lines of evidence approach for several passerine species will help further reduce the uncertainty which is often inherent in the risk assessment process, and determine the risk of several passerine species breeding in the Tittabawassee River floodplain.

Permits, Approvals, and Funding

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

Additionally, James Dastyck and Steven Kahl of the US Fish and Wildlife Service Shiawassee National Wildlife Refuge granted approval to access to the refuge property, the Saginaw County Park and Tittabawassee Township Park rangers granted access to Tittabawassee Township Park and Freeland Festival Park, Tom Lenon and Dick Touvell of the Chippewa Nature Center granted property access, and Michael Bishop of Alma College provided oversight as the Master Bander. More than 50 cooperating landowners throughout the research area granted access to their property, making this research possible. Funding was provided through an unrestricted grant from The Dow Chemical

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CHAPTER 2

Passerine exposure to primarily PCDFs and PCDDs in the river floodplains near Midland, Michigan, USA

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Abstract

House wren (*Troglodytes aedon*), tree swallow (*Tachycineta bicolor*), and eastern bluebird (*Sialia sialis*) tissues collected in study areas (SAs) downstream of Midland, Michigan, USA contained concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) greater than in upstream reference areas (RAs) in the region. The sum of concentrations of PCDD/DFs (ΣPCDD/DFs) in eggs of house wrens and eastern bluebirds from SAs were 4- to 22-fold greater compared to those from RAs, while concentrations in tree swallow eggs were similar among areas. Mean house wren and eastern bluebird ΣPCDD/DFs and sum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($\Sigma TEQs_{WHO\text{-}Avian}$), based on 1998 WHO avian toxic equivalency factors, egg concentrations ranged from 860 (430) to 1500 (910) ng/kg wet weight (ww) and 470 (150) to 1100 (510) ng/kg ww, respectively at the most contaminated study areas along the Tittabawassee River, while mean concentrations in tree swallow eggs ranged from 280 (100) to 760 (280) ng/kg ww among all locations. Concentrations of ΣPCDD/DFs in nestlings of all studied species at SAs were 3- to 50-fold greater compared to RAs. Mean house wren, tree swallow, and eastern bluebird nestling concentrations of ΣPCDD/DFs and $\Sigma TEQs_{WHO\text{-}Avian}$ ranged from 350 (140) to 610 (300) ng/kg ww, 360 (240) to 1100 (860) ng/kg ww, and 330 (100) to 1200 (690) ng/kg ww, respectively at SAs along the Tittabawassee River. Concentrations of $\Sigma TEQs_{WHO\text{-}Avian}$ were positively correlated with ΣPCDD/DF concentrations in both eggs and nestlings for all species studied. Profiles of relative concentrations of individual congeners were dominated by furan congeners (69–84%), primarily 2,3,7,8-tetrachlorodibenzofuran and

2,3,4,7,8-pentachlorodibenzofuran, for all species at SAs on the Tittabawassee and Saginaw rivers, but were dominated by dioxin congeners at upstream RAs.

Introduction

Concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) in soil and sediment in portions of the Tittabawassee and Saginaw rivers and associated floodplain downstream of Midland, Michigan (USA) are greater than the background concentration for the region (Hilscherova et al. 2003). Potential sources of the PCDD/DFs are historical production of organic chemicals and on-site storage and disposal, prior to the establishment of modern waste management protocols (Amendola and Barna 1986). The congener profile of the PCDD/DFs is dominated by 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) which is consistent with the waste stream of a chloralkali plant using a graphite-electrode process (Rappe et al. 1991; Svensson 1993). The lipophilic nature and slow degradation rates of these compounds (Mandal 2005), combined with consistent inundation of the floodplain, led to the continued presence of PCDD/DFs in floodplain soils and sediments.

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

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

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

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

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

The portion of the research described here focused on tissue-based exposure analyses. Results for the dietary-based exposure (Fredricks et al. 2009a) and nest productivity

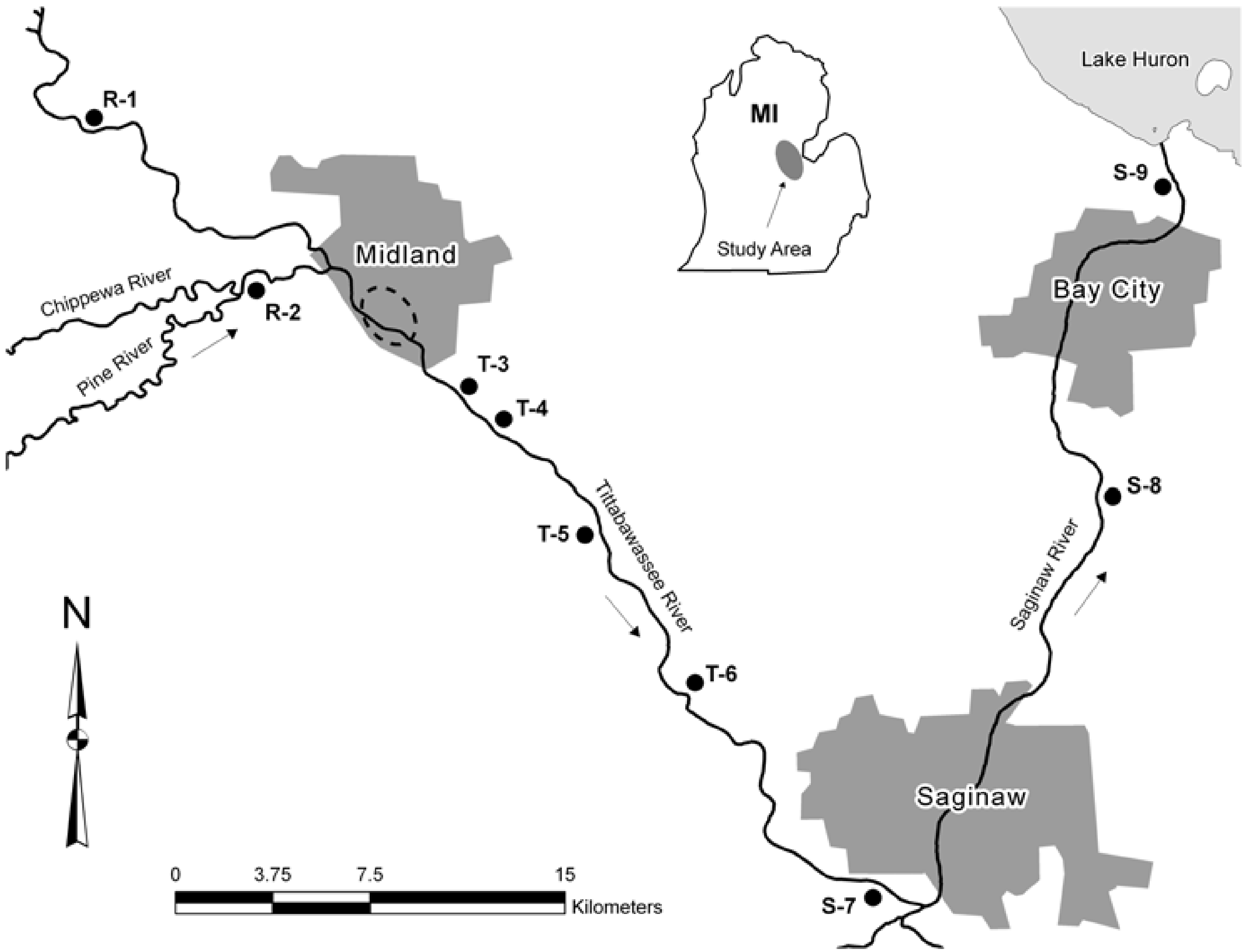
(Fredricks et al. 2009b) are reported elsewhere. Incorporation of all three lines of evidence into an ecological risk assessment will eventually lead to informed decisions about the potential impact(s) of on-site exposure and will aid in both the planning and evaluation of effective remedial actions.

Methods

Site description

The research was conducted on the Tittabawassee, Chippewa, and Saginaw rivers, in the vicinity of Midland, Michigan (Figure 2.1). The reference areas (RAs) were located upstream of the putative sources of PCDD/DF (Hilscherova et al. 2003) on the Tittabawassee (R-1) and Chippewa (R-2) rivers (Figure 2.1). The area downstream of the putative PCDD/DF sources, defined as the study area (SA), includes approximately 72 km of the Tittabawassee and Saginaw rivers. The SA stretched from the upstream boundary, defined as the low-head dam near Midland, Michigan, to where the Saginaw River enters Saginaw Bay. Throughout the SA, the Tittabawassee River is free flowing to the confluence with the Saginaw River and eventually Saginaw Bay. The SA consisted of two areas: the Tittabawassee River study areas, which included four locations (T-3 to T-6), and the Saginaw River study areas, which included two locations (S-7 and S-9). S-7 is located on a peninsula between the Tittabawassee and Saginaw rivers just upstream of their confluence. The six SAs were selected based on availability of landowner access to the sites and expected high end exposure based on a previous study that measured soil and sediment concentrations (Hilscherova et al. 2003).

Figure 2.1. 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 and S-9) were monitored from 2005–2007. Direction of river flow is designated by arrows; suspected source of contamination is enclosed in the dashed oval.



Lake Huron
MI
Study Area
R-1
R-2
Chippewa River
Pine River
Midland
T-3
T-4
T-5
T-6
Tittabawassee River
S-7
S-8
S-9
Saginaw River
Bay City
Saginaw
N
0
3.75
7.5
15
Kilometers

Nest boxes

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

Tissue collection

Both eggs and nestlings were collected for quantification of PCDD/DFs. Nest boxes were randomly selected from the active nest boxes at a given location for either live egg or nestling collections but not both. Fresh egg mass was determined on the date laid. Abandoned and addled eggs were collected for possible quantification of PCDD/DF congeners and determination of degree of development after no activity for seven days (3 to 4 d post hatch for addled eggs) or by the presence of new nesting material. Addled eggs were defined as those that failed to hatch 3 to 4 d post hatch of the remainder of the clutch. Eggs were individually stored wrapped in chemically cleaned foil in a chemically clean glass jar (I-CHEM, Rockwood, TN) at ambient temperature in the field and refrigerated at 4 °C. In the laboratory, collected eggs were opened around the girth with a chemically cleaned scalpel blade and assessed for stage of development and the presence of any abnormalities (Giesy et al. 1994; Larson et al. 1996). Nestlings (1 per box) were collected at 10-d post-hatch for house wrens or 14-d post-hatch for eastern bluebirds and tree swallows, and euthanized via cervical dislocation. Nestlings were stored in similar glass jars on wet ice in the field and at -20 °C until analyses. In the laboratory, collected nestlings were homogenized without feathers, bill, legs, and gizzard and crop contents with a chemically cleaned stainless steel Omni-mixer® (Omni International, Marietta, GA). The homogenates were stored at -20 °C until extraction.

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

Identification and quantification of PCDD/DF congeners

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

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

Statistical analyses

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

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

contaminants or among multiple eggs sampled from the same nesting attempt for clutch variability trends.

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

Results

ΣPCDD/DF, ΣPCB, and ΣDDX concentrations

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

Concentrations of ΣPCDD/DF in eggs of both house wrens and eastern bluebirds were significantly different among sampling locations while concentrations of ΣPCDD/DF in eggs of tree swallows were not (Table 2.1). Mean concentrations of ΣPCDD/DFs in house wren and eastern bluebird eggs were 10- to 19-fold and 4- to 16-fold greater at Tittabawassee River SAs than RAs, respectively, while house wren eggs at Saginaw River SAs tended to be intermediate between the two and comparisons at this location were not possible for eastern bluebird eggs due to a limited sample size. Maximum concentration of ΣPCDD/DF in eggs of house wrens, tree swallows, and eastern bluebirds were 7200 ng/kg at T-4, 2000 ng/kg at R-1, and 2400 ng/kg at T-6, respectively.

Concentrations of ΣPCDD/DF in nestlings of house wrens, tree swallows, and eastern bluebirds were significantly different between sampling locations (Table 2.2). Mean concentrations of ΣPCDD/DFs in house wren, tree swallow, and eastern bluebird nestlings were 15- to 49-fold, 4- to 23-fold, and 7- to 45-fold greater at Tittabawassee River SAs than RAs, respectively, while house wren and tree swallow nestlings at

Table 2.1. Total concentrations of furans and dioxins (ΣPCDD/DF) and TEQs$_{WHO\text{-}Avian}$ in eggs[a] of house wrens, tree swallows and eastern bluebirds collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Values[b] (ng/kg ww) are given as the geometric mean with the sample size given in parentheses (*n*) over the 95% confidence interval.

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

[a] Eggs include both live and addled eggs

[b] Values were rounded and represent only two significant figures

[c] Means identified with the same letter are not significantly different among locations (across) at the *p*=0.05 level using Bonferroni means separation test

[d] TEQ$_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values

Table 2.1. (Continued)

[e] Range reported for sites with only two samples. Sites were not included in the between location statistical comparisons
[f] N/A = no samples collected from this location

Table 2.2. Total concentrations of furans and dioxins (ΣPCDD/DF) and $TEQs_{WHO\text{-}Avian}$ in nestlings[a] of house wrens, tree swallows, and eastern bluebirds collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Values[b] (ng/kg ww) are given as the geometric mean with the sample size given in parentheses (n) over the 95% confidence interval.

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

[a] HW NS were collected on day 10, TS and EB NS were collected on day 14 (hatch=day 0)

[b] Values were rounded and represent only two significant figures

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

[d] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values

Table 2.2. (Continued)

[e] N/A = no samples collected from this location
[f] Range reported for sites with only two samples. Sites were not included in the between location statistical comparisons

Saginaw River SAs tended to be intermediate between the other two SAs and comparisons at this area were not possible for eastern bluebird eggs due to a limited sample size. Maximum concentration of ΣPCDD/DF in nestlings of house wrens, tree swallows, and eastern bluebirds occurred at T-6 and were 1700 ng/kg, 7300 ng/kg, and 2100 ng/kg, respectively.

Concentrations of ΣPCDD/DF in live and addled eggs of tree swallows were significantly different at the RAs ($t=3.08$ $p=0.0095$), but not at SAs. ΣPCDD/DF concentrations in live and addled eggs were not significantly different for house wrens or eastern bluebirds. Tree swallow addled egg ΣPCDD/DF concentrations were 2-fold greater than live eggs collected at RAs (Figure 2.2).

Concentrations of ΣPCDD/DF in tree swallow eggs were correlated with egg lay order ($R=0.61284$, $p=0.0198$, $n=14$) at the RAs, but not at the SAs. ΣPCDD/DF concentrations in eggs were not correlated with egg lay order for house wrens or eastern bluebirds. The correlation between concentrations of ΣPCDD/DF and date laid were only significant for tree swallow eggs at the Saginaw River SA ($R=0.56296$, $p=0.0452$, $n=13$). Concentrations of ΣPCDD/DF were individually correlated with collection day in nestlings of house wrens, tree swallows, and eastern bluebirds at RAs ($R=-0.70022$, $p=0.0112$, $n=12$; $R=0.70403$, $p=0.0106$, $n=12$; $R=0.68281$, $p=0.0144$, $n=12$; respectively), while all correlations were not significant at downstream SAs. For the clutches analyzed, the within-clutch variability of concentrations of ΣPCDD/DF in eggs varied by only 10–38% across species and sites (Table 2.3).

Concentrations of ΣPCBs in eggs were greatest for tree swallows, intermediate for house wrens, and least for eastern bluebirds (Table 2.4). Concentrations of ΣDDXs were

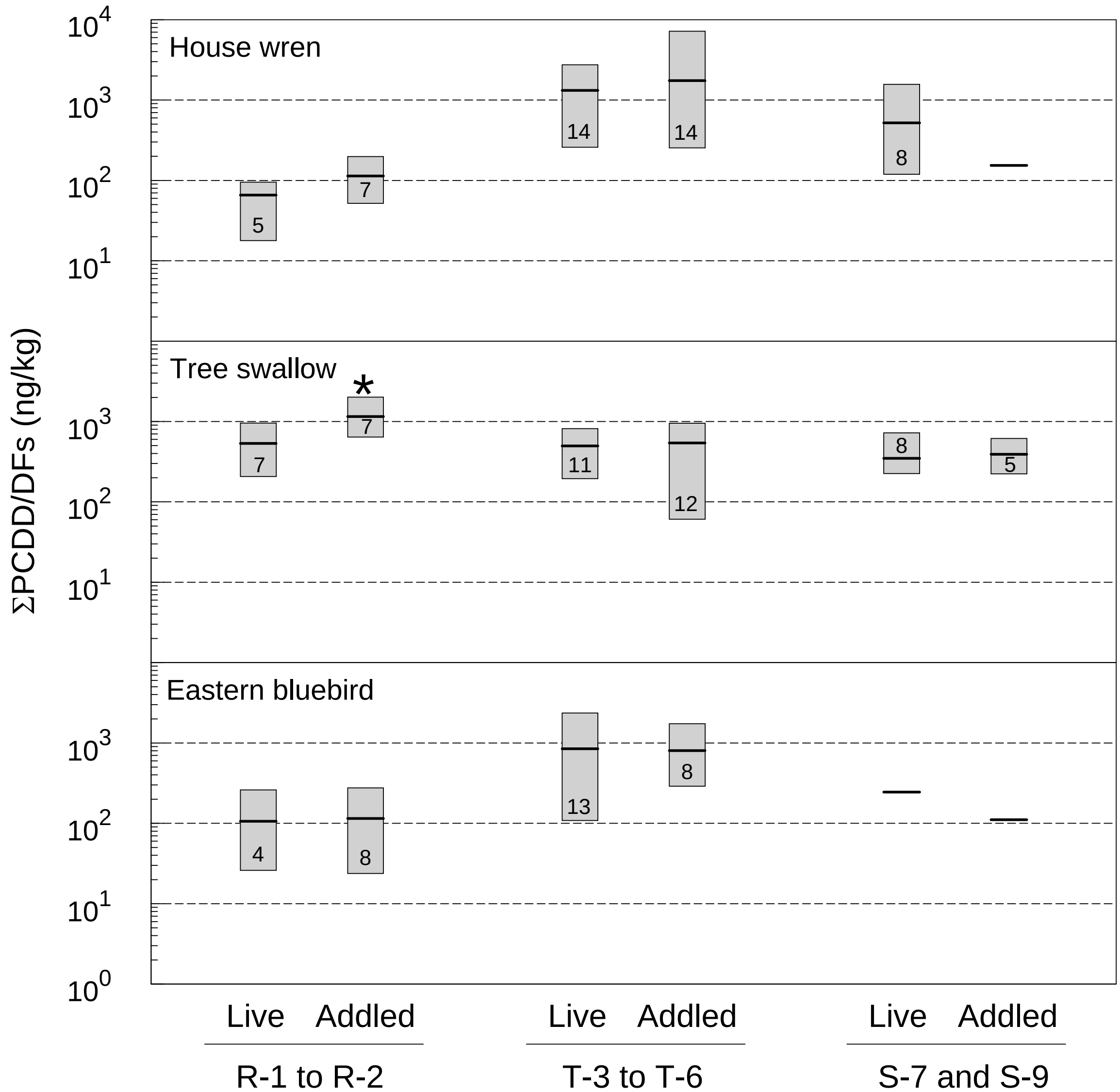


Figure 2.2. Range and mean of ΣPCDD/DF concentrations in live and addled eggs of house wrens, tree swallows, and eastern bluebirds collected in 2005–2007 near Midland, Michigan. Sample size is indicated for each area with at least two samples collected. R-1 to R-2=Reference Areas; T-3 to T-6=Tittabawassee River Study Areas; S-7 and S-9=Saginaw River Study Areas; p<0.05*.

Table 2.3. Within clutch variability of total concentrations of furans and dioxins (ΣPCDD/DF) and TEQs$_{\text{WHO-Avian}}$[a] in eggs of house wrens, tree swallows and eastern bluebirds collected during 2005-2007 from the Chippewa and Tittabawassee River floodplains, Midland, Michigan, USA. ΣPCDD/DF (ng/kg ww) with egg type given in parentheses over TEQ$_{\text{WHO-Avian}}$ with egg number laid given in parentheses.

	Clutch initiation[b]	Site[c]	E1[d,e]	E2	E3	E4	E5	Percent difference[f]
House wren								
Clutch 1	21Jun06	R-1	240 (LE)[g]	150 (AE)	200 (AE)			37
			51 (2)[h]	32 (5)	30 (7)			41
Clutch 2	10May06	R-1	77 (LE)	73 (AE)	60 (AE)			21
			8.4 (1)	8.2 (3)	9.6 (5)			15
Clutch 3	03Jun07	T-4	1400 (AE)	1600 (AE)	1700 (AE)			19
			630 (1)	480 (3)	780 (4)			39
Clutch 4	24May06	T-5	330 (AE)	320 (AE)	240 (AE)	250 (AE)		27
			140 (1)	140 (3)	100 (4)	100 (5)		28
Tree swallow								
Clutch 1	09May06	R-1	430 (LE)	370 (LE)	330 (LE)			23
			120 (1)	110 (2)	110 (3)			7
Clutch 2	30May05	R-2	510 (LE)	550 (AE)	660 (AE)	740 (AE)	780 (AE)	34
			270 (1)	290 (2)	340 (3)	370 (4)	370 (5)	27
Clutch 3	08May06	T-3	690 (AE)	670 (LE)	560 (AE)			20
			210 (1)	210 (2)	190 (3)			9
Clutch 4	19May06	T-6	1000 (AE)	1500 (AE)	1700 (AE)			38
			690 (2)	1100 (3)	1200 (4)			42
Eastern bluebird								
Clutch 1	02Jun06	R-1	24 (AE)	25 (AE)	24 (AE)	27 (AE)		14
			6.8 (1)	6.4 (2)	5.9 (3)	6.4 (4)		14
Clutch 2	30May07	R-2	120 (AE)	99 (AE)	87 (AE)			25

Table 2.3. (Continued)

			28 (1)	27 (3)	25 (5)			9
Clutch 3	14May06	T-3	380 (LE)	390 (LE)	360 (LE)	350 (LE)	380 (LE)	29
			170 (1)	170 (2)	170 (3)	160 (4)	180 (5)	38
Clutch 4	27Apr07	T-3	210 (AE)	300 (AE)	280 (AE)			10
			100 (1)	170 (4)	160 (4)			9
Clutch 5	01Aug05	T-6	1400 (AE)	1600 (AE)	1400 (AE)			11
			1000 (2)	1200 (2)	1000 (3)			11

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values

[b] Clutch Initiation is the day the first egg was discovered

[c] R-1–R-2 are Reference Areas and T-3–T-6 are Tittabawassee River Study Areas

[e] E1–E5 indicate individual eggs analyzed per clutch

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

[g] Egg Type: LE=Live egg and AE=Addled egg

[h] Egg #: Numbered in order as laid starting with 1 (if two eggs have the same number they were both found new so lay order is unknown)

Table 2.4. Concentrations of selected co-contaminants in eggs of house wrens, tree swallows and eastern bluebirds collected during 2005-2007 from the Chippewa and Tittabawassee River floodplains, Midland, Michigan, USA. Values of $TEQs_{WHO\text{-}Avian}$ are presented in ng/kg ww and PCBs and DDXs[a] are presented in mg/kg ww.

	Site[b]	Egg type[c]	Egg #[d]	ΣPCBs TEQs[e,f]	ΣPCDD/DFs TEQs	ΣPCBs[g]	2'4'-DDT	4'4'-DDE	4'4'-DDT	ΣDDXs
House wren										
Sample 1[h]	T-3	LE	1	12	620	1.3E[i] - 2	6.4E - 5	6.6E - 2	8.9E - 4	6.7E - 2
Sample 2	T-4	AE	2	8.0	180	3.5E - 2	4.7E - 5	3.0E - 2	2.9E - 4	3.1E - 2
Tree Swallow										
Sample 1	R-2	LE	1	62	110	2.1E - 2	1.1E - 3	1.3E - 1	4.2E - 3	1.4E - 1
Sample 2	T-4	AE	5	520	580	2.3E - 1	7.4E - 4	2.7E - 1	7.0E - 3	2.8E - 1
Sample 3	T-6	LE	3	160	540	8.0E - 2	4.4E - 5	7.2E - 1	1.7E - 3	7.2E - 1
Eastern bluebird										
Sample 1	R-1	AE	1	0.57	6.8	1.3E - 3	2.8E - 5	1.0E - 2	2.3E - 4	1.0E - 2
Sample 2	R-2	AE	3	0.73	27	3.7E - 3	2.0E - 5	1.8E - 1	8.2E - 4	1.8E - 1
Sample 3	T-3	LE	1	0.68	170	2.6E - 3	9.0E - 6	6.8E - 2	3.9E - 3	7.1E - 2
Sample 4	T-3	AE	4	0.73	170	5.1E - 3	1.6E - 5	7.2E - 2	1.5E - 3	7.4E - 2
Sample 5	T-6	LE	2	0.93	540	6.1E - 3	2.0E - 5	1.6E - 1	3.3E - 3	1.6E - 1
Sample 6	T-6	AE	2	2.1	1000	6.2E - 3	2.5E - 5	7.7E - 2	8.3E - 4	7.8E - 2

[a] ΣDDXs=sum of dichloro-diphenyl-trichloroethane (2',4' and 4',4' DDT isomers) and dichloro-diphenyl-dichloroethylene (4',4'-DDE)

[b] R-1–R-2 are Reference Areas and T-3–T-6 are Tittabawassee River Study Areas

[c] Egg Type: LE=Live egg and AE=Addled egg Clutch Initiation is the day the first egg was discovered

[d] Egg #: Numbered in order as laid starting with 1R-1–R-2 are Reference Areas and T-3–T-6 are Tittabawassee River Study Areas

[e] Values were rounded and represent only two significant figures

[f] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values

Table 2.4. (Continued)

[g] ΣPCBs included only the 12 non- and mono-*ortho*-substituted congeners
[h] Each sample is an individual egg from unique clutches
[i] $E = \times 10^{n}$

greatest in tree swallow eggs and were primarily composed of 4', 4'-dichloro-diphenyl-dichloroethylene (Table 2.4).

TCDD equivalents ($TEQ_{WHO\text{-}Avian}$)

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

$TEQ_{WHO\text{-}Avian}$ concentrations in eggs of both house wrens and eastern bluebirds were significantly different among sampling locations while $TEQ_{WHO\text{-}Avian}$ concentrations in eggs of tree swallows were not (Table 2.1). Mean $TEQ_{WHO\text{-}Avian}$ concentrations in house wren and eastern bluebird eggs were 15- to 91-fold and 5- to 46-fold greater at Tittabawassee River SAs than RAs, respectively, while house wren eggs at Saginaw River SAs tended to be intermediate between the two and comparisons at this location were not possible for eastern bluebird eggs due to a limited sample size. Maximum $TEQ_{WHO\text{-}Avian}$ concentrations in eggs of house wrens, tree swallows, and eastern bluebirds were 2300 ng/kg at T-3, 730 ng/kg at R-1 and 1000 ng/kg at T-6, respectively.

Concentrations of $TEQ_{WHO\text{-}Avian}$ in nestlings of house wrens, tree swallows, and eastern bluebirds were significantly different between sampling locations (Table 2.2). Mean $TEQ_{WHO\text{-}Avian}$ concentrations in house wrens, tree swallows, and eastern bluebirds nestlings were 21- to 105-fold, 6- to 58-fold, and 15- to 276-fold greater at Tittabawassee River SAs than RAs, respectively, while house wren and tree swallow nestlings at Saginaw River SAs tended to be intermediate between the other two SAs and comparisons at this area were not possible for eastern bluebird eggs due to a limited sample size. Maximum $TEQ_{WHO\text{-}Avian}$ concentrations in nestlings of house wrens, tree swallows, and eastern bluebirds occurred at T-6 and were 1200 ng/kg, 6000 ng/kg, and 1400 ng/kg, respectively.

Concentrations of $TEQ_{WHO\text{-}Avian}$ in live and addled eggs presented the same trends as concentrations of ΣPCDD/DF (Figure 2.2) for all studied species. $TEQ_{WHO\text{-}Avian}$ concentrations in addled tree swallow eggs were greater than in live eggs at RAs (t=3.52 p=0.0042). Concentrations of $TEQ_{WHO\text{-}Avian}$ in tree swallow eggs were correlated with egg lay order (R=0.60047, p=0.0232, n=14) at the RAs, but not at the SAs. $TEQ_{WHO\text{-}Avian}$ concentrations in eggs of house wrens and eastern bluebirds were not correlated with egg lay order. Concentrations of $TEQ_{WHO\text{-}Avian}$ in eggs and nestlings of all species were not correlated with date laid or collection day, respectively, across all areas. For the clutches analyzed, the within-clutch variability of concentrations of $TEQ_{WHO\text{-}Avian}$ in eggs varied by only 7–42% across species and sites (Table 2.3). Concentrations of ΣPCB $TEQ_{WHO\text{-}Avian}$ in eggs of tree swallows comprised from 23–47% of the $\Sigma TEQ_{WHO\text{-}Avian}$, whereas, in house wren and eastern bluebird eggs ΣPCB $TEQ_{WHO\text{-}Avian}$ concentrations only comprised <1–8% (Table 2.4).

Congener patterns

Relative proportions of PCDD/DF concentrations contributed by individual congeners varied between the eggs and nestlings as well as among species and sampling areas. Congener profiles were characterized by principle component analysis (PCA) by relative orderings of PCDD/DF concentrations normalized to the ΣPCDD/DF concentration. The PCA model that included two principle components (PC1 and PC2) explained 85% of the total variance. All samples collected in RAs had negative greatest eigenvectors for both PC1 and PC2, while tree swallow samples were separated by positive vectors for PC1 (loading score of 0.89 for 2,3,7,8-TCDF). House wren and eastern bluebird had positive vectors for PC2 [loading score of 0.84 for 2,3,4,7,8-pentachlorodibenzofuran (PeCDF)], and negative vectors for PC1 (Figure 2.3).

For all three species, dioxins dominated the congener profile at RAs and furans dominated at the SAs. For example, at RAs, tree swallow mean egg PCDD/DF concentration congener profiles were dominated by 79% dioxin congeners compared to SAs that only had 44% (Figure 2.4). For all species studied, congener profiles of eggs and nestlings at Saginaw River SAs were similar to those at Tittabawassee River SAs (Figure 2.5). Mean PCDD/DF congener profiles of egg and nestling house wrens and eastern bluebirds were dominated by a combination of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF at Tittabawassee River SAs (Figure 2.4). An even larger proportion of the total was 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF for tree swallows at the SAs. Mean nestling PCDD/DF concentration congener profiles for all species at Tittabawassee River SAs were composed of between 69 and 84% furan congeners with 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF making up 35–60% of the total. The majority of congeners were above the detection limit in over 50% of samples with the exception of the dioxin-like congeners 1,2,3,7,8,9-hexachlorodibenzofuran, 1,2,3,4,7,8,9-heptachlorodibenzofuran, 1,2,3,4,6,7,8,9-octachlorodibenzofuran (Supplemental Information: Tables 2.5–2.10).

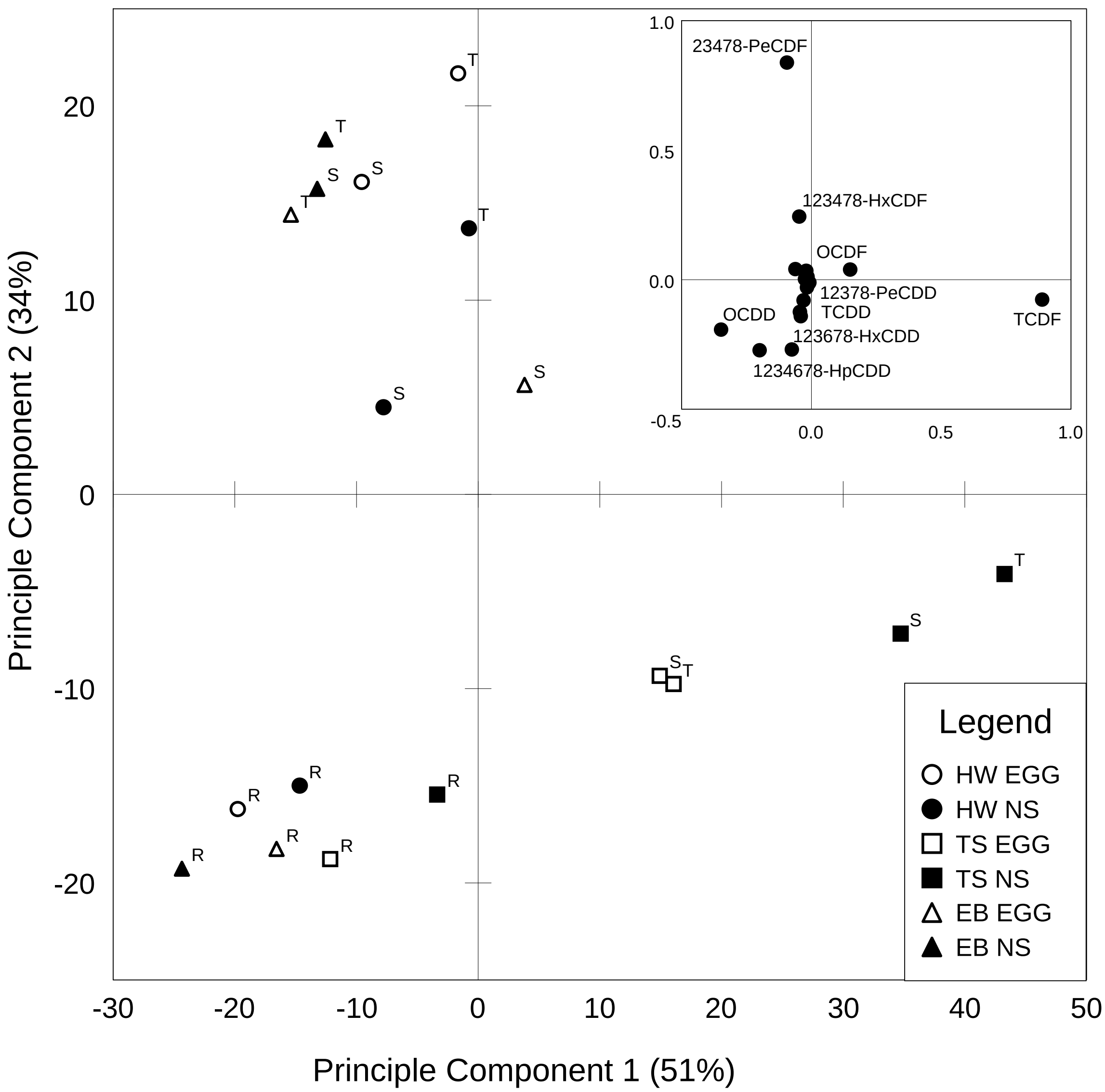
Discussion

ΣPCDD/DF, ΣPCB, and ΣDDX concentrations

Concentrations of ΣPCDD/DF in all passerine tissues collected except tree swallow eggs were greatest at Tittabawassee River SAs while those from Saginaw River SAs had intermediate concentrations and those from RAs were the least. Concentrations of ΣPCDD/DF in tree swallow eggs were similar among reference and study areas. Most accumulation studies of chlorinated hydrocarbon residues in passerines included PCBs and DDXs. A single study investigated 2,3,7,8-TCDD exposure in tree swallow tissues (Custer et al. 2005). Mean concentrations of 2,3,7,8-TCDD in eggs and nestlings of tree swallows collected from contaminated areas in the Woonasquatucket River floodplain, Rhode Island, USA ranged from 310 to 1000 ng/kg ww and 570 to 990 ng/kg ww, respectively (Custer et al. 2005) and were similar to the ΣPCDD/DF concentrations in eggs and nestlings for all three species from the current study. The same study reported a maximum concentration of 2,3,7,8-TCDD in a tree swallow nestling that was four times less than the maximum level of ΣPCDD/DF observed in the current study: 7300 ng/kg in a tree swallow nestling collected at T-6. To our knowledge this sample contains the greatest measured concentration of ΣPCDD/DF reported in passerine bird tissues.

Similar concentrations of ΣPCDD/DF in eggs of tree swallows at RAs and SAs from the current study do not align with site-specific sediment trends. In contrast, ΣPCDD/DF

Figure 2.3. Principle component analysis of PCDD/DF concentration congener profiles in eggs and nestlings of house wrens (HW), tree swallows (TS), and eastern bluebirds (EB) collected in 2005–2007 near Midland, Michigan. Individual PCDD/DF congener loading scores for each principle component is depicted in the inset. R=Reference Area; T=Tittabawassee River Study Area; S=Saginaw River Study Areas; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin.



Principle Component 1 (51%)
Principle Component 2 (34%)
Legend
HW EGG
HW NS
TS EGG
TS NS
EB EGG
EB NS
23478-PeCDF
123478-HxCDF
OCDF
12378-PeCDD
TCDD
TCDF
OCDD
123678-HxCDD
1234678-HpCDD

Figure 2.4. Mean congener percent contributions in eggs and nestlings of house wrens, tree swallows, and eastern bluebirds collected in 2005–2007 near Midland, Michigan. R-1 to R-2=Reference Area; T-3 to T-6=Tittabawassee River Study Area; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin.

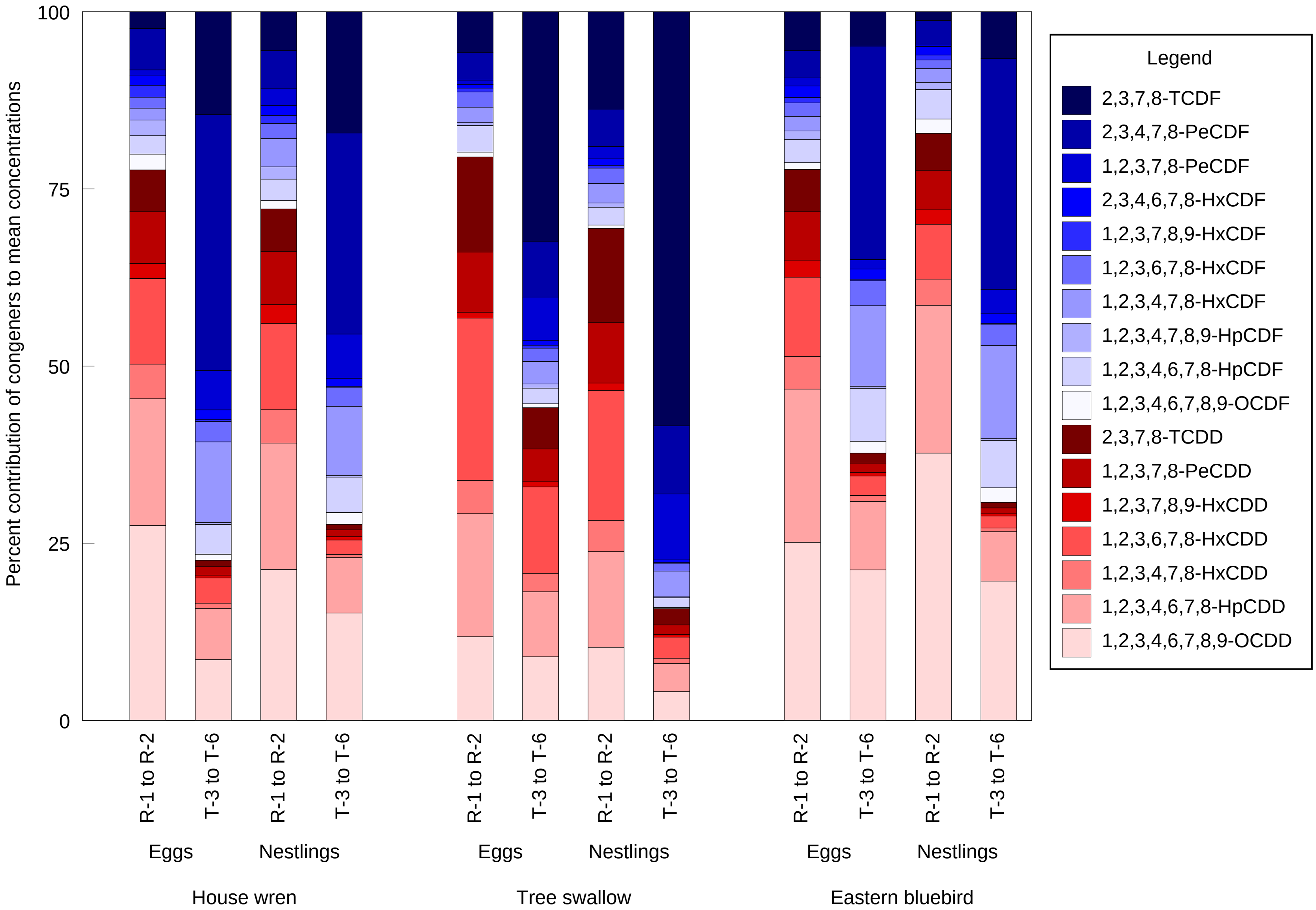
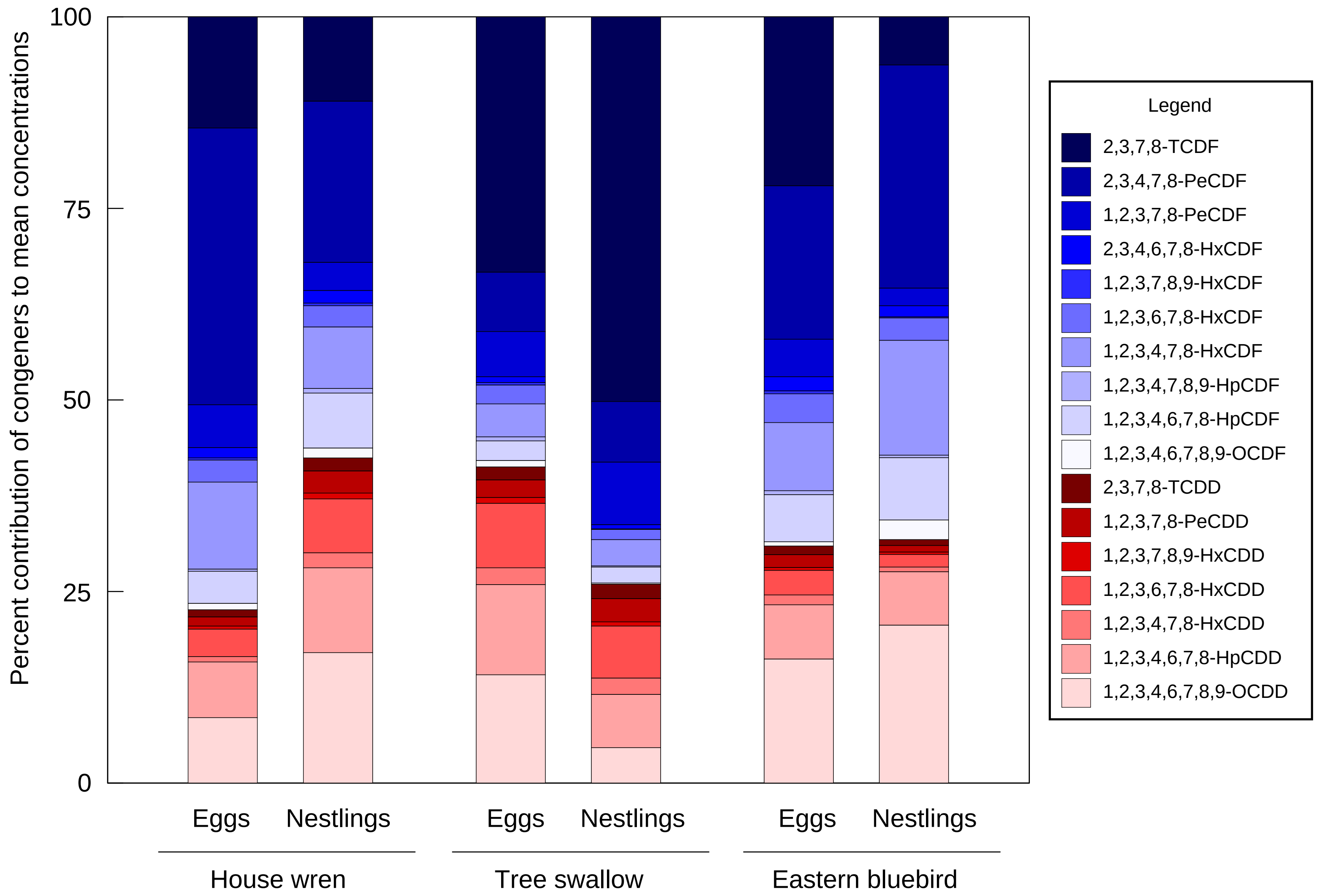

Percent contribution of congeners to mean concentrations
100
75
50
25
0
R-1 to R-2
T-3 to T-6
R-1 to R-2
T-3 to T-6
Eggs
Nestlings
House wren
R-1 to R-2
T-3 to T-6
R-1 to R-2
T-3 to T-6
Eggs
Nestlings
Tree swallow
R-1 to R-2
T-3 to T-6
R-1 to R-2
T-3 to T-6
Eggs
Nestlings
Eastern bluebird
Legend
2,3,7,8-TCDF
2,3,4,7,8-PeCDF
1,2,3,7,8-PeCDF
2,3,4,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF
1,2,3,6,7,8-HxCDF
1,2,3,4,7,8-HxCDF
1,2,3,4,7,8,9-HpCDF
1,2,3,4,6,7,8-HpCDF
1,2,3,4,6,7,8,9-OCDF
2,3,7,8-TCDD
1,2,3,7,8-PeCDD
1,2,3,7,8,9-HxCDD
1,2,3,6,7,8-HxCDD
1,2,3,4,7,8-HxCDD
1,2,3,4,6,7,8-HpCDD
1,2,3,4,6,7,8,9-OCDD

Figure 2.5. Mean congener percent contributions in eggs and nestlings of house wrens, tree swallows, and eastern bluebirds collected in 2006–2007 along the Saginaw River (S-7 and S-9) near Midland, Michigan. TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin.


Percent contribution of congeners to mean concentrations
100
75
50
25
0
Eggs
Nestlings
Eggs
Nestlings
Eggs
Nestlings
House wren
Tree swallow
Eastern bluebird
Legend
2,3,7,8-TCDF
2,3,4,7,8-PeCDF
1,2,3,7,8-PeCDF
2,3,4,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF
1,2,3,6,7,8-HxCDF
1,2,3,4,7,8-HxCDF
1,2,3,4,7,8,9-HpCDF
1,2,3,4,6,7,8-HpCDF
1,2,3,4,6,7,8,9-OCDF
2,3,7,8-TCDD
1,2,3,7,8-PeCDD
1,2,3,7,8,9-HxCDD
1,2,3,6,7,8-HxCDD
1,2,3,4,7,8-HxCDD
1,2,3,4,6,7,8-HpCDD
1,2,3,4,6,7,8,9-OCDD

concentrations in tree swallow nestlings collected in the same areas mirror the site-specific sediment trends as expected. These results are similar to those for ΣPCB concentrations in tree swallow eggs collected from an upstream reference site along the Champlain Canal that were similar to those collected from downstream sites with known contamination along the Hudson River, New York, USA (Secord et al. 1999). In that study, concentrations in nestlings were less at reference areas, which is similar to the findings in the current study. One possible explanation for these observations is that migrating swallows follow aquatic systems (Butler 1988) and could accumulate PCDD/DFs in route. Maternal contaminant deposition from body burdens may provide another explanation. Maternal transfer of contaminants to eggs has been shown to vary depending on overwintering areas in black-crowned night-herons (Henny and Blus 1986). Thus, adult female tree swallows could be exposed to concentrations of ΣPCDD/DF along migration or on wintering grounds universally, but previous research demonstrated that ring-necked pheasant hens (*Phasianus colchicus*) were only able to translocate approximately 1% of their cumulative dosage amount to each egg (Nosek et al. 1992b). Since the congener profiles in tree swallow eggs were different between RAs and SAs, the most plausible possibility is that, prior to breeding, foraging ranges of swallows at upstream RAs temporarily include a proximal contaminated site. This explanation seems most reasonable since adult tree swallows arrive at breeding areas to defend breeding territories several weeks prior to clutch initiation (Stutchbury and Robertson 1987) and have slightly larger foraging ranges than during brood rearing (Quinney and Ankney 1985). Additionally, most passerines are considered income-breeders (meaning that the majority of resources for egg production are acquired through the daily diet during egg development), and this further confirms the hypothesis that tree swallow females are likely traveling to a proximal contaminated site during egg production at RAs (Langin et al. 2006; Nager 2006).

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

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

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

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

TCDD equivalents ($TEQ_{WHO\text{-}Avian}$)

$TEQ_{WHO\text{-}Avian}$ concentrations in eggs and nestlings of house wrens, tree swallows, and eastern bluebirds were greater at downstream study areas, and, like ΣPCDD/DF concentrations, they were greatest at the T-6 location. One possible explanation for consistently greatest values at the T-6 location involves the natural hydrology of the Tittabawassee River. When at flood stage, the river flows across the large bends near T-6 instead of following the normal river channel (Figure 2.1). The water loses momentum and energy quickly and deposits large amounts of sediment over those areas, creating a "sink" location for sediment-bound contaminants.

Based on site-specific contamination and a gradient of exposures among locations, correlations were expected between concentrations of ΣPCDD/DF and $TEQ_{WHO\text{-}Avian}$. $TEQ_{WHO\text{-}Avian}$ and ΣPCDD/DF concentrations for eggs and nestlings were positively correlated for all study species (Figure 2.6). This was due to the consistent prevalence of three congeners with high $TEF_{WHO\text{-}Avian}$ values at SAs. Three PCDD/DF congeners (2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin) have $TEF_{WHO\text{-}Avian}$ (van den Berg et al. 1998) values equivalent to TCDD. Combined with TCDD, these four congeners make up between 85–95% of the $TEQ_{WHO\text{-}Avian}$ concentrations for both eggs and nestlings at the Tittabawassee and Saginaw River SAs. Individual congener correlations for the concentrations of 17 PCDD/DFs and $TEQs_{WHO\text{-}Avian}$ for eggs and nestlings by species were all highly correlated (unpublished data). Strong positive correlations indicate a site-specific contaminant gradient among samples across study areas.

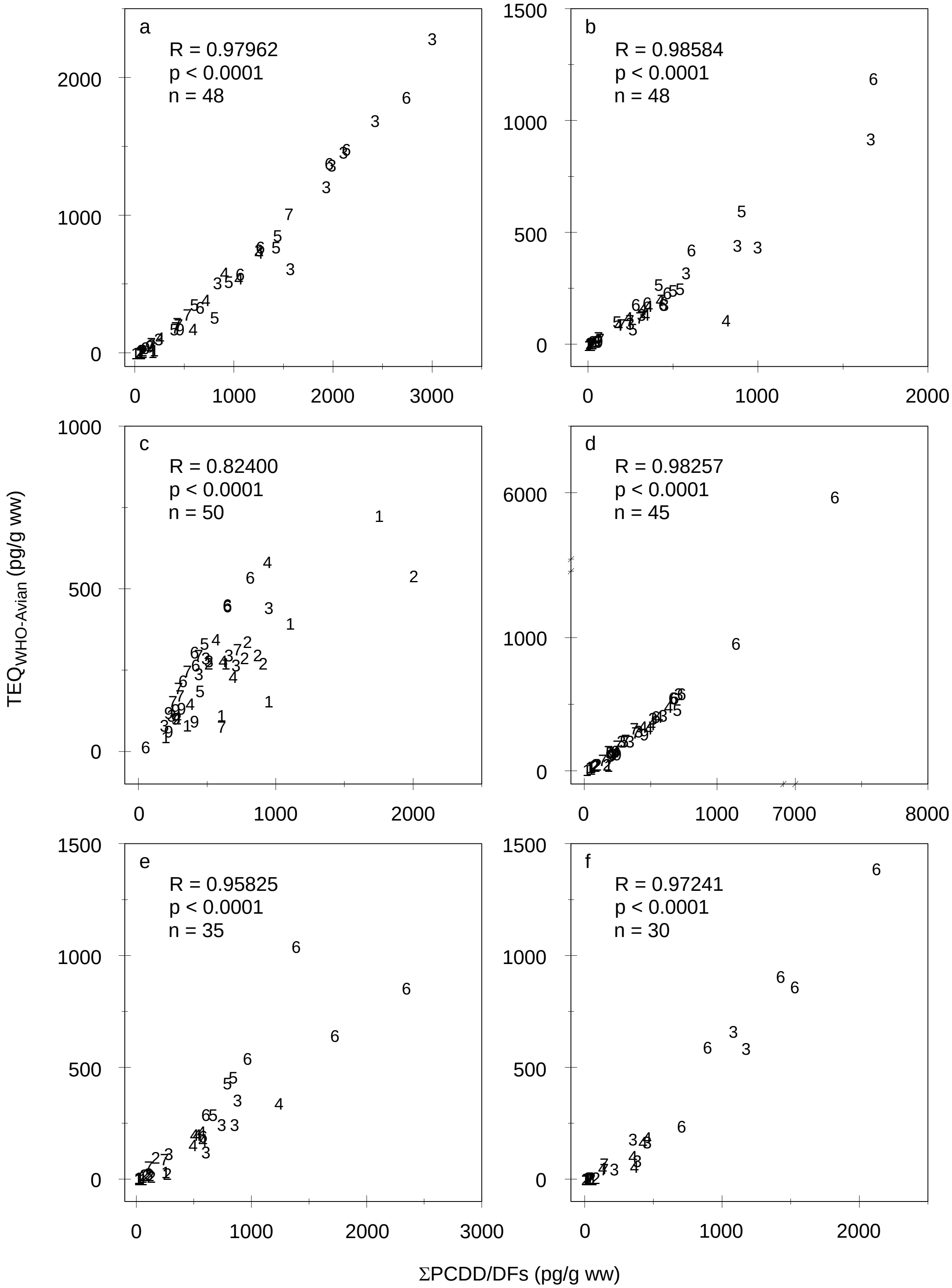
An egg collected from a house wren nest at T-4 contained the highest measured concentration of ΣPCDD/DF from this study (7200 ng/kg). The primary constituent was OCDD and the concentration of TEQ was only 350 ng/kg $TEQ_{WHO\text{-}Avian}$, which was made up of congeners that represented only 5% of the ΣPCDD/DF concentration. The egg was collected in mid-May 2005, was the third egg laid in the clutch, and the adult female was never recaptured again on-site. For comparison, at RAs egg and nestling mean percent ΣPCDD/DF of $TEQ_{WHO\text{-}Avian}$ concentrations ranged from 23–33% and 16.5–42.2%, respectively, while at study sites egg and nestling means ranged from 40–55% and 39–73%, respectively. Due to this discrepancy, this egg was removed from correlations between $TEQ_{WHO\text{-}Avian}$ and ΣPCDD/DF concentrations as an outlier (Figure 2.6). The nestling tree swallow that had the greatest concentration of ΣPCDD/DF also had the greatest concentration of $TEQ_{WHO\text{-}Avian}$ of 6000 ng/kg. Unlike the egg sample from the house wren that was dropped from the correlation analyses, the congener pattern of this sample correlated well with the other tree swallow samples (Figure 2.6).

Congener patterns

Concentrations of ΣPCDD/DF in tree swallow eggs were similar at RAs compared to SAs but not so for the other two species (Table 2.1). The congener profile for tree swallows however was dominated by dioxins at RAs, compared to furans at SAs (Figure 2.4). As expected, concentrations of ΣPCDD/DF and profiles of relative proportions of congeners in tree swallow nestlings had greater concentrations at SAs that were dominated by furan congeners. Moreover PCA loading scores for the first and second components separated relative congener proportions of ΣPCDD/DF concentrations by species and study location (Figure 2.3). These details support the hypothesis that adult females, prior to breeding, are potentially exposed to primarily PCDDs at a proximal contaminated site near the RAs. Since foraging ranges are limited for adults feeding nestlings (Quinney and Ankney 1985), nestling dietary exposure at RAs would not include the proximal contaminated site. This explains why tree swallow nestlings have near background ΣPCDD/DF concentrations and a similar congener profile compared to other samples collected at RAs.

Previous research on concentrations of ΣPCDD/DF on the Tittabawassee River has shown that soil and sediment congener profiles are dominated by PCDF congeners

Figure 2.6. Correlation plots of ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ for a. house wrens eggs, b. house wren nestlings, c. tree swallow eggs, d. tree swallow nestlings (note: axis breaks), e. eastern bluebird eggs, and f. eastern bluebird nestlings collected in 2005-2007 near Midland, Michigan with indications of R- and *p*-values and sample size. 1=R-1; 2=R-2; 3=T-3; 4=T-4; 5=T-5; 6=T-6; 7=S-7; 9=S-9.


a
R = 0.97962
p < 0.0001
n = 48
b
R = 0.98584
p < 0.0001
n = 48
c
R = 0.82400
p < 0.0001
n = 50
d
R = 0.98257
p < 0.0001
n = 45
e
R = 0.95825
p < 0.0001
n = 35
f
R = 0.97241
p < 0.0001
n = 30
TEQWHO-Avian (pg/g ww)
ΣPCDD/DFs (pg/g ww)

(Hilscherova et al. 2003). As expected, at downstream SAs, ΣPCDD/DF congener profiles in passerine egg and nestling samples had a similar congener pattern to site-specific soils and sediments (Hilscherova et al. 2003) for all three species (Figure 2.4). Interestingly, the reasons why tree swallow egg and nestling congener profiles at SAs contained greater percentages of 2,3,7,8-TCDF compared to tissues of terrestrial foraging house wrens and eastern bluebirds were probably associated with different dietary exposures. On the Woonasquatucket River, adult tree swallow females deposited greater concentrations of TCDD in eggs (Custer et al. 2005) compared to proximal sites with lesser TCDD concentrations. At that site however, terrestrial foraging species were not studied so differences in tissue accumulation for TCDD for terrestrial foraging passerines is largely unknown. Though, it is possible that there are not only species-specific but also congener-specific accumulation, sequestration, metabolism, and deposition differences that could account for the differences (Kubota et al. 2006).

Conclusions

Overall, egg and nestling exposures for house wrens, tree swallows, and eastern bluebirds were greater downstream of Midland than upstream and the downstream congener pattern was dominated by furan congeners, rather than PCDDs that were dominant upstream. Eggs of tree swallows at RAs had ΣPCDD/DF and $TEQ_{WHO\text{-}Avian}$ concentrations that were similar to SAs, albeit primarily based on PCDD congeners, compared to the PCDF congeners associated with eggs collected from SAs. Despite anomalies associated with tree swallow egg concentrations at RAs, nestling concentrations of both ΣPCDD/DF and $TEQ_{WHO\text{-}Avian}$ in all species studied were less at

RAs compared to SAs. We stress the importance of site-specific tissue exposure monitoring, and, due to the potentially different sources to each, the necessity of both egg and nestling samples. To our knowledge this is the first site-specific study of passerines exposed to elevated concentrations of mixtures dominated by furan congeners. Co-contaminants, including DDXs and PCBs, were generally at background levels for all three species studied based on egg data, with the exception of ΣPCB $TEQ_{WHO\text{-}Avian}$ in tree swallows. However, because only a small subset of tree swallow eggs was analyzed for PCBs, there is some uncertainty associated with this conclusion. Overall, based on egg and nestling tissue concentrations, passerine birds breeding in the Tittabawassee River floodplain downstream of Midland, Michigan, have significant exposure to ΣPCDD/DFs. Subsequent manuscripts will discuss implications of these results by incorporating data from dietary exposure (Fredricks et al. 2009a) and productivity (Fredricks et al. 2009b) into terrestrial-based (Fredricks et al. 2009c) and aquatic-based risk assessments of passerines nesting near Midland, Michigan.

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Freeland Festival Park, Tom Lenon and Dick Touvell of the Chippewa Nature Center for assistance and property access, and Michael Bishop of Alma College for his key contributions to our banding efforts as our Master Bander. We acknowledge the more than 50 cooperating landowners throughout the research area who granted us access to their property, making this research possible. Prof. Giesy was supported by the Canada Research Chair program and an at large Chair Professorship at the Department of Biology and Chemistry and Research Centre for Coastal Pollution and Conservation, City University of Hong Kong. Funding was provided through an unrestricted grant from The Dow Chemical Company, Midland, Michigan to J.P. Giesy and M.J. Zwiernik of Michigan State University.

Animal Use

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

Supplemental Information

Table 2.5. Concentrations of furan and dioxin congeners in house wren eggs[a] collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Values[b] (ng/kg ww) are given as the arithmetic mean ± SD over the range.

	Reference Areas		Study Areas					
	R-1	R-2	T-3	T-4	T-5	T-6	S-7	S-9
Chemical[c]	*n*=6	*n*=6	*n*=9	*n*=7	*n*=6	*n*=6	*n*=6	*n*=3
2378-TCDF	0.77	4.0±3.6	170±140	150±110	180±110	220±300	34±17	27±30
		1.1–8.7	50–500	40–330	54–360	33–830	15–59	8.4–61
	5 ND	1 ND						
23478-PeCDF	3.2±1.7	9.3±14	890±630	220±120	260±190	790±540	280±310	36±21
	1.0–5.6	2.2–38	31–2100	63–350	88–540	280–1600	55–900	22–61
	1 ND							
12378-PeCDF		0.56	68±58	54±40	77±51	78±93	12±6.1	8.7±11
			18–210	17–110	28–160	15–270	5.8–23	1.9–21
	6 ND	5 ND						
234678-HxCDF			26±13	12±3.2	15±12	25±18	9.9±8.5	3.5±3.2
	1.1–2.6	0.64–0.91	3.1–48	9.2–17	4.4–36	11–52	2.8–27	1.7–7.2
	4 ND	4 ND		1 ND				
123789-HxCDF			2.2±0.88					
			1.6–3.2		2.2–3.0	1.0–1.5		
	6 ND	6 ND	6 ND	7 ND	4 ND	4 ND	6 ND	3 ND
123678-HxCDF	1.6±0.96		52±28	24±11	28±18	54±35	21±22	5.9±4.4
	0.93–2.7	0.76–1.1	4.2–93	7.4–40	9.6–57	25–100	4.6–65	2.9–11
	3 ND	4 ND						
123478-HxCDF	1.9±0.98		210±100	91±46	100±76	230±160	82±86	13±11

Table 2.5. (Continued)

	0.85–2.8	1.0–1.2	19–350	29–160	34–230	98–460	18–250	6.0–26
	3 ND	4 ND						
1234789-HpCDF			3.0±1.4	3.1±2.9	2.5	2.4±0.42		
			1.5–4.8	0.83–6.4		1.9–2.6		
	6 ND	6 ND	5 ND	4 ND	5 ND	3 ND	6 ND	3 ND
1234678-HpCDF	4.1	3.0±0.46	53±27	63±72	46±27	49±9.3	35±17	16±20
		2.5–3.4	19–120	13–220	22–88	36–62	9.8–63	4.3–40
	5 ND	3 ND						
12346789-OCDF			12±11	17±20		8.9±5.4	4.4±2.3	3.6±5.1
			4.3–40	3.7–51	11–13	2.8–15	1.1–8.4	0.56–9.4
	6 ND	6 ND		2 ND	4 ND	1 ND		
2378-TCDD	3.3±4.4	7.0±1.7	11±6.5	9.8±2.6	8.0±2.3	9.0±3.3	3.7±1.6	14±14
	0.80–10	4.2–9.3	5.3–22	6.6–14	3.9–11	5.3–13	2.0–6.4	5.0–30
	2 ND							
12378-PeCDD	5.6±4.0	7.6±3.0	11±7.4	13±7.6	18±22	10±2.3	6.9±4.5	14±6.8
	1.7–12	5.0–14	3.7–24	6.2–29	4.7–63	7.1–12	3.1–16	8.3–22
	1 ND							
123789-HxCDD		1.2	4.5±3.2	13±20	7.7±6.5	3.4±0.79	2.0±0.59	
	3.0–5.5		2.1–9.2	2.5–49	3.4–15	2.4–4.3	1.0–2.5	1.5–7.4
	4 ND	5 ND	5 ND	2 ND	3 ND	2 ND	1 ND	1 ND
123678-HxCDD	10±7.8	13±10	25±16	190±440	49±73	22±7.7	16±8.4	29±15
	2.0–23	7.1–34	5.1–51	12–1200	14–200	13–32	6.7–32	13–44
123478-HxCDD	6.3±4.0	5.0±3.4	7.0±4.6	8.9±7.6	16±22	4.8±0.85	3.8±1.9	7.6±3.9
	0.78–9.9	2.6–11	3.5–15	3.3–24	4.5–55	3.8–6.1	2.0–7.2	3.1–10
	2 ND	1 ND	4 ND	1 ND	1 ND	1 ND		
1234678-HpCDD	18±15	15±7.2	68±45	520±1200	54±24	56±15	35±10	29±22
	3.6–41	6.1–24	21–170	22–3300	31–99	34–75	16–44	14–54
12346789-OCDD	41±41	16±4.4	110±83	320±670	67±15	81±28	49±13	33±23

Table 2.5. (Continued)

	4.6–110	8.7–22	42–320	25–1900	50–89	43–110	24–59	18–60

[a] Eggs include both live and addled eggs

[b] Values were rounded and represent only two significant figures

[c] TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin

Table 2.6. Concentrations of furan and dioxin congeners in house wren nestlings collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Values[a] (ng/kg ww) are given as the arithmetic mean ± SD over the range.

	Reference Areas		Study Areas					
	R-1	R-2	T-3	T-4	T-5	T-6	S-7	S-9
Chemical[b]	*n*=6	*n*=6	*n*=7	*n*=7	*n*=6	*n*=6	*n*=6	*n*=4
2378-TCDF	1.2±1.9	1.4±0.90	97±56	28±8.6	120±110	170±170	21±12	5.7±2.0
	0.23–4.7	0.52–2.8	47–190	20–42	20–340	37–500	11–37	3.9–8.2
	1 ND	1 ND						
23478-PeCDF	0.84±0.58	1.3±0.37	240±220	100±36	120±77	210±220	69±51	5.8±0.84
	0.34–2.0	0.83–1.9	45–680	63–160	23–240	79–650	13–150	4.7–6.8
12378-PeCDF	0.43±0.64	0.64±0.44	44±32	13±3.3	40±33	59±68	8.8±4.5	1.6±1.2
	0.12–1.6	0.19–1.3	13–99	9.4–18	9.5–100	14–190	4.6–16	0.50–2.8
	1 ND							1 ND
234678-HxCDF	0.30±0.071	0.36±0.067	10±8.8	4.0±1.2	4.3±2.1	8.3±8.2	4.3±3.1	0.70±0.18
	0.23–0.36	0.30–0.43	2.5–28	2.4–5.9	1.9–7.2	3.1–25	1.2–9.7	0.50–0.93
	3 ND	1 ND						
123789-HxCDF			0.94±0.72		0.78	1.3±1.2	0.51	
			0.31–1.9			0.54–2.7		
	6 ND	6 ND	3 ND	7 ND	5 ND	3 ND	5 ND	4 ND
123678-HxCDF	0.35±0.090	0.59±0.24	23±18	10±3.8	10±4.9	18±18	7.4±5.4	1.3±0.37
	0.28–0.48	0.31–1.0	5.7–55	5.0–17	4.1–15	8.3–54	2.0–17	0.91–1.7
	2 ND							
123478-HxCDF	0.47±0.19	1.6±2.1	82±59	36±14	38±22	63±55	25±18	2.1±0.54
	0.29–0.68	0.27–5.8	21–170	20–57	14–62	31–170	6.8–56	1.7–2.9
	2 ND							
1234789-HpCDF			2.2±1.1	1.1±0.47	1.7±0.43	1.8±1.3		
			0.77–3.6	0.41–1.7	1.4–2.2	0.87–3.6	0.54–1.3	

Table 2.6. (Continued)

	6 ND	6 ND	1 ND	2 ND	3 ND	2 ND	4 ND	4 ND
1234678-HpCDF	0.39±0.14	0.81±0.27	36±20	22±13	22±13	20±11	19±14	3.3±2.4
	0.27–0.59	0.44–1.2	16–66	7.9–49	8.9–40	9.5–40	5.2–40	1.8–6.8
	2 ND							
12346789-OCDF		0.20	17±17	7.6±5.5	6.6±5.1	6.1±4.9	4.0±2.9	0.93±0.84
			4.9–52	1.8–17	1.4–14	2.6–15	2.4–8.4	0.25–1.9
	6 ND	5 ND					2 ND	1 ND
2378-TCDD	0.63±0.26	1.8±0.34	3.6±1.2	3.4±1.0	3.2±1.6	2.5±0.58	1.3±0.59	1.9±0.91
	0.40–1.1	1.2–2.1	2.3–5.5	2.0–4.6	2.1–6.5	1.4–2.9	0.49–1.9	0.97–3.2
12378-PeCDD	1.1±0.37	1.8±0.37	3.7±1.2	3.9±1.4	5.6±5.5	2.7±0.47	2.0±0.96	3.4±1.9
	0.56–1.6	1.3–2.3	2.6–6.0	1.9–5.7	2.6–16	2.0–3.5	0.80–3.2	1.5–5.9
123789-HxCDD	0.56±0.32	0.73±0.21	1.8±0.88	2.4±2.0	2.8±2.9	1.2±0.34	1.4±0.41	0.69±0.27
	0.35–1.1	0.48–0.89	1.1–3.4	0.88–6.9	1.0–8.0	0.86–1.8	0.90–1.9	0.44–0.98
	1 ND	3 ND	1 ND		1 ND		2 ND	1 ND
123678-HxCDD	2.0±1.4	2.7±0.92	9.2±4.1	6.9±2.8	12±13	5.8±1.2	5.7±3.8	7.9±4.8
	0.65–4.8	1.8–4.1	5.2–16	3.6–11	4.3–39	3.6–6.8	2.1–12	4.0–15
				1 ND				
123478-HxCDD	0.83±0.45	0.95±0.26	2.0±0.8	1.6±0.61	2.9±2.9	1.3±0.31	1.3±0.31	2.3±1.7
	0.32–1.7	0.55–1.2	1.2–3.5	0.96–2.7	1.2–8.1	0.68–1.5	0.94–1.6	1.1–4.6
			1 ND	1 ND	1 ND		1 ND	
1234678-HpCDD	2.6±1.2	4.5±1.8	48±35	64±110	29±16	23±11	19±11	8.3±3.4
	1.7–5.0	1.8–7.5	20–110	15–310	11–56	9.2–36	5.1–36	4.7–13
12346789-OCDD	3.3±1.8	5.2±1.8	110±58	83±85	58±28	52±32	38±24	10±4.6
	1.4–6.5	2.6–8.0	59–200	26–270	16–97	15–93	8.6–69	4.0–14

[a] Values were rounded and represent only two significant figures

Table 2.6. (Continued)

[b] TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin

Table 2.7. Concentrations of furan and dioxin congeners in tree swallow eggs[a] collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Values[b] (ng/kg ww) are given as the arithmetic mean ± SD over the range.

	Reference Areas		Study Areas					
	R-1	R-2	T-3	T-4	T-5	T-6	S-7	S-9
Chemical[c]	*n*=7	*n*=7	*n*=8	*n*=6	*n*=2	*n*=7	*n*=7	*n*=6
2378-TCDF	42±35	52±22	110±55	170±140		240±130	150±76	76±21
	10–95	23–93	56–200	53–420	130–250	1.1–380	1.6–230	39–95
23478-PeCDF	31±30	35±15	32±15	43±22		50±34	43±16	13±3.4
	6.0–89	13–63	13–57	16–79	35–61	2.6–100	24–64	9.7–18
12378-PeCDF	8.5±4.6		18±13	42±25		56±28	36±9.8	12±4.2
	4.1–13	4.8–6.3	4.5–43	18–82	27–61	31–100	28–53	7.1–17
	4 ND	5 ND	2 ND	1 ND		1 ND	1 ND	2 ND
234678-HxCDF	7.4±2.5	4.7±3.4	4.1±3.3	3.7±1.6		3.6±2.0	3.2±1.6	2.8±1.5
	4.5–9.0	1.4–8.2	1.2–8.6	2.3–6.2	2.1–3.6	1.4–4.8	1.7–5.9	1.8–5.0
	4 ND	4 ND	4 ND	1 ND		4 ND	1 ND	2 ND
123789-HxCDF	7 ND	7 ND	8 ND	6 ND	2 ND	7 ND	7 ND	6 ND
123678-HxCDF	15±12	22±14	12±7.7	13±11		6.5±3.5	6.9±4.8	11±10
	3.8–38	5.6–39	2.9–28	4.6–34	4.2–5.9	1.2–13	3.1–17	3.2–27
123478-HxCDF	19±21	18±14	13±6.3	21±7.6		18±10	24±29	6.6±3.3
	6.4–60	8.0–46	5.3–20	9.7–28	13–14	1.1–31	11–89	4.1–11
	1 ND	1 ND	2 ND					1 ND
1234789-HpCDF				1.8±1.8				
				0.55–3.9				
	7 ND	7 ND	8 ND	3 ND	2 ND	7 ND	7 ND	6 ND
1234678-HpCDF	34±43	36±53	15±9.9	15±6.0	6.2	7.4±4.0	14±13	7.3±4.7
	10–130	4.8–160	5.3–32	9.6–25		1.6–15	6.7–43	2.5–13
				1 ND	1 ND			1 ND

Table 2.7. (Continued)

12346789-OCDF		10±17	4.4±3.5	3.8±3.0	4.0		7.8±14	
	3.8–46	0.93–35	1.2–8.0	1.9–8.2		1.1–3.2	0.79–37	1.1–2.2
	5 ND	3 ND	5 ND	2 ND	1 ND	5 ND	1 ND	4 ND
2378-TCDD	100±100	130±54	61±40	31±38		7.7±3.4	5.4±1.5	6.4±1.6
	15–300	44–220	2.8–120	7.6–110	6.7–8.4	3.6–13	3.4–7.5	3.2–7.6
							1 ND	
12378-PeCDD	70±71	78±35	37±22	27±20		11±5.0	9.5±7.6	10±3.2
	12–220	23–140	4.9–60	7.6–52	7.5–13	5.1–19	3.7–25	8.1–15
							1 ND	2 ND
123789-HxCDD	7.7±2.9	5.4±3.4	3.1±1.6	5.3±2.9		2.6±0.82	2.5±1.7	3.2±0.60
	3.5–12	2.1–8.9	1.7–5.3	3.8–11	2.1–5.2	2.1–3.6	1.2–5.9	2.5–3.9
		4 ND	4 ND	1 ND		4 ND	1 ND	2 ND
123678-HxCDD	210±190	200±120	88±55	70±48		32±21	33±59	32±16
	31–610	52–440	13–180	19–140	8.9–140	9.3–67	6–170	14–60
123478-HxCDD	39±33	41±23	19±11	17±11		6.4±2.9	6.4±5.7	9.8±6.6
	9.2–110	11–85	3.6–34	5.7–34	4.6–11	3.3–11	2.2–18	4.8–22
1234678-HpCDD	140±67	130±120	60±45	61±28		19±7.9	31±24	51±20
	55–210	28–380	21–150	33–110	17–41	11–34	15–67	32–88
12346789-OCDD	77±71	110±87	49±32	59±31		19±9.3	66±95	46±22
	31–230	38–280	16–99	31–110	27–37	10–38	16–280	26–80

[a] Eggs include both live and addled eggs

[b] Values were rounded and represent only two significant figures

[c] TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin

Table 2.8. Concentrations of furan and dioxin congeners in tree swallow nestlings collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Values[a] (ng/kg ww) are given as the arithmetic mean ± SD over the range.

	Reference Areas		Study Areas					
	R-1	R-2	T-3	T-4	T-5	T-6	S-7	S-9
Chemical[b]	*n*=6	*n*=6	*n*=6	*n*=6	*n*=3	*n*=6	*n*=6	*n*=6
2378-TCDF	6.9±1.7	15±3.9	280±120	260±97	220±130	1200±1700	170±74	110±53
	4.2–9.1	11–22	170–490	96–370	110–360	330–4600	65–270	87–220
23478-PeCDF	3.4±1.3	5.8±0.91	43±17	51±21	40±33	260±480	29±11	16±9.2
	1.1–4.9	4.2–6.7	23–68	24–83	18–78	45–1200	14–43	11–35
12378-PeCDF	0.86±0.37	2.2±0.50	37±13	45±18	49±44	220±370	30±12	16±5.9
	0.55–1.4	1.7–3.0	19–55	21–71	20–99	55–990	13–44	13–28
	2 ND							
234678-HxCDF	0.69±0.48	1.1±0.20	2.1±0.92	2.8±0.65	2.6±2.1	9.3±17	1.4±0.47	1.5±0.45
	0.25–1.6	0.88–1.4	1.0–3.7	2.1–3.8	1.3–5.0	1.5–43	0.83–2.2	1.1–2.4
		1 ND						
123789-HxCDF				0.29		2.3		
	6 ND	6 ND	6 ND	5 ND	3 ND	5 ND	6 ND	6 ND
123678-HxCDF	1.7±1.1	2.3±0.67	5.7±2.2	5.6±0.92	5.3±4.7	15±20	3.3±0.94	3.6±0.69
	0.40–3.7	1.8–3.5	2.7–9.1	3.7–6.3	2.4–11	5.1–57	2.3–4.7	2.8–4.8
123478-HxCDF	1.7±0.72	3.3±1.2	18±6.5	18±5.8	20±19	62±92	11±4.4	8.2±3.6
	0.69–2.9	2.0–5.1	7.9–27	8.4–24	8.1–42	20–250	6.4–18	6.2–15
1234789-HpCDF	0.55					0.85		
			0.37–0.77	0.41–0.75				
	5 ND	6 ND	4 ND	4 ND	3 ND	5 ND	6 ND	6 ND
1234678-HpCDF	3.1±4.4	3.0±2.9	7.8±2.7	7.3±1.8	8.9±8.6	8.1±4.2	4.6±1.6	6.7±4.2
	0.61–11	1.0–8.7	3.4–10	4.5–9.4	3.1–19	5.3–16	2.3–6.5	2.9–15

Table 2.8. (Continued)

	1 ND							
12346789-OCDF	3.5	0.97	1.1±0.49	1.2±0.45	0.82±0.68	0.87±0.15		0.96±1.3
			0.45–1.5	0.51–1.7	0.30–1.6	0.76–1.1	0.21–0.42	0.30–3.4
	5 ND	5 ND	2 ND	1 ND		2 ND	4 ND	1 ND
2378-TCDD	8.8±4.4	15±3.4	16±5.6	12±3.9	4.1±1.2	10±3.5	3.0±1.1	6.8±0.50
	2.0–15	11–21	8.7–25	8.2–20	3.2–5.4	6.4–17	1.4–4.6	6.2–7.5
12378-PeCDD	5.9±3.0	9.6±2.0	9.7±4.1	7.7±3.3	3.8±0.87	4.9±1.1	2.5±0.29	13±2.6
	1.5–11	7.9–13	6.3–18	4.7–14	2.8–4.3	3.7–6.9	2.2–3.0	8.9–16
123789-HxCDD	1.2±1.6	1.4±0.64	2.4±1.1	2.1±0.30	1.8±0.34	1.6±0.34	1.0±0.28	1.7±0.33
	0.36–4.5	1.0–2.2	1.3–4.0	1.8–2.6	1.5–2.2	1.2–1.9	0.75–1.4	1.3–2.3
		3 ND	2 ND	1 ND		2 ND		
123678-HxCDD	14±8.0	20±6.5	18±8.4	16±8.2	14±7.3	9.1±2.9	5.2±0.51	29±4.9
	2.5–27	15–32	9.1–34	11–32	5.1–18	5.8–14	4.5–5.9	22–34
123478-HxCDD	3.3±2.2	5.0±1.8	4.9±2.8	4.5±2.4	2.4±0.66	2.4±0.63	1.5±0.30	9.1±2.1
	0.86–7.3	3.6–8.6	2.3–10	2.9–9.2	1.7–2.8	1.7–3.1	1.1–2.0	5.9–12
1234678-HpCDD	13±17	16±13	20±7.8	23±7.3	18±6.7	20±7.8	10±3.6	26±6.6
	2.9–48	6.6–41	7.2–30	17–37	12–25	14–36	5.7–14	19–35
12346789-OCDD	12±17	10±6.0	19±6.8	24±4.7	18±13	28±17	13±8.5	13±11
	2.6–46	5.9–22	6.9–27	16–28	8.5–33	19–61	3.9–27	5.8–35

[a] Values were rounded and represent only two significant figures

[b] TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin

Table 2.9. Concentrations of furan and dioxin congeners in eastern bluebird eggs[a] collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Values[b] (ng/kg ww) are given as the arithmetic mean ± SD over the range.

	Reference Areas		Study Areas					
	R-1	R-2	T-3	T-4	T-5	T-6	S-7	S-9
Chemical[c]	*n*=6	*n*=6	*n*=6	*n*=6	*n*=3	*n*=6	*n*=2	*n*=0[d]
2378-TCDF	6.1		11±5.6	22±12	86±110	71±45		
		0.44–77	4.2–18	11–40	11–210	8.3–120	6.4–46	
	5 ND	4 ND						
23478-PeCDF	2.4±2.2	5.2±3.3	150±100	160±52	270±100	480±310		
	1.0–6.2	2.6–12	14–290	120–270	190–380	170–970	10–76	
	1 ND							
12378-PeCDF		13	4.5±2.6	6.0±2.0	17±19	19±8.1		
			2.3–7.5	3.0–8.7	3.4–38	5.1–27	2.5–9.7	
	6 ND	5 ND	1 ND					
234678-HxCDF	2.0±2.1	1.6±0.43	9.2±5.3	8.6±3.2	9.3±1.9	19±9.1		
	0.72–5.1	0.95–2.2	1.6–16	5.4–15	7.3–11	7.9–30	0.72–7.6	
	2 ND							
123789-HxCDF	6 ND	6 ND	6 ND	6 ND	3 ND	6 ND	2 ND	
123678-HxCDF	2.0±1.9	2.3±1.2	21±14	23±9.5	27±10	45±19		
	0.87–4.8	1.7–4.7	2.3–39	16–42	18–38	26–75	1.5–15	
	2 ND							
123478-HxCDF	3.3±4.3	3.1±1.4	84±57	62±14	64±34	160±100		
	0.63–8.3	2.3–6.0	6.1–150	41–81	31–99	76–350	2.3–39	
	3 ND							
1234789-HpCDF					1.8			
			2.5–2.7	1.6–2.8		1.7–3.2		
	6 ND	6 ND	4 ND	4 ND	2 ND	4 ND	2 ND	

1234678-HpCDF	4.1±3.6	5.6±3.9	58±35	54±16	37±4.0	78±69	
	1.8–8.3	2.6–13	5.7–83	37–82	32–40	20–170	3.3–23
	3 ND						
12346789-OCDF		0.78±0.49	13±4.8	17±9.9	9.2±4.2	19±19	2.2
		0.47–1.4	5.3–17	7.6–36	4.7–13	3.2–45	
	6 ND	3 ND	1 ND			1 ND	1 ND
2378-TCDD	3.0±2.0	7.8±2.7	7.3±2.5	9.9±1.5	11±3.7	9.9±5.6	
	1.1–6.6	4.0–11	4.3–11	7.1–11	8.1–15	4.6–16	1.6–2.1
12378-PeCDD	5.0±6.0	8.3±2.2	7.4±2.1	7.8±2.0	9.8±1.4	7.9±3.5	3.4
	1.5–17	4.2–11	4.9–9.8	6.1–11	8.9–11	3.0–12	
							1 ND
123789-HxCDD	5.1±5.6	3.1±1.5	3.1±1.1	3.8±1.5	3.9±0.89	6.2±1.4	
	1.7–12	0.90–5.4	1.9–4.3	2.5–6.7	3.1–4.8	4.7–7.5	
	3 ND		1 ND			3 ND	2 ND
123678-HxCDD	10±16	15±6.0	16±5.5	17±3.0	23±3.9	20±9.1	
	2.4–43	8.9–24	8.1–23	14–21	20–27	8.1–29	4.6–5.4
123478-HxCDD	4.2±6.2	5.4±1.5	5.4±1.7	4.3±0.82	6.0±0.30	6.0±2.3	
	1.2–17	3.2–7.6	3.4–7.1	2.9–5.0	5.6–6.2	3.4–8.5	1.7–2.6
1234678-HpCDD	20±27	34±33	61±31	73±39	69±1.7	89±66	
	4.9–73	10–100	20–96	52–150	68–71	23–180	7.0–19
1234678-HpCDD	20±27	34±33	61±31	73±39	69±1.7	89±66	
	4.9–73	10–100	20–96	52–150	68–71	23–180	7.0–19
12346789-OCDD	18±20	38±29	130±81	180±150	120±6.8	250±240	
	5.6–57	12–92	27–240	89–490	120–130	49–630	17–42

[a] Eggs include both live and addled eggs

[b] Values were rounded and represent only two significant figures

[c] TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF =

Table 2.9. (Continued)

heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin

[d] No samples collected

Table 2.10. Concentrations of furan and dioxin congeners in eastern bluebird nestlings collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. Values[a] (ng/kg ww) are given as the arithmetic mean ± SD over the range.

	Reference Areas		Study Areas					
	R-1	R-2	T-3	T-4	T-5	T-6	S-7	S-9
Chemical[b]	*n*=6	*n*=6	*n*=6	*n*=5	*n*=0[c]	*n*=5	*n*=2	*n*=0
2378-TCDF	1.2	0.57±0.10	63±120	6.0±5.1		130±140		
		0.44–0.69	1.9–310	2.1–12		13–370	2.1–17	
	5 ND	2 ND						
23478-PeCDF	0.57±0.20	1.6±0.39	200±180	96±56		630±440		
	0.32–0.91	1.1–2.0	30–510	42–160		190–1200	41–47	
12378-PeCDF	0.12		33±64	3.3±2.3		66±59		
		0.080–0.42	1.4–160	1.5–6.6		7.6–160	1.3–5.7	
	5 ND	4 ND						
234678-HxCDF	0.37±0.092	0.64±0.15	8.2±5.3	5.1±2.0		21±12		
	0.27–0.49	0.52–0.85	2.9–15	2.6–7.7		6.2–36	2.0–2.5	
	1 ND	2 ND						
123789-HxCDF						0.92±0.16		
						0.75–1.1		
	6 ND	6 ND	6 ND	5 ND		2 ND	2 ND	
123678-HxCDF	0.42±0.19	0.68±0.18	16±11	12±6.6		49±35		
	0.23–0.69	0.46–0.89	5.3–34	4.4–19		11–97	4.1–4.7	
	1 ND	2 ND						
123478-HxCDF	0.35±0.076	1.1±0.37	65±61	47±22		250±170		
	0.23–0.41	0.55–1.6	17–180	22–77		72–470	22–24	
	1 ND							
1234789-HpCDF			2.5±1.9	1.5±0.67		2.6±1.4		

Table 2.10. (Continued)

			1.1–4.7	0.76–2.0		0.71–4.6	
	6 ND	6 ND	3 ND	2 ND			2 ND
1234678-HpCDF	1.5±1.4	1.8±0.75	35±20	34±15		38±15	
	0.27–4.0	0.58–2.8	21–71	13–53		12–50	8.0–16
12346789-OCDF	1.1	0.39	12±9.7	11±4.6		10±6.2	
			3.5–30	4.1–17		2.7–17	3.2–4.6
	5 ND	5 ND					
2378-TCDD	0.69±0.41	2.8±1.4	5.0±1.6	3.3±1.0		2.6±0.54	
	0.22–1.4	0.80–5.0	2.5–6.9	2.1–4.4		2.1–3.5	1.1–1.3
12378-PeCDD	0.96±0.26	2.6±0.85	5.3±2.0	3.2±0.61		2.7±0.34	
	0.73–1.4	1.1–3.5	2.6–7.8	2.5–3.8		2.4–3.2	1.2–1.5
123789-HxCDD	0.79±0.45	0.88±0.18	2.2±0.76	1.2±0.57		1.3±0.54	0.53
	0.44–1.6	0.63–1.1	1.3–3.2	0.65–2.2		0.92–2.1	
	1 ND	1 ND				1 ND	1 ND
123678-HxCDD	1.9±0.90	3.2±1.0	10±2.4	7.2±2.4		5.7±0.99	
	1.0–3.6	1.5–4.7	6.3–14	3.8–10		4.7–7.2	2.5–2.5
123478-HxCDD	0.85±0.18	1.5±0.50	3.0±1.0	2.0±0.66		1.9±0.21	
	0.63–1.2	0.62–2.1	1.5–4.4	1.6–3.2		1.6–2.2	0.90–1.1
1234678-HpCDD	7.9±7.7	7.7±3.6	43±11	31±13		29±14	
	2.0–23	2.4–13	25–54	12–47		16–53	9.3–12
12346789-OCDD	12±9.4	19±17	110±51	91±40		100±68	
	3.2–27	5.8–52	62–200	29–140		40–220	26–36

[a] Values were rounded and represent only two significant figures

[b] TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin

Table 2.10. (Continued)

[c] No samples collected

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CHAPTER 3

Dietary exposure of three passerine species to PCDD/DFs from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA

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Abstract

Dietary exposure of house wrens (*Troglodytes aedon*), tree swallows (*Tachycineta bicolor*), and eastern bluebirds (*Sialia sialis*) to polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) near Midland, Michigan (USA) was evaluated based on site-specific data, including concentrations of residues in bolus samples and individual invertebrate orders, and dietary compositions by study species. Site-specific dietary compositions for the three species were similar to those reported in the literature, but differed in the relative proportions of some dietary items. Oligocheata (non-depurated) and Brachycera (Diptera) contained the greatest average concentrations of ΣPCDD/DFs of the major site-specific dietary items collected via food web-based sampling. Average ingestion of ΣPCDD/DFs from site-specific bolus-based and food web-based dietary concentrations for nestlings at study areas (SAs) was 6- to 20-fold and 2- to 9-fold greater than at proximally located reference areas (RAs), respectively. Average ingestion of total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) from site-specific bolus-based and food web-based dietary concentrations for nestlings at SAs was 31- to 121-fold and 9- to 64-fold greater than at proximally located RAs, respectively. Estimates of ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ tissue concentrations based on nestling dietary exposures were greater than those measured. Plausible explanations include nestling metabolism of 2,3,7,8-tetrachlorodibenzofuran and assimilation rates of less than the 70% assumed to occur over the nestling growth period. Profiles of the relative concentrations of individual PCDD/DF congeners in samples of invertebrates and bolus at SAs on the Tittabawassee River downstream of the source of

contamination were dominated by 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (22 to 44%) and 2,3,7,8-tetrachlorodibenzofuran (18 to 50%).

Keywords: furans; dioxins; bolus; birds; TEQs; invertebrates

Introduction

Site-specific dietary exposure to polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) was determined for three cavity-nesting, insectivorous passerine species downstream of Midland, Michigan (USA). Tree swallows (*Tachycineta bicolor*), which eat primarily emergent aquatic invertebrates (McCarty 1997; McCarty and Winkler 1999; Mengelkoch et al. 2004), have been shown to have exposure links to contaminated sediments (Custer et al. 1998; Echols et al. 2004; Maul et al. 2006; Neigh et al. 2006c; Papp et al. 2007; Smits et al. 2005). House wrens (*Troglodytes aedon*) and eastern bluebirds (*Sialia sialis*) have been used to assess the contaminant exposure of terrestrial insectivores at locations with contaminated soils (Neigh et al. 2006a). Both species primarily feed on terrestrial invertebrates (Beal 1915; Guinan and Sealy 1987), however they have different habitat preferences and foraging strategies which may influence contaminant exposure.

PCDFs, and to a lesser extent PCDDs, are present at elevated concentrations in the Tittabawassee and Saginaw rivers downstream of Midland, Michigan, and appear to have originated from the production, storage, and disposal of organic chemicals prior to the establishment of modern waste management protocols (USEPA, Amendola and Barna 1986). Total concentrations of PCDD/DFs (ΣPCDD/DFs) in floodplain soils and sediments, from the study area (SA), ranged from 1.0×10^2 to 5.4×10^4 ng/kg dw, while mean ΣPCDD/PCDF concentrations in soils and sediments in the reference area (RA) upstream of Midland were 10- to 20-fold less (Hilscherova et al. 2003).

Usually, PCDD/DFs, polychlorinated biphenyls (PCBs), and similar chlorinated hydrocarbons occur in the environment as mixtures. The mixture of chlorinated hydrocarbons in the SA is dominated by a few PCDF congeners, which makes it distinctive compared to other locations contaminated with PCB mixtures or PCDDs (Custer et al. 2002; Custer et al. 2005; Custer et al. 2006; Froese et al. 1998; Harris and Elliott 2000; Neigh et al. 2006b; Neigh et al. 2006c; Secord et al. 1999; Shaw 1983; Smits et al. 2005; Spears et al. 2008). 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 (ATS 2007; ATS 2009).

PCDFs and related chlorinated hydrocarbons are persistent and lipophilic (Mandal 2005), and have a great potential to accumulate through the food web (Blankenship et al. 2005; Custer et al. 1998; Kay et al. 2005; Maul et al. 2006; Russell et al. 1999; Smits et al. 2005; Wan et al. 2005). Because of the elevated soil and sediment concentrations in the SA (Hilscherova et al. 2003), and low avian dietary exposure thresholds (Custer et al. 2005; Eisler 2000; Nosek et al. 1992), the authors investigated the potential for the accumulation of PCDFs and PCDDs from invertebrates to resident insectivorous birds.

Complex relationships exist between site-specific contaminant concentrations, dietary exposure pathways, and resulting tissue concentrations. In particular, concentrations of chlorinated hydrocarbons and the congener profiles of the relative concentrations of congeners have been shown to be site-specific (Custer and Read 2006; Maul et al. 2006; Papp et al. 2007). Site-specific dietary composition is related to the prevailing invertebrate abundance (Custer et al. 2005; Echols et al. 2004; Neigh et al. 2006a; Nichols et al. 1995; Quinney and Ankney 1985; Smits et al. 2005; Wayland et al. 1998) and the timing of nest initiation (Custer et al. 1998; Maul et al. 2006; Papp et al. 2007), especially for tree swallows that prey primarily on emergent aquatic invertebrates to feed nestlings (Blancher and McNicol 1991; Johnson and Lombardo 2000; McCarty and Winkler 1999). Site-specific residue concentrations in egg and nestling tissues have been studied for a variety of chlorinated hydrocarbons and species (Ankley et al. 1993; Bishop et al. 1995; Custer et al. 1998; Custer et al. 2003; Custer et al. 2005; Froese et al. 1998; Henning et al. 2003; Neigh et al. 2006a; Neigh et al. 2006b; Neigh et al. 2006c; Spears et al. 2008). Fewer studies have investigated accumulation via the diet or determined uptake rates (Echols et al. 2004; Nichols et al. 1995; Nichols et al. 2004).

The primary objectives of this study were to characterize dietary exposure of adults and nestlings of three insectivorous passerine species representing different feeding guilds with different pathways of exposure to PCDD/DFs, and to compare concentrations and congener profiles of composited site-specific food web-based dietary samples to bolus samples. Bolus samples are actual dietary samples collected by the adult birds for comparison to the dietary concentrations estimated using residue concentrations of invertebrates collected at each site. By comparing the bolus-based dietary samples with the food web-based dietary estimates based on site-specific percent dietary compositions and concentrations in food web items, it can be determined whether invertebrates collected in the BSAs are truly representative of the congener profile and concentrations fed to on-site nestlings. A secondary objective was to determine how well estimates of dietary exposure based on site-specific dietary composition and accumulation factors from the literature correspond to measured concentrations in nestlings (Fredricks et al. 2009a).

The study examined four endpoints: 1) site-specific dietary composition by species; 2) concentrations of ΣPCDD/DF and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents ($TEQ_{WHO\text{-}Avian}$) based on World Health Organization (WHO) TCDD equivalency factors for birds ($TEF_{WHO\text{-}Avian}$) (van den Berg et al. 1998) in food web-based composited invertebrate samples and bolus samples for each species; 3) spatial and species-specific trends in concentrations; and 4) patterns of relative concentrations of individual congeners. Comparisons of congener-specific concentrations stratified by feeding pathway and area were made to provide insight into the contaminants source and species-specific exposures. This information can be used to estimate exposure of other species to the contaminants.

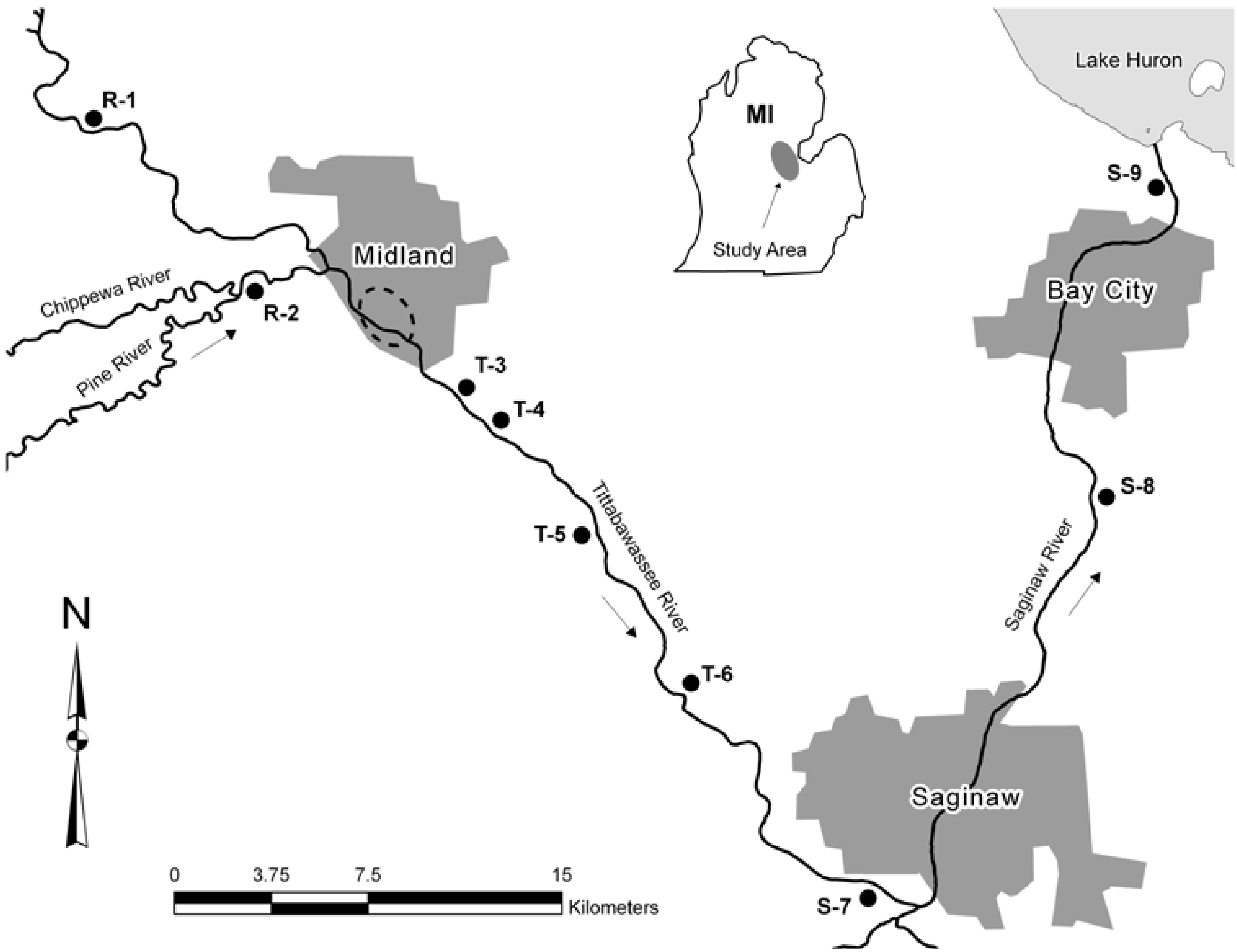
The portion of the study described here focuses on dietary-based exposure analyses. Results for tissue-based exposure (Fredricks et al. 2009a) and nest productivity (Fredricks et al. 2009b) are reported elsewhere. The incorporation of three lines of evidence into a multiple lines of evidence assessment of ecological risk (Fairbrother 2003) will provide site-specific information to make informed decisions about the potential impact(s) of contaminants and will aid in both the planning and evaluation of effective remedial actions.

Methods

Site description

Study locations were selected on the Tittabawassee, Chippewa, and Saginaw rivers in the vicinity of Midland, Michigan (Figure 3.1). Nest boxes were placed and prey items were collected from within the 100-year floodplain of the individual rivers. Two RAs

Figure 3.1. 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 S-9) were monitored from 2005–2007. Direction of river flow is designated by arrows; suspected source of contamination is enclosed the dashed oval.


R-1
Midland
Chippewa River
R-2
Pine River
T-3
T-4
T-5
Tittabawassee River
T-6
MI
Study Area
Lake Huron
S-9
Bay City
S-8
Saginaw River
Saginaw
S-7
N
0
3.75
7.5
15
Kilometers

were located upstream of the putative sources of PCDD/DFs (Hilscherova et al. 2003) on the Tittabawassee (R-1) and Chippewa (R-2) Rivers (Figure 3.1). Study areas downstream of the putative sources of PCDD/DFs include approximately 72 km of free flowing river from the upstream boundary defined as the low-head dam near Midland, Michigan through the confluence of the Tittabawassee and Saginaw rivers to where the Saginaw River enters Saginaw Bay. The SAs within the Tittabawassee River area included four locations (T-3 to T-6) approximately equally spaced, and locations (S-7 to S-9) which are at the initiation, median, and terminus of the Saginaw River. The seven SAs (T-3 to S-9) were selected based on the necessity to discern spatial trends, ability to gain access privileges, and maximal receptor exposure potential based on floodplain width and measured soil and sediment concentrations (Hilscherova et al. 2003). Nest box trails within RAs and SAs each contained between 30 and 60 nest boxes and spanned a continuous foraging area of between 1 and 3 km of river. S-8 was an exception and was only used for sediment and dietary food web sampling. No studies of birds were conducted at this location.

Tissue collections

Detailed site descriptions and protocols for collecting and handling samples of eggs and nestlings have been previously presented (Fredricks et al. 2009a). Briefly, nest boxes were monitored daily from mid-April through the end of the breeding season (2005–2007). Eggs and nestlings were collected for quantification of PCDD/DFs. Nest boxes were randomly selected from the active nest boxes at a given location for either live egg or nestling collections but not both. Abandoned and addled eggs were salvaged opportunistically. Fresh and addled eggs were collected from 49 house wren, 50 tree swallow, and 35 eastern bluebird nests during the 2005–2007 breeding seasons. Nestlings were collected from 48 house wren nests at 10-d post hatch, while nestlings were collected from 45 tree swallow and 30 eastern bluebird nests at 14-d post hatch. Adult passerines were not collected for quantification of residues because part of the research focused on a long-term adult and nestling survival and recruitment.

Measurement endpoints associated with productivity (Fredricks et al. 2009b) included egg mass, clutch size, hatching success, fledgling success, total productivity, and nestling growth. Masses of nestlings were made 3-, 6-, 9-, and 10-d post hatch for house wrens, and 4-, 8-, 12-, and 14-d post hatch for tree swallows and eastern bluebirds. Species- and site-specific nestling growth curves were utilized in the dietary exposure assessments presented.

Food web sampling

Collection of invertebrates occurred at nine predetermined biological sampling areas (BSAs) that were located within the RA and SA locations. Each BSA included two 30 m × 30 m grids proximal to the river bank, one for terrestrial sampling and one for aquatic sampling. Study area BSA locations were selected based on maximizing the potential for collecting food items with the greatest contaminant concentrations for a given nest box trail given the available soil and sediment data.

Site-specific invertebrate sampling took place 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. Temporal variation in the composition of sampled species and potential concentration differences were accounted for by collecting samples at multiple times throughout the breeding season (mid-May, June, and August). During each sampling period all samples for that period were collected within a six-day window to minimize temporal variation. All collected invertebrates were transferred to a labeled, chemically clean glass jar (I-CHEM, Rockwood, TN). Sampling was terminated upon the collection of approximately 5 g or more biomass per order. Samples were stored on wet ice while in the field and transferred to a -20 ºC freezer until categorization and homogenization. Sampling methods were designed to target aquatic emergent insects (AEIs), benthic invertebrates (BIs), and terrestrial invertebrates (TIs) through specific methodologies that maximized invertebrate biomass and diversity of collected samples.

Collection of aquatic emergents

Aquatic emergent insects were collected with a modified form of aerial trap called a light screen, which targeted flying adult aquatic insects. Collections began at dusk and continued for 1 to 3 h at each location or until invertebrate encounter frequency declined. A metal halide light attracted invertebrates to a white sheet and invertebrates were collected in Insect Vac Collection Chambers and Insect Vacs (Bioquip Products, Rancho Dominguez, CA). Aerial nets were used to collect airborne invertebrates that did not land on the sheet.

Collection of benthic invertebrates

Aquatic macroinvertebrates were collected by use of several different methods, depending on the habitat type at each collection location. Sampling methods used included individual picking with forceps from submerged woody debris; cobble and aquatic vegetation; sieve bucket; and D-frame kick net. Sampling occurred during daylight hours at all locations.

Collection of soil, terrestrial plants and invertebrates

Sampling techniques used to collect soil and terrestrial plants and invertebrates at each location employed a four-tiered approach: 1) a 1 m x 1 m plot was randomly selected from within a BSA and one or more composite plant samples were collected based on plant diversity; 2) soil was excavated by hand-digging to a depth of 15 cm; a soil composite sample and all resident Oligocheata were collected; 3) forceps were used to collect surface-dwelling terrestrial invertebrates from the soil surface and leaf litter; and 4) aerial or plant perching invertebrates were collected using sweep nets and/or aerial invertebrate nets. Oligocheata were rinsed in distilled water prior to chemical analyses.

Sorting of invertebrates

Invertebrates were categorized taxonomically to the order level for each life stage collected during each sampling period per site. For emergent insects the order Diptera was divided into the sub-orders Nematocera (primarily aquatic) and Brachycera (primarily terrestrial) to account for possible exposure differences during the larval life stages. Samples were then homogenized and stored at -20 ºC until extraction.

Sampling of food bolus

Dietary food items were collected as bolus samples from nestling house wrens, tree swallows, and eastern bluebirds following the methods described by Mellott and Woods (1993). Briefly, nestlings between the ages of 3- to 9-d post-hatch for house wrens or 4- to 12-d post-hatch for tree swallows and eastern bluebirds were fitted with a black electrical cable-tie (10.2 cm size) around the base of their neck. The use of a cable-tie allows finite adjustments and maximizes collection potential while minimizing bias associated with loss of smaller invertebrates (McCarty and Winkler 1991) and nestling mortalities. Ligatured nestlings were observed for 20–30 s to observe for unnatural behaviors such as pronounced gasping or struggling. Bolus sampling occurred throughout the day to account for any temporal variation in invertebrate abundance or activity. Bolus samples were collected from nestlings 1 h after ligature application. Bolus material found in the nest cavity was also collected. Nests were randomly selected for bolus sampling and nests were not sampled on consecutive days. Nest boxes were concurrently sampled for nestling growth and reproductive parameters. Both this and other studies have shown that there is no discernable difference in growth of nestlings from boxes that do or do not have samples of boluses collected (Neigh et al. 2006a). Additionally, adult tree swallows deliver food to the nest approximately18 times/h throughout the day (McCarty 2002) so dietary sampling should only represent a small portion of a nestlings' daily food requirements.

Estimation of site-specific diet

The site-specific diet was based on the relative proportion of the total mass represented by each invertebrate order identified in the bolus samples. Invertebrates in each bolus sample were classified to order and the total number and mass of each order was recorded for each sample. The site-specific diet obtained from the bolus samples was used in the estimate of food web-based dietary exposure described subsequently.

Bolus sample residue analysis

Where possible, bolus samples were recombined for residue analyses based on the clutch from which they were collected. For clutches lacking sufficient biomass of bolus for residue analyses, bolus samples were combined within a study area until the necessary biomass was obtained. Due to limited biomass from house wren bolus samples at R-1, samples from 2005 and 2006 were combined.

Identification and quantification of PCDD/DF congeners

Concentrations of seventeen 2,3,7,8-substituted PCDD/DF congeners were measured in all invertebrate and bolus samples that had enough biomass to meet analytical requirements. PCDD/DFs were quantified in accordance with EPA Method 8290 with minor modifications (U.S. Environmental Protection Agency (USEPA) 1998). Briefly, samples were homogenized with anhydrous sodium sulfate and Soxhlet extracted in hexane:dichloromethane (1:1) for 18 h. Before extraction, known amounts of ^{13}C-labeled analytes were added to each sample as internal standards. The extraction solvent was exchanged to hexane and the extract was concentrated to 10 mL. Ten percent of this extract was removed for lipid content determination. Extracts were initially purified by treatment with concentrated sulfuric acid. The extract was then passed through a silica gel column containing silica gel and sulfuric acid silica gel and eluted with hexane. The extract received additional column chromatography by elution through acidic alumina, which resulted in two fractions. The first elution contained most of the PCBs and pesticide compounds, while the second fraction contained dioxins and furans. This second fraction was then passed through a carbon column packed with 1 g of activated carbon-impregnated silica gel. The first fraction of the silica gel, eluted with various solvent mixtures, was combined with the first fraction from the acidic alumina column and was retained for possible co-contaminant analyses. The second fraction of the silica gel, eluted with toluene, contained the 2,3,7,8-substituted PCDD/DFs. Components were analyzed using HRGC-HRMS, a Hewlett-Packard 6890 GC (Agilent Technologies, Wilmington, DE) connected to a MicroMass® high resolution mass spectrometer (Waters Corporation, Milford, MA). PCDF and PCDD congeners were separated on a DB-5 capillary column (Agilent Technologies, Wilmington, DE) coated at 0.25 μm (60 m x 0.25 mm i.d.). The mass spectrometer was operated at an EI energy of 60 eV and an ion current of 600 μA. Congeners were identified and quantified by use of single ion monitoring (SIM) at the two most intensive ions of the molecular ion cluster. Concentrations of 2,3,7,8-tetrachlorodibenzofuran (TCDF) were confirmed by using a DB-225 (60 m x 0.25 mm i.d., 0.25 μm film thickness) column (Agilent Technologies, Wilmington, DE). Chemical analyses included pertinent quality assurance practices, including matrix spikes, blanks, and duplicates.

Dietary exposure calculations

Dietary exposures of adult and nestling house wrens, tree swallows, and eastern bluebirds were estimated using the U.S. Environmental Protection Agency (USEPA) Wildlife Exposure Factors Handbook (WEFH; USEPA 1993) equations for passerine birds. Major factors influencing dietary exposure included daily food intake rate [IR; g ww food/g body weight (BW)/d], nestling or adult mass (BW), dietary concentrations (C), and uncertainty factors associated with availability of contaminants for absorption to the bird from the diet. USEPA WEFH equation 3-4 was used to calculate IR. Other equations to estimate food ingestion rate based on bioenergetics techniques (Nichols et al. 1995; Nichols et al. 2004) resulted in lesser estimates of intake, which could lead to an underestimate of potential exposure.

Site-specific mean (± standard deviation) adult masses used to calculate IR for house wrens, tree swallows, and eastern bluebirds were 11.2 g (±1.3; n=349), 20.9 g (±1.6; n=235), and 31.2 g (±2.1; n=83), respectively. Species-specific nestling growth curves (Fredricks et al. 2009b) were used to estimate daily IR over the nesting period. Species-specific dietary concentrations in food items were estimated using two methods: 1) food web-based diet: multiplying study-specific dietary compositions for major (>1% by mass) prey items by respective area-specific (R-1 to R-2; T-3 to T-6; S-7 to S-9) average, minimum, and maximum concentrations of ΣPCDD/DFs in associated prey items for each study species, and 2) bolus-based diet; area-specific average, minimum, and maximum concentrations from actual bolus samples collected from nestlings of each species studied. Minimum and maximum concentrations were chosen to cover the range of possible invertebrate concentrations found on site, which the authors expected to include the worst-case scenario for dietary exposure.

Assimilation efficiency (AE) has been estimated to be 70 to 90%, based on the results of a study of PCB exposures 70% was used (Nichols et al. 2004). Potential average daily dose (ADD_{pot}; ng/kg/d) was calculated using equation 4-3 (USEPA 1993) assuming that 100% of the foraging range for each species was within the associated study area (McCarty and Winkler 1999). Total ingestion of ΣPCDD/DFs was estimated for house wren nestlings up to 10 d post-hatch, and for tree swallow and eastern bluebird nestlings up to 14 d post-hatch, since these are the respective days that nestlings were collected. Predictions of nestling body burdens incorporated maternal transfer of contaminants to the nestlings via the egg. Dietary exposure estimates apply only to the nesting period for both adults and nestlings because foraging habits and range are likely more variable outside the nesting period.

Statistical analyses

Total concentrations of the seventeen 2,3,7,8-substituted PCDD/DF congeners are reported as the sum of all congeners (ng/kg wet weight (ww)). Individual congeners that were less than the limit of quantification were assigned a value of half the sample method detection limit. Concentrations of $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) were calculated for PCDD/DFs by summing the product of the concentration of each congener, multiplied by its avian $TEF_{WHO\text{-}Avian}$ (van den Berg et al. 1998). Samples of invertebrates from the food web were composites of all individuals of an order collected per location per sampling period. Arithmetic means (range) are presented (Tables 3.1 and 3.2).

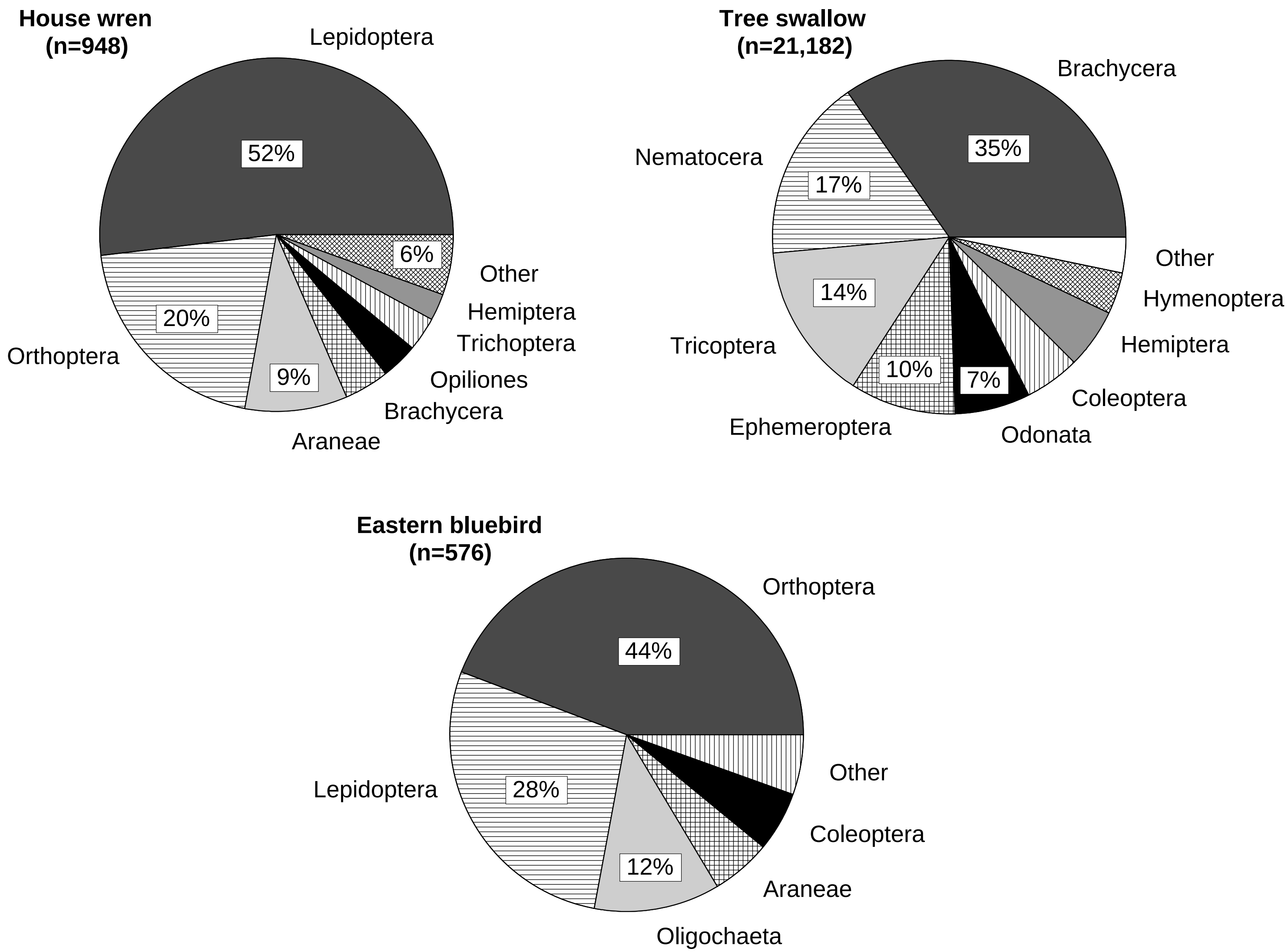
Statistical analyses were performed using SAS® software (Release 9.1; SAS Institute Inc., Cary, NC, USA). Prior to the use of parametric statistical procedures, normality was evaluated using the Shapiro-Wilks test and the assumption of homogeneity of variance was evaluated using the Levene's test. Values that were not normally distributed were transformed using the natural log (ln) of (x+1) before statistical analyses. Concentrations in bolus samples by species studied and invertebrates by orders were tested for effects of study area on concentrations of both ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$. PROC GLM was used to make comparisons for invertebrate orders when represented in the RA, Tittabawassee River SA, and Saginaw River SA. When significant differences among locations were indicated, Bonferroni's *t*-test was used make comparisons between study areas. PROC TTEST was used to make comparisons between bolus samples by species studied collected at RAs and SAs. Differences were considered to be statistically significant at $p < 0.05$.

Results

Dietary composition

Sampling of boluses resulted in the collection of at least one invertebrate from nestlings in 86%, 93%, and 86% of attempted bolus sampling events at selected house wren (n=135), tree swallow (n=96), and eastern bluebird (n=51) nesting attempts, respectively. Sampling intensity and success were greatest in 2006, in part because of additional collection locations (S-7 and S-9) combined with greater sampling proficiency. The greatest number of individual invertebrates collected was from tree swallow nestlings, while the fewest were from eastern bluebird nestlings (Figure 3.2). Eastern

Figure 3.2. Percent mass dietary compositions for house wrens, tree swallow, and eastern bluebirds collected in 2004–2006 from Chippewa, Tittabawassee, and Saginaw River floodplains near Midland, Michigan, USA. Percentages for orders over 5% and invertebrate sample size by species are indicated.


House wren
(n=948)
Lepidoptera
52%
6%
Other
Hemiptera
Trichoptera
Opiliones
Brachycera
Araneae
9%
20%
Orthoptera
Tree swallow
(n=21,182)
Brachycera
35%
Nematocera
17%
Other
Hymenoptera
Hemiptera
Coleoptera
Odonata
7%
10%
Ephemeroptera
Tricoptera
14%
Eastern bluebird
(n=576)
Orthoptera
44%
Other
Coleoptera
Araneae
Oligochaeta
12%
28%
Lepidoptera

bluebirds consume fewer large invertebrates, tree swallows consume greater numbers of small invertebrates, and house wrens are intermediate. Invertebrate orders represented in bolus samples include Amphipoda, Araneae, Coleoptera, Diptera, Dermaptera, Diplopoda, Ephemeroptera, Gastropoda, Hemiptera, Hymenoptera, Isopoda, Lepidoptera, Mantodea, Neuroptera, Odonata, Oligocheata, Opiliones, Orthoptera, Plecoptera, Thysanura and Trichoptera. Stones/grit, vegetation, and egg shell fragments were also identified in bolus samples.

Samples of boluses delivered by adults to nestling house wrens, tree swallows, and eastern bluebirds contained 22,706 individual invertebrates. Site-specific bolus samples contained 95% and 94% terrestrial invertebrates by mass for house wren and eastern bluebird nestlings, respectively, while only 43% of invertebrates sampled from tree swallow nestlings were of terrestrial origin (Figure 3.2). Dipterans composed 52% of bolus samples by mass from tree swallow nestlings (Figure 3.2). Nestling house wrens and eastern bluebirds were fed primarily Lepidoptera and Orthoptera, respectively, and secondarily Orthoptera and Lepidoptera, respectively (Figure 3.2). Oligocheata composed a greater percentage of nestling eastern bluebird diets in several clutches at T-4 in both 2005 and 2006, as opposed to all other locations in which it was only a very minor dietary component. Generally, nestling tree swallows were fed fewer Trichoptera and more Coleoptera at downstream SAs relative to upstream RAs. Otherwise, species-specific dietary compositions between study sites were similar.

ΣPCDD/DFs and TEQs$_{WHO\text{-}Avian}$

Concentrations of ΣPCDD/DFs and TEQs$_{WHO\text{-}Avian}$ in the majority of invertebrate orders were different among study areas, except concentrations of ΣPCDD/DFs were spatially similar for the orders Araneae and Orthoptera. Eight invertebrate orders were classified as "important" based on dietary composition for each species studied, and composed approximately 92%, 76%, and 90% of the invertebrates consumed on-site by nestling house wrens, tree swallows, and eastern bluebirds, respectively (Figure 3.2). Mean concentrations of ΣPCDD/DFs and TEQs$_{WHO\text{-}Avian}$ in primarily aquatic orders were <1- to 10-fold and 5- to 59-fold greater at Tittabawassee River SAs than RAs, respectively, while concentrations at Saginaw River SAs were intermediate. Mean concentrations of ΣPCDD/DFs and TEQs$_{WHO\text{-}Avian}$ in primarily terrestrial orders were <1- to 29-fold and 7- to 220-fold greater at Tittabawassee River SAs than RAs, respectively, while concentrations at Saginaw River SAs were intermediate. Concentrations of ΣPCDD/DFs in the primarily aquatic invertebrate orders of Trichoptera and Nematocera were significantly greater at SAs ($F=75.76$ $p<0.0001$ and $F=10.74$ $p=0.0055$, respectively) compared to RAs, while concentrations in Ephemeroptera were similar. One Ephemeroptera sample from R-1 stood out with a ΣPCDD/DFs concentration of 2.5×10^3 ng/kg, however this difference from other samples was no longer apparent when the sample contaminant burden was evaluated as ΣTEQs$_{WHO\text{-}Avian}$ (3.0×10^1 ng/kg). Concentrations of ΣPCDD/DFs in the primarily terrestrial invertebrate orders of Brachycera, Lepidoptera, and Oligocheata were significantly greater at SAs ($F=5.66$ $p=0.0447$, $F=17.63$ $p=0.0005$, and $F=200.53$ $p<0.0001$, respectively) compared to RAs, while concentrations in Araneae and Orthoptera were similar. Concentrations of $TEQs_{WHO\text{-}Avian}$ for all invertebrate orders that were predominant in the diets were significantly greater at SAs compared to RAs. Maximum concentrations of $TEQs_{WHO\text{-}Avian}$ ranged from 2.3×10^1 ng/kg at T-3 in Nematocera to 1.4×10^3 ng/kg at T-3 in Oligocheata (additional descriptive statistics are presented for predominant orders by site in supplemental information; Tables 3.3 to 3.10).

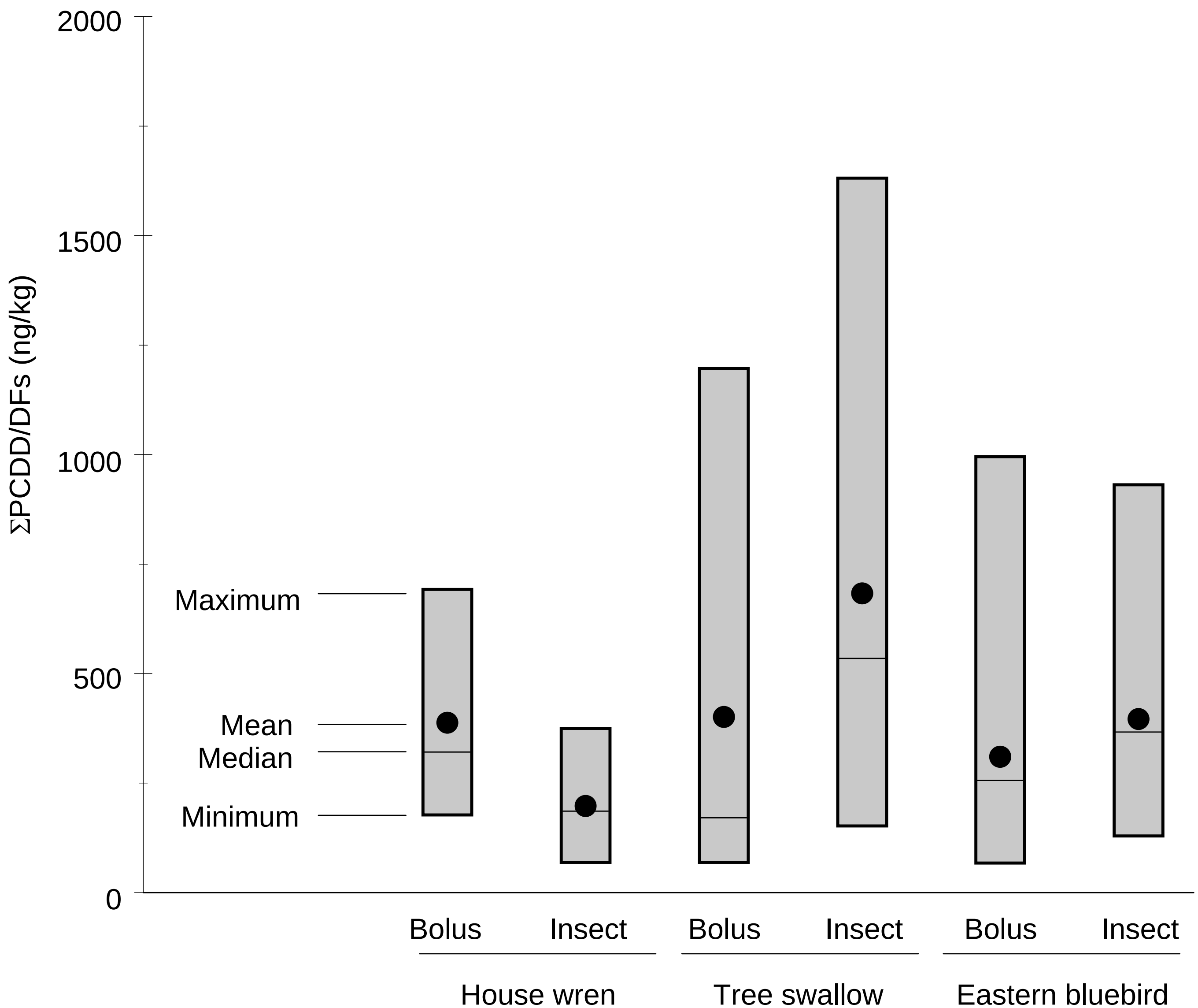
Minimum, mean, and median concentrations of $TEQs_{WHO\text{-}Avian}$ followed a similar trend as ΣPCDD/DFs for all species. Mean concentrations of $TEQs_{WHO\text{-}Avian}$ in food web-based dietary estimates for house wrens, tree swallows, and eastern bluebirds were 53-, 40-, and 72-fold greater at Tittabawassee River SAs than RAs, respectively. The maximum concentration of $TEQs_{WHO\text{-}Avian}$ in food web-based dietary estimates for house wrens, tree swallows, and eastern bluebirds were 1.6×10^2 ng/kg, 7.6×10^2 ng/kg, and 2.3×10^2 ng/kg, respectively.

Concentrations of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ in samples of boluses collected from nestling house wrens, tree swallows, and eastern bluebirds were greater at Tittabawassee River SAs compared to RAs. Mean concentrations of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ in samples of boluses collected from nestlings from all three species ranged from 6- to 21-fold and 41- to 136-fold greater at Tittabawassee River SAs than RAs, respectively. The maximum concentrations of $TEQs_{WHO\text{-}Avian}$ in bolus samples collected from nestling house wrens, tree swallows, and eastern bluebirds occurred at T-6 and were 4.7×10^2 ng/kg, 9.5×10^2 ng/kg, and 5.7×10^2 ng/kg, respectively.

Dietary concentrations of ΣPCDD/DFs in food web-based dietary estimates varied by species when compared to bolus-based dietary estimates. Mean and median concentrations of ΣPCDD/DFs in food web-based dietary estimates were greater for tree swallows (1.7- and 3.1-fold, respectively), similar for eastern bluebirds, and lesser for house wrens (2.0- and 1.7-fold, respectively) compared to those in bolus-based dietary estimates (Figure 3.3).

Relative proportions of mean PCDD/DF concentrations contributed by individual congeners for invertebrates collected during food web sampling varied among sampling areas and among individual invertebrate orders. At RAs mean PCDD/DF congener profiles for invertebrates were dominated by 51 to 78% 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD) compared to SAs that only contained 19 to 47%. Mean PCDD/DF congener profiles for Trichoptera, Nematocera, Ephemeroptera and Brachycera were dominated by 2,3,7,8-TCDF at Tittabawassee River SAs, while Oligocheata averaged only 14% 2,3,7,8-TCDF (Figure 3.4). Five congeners contributed 77 to 90% of relative proportions of PCDD/DF congener concentrations in bolus-based and food web-based dietary estimates at both RAs and SAs. Relative proportions of PCDD/DF concentrations contributed by TCDF were 17% and 14% lower for food web-based dietary estimates compared to bolus-based estimates for tree swallows and eastern bluebirds, respectively, while house wren proportions were similar. Relative proportions of PCDD/DF concentrations in bolus samples for all species varied by site with T-6 generally having 20% greater 2,3,7,8-TCDF than other Tittabawassee River SAs (Figure 3.5).

Figure 3.3. Comparison of ranges, median, and mean ΣPCDD/DF concentrations (ng/kg) of site-specific bolus-based (Bolus) and food web-based (Insect) dietary exposure estimates for house wren, tree swallow, and eastern bluebird diets collected in 2004–2006 at Tittabawassee River study areas (T-3 to T-6) downstream of Midland, Michigan, USA. Insect was calculated from composite samples of invertebrates from food web collections weighted by dietary composition.



ΣPCDD/DFs (ng/kg)
2000
1500
1000
500
0
Maximum
Mean
Median
Minimum
Bolus
Insect
Bolus
Insect
Bolus
Insect
House wren
Tree swallow
Eastern bluebird

Figure 3.4. Percent mean ΣPCDD/DF congener profiles for predominant dietary aquatic and terrestrial invertebrate orders for house wrens, tree swallows, and eastern bluebirds collected during 2004 at Tittabawassee River study areas (T-3 to T-6) downstream of Midland, Michigan, USA. Mean ± SD ΣPCDD/DF concentrations presented by order; scale on the y-axis varies. Sample size indicates number of composite invertebrate samples from food web collections. TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF= hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin.

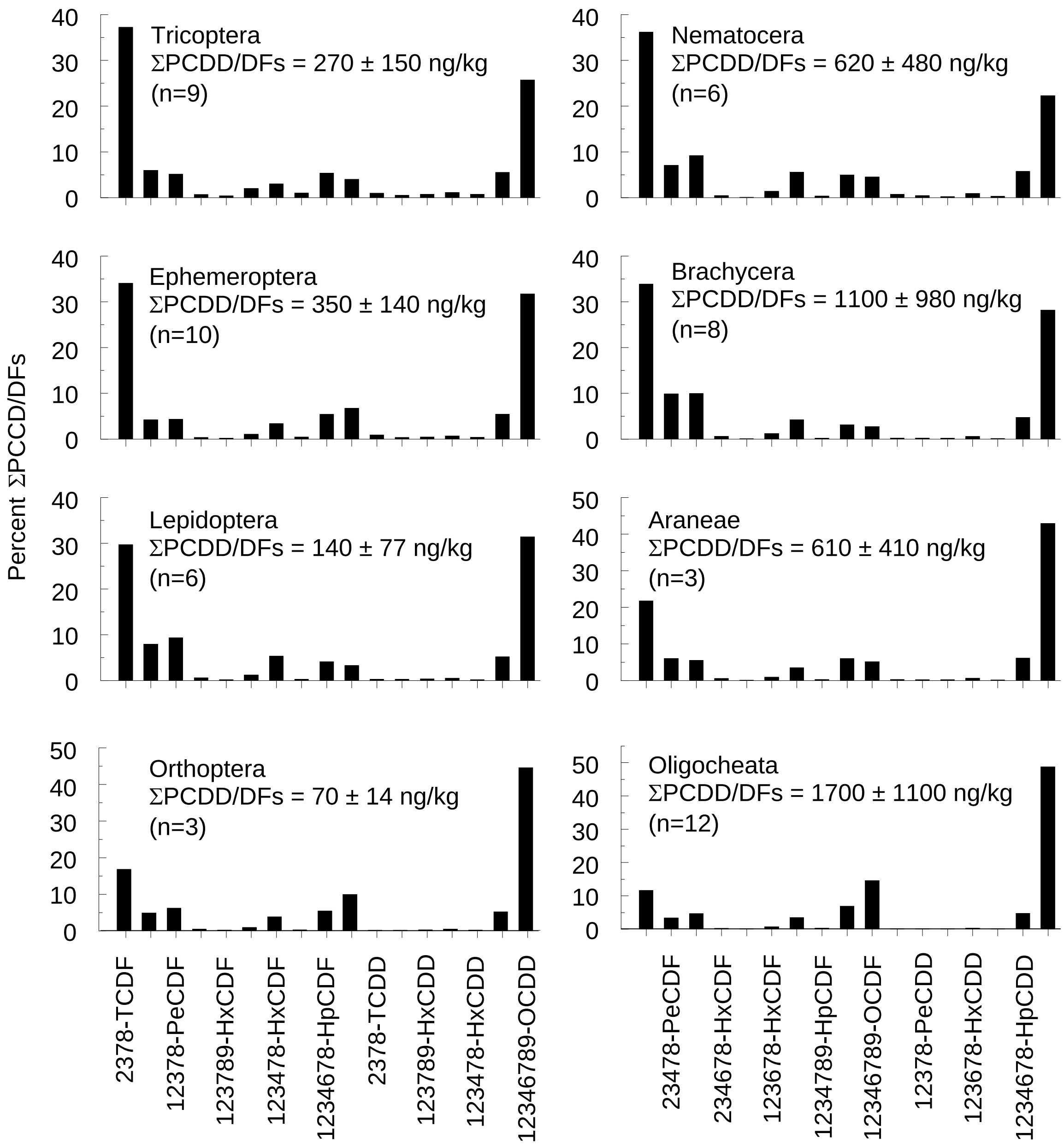
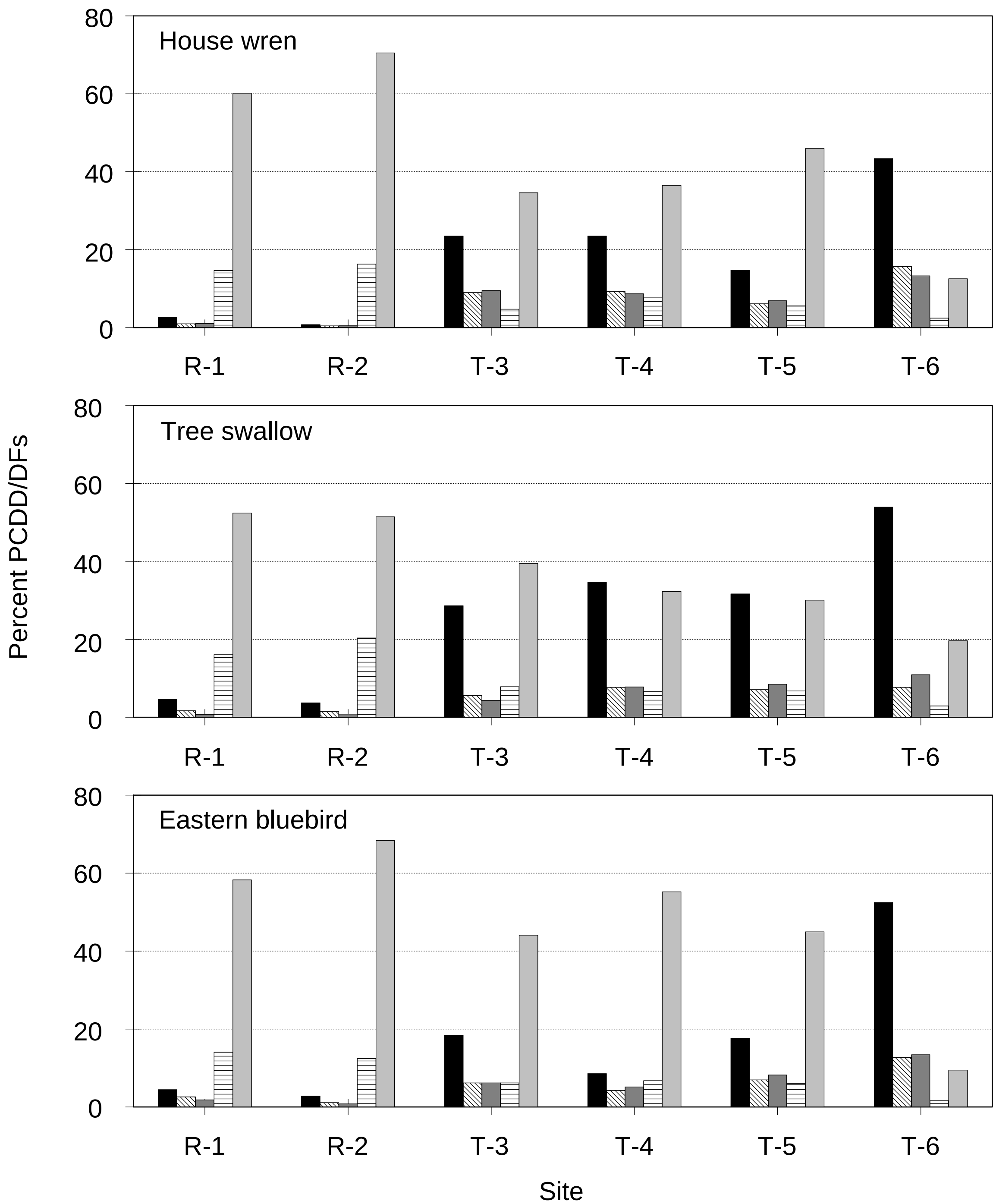

Percent ΣPCCD/DFs
Tricoptera
ΣPCDD/DFs = 270 ± 150 ng/kg
(n=9)
Nematocera
ΣPCDD/DFs = 620 ± 480 ng/kg
(n=6)
Ephemeroptera
ΣPCDD/DFs = 350 ± 140 ng/kg
(n=10)
Brachycera
ΣPCDD/DFs = 1100 ± 980 ng/kg
(n=8)
Lepidoptera
ΣPCDD/DFs = 140 ± 77 ng/kg
(n=6)
Araneae
ΣPCDD/DFs = 610 ± 410 ng/kg
(n=3)
Orthoptera
ΣPCDD/DFs = 70 ± 14 ng/kg
(n=3)
Oligocheata
ΣPCDD/DFs = 1700 ± 1100 ng/kg
(n=12)
2378-TCDF
12378-PeCDF
123789-HxCDF
123478-HxCDF
1234678-HpCDF
2378-TCDD
123789-HxCDD
123478-HxCDD
12346789-OCDD
23478-PeCDF
234678-HxCDF
123678-HxCDF
1234789-HpCDF
12346789-OCDF
12378-PeCDD
123678-HxCDD
1234678-HpCDD

Figure 3.5. Percent mean ΣPCDD/DF congener profiles for dominant congeners in nestling house wren, tree swallow, and eastern bluebird bolus samples by site collected from the Chippewa and Tittabawassee Rivers floodplains in 2004–2007, Midland, Michigan, USA. 2378-tetrachlorodibenzofuran (black); 23478-pentachlorodibenzofuran (angled stripe); 12378-pentachlorodibenzofuran (dark grey); 1234678-heptachlorodibenzo-*p*-dioxin (horizontal stripe); 12346789-octachlorodibenzo-*p*-dioxin (light grey); n=1 for house wren at R-1 and tree swallow at T-5; n=3 for eastern bluebird at T-3 and T-6; n=4 for tree swallow at R-2; and n=2 for all other sites.


House wren
Tree swallow
Eastern bluebird
Percent PCDD/DFs
Site
R-1
R-2
T-3
T-4
T-5
T-6
0
20
40
60
80

Potential average daily dose

ADD_{pot} for adult house wrens, tree swallows, and eastern bluebirds were greater at SAs compared to RAs when based on either bolus sample concentrations or food web-based concentrations for both ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$. ADD_{pot} based on bolus sample concentrations of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ for adult house wrens, tree swallows, and eastern bluebirds were 14- and 136-fold, 6- and 41-fold, and 21- and 125-fold greater at Tittabawassee River SAs than RAs, respectively (Table 3.1). ADD_{pot} based on food web-based concentrations of ΣPCDD/DFs or $TEQs_{WHO\text{-}Avian}$ for adult house wrens, tree swallows, and eastern bluebirds were 5- and 45-fold, 5- and 41-fold, and 9- and 70-fold greater at the Tittabawassee River SAs than RAs, respectively (Table 3.1). ADD_{pot} based on food web-based concentrations of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ for adults of all studied species were less for Saginaw River SAs compared to Tittabawassee River SAs (Table 3.1). ADD_{pot} ranges for concentrations of both ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ overlapped for adult tree swallows based either on bolus-based or food web-based dietary concentrations, whereas ADD_{pot} ranges were greater for bolus-based compared to food web-based dietary concentrations for both house wren and eastern bluebird adults (Table 3.1).

Total ingestion of residues by nestlings

Total ingestion of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ for nestling house wrens, tree swallows, and eastern bluebirds was greater at SAs compared to RAs for either bolus-based or food web-based diets. Average total ingestion of ΣPCDD/DFs and $TEQs_{WHO\text{-}}$

$_{Avian}$ for bolus-based diets for nestling house wrens, tree swallows, and eastern bluebirds were 14- and 163-fold, 6- and 38-fold, and 21- and 114-fold greater at Tittabawassee River SAs than RAs, respectively (Table 3.2). Average total ingestion of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ for food web-based diets for nestling house wrens, tree swallows, and eastern bluebirds were 5- and 39-fold, 5- and 38-fold, and 9- and 55-fold greater at Tittabawassee River SAs than RAs, respectively (Table 3.2). Ranges of total ingestion of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ for nestlings of all species were comparable between bolus-based or food web-based dietary exposures (Table 3.2).

Predictions of nestling body burdens (ΣPCDD/DFs) at fledge based on bolus ADD_{pot} were greater than those measured (Fredricks et al. 2009a) for all species studied regardless of study area. Nestling body burdens equaled the sum of the total ingestion of bolus-based dietary ΣPCDD/DFs over the nesting period and measured average total ΣPCDD/DFs per egg divided by the average nestling mass (Fredricks et al. 2009b). Mean ± SD total pg ΣPCDD/DFs per egg of house wren, tree swallow, and eastern bluebird eggs was $1.2 \times 10^2 \pm 7.8 \times 10^1$ (*n*=12), $1.3 \times 10^3 \pm 7.6 \times 10^2$ (*n*=14), and $2.8 \times 10^2 \pm 2.2 \times 10^2$ (*n*=12) in the RAs and $2.2 \times 10^3 \pm 1.4 \times 10^3$ (*n*=21), $1.9 \times 10^3 \pm 1.6 \times 10^3$ (*n*=28), and $8.7 \times 10^2 \pm 3.9 \times 10^2$ (*n*=23) in the SAs, respectively (Fredricks et al. 2009a). Mean ± SD concentration (ng/kg ww) of ΣPCDD/DFs in nestlings of house wrens, tree swallows, and eastern bluebirds at reference areas and Tittabawassee River SAs was $2.0 \times 10^1 \pm 7.5 \times 10^0$ (*n*=12) and $5.6 \times 10^2 \pm 4.0 \times 10^2$ (*n*=26), $9.4 \times 10^1 \pm 4.9 \times 10^1$ (*n*=12) and $8.7 \times 10^2 \pm 1.5 \times 10^3$ (*n*=21), and $3.8 \times 10^1 \pm 2.4 \times 10^1$ (*n*=12) and $7.6 \times 10^2 \pm 5.7 \times 10^2$ (*n*=16), respectively

Table 3.1. Potential average (range) ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ daily dose (ADD_{pot}; ng/kg body weight/d) calculated from site-specific bolus-based and food web-based dietary exposure for adult house wrens, tree swallows, and eastern bluebirds breeding during 2004–2006 within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA.

	Bolus		Food web	
Study Area	ΣPCDD/DFs	$TEQ_{WHO\text{-}Avian}$[a]	ΣPCDD/DFs	$TEQ_{WHO\text{-}Avian}$
House wren				
R-1 and R-2[b]	26 (12–45)[c,d]	1.1 (0.73–1.7)	36 (10–76)	1.5 (0.54–3.0)
T-3 to T-6	360 (160–630)	150 (38–430)	180 (64–340)	68 (13–140)
S-7 and S-9	–[e]	–	140 (20–710)	16 (5.9–34)
Tree swallow				
R-1 and R-2	53 (16–90)	4.9 (1.4–8.8)	120 (20–370)	6.1 (1.3–13)
T-3 to T-6	340 (58–1000)	200 (24–800)	570 (130–1400)	250 (34–630)
S-7 and S-9	–	–	200 (130–370)	70 (40–120)
Eastern bluebird				
R-1 and R-2	12 (4.2–20)	0.88 (0.44–1.9)	35 (10–92)	1.1 (0.47–2.2)
T-3 to T-6	250 (54–790)	110 (13–450)	310 (100–740)	77 (24–180)
S-7 and S-9	–	–	240 (84–720)	41 (6.2–110)

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values

[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area

[c] Values were rounded and represent only two significant figures

[d] Food ingestion rate was calculated from equations in The Wildlife Exposure Factors Handbook (US EPA 1993)

[e] Residue analyses were not conducted on bolus collected invertebrates at S-7 and S-9

Table 3.2. Average (range) total ingestion of ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) determined from site-specific bolus-based and food web-based dietary exposure for nestling house wrens, tree swallows, and eastern bluebirds within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

	Bolus		Food web	
Study Area	ΣPCDD/DFs	$TEQ_{WHO\text{-}Avian}$[a]	ΣPCDD/DFs	$TEQ_{WHO\text{-}Avian}$
House wren[b]				
R-1 and R-2[c]	180[d,e] (82–310)	5.7 (4.5–9)	250 (73–530)	8.7 (3.2–18)
T-3 to T-6	2500 (1100–4400)	930 (240–2400)	1300 (440–2400)	340 (68–410)
S-7 and S-9	–[f]	–	980 (140–4900)	87 (40–190)
Tree swallow				
R-1 and R-2	530 (150–890)	40 (11–75)	1200 (200–3700)	52 (9.6–120)
T-3 to T-6	3300 (580–10000)	1500 (160–3600)	5700 (1300–14000)	2000 (260–2000)
S-7 and S-9	–	–	1900 (1300–3700)	550 (330–970)
Eastern bluebird				
R-1 and R-2	120 (44–200)	7.2 (3.9–16)	360 (110–950)	9.2 (4.2–19)
T-3 to T-6	2500 (560–8100)	820 (110–3300)	3200 (1100–7600)	510 (220–500)
S-7 and S-9	–	–	2500 (870–7400)	380 (32–1100)

Table 3.2. (Continued)

[a] $TEQ_{\text{WHO-Avian}}$ were calculated based on the 1998 avian WHO TEF values

[b] HW = 10-d nesting period; TS and EB = 14-d nesting period

[c] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area

[d] Values were rounded and represent only two significant figures

[e] Food ingestion rate was calculated from equations in The Wildlife Exposure Factors Handbook (USEPA 1993)

[f] Residue analyses were not conducted on bolus collected invertebrates at S-7 and S-9

(Fredricks et al. 2009a). Maternal transfer of total ΣPCDD/DFs per egg contributed less than 13% of the predicted nestling body burdens at fledge for all species studied regardless of study area or diet type. Therefore predictions of nestling body burdens (Figure 3.6) were largely determined by dietary ingestion of ΣPCDD/DFs and selection of dietary exposure estimates.

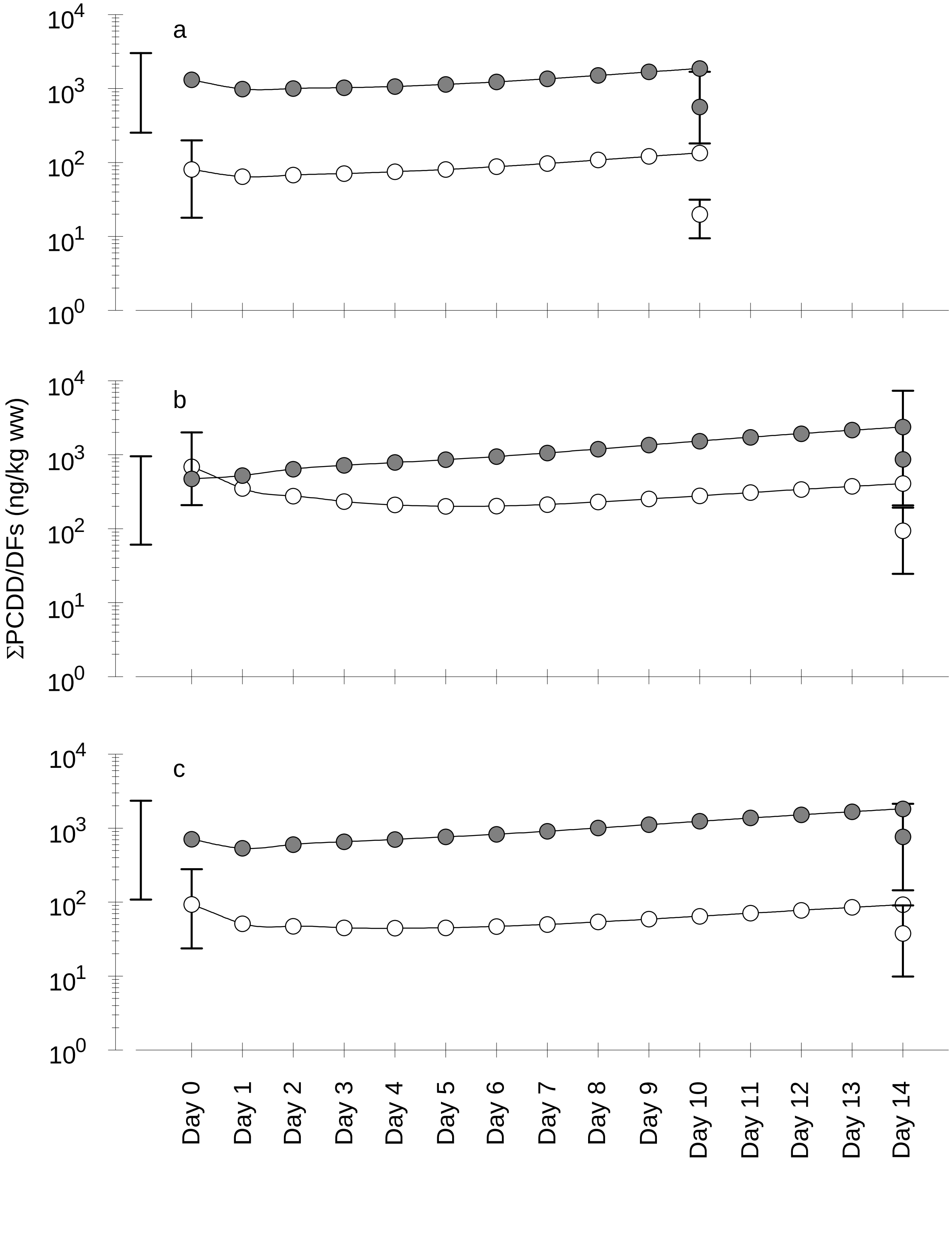
Discussion

Dietary composition

Passerines have a limited foraging range when feeding nestlings (Pinkowski 1977; Quinney and Ankney 1985) and preferentially may select the most beneficial invertebrates rather than the most abundant (Guinan and Sealy 1987; McCarty and Winkler 1999; Quinney and Ankney 1985). Prey selection also depends on the age of the nestlings (Luttenton 1989; Morton 1984; Pinkowski 1978), with smaller invertebrates with less chitin selected to facilitate growth and digestion for younger nestlings.

Bolus sampling was initiated 3–4 d post-hatch, which possibly limited the presence of some orders from our analyses. Additionally, incidental soil and sediment ingestion were not considered for dietary exposure for several reasons: 1) DI-water rinsed non-depurated Oligocheata were used and likely represent the most probable source of soil exposure, 2) field collected invertebrates were not rinsed prior to analyses so any incidental soil or sediment would be included in their analyses, 3) concentrations of ΣPCDD/DF in soil and sediment were greater than those in invertebrates and even small percentages could skew estimated dietary exposures, and 4) PCDD/DFs are less bioavailable when bound to soil

Figure 3.6. Predicted nestling body burdens based on adjusted dietary accumulation of ΣPCDD/DFs from mean bolus-based concentrations and food intake equations (line connected points), and mean with range concentrations in eggs and nestlings for a) house wrens, b) tree swallows, and c) eastern bluebirds collected in the Chippewa and Tittabawassee River floodplains during 2004–2007 near Midland, Michigan, USA. Predicted nestling body burdens were adjusted based on 0.7 assimilation efficiency (Nichols et al. 2004). Open symbols are from reference areas (R-1 and R-2); closed symbols are from Tittabawassee River study areas (T-3 to T-6); egg concentration ranges from Tittabawassee River study areas are offset.



a
b
c
10^4
10^3
10^2
10^1
10^0
ΣPCDD/DFs (ng/kg ww)
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14

or sediment and are likely not assimilated to the same degree as in biota (Alexander 2000; Budinsky et al. 2008; Froese et al. 1998; Stephens et al. 1995).

Dietary items collected in site-specific bolus samples from nestling house wrens, tree swallows, and eastern bluebirds represented similar orders as expected from literature-based diets, while relative proportions of invertebrate orders varied. When feasible, it is important to quantify site-specific dietary composition since variations in prey selection can influence dietary exposure estimates (Neigh et al. 2006a; Papp et al. 2007; Smits et al. 2005). Literature-based estimates of tree swallow ingestion of Dipterans were 15-20% [wet weight; ww (Johnson and Lombardo 2000; McCarty and Winkler 1999; Neigh et al. 2006a)] compared to 52% in the current study. Nestling eastern bluebirds from this study were fed primarily Orthoptera (44% ww) and Lepidoptera (28% ww), while nestlings from other studies in Michigan were fed primarily Orthoptera (45% ww) and Trichoptera [30% ww (Neigh et al. 2006a)], and Lepidoptera (40% ww), Orthoptera (20% ww), and Oligocheata [20% ww (Pinkowski 1978)]. Similar numbers of Lepidoptera larvae and adults were fed to house wren nestlings (52%) compared to nestlings in Ohio (57%; Luttenton 1989), but were greater than numbers fed to house wren nestlings in Illinois (22%; Morton 1984) and Michigan (30%; Neigh et al. 2006a). However, despite the lower frequency of Lepidoptera in the Michigan study, the order still accounted for approximately 80% of the diet by mass (Neigh et al. 2006a).

There was a greater prevalence of Trichoptera in the diet of tree swallows at upstream RAs compared to downstream SAs. However, Trichoptera abundance was not quantified at study sites, tree swallow foraging and dietary composition is related to prey abundance (McCarty 1997). Therefore, the lesser Trichoptera abundance at SAs was likely due to site-specific habitat and river flow differences. Trichoptera depend on suitable riverbed substrates for attachment (Wiggins 1996), and downstream SA substrates are dominated by a sandy bottom with limited submerged vegetation or debris. Additionally, increased sedimentation and decreased water quality at SAs could also influence the presence of Trichoptera since they are considered a sensitive order to these types of disturbance (Hachmöller et al. 1991).

Eastern bluebird foraging on Oligocheata was primarily limited to T-4 in both 2005 and 2006. Similar habitat characteristics and rainfall patterns were present in the vicinity of nest boxes at other sites in both the RAs and SAs. Pair/individual feeding specialization did not appear to be a factor as different adult pairs bred at T-4 between years. It is possible that soil conditions at T-4 contributed to greater Oligocheata presence than at other sites, but specific measurements were not made. A similar pattern of hit-or-miss Oligocheata foraging by eastern bluebirds has been observed in other Michigan studies. On the Kalamazoo River Oligocheata were not collected in bolus samples from nestling eastern bluebirds (Neigh et al. 2006a), but were identified in adult feeding trips and bolus samples from eastern bluebirds in Macomb County (Pinkowski 1978). In the current study adult eastern bluebirds that fed Oligocheata to nestlings did so early in the nesting season and Oligocheata were limited to the first brood, which was similar to observations in previous research (Pinkowski 1978).

ΣPCDD/DFs and TEQs$_{WHO\text{-}Avian}$

Concentrations of ΣPCDD/DF and TEQs$_{WHO\text{-}Avian}$ varied among invertebrate orders at Tittabawassee River SAs. Since food web-based invertebrate samples represented composite samples of multiple individuals, there was likely some small-scale spatial integration due to movement of invertebrates into the sampling grids from nearby areas. General relationships at SAs indicated some large-scale spatial trends. Invertebrates at T-6 consistently contained maximum concentrations as compared to other SAs.

Habitat use by invertebrates also resulted in concentration differences between and within orders. Of the predominant invertebrate orders that made up the majority of the dietary composition for all three study species, terrestrial orders had the greatest concentrations (Brachycera and Oligocheata). Within one order, terrestrial Dipterans (Brachycera) had 2-times greater ΣPCDD/DF concentrations and $TEQs_{WHO\text{-}Avian}$ than aquatic Dipterans (Nematocera) at SAs. Concentrations in Lepidoptera and Orthoptera, major contributors to both house wren and eastern bluebird diets (72% for both), were the lowest of all the predominant orders. Sex-specific differences within invertebrate orders were beyond the scope of our research, but Maul et al. (2006) separated the genus *Chironomus* from other Nematocerans and analyzed the sexes separately for PCB concentrations. Male *Chironomus* had greater concentrations compared to females and differences were attributed to sex-dependent life-history factors. It is likely that similar subtle differences in order-specific life-history factors can influence exposure and assimilation of PCDD/DFs.

Similar to individual invertebrate orders, bolus samples collected from all species had greatest residue concentrations at T-6. Additionally, relative proportions of mean 2,3,7,8-TCDF concentrations in bolus samples were greatest at T-6 for all study species. The relative proportion of 2,3,7,8-TCDF increases in bolus samples from T-3 to T-6, which mirrors the pattern of increasing ΣPCDD/DF concentrations in bolus samples (Figure

3.5). These trends are similar to house wren, tree swallow, and eastern bluebird tissues collected from the same sites (Fredricks et al. 2009a). One possible explanation for greater values in both food web items and bolus samples at the T-6 location involves the natural hydrology of the Tittabawassee River. When at flood stage, the river flows across the large bends near T-6 instead of following the normal river channel (Figure 3.1). The water loses momentum and energy quickly and deposits large amounts of sediment over those areas, creating a "sink" location for sediment bound contaminants.

Food web dietary exposure estimates were based on site-specific dietary composition, derived from bolus sample composition, combined with concentrations in respective invertebrate orders. While ranges of ΣPCDD/DF concentrations (Figure 3.3) and $TEQs_{WHO\text{-}Avian}$ estimated dietary exposure varied among species, similar concentration trends were present between ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$. In eastern bluebirds, however, the lesser $TEQ_{WHO\text{-}Avian}$ values in Orthoptera and Oligocheata samples (Figure 3.4) resulted in lower food web-based dietary $TEQs_{WHO\text{-}Avian}$ than the ΣPCDD/DF concentrations would suggest due to the relatively low TCDD potency associated with these orders. Approximately 50% of the ΣPCDD/DF concentrations in eastern bluebird food web-based dietary exposure estimates were from Oligocheata, which only occurred in a few nests at T-4. The presence of Oligocheata explains the difference between ΣPCDD/DF concentrations and $TEQs_{WHO\text{-}Avian}$ food web-based dietary exposure estimates.

For house wrens and eastern bluebirds, the range of food web-based dietary exposure estimates was less than the exposure estimates based on bolus samples, while tree swallows had similar ranges. Species-specific foraging strategies between tree swallows and the terrestrial foraging species, combined with sampling protocols for food web collections can account for this difference. Tree swallows primarily forage near or over bodies of water (McCarty 1997; McCarty and Winkler 1999), while house wrens and eastern bluebirds forage in close proximity to their nest box (Guinan and Sealy 1987; Pinkowski 1977). Since food web collections occurred in one location (30 m × 30 m) per site adjacent to the river, it is unlikely that terrestrial foraging species would forage only in the exact same area. Tree swallows, meanwhile, are drawn to the river to feed where, due to river dynamics, concentrations are expected to be more uniform. PCB concentration differences between dietary samples (bolus or gut contents) and site-specific invertebrate samples (collected with nets) have been previously documented for passerines (Echols et al. 2004; Maul et al. 2006; Smits et al. 2005).

Exposure to PCDFs and PCDDs on both a ΣPCDD/DF and $TEQ_{WHO\text{-}Avian}$ basis of food web invertebrates and bolus samples from the current study was similar to or greater than that from other sites contaminated with chlorinated hydrocarbons. Stomach contents from tree swallow nestlings and pipers from the Woonasquatucket River floodplain contained 71 to 219 ng/kg ww 2,3,7,8-TCDD (Custer et al. 2005), while samples from the primarily PCB contaminated Housatonic River area (Custer et al. 2003) had only a few PCDD/DF congeners with detectable concentrations (2,3,7,8-TCDF, 17 to 38 ng/kg ww; 1,2,3,7,8-pentachlordibenzofuran (PeCDF), 15 to 142 ng/kg ww; 1,2,3,4,6,7,8-heptachlorodibenzofuran, 14 to 30 ng/kg ww). Tree swallows primarily exposed to PCBs in southern Illinois had dietary $TEQ_{WHO\text{-}Avian}$ concentrations that ranged from 0.52 to 35 ng/kg ww for food web samples and averaged 4.4 ng/kg ww in stomach contents (Maul et al. 2006).

Average potential daily dose

To gauge exposure and facilitate future assessment for reproductive risks, ADD_{pot} was estimated for adult passerines while breeding on-site. The $TEQ_{WHO\text{-}Avian}$ dietary exposures reported here for the Tittabawassee River are similar to $TEQ_{WHO\text{-}Avian}$ dietary exposure estimates reported for insectivorous passerines from the PCB contaminated Kalamazoo River, Michigan, USA (Neigh et al. 2006a). Adult tree swallows (Kay et al. 2005) and house wrens (Blankenship et al. 2005) had 64% and 56% greater ΣPCB body burdens, respectively, than nestlings from the same study sites on the Kalamazoo River. For the current study, adult passerines were not collected for residues analyses because of the concurrent long-term monitoring study of adult and nestling survival, but based on the study from the Kalamazoo River greater adult body burdens would be expected. The relative proportion of ΣPCDD/DF to $TEQ_{WHO\text{-}Avian}$ for adult ADD_{pot} for bolus-based and food web-based dietary exposures ranged from 4.2 to 9.2% and 2.0 to 3.2% at RAs, respectively, while at SAs they ranged from 42 to 59% and 20 to 38%, respectively. Higher percentages at SAs are due to greater proportions of primarily 2,3,7,8-TCDF and secondarily 2,3,4,7,8-PeCDF in the congener profiles at those sites.

Total ingestion of residues by nestlings

Predicted nestling body burdens of PCDD/DFs prior to fledge were greater than measured nestling tissue concentrations (Fredricks et al. 2009a) regardless of study area. The most plausible explanations for this discrepancy are overestimates of the daily dietary exposures or overestimates of residue assimilation efficiency or both. The predicted total ingestion by nestlings was based on a mean daily dietary dose that was calculated using residue concentrations in bolus samples. The bolus sample method was selected because bolus samples were composed of the invertebrates selected by the adult passerines on-site. When minimum values for bolus samples were used, instead of mean values, to estimate nestling body burdens at Tittabawassee River SAs the predicted values were similar to the measured mean nestling tissue concentrations.

The second and more likely explanation of the overestimation of predicted nestling body burdens from bolus-based exposure estimates is that the assimilation efficiency for the site-specific mixture of PCDD/DFs is less than the 0.7 suggested for PCBs (Nichols et al. 2004). Differential metabolism of residues by embryos and nestlings or selective sequestration of specific PCDD/DF congeners could account for some of the difference between the predicted nestling body burdens from bolus-based exposure estimates and those measured in nestlings. The fact that house wren and eastern bluebird nestlings contained lesser percentages of 2,3,7,8-TCDF, and greater percentages of 2,3,4,7,8-PeCDF compared to dietary exposure estimates suggests a dynamic metabolism or sequestration mechanism. Similarly, related field and laboratory studies have noted a short half life for 2,3,7,8-TCDF in developing embryos and growing chicks that resulted in significantly lesser tissue concentrations than would have been expected (Zwiernik, Personal Communication). Based on comparisons of congener specific adult biomagnification factors in herring gulls (*Larus argentatus*), TCDF was determined to be rapidly metabolized as opposed to 2,3,4,7,8-PeCDF in which metabolism was determined to be variable and possibly linked to species specific differences in distribution or metabolism (Braune and Norstrom 1989). Previous research on mallards (*Anas* *platyrhynchos*; Norstrom et al. 1976) and bald eagles (*Haliaeetus leucocephalus*; Elliot et al. 1996) have discussed similar trends in metabolism for PCDF congeners. Alternatively, the predicted PCB concentrations based on the diet and those measured in tree swallow nestlings along the Saginaw River, Michigan were similar based on the assimilation efficiency of 0.7 (Echols et al. 2004). Those authors suggested that differential metabolism of PCBs was not very important in nestlings. Potential explanations for the differences between house wren and eastern bluebird nestlings and dietary exposure estimates could be unique PCDD/DF bioavailability from terrestrial invertebrates. However, these adjustments still cannot fully explain the differences observed between expected and observed nestling concentrations.

Conclusions

Estimates of dietary exposure for both adult and nestling passerines were greater at Tittabawassee River SAs compared to RAs, while exposure at Saginaw River SAs was intermediate. Though few studies have investigated avian dietary exposure of PCDD/DFs, based on $TEQs_{WHO\text{-}Avian}$ our results were in line with other contaminated sites. Estimates of the dietary exposure of nestlings were greater than the measured concentrations of ΣPCDD/DF in nestlings (Fredricks et al. 2009a). Metabolism of compounds prior to hatch, possible overestimation of food intake rates, positively skewed concentration data in the diet at SAs, and assimilation of less than 70% of the compounds from the diet all likely led to greater predicted relative to measured concentrations in nestlings. Characterizing dietary exposure for passerine birds with either bolus-based sample analyses or food web-based dietary analyses involves labor intensive sampling procedures. Our results indicate that both methods provide similar exposure estimates that varied by species. Due to efficient bolus collection via cable-ties, collection of true site-specific dietary items, and the time savings associated with not sorting collected invertebrates to order for analytical analyses the authors recommend future dietary exposure assessments to use bolus sampling for passerines when feasible. Additional manuscripts will discuss implications of these results by incorporating data from tissue exposure (Fredricks et al. 2009a) and productivity (Fredricks et al. 2009b) into aquatic (Fredricks et al. 2009c) and terrestrial (Fredricks et al. 2009d) passerine risk assessments.

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Animal Use

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

Supplemental Information

Table 3.3. Descriptive statistics for ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) by site from food web invertebrate collections for Araneae samples collected within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

		ΣPCDD/DFs		$TEQ_{WHO\text{-}Avian}$[a]	
Site[b]	n	mean±SD (range)	CLMs	mean±SD (range)	CLMs
R-1	2	(36–45)[c,d]	–	(0.99–1.8)	–
R-2	1	360	–	4.5	–
T-4	2	(260–510)	–	(43–110)	–
T-6	1	1100	–	380	–

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area
[c] Values were rounded and represent only two significant figures
[d] Mean, SD, and CLMs were not reported for sites with less than 3 samples

Table 3.4. Descriptive statistics for ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) by site from food web invertebrate collections for Lepidoptera samples collected within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

		ΣPCDD/DFs		$TEQ_{WHO\text{-}Avian}$ [a]	
Site[b]	n	mean±SD (range)	CLMs	mean±SD (range)	CLMs
R-1	4	12±3.9 (8.1–17)[c]	6.2–19	0.95±0.41 (0.52–1.4)	0.29–1.6
R-2	3	22±2.8 (20–25)	15–29	1.1±0.41 (0.73–1.5)	0.037–2.1
T-3	1	57[d]	–	9.3	–
T-4	1	240	–	85	–
T-5	2	(140–180)	–	(87–98)	–
T-6	2	(42–180)	–	(11–47)	–
S-7	3	540±740 (110–1400)	-1300–2400	25±10 (16–36)	-0.15–51
S-8	2	(18–41)	–	(4.5–16)	–
S-9	2	(39–51)	–	(3.1–5.6)	–

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area
[c] Values were rounded and represent only two significant figures
[d] Mean, SD, and CLMs were not reported for sites with less than 3 samples

Table 3.5. Descriptive statistics for ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) by site from food web invertebrate collections for Nematocera samples collected within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

		ΣPCDD/DFs		$TEQ_{WHO\text{-}Avian}$[a]	
Site[b]	n	mean±SD (range)	CLMs	mean±SD (range)	CLMs
R-1	2	(20–42)[c,d]	–	(1.7–3.7)	–
R-2	1	130	–	9.1	–
T-3	2	(140–170)	–	(40–56)	–
T-4	2	(310–1200)	–	(130–520)	–
T-5	1	800	–	500	–
T-6	1	1100	–	470	–
S-7	2	(170–250)	–	(100–160)	–
S-8	2	(110–140)	–	(56–74)	–
S-9	3	200±49 (160–260)	82–330	76±23 (58–100)	20–130

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area
[c] Values were rounded and represent only two significant figures
[d] Mean, SD, and CLMs were not reported for sites with less than 3 samples

Table 3.6. Descriptive statistics for ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) by site from food web invertebrate collections for Oligocheata (non-depurated) samples collected within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

		ΣPCDD/DFs		$TEQ_{WHO\text{-}Avian}$[a]	
Site[b]	n	mean±SD (range)	CLMs	mean±SD (range)	CLMs
R-1	4	54±20 (31–74)[c]	23–85	1.3±0.92 (0.53–2.6)	-0.16–2.8
R-2	2	(56–86)[d]	–	(1–2.8)	–
T-3	2	(1600–1800)	–	(200–230)	–
T-4	4	1100±440 (560–1500)	370–1800	280±190 (130–550)	-20–580
T-5	2	(1300–1700)	–	(120–310)	–
T-6	4	2500±1700 (950–4700)	-150–5100	330±97 (190–410)	180–490
S-7	2	(1500–2600)	–	(300–920)	–
S-9	2	(810–1400)	–	(44–68)	–

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area
[c] Values were rounded and represent only two significant figures
[d] Mean, SD, and CLMs were not reported for sites with less than 3 samples

Table 3.7. Descriptive statistics for ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) by site from food web invertebrate collections for Orthoptera samples collected within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

		ΣPCDD/DFs		$TEQ_{WHO\text{-}Avian}$ [a]	
Site[b]	n	mean±SD (range)	CLMs	mean±SD (range)	CLMs
R-1	2	(12–91)[c,d]	–	(0.57–0.63)	–
R-2	1	10	–	1.9	–
T-3	1	55	–	23	–
T-4	2	(74–82)	–	(12–14)	–
S-7	2	(5.7–85)	–	(2.1–18)	–
S-9	1	5.6	–	1.1	–

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area
[c] Values were rounded and represent only two significant figures
[d] Mean, SD, and CLMs were not reported for sites with less than 3 samples

Table 3.8. Descriptive statistics for ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) by site from food web invertebrate collections for Trichoptera samples collected within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

		ΣPCDD/DFs		$TEQ_{WHO\text{-}Avian}$ [a]	
Site[b]	n	mean±SD (range)	CLMs	mean±SD (range)	CLMs
R-1	5	24±13[c] (13–40)	8.3–40	1.4±0.98 (0.71–3)	0.23–2.7
R-2	5	55±68 (13–180)	-29–140	9.3±16 (0.93–37)	-10–29
T-3	3	170±40 (140–220)	71–270	48±27 (21–74)	-18–110
T-4	4	320±200 (150–520)	7.8–630	100±68 (61–200)	-8.2–210
T-5	4	210±110 (64–310)	34–390	85±63 (22–170)	-16–190
T-6	3	390±180 (230–580)	-54–840	160±96 (91–270)	-79–400
S-7	3	410±170 (250–590)	-11–830	230±140 (130–390)	-110–570
S-8	2	(170–220)[d]	–	(97–120)	–
S-9	1	160	–	58	–

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area
[c] Values were rounded and represent only two significant figures
[d] Mean, SD, and CLMs were not reported for sites with less than 3 samples

Table 3.9. Descriptive statistics for ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) by site from food web invertebrate collections for Brachycera samples collected within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

		ΣPCDD/DFs		$TEQ_{WHO\text{-}Avian}$ [a]	
Site[b]	n	mean±SD (range)	CLMs	mean±SD (range)	CLMs
R-1	1	30[c,d]	–	1.5	–
R-2	1	300	–	13	–
T-3	2	(380–2800)	–	(43–1400)	–
T-4	2	(280–1200)	–	(96–490)	–
T-5	3	1300±900 (500–2300)	-940–3500	640±540 (250–1200)	-690–2000
T-6	1	190	–	100	–
S-9	1	150	–	67	–

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area
[c] Values were rounded and represent only two significant figures
[d] Mean, SD, and CLMs were not reported for sites with less than 3 samples

Table 3.10. Descriptive statistics for ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) by site from food web invertebrate collections for Ephemeroptera samples collected within the Chippewa, Tittabawassee, and Saginaw River floodplains during 2004–2006, Midland, Michigan, USA.

		ΣPCDD/DFs		$TEQ_{WHO\text{-}Avian}$ [a]	
Site[b]	n	mean±SD (range)	CLMs	mean±SD (range)	CLMs
R-1	2	(54–2500)[c,d]	–	(30–32)	–
R-2	3	84±38 (60–130)	-10–180	8.4±9.4 (2.8–19)	-15–32
T-3	2	(250–260)	–	(42–130)	–
T-4	2	(510–620)	–	(210–220)	–
T-5	4	250±62 (190–330)	160–350	84±75 (37–200)	-36–200
T-6	2	(400–480)	–	(220–290)	–
S-7	1	340	–	150	–
S-8	1	110	–	37	–

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area
[c] Values were rounded and represent only two significant figures
[d] Mean, SD, and CLMs were not reported for sites with less than 3 samples

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CHAPTER 4

Reproductive success of house wrens, tree swallows, and eastern bluebirds exposed to elevated concentrations of PCDFs in a river system downstream of Midland, Michigan, USA

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Abstract

Elevated concentrations of polychlorinated dibenzofurans (PCDFs) in soils and sediments downstream of Midland, Michigan initiated a site-specific assessment of passerine health including reproductive parameters reported here. From 2005 through 2007, house wren, tree swallow, and eastern bluebird nests were monitored daily at study areas (SAs) downstream of Midland, Michigan and at upstream reference areas (RAs). Concurrent studies were conducted to investigate tissue-based and dietary-based exposure of passerines to concentrations of ΣPCDD/DFs and 2,3,7,8-TCDD equivalents (TEQ). Overall reproductive parameters for the three passerine species studied were similar or greater at downstream SAs compared to upstream RAs. Specifically, hatching success was consistent among years, species, locations, and between EARLY and LATE nesting attempts. Of all initiated clutches, 66% (n=427), 73% (n=245), and 64% (n=122) successfully fledged at least one nestling for house wrens, tree swallows, and eastern bluebirds, respectively. Adult females were banded with or identified by a unique US FWS band at 91%, 98%, and 96% of clutches that hatched nestlings for house wrens, tree swallows, and eastern bluebirds, respectively. As a result, overall reproductive output was determined for individually identified females within and among seasons for the study period. Tree swallow females at a Saginaw River SA had significantly greater production of nestlings over the course of the study than those at other study areas. Site-specific adult and fledgling recapture percentages were consistent but limited by study duration and study area range.

Keywords: Tittabawassee River; furans; dioxins; passerines

Introduction

Portions of the Tittabawassee and Saginaw rivers and associated floodplain downstream of Midland, Michigan (USA) have concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) in soil and sediment that are greater than the background concentrations for the region [1]. Sum concentrations of the 17 2,3,7,8-substituted PCDD/DFs (ΣPCDD/DFs) in floodplain soils and sediments, from the study area (SA) downstream of Midland, Michigan ranged from 1.0×10^2 to 5.4×10^4 ng/kg dry weight (dw), respectively, while mean ΣPCDD/DF concentrations in soils and sediments upstream of Midland were 10- to 20-fold less [1]. The elevated PCDD/DFs are likely associated with the historical production of organic chemicals and the on-site storage and disposal of manufacturing bi-products, prior to the establishment of modern waste management protocols [2]. The lipophilic nature and slow degradation rates of these compounds [3], combined with consistent inundation of the floodplain, led to the historical dispersion of PCDD/DFs throughout floodplain soils and sediments.

PCDD/DFs, polychlorinated biphenyls (PCBs), and similar chlorinated hydrocarbons occur in the environment as mixtures. The mixture of chlorinated hydrocarbons in the SA is dominated by a few PCDF congeners, which makes it distinct from most other locations that are contaminated with PCBs or PCDDs [4-16]. The primary toxicological response to dioxin-like compounds is mediated through the aryl hydrocarbon receptor (AhR) and effects include carcinogenicity, immuno-toxicity, and adverse effects on reproduction, development, and endocrine functions [17]. In particular, AhR-mediated compounds have been shown to decrease hatching success, adult attentiveness, and immune function of passerine species [5,18]. Recent findings suggest the magnitude of response could be species-dependent for birds [19,20].

Three cavity-nesting passerine bird species were selected for this study to provide data for a site-specific ecological risk assessment of the Tittabawassee and Saginaw rivers and the associated floodplain downstream of Midland, Michigan using the multiple-lines of evidence approach described by Fairbrother [21]. Tree swallows (*Tachycineta bicolor*), which eat primarily emergent aquatic invertebrates [22], have been shown to have exposure links to contaminated sediments [10,15,23-26], and have been extensively utilized in field studies [5,7,11,14,24]. House wrens (*Troglodytes aedon*) and eastern bluebirds (*Sialia sialis*) have been used to assess the reproductive success of terrestrial insectivores at locations with PCB contaminated soils [12]. Several other studies investigating the effects of contaminants on songbirds have used eastern bluebirds and house wrens [10,27-29].

The primary objectives of the study described herein were to assess the nesting success of three insectivorous passerine species exposed to PCDD/DFs through both terrestrial and aquatic pathways. Additional comparisons were made based on individually identified adult females, age of adult females, between study areas, and for both EARLY and LATE nesting attempts.

The incorporation of "multiple lines of evidence" [21,30] into an ecological risk assessment including information on concentrations of residues of concern in eggs and juvenile birds [31] and in the diet [32], will help provide site-specific information to make informed decisions about the potential impact(s) of contaminants and aid in the planning and evaluation of effective remedial actions.

Methods

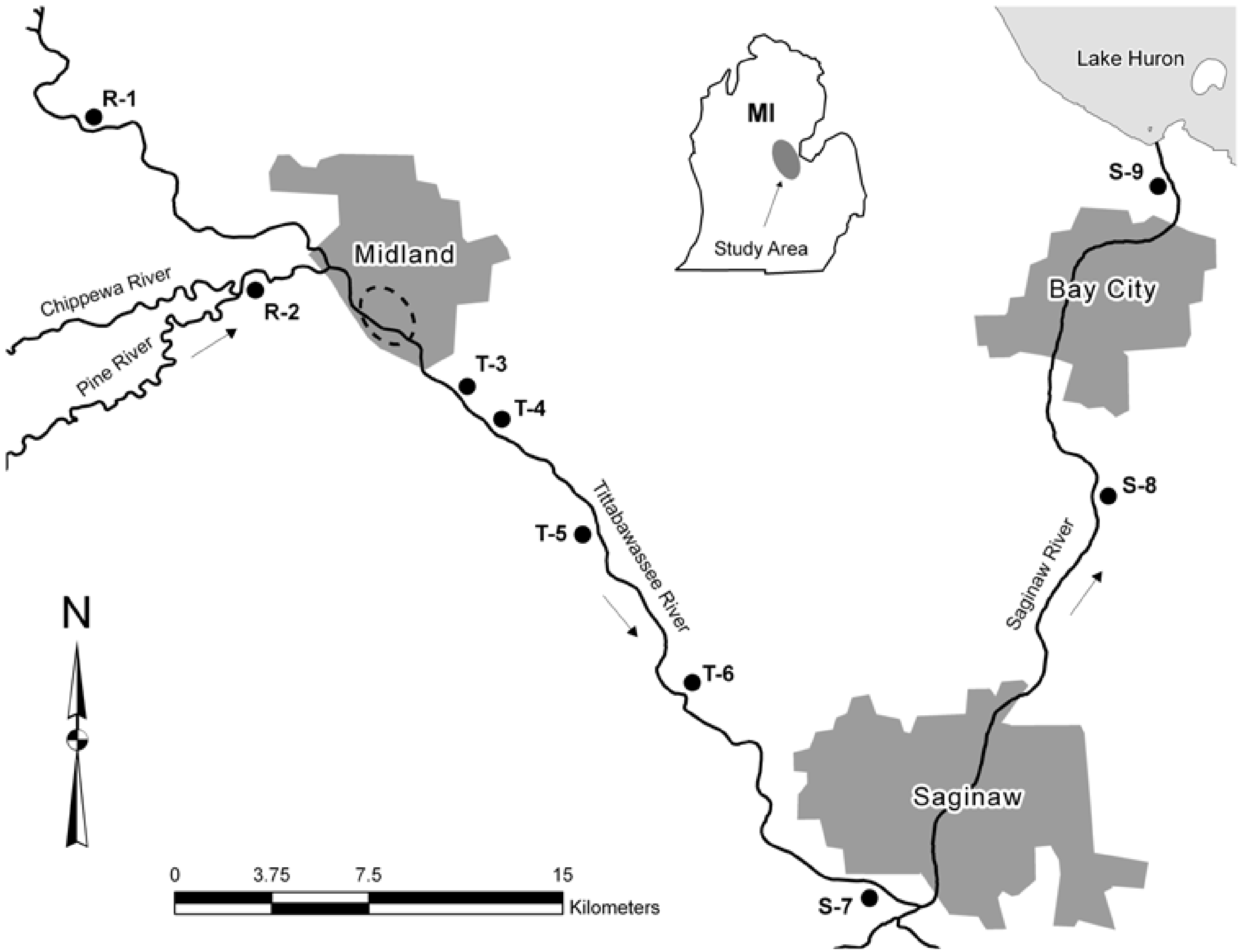
Site details

Study locations were selected on the Tittabawassee, Chippewa, and Saginaw rivers in the vicinity of Midland, Michigan (Figure 4.1). Nest boxes were located within the 100-year floodplain of the individual rivers. Two reference areas (RAs) were positioned upstream of the putative sources of PCDD/DFs [1] on the Tittabawassee (R-1) and Chippewa (R-2) rivers (Figure 4.1). Study areas downstream of the putative sources of PCDD/DFs include approximately 72 km of free flowing river from the upstream boundary, defined as the low-head dam near Midland, Michigan, through the confluence of the Tittabawassee and Saginaw rivers to where the Saginaw River enters Saginaw Bay. The SAs within the Tittabawassee River area included four locations (T-3 to T-6) approximately equally spaced, and locations (S-7 to S-9) which are approximately at the initiation, median, and terminus of the Saginaw River. The seven SAs (T-3 to S-9) were selected 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 [1]. Individual nest box trails within RAs and SAs each contained between 30 and 60 nest boxes that spanned a continuous foraging area of between 1 and 3 km. S-8 was the exception and was only used for sediment and dietary food web sampling. No studies of birds were conducted at this location.

Nest success measurements

Standard passerine nest boxes [31] with wire mesh predator guards around the entrance hole were mounted to a greased metal post and placed at RAs and Tittabawassee

Figure 4.1. 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 S-9) were monitored from 2005–2007. Direction of river flow is designated by arrows; suspected source of contamination is enclosed the dashed oval.



R-1
Midland
Chippewa River
R-2
Pine River
T-3
T-4
T-5
Tittabawassee River
T-6
MI
Study Area
Lake Huron
S-9
Bay City
S-8
Saginaw River
Saginaw
S-7
N
0
3.75
7.5
15
Kilometers

River SAs in 2004, and the Saginaw River SAs in 2005. Monitoring began one year subsequent to placement and continued through 2007 at all sites. Boxes were placed in species-specific micro-habitats at each site to concurrently maximize occupancy at study sites by house wrens, tree swallows, and eastern bluebirds. Boxes were monitored daily after clutch initiation through incubation and then near the expected hatch day for each species. Warm clutches with similar number of eggs on two subsequent visits indicated clutch completion. Eggs were individually identified and massed on the date laid. Clutches were considered abandoned if there was no adult activity for 7 d, or new nesting material was added over cold eggs. Hatch day (brood day 0) was determined as the day when the majority (≥50%) of the eggs in a clutch had hatched. Eggs in which concentrations of residues were quantified were collected after clutch size was noted. Therefore, clutch size was not adjusted for egg sampling. However, hatching success, fledging success, and productivity measurements were calculated based on an adjusted clutch size since the fertility and hatchability of the collected egg was unknown at collection. Additionally, brood size and number of fledglings were predicted based on the adjusted hatching success and productivity, respectively.

Individual nestlings were massed multiple times for each brood during 2005 and 2006 for house wren and tree swallows, while eastern bluebirds were also measured in 2007. Masses of house wrens were measured 3, 6, 9, and 10 d post-hatch, while tree swallow and eastern bluebird masses were determined 4, 8, 12, and 14 d post-hatch. Mass gained per day was calculated as the third minus the first mass measurement divided by the days between. Logistic growth curves were fit to nestling masses over the nesting period [33,34], and growth rate constants were estimated using site-specific asymptotic values based on nestling masses [house wren, 12 g (n=205 broods); tree swallow, 28 g (n=133 broods); eastern bluebird, 32 g (n=76 broods)]. Since the initial mass on brood day 0 was not measured, the mean egg mass by species was used. Nestlings were collected for measurement of residues either 10 d post-hatch for house wrens or 14 d post-hatch for tree swallows and eastern bluebirds. Gender of eastern bluebird nestlings was determined by feather color between 12 to 14 d post-hatch, while house wren and tree swallow nestlings were monomorphic and gender was not determined.

House wren, tree swallow, and eastern bluebird nestlings and adults were banded with US Fish and Wildlife Service aluminum leg bands throughout the study. Nestlings were banded on the first day that mass was determined for all species except if house wren nestlings were less than 5 g in which case they were banded when sufficiently developed. Adults were opportunistically caught (usually incubating eggs) during routine box monitoring if they failed to depart the box as the researchers approached. More commonly adults were actively trapped by researchers during brood rearing to prevent stressing incubating females into abandoning a clutch [35]. Gender and age of adults were determined to the best resolution feasible [36,37], which for house wrens and male tree swallows was after-hatch-year (AHY) while for eastern bluebirds and female tree swallows second-year (SY) and after-second-year (ASY) birds could be distinguished. During routine handling nestlings and adults were monitored for gross external morphological abnormalities.

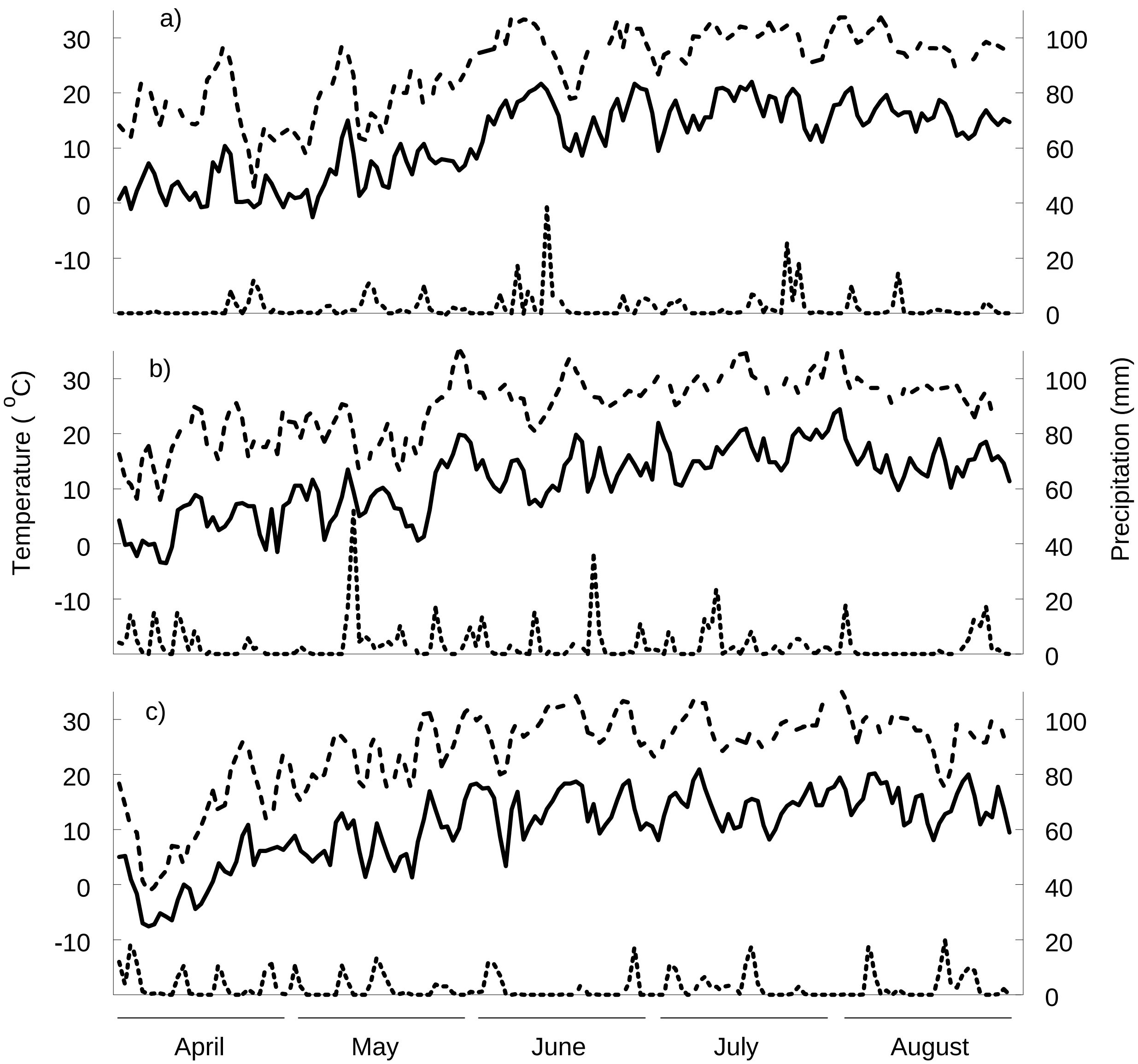
Meteorological measurements

Measurements of weather conditions were compiled from three stations located in Sanford (WxSrcID: 25488), Midland (WxSrcID: 15505), and Saginaw (WxSrcID: 15488) from The Weather Warehouse website (http://weather-warehouse.com) for all years of the study. Locations were chosen to best represent spatial variability in weather associated with such a large study area. Daily minimum, mean, and maximum temperatures in addition to total precipitation were compiled from the available data (supplemental information Figure 4.2). Average minimum, mean, and maximum temperatures, as well as the overall minimum and maximum temperatures, and total precipitation were compiled for individual nesting attempts from clutch initiation through the fledge date from the nearest weather station.

Statistical analyses

Statistical analyses were performed using SAS® software (Release 9.1; SAS Institute Inc., Cary, NC, USA). The experimental unit for measurements associated with eggs, nestlings, and nest success was the nest box, since individual measurements within a clutch cannot be considered independent [38]. Similarly nest success measurements were reported on a per nesting attempt basis, thus making each attempt per box a separate experimental unit [39]. Prior to the use of parametric statistical procedures, normality was evaluated using the Shapiro-Wilks test and the assumption of homogeneity of variance was evaluated using Levene's test. Nest parameters that were not normally distributed were ranked prior to statistical analyses. PROC GLM was used for comparisons and when significant differences among locations were indicated,

Figure 4.2. Mean precipitation (dotted line), minimum (solid line) and maximum (dashed line) temperatures for a) 2005, b) 2006, and c) 2007 recorded at three sites across study locations near Midland, Michigan, USA.


a)
b)
c)
Temperature (oC)
Precipitation (mm)
30
20
10
0
-10
100
80
60
40
20
0
April
May
June
July
August

Bonferroni's *t*-test was used to compare individual locations. PROC NLIN was used to fit growth curves based on nestling masses. Differences were considered to be statistically significant at p <0.05.

Results

Reproductive success

Adult house wrens, tree swallows and eastern bluebirds initiated nests at each study site with the exception of S-9 in which no eastern bluebirds nested. From 2005–2007 at all study sites 427, 245, and 122 clutches were initiated by house wrens, tree swallows, and eastern bluebirds, respectively (Table 4.1). Of all initiated clutches, 66%, 73%, and 64% successfully fledged at least one nestling for house wrens, tree swallows, and eastern bluebirds, respectively. Clutch initiations were spread across study sites such that most sites had approximately 10% of the overall nesting attempts for each species, but exceptions included: 20% and 2% for house wrens at T-5 and S-9, respectively; 19%, 21%, and 3% for tree swallows at R-2, T-3, and T-5, respectively; and 20%, 26%, and 4% for eastern bluebirds at R-2, T-3, and S-7, respectively. Nests that were preyed upon or abandoned comprised the majority of nests that were not successful (Table 4.1). Predation events were categorized by criteria defined by Etterson et al. [40], and for all studied species was primarily from competition with other studied species for a nest box or due to predation by mammals. Snakes only preyed upon nests at S-7, which accounted for 4%, 5%, and 10% of the total predation occurrences at all sites for house wrens tree swallows, and eastern bluebirds, respectively.

Table 4.1. Nesting attempt outcomes and percentages of initiated clutches for house wrens, tree swallows, and eastern bluebirds nesting in the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA.

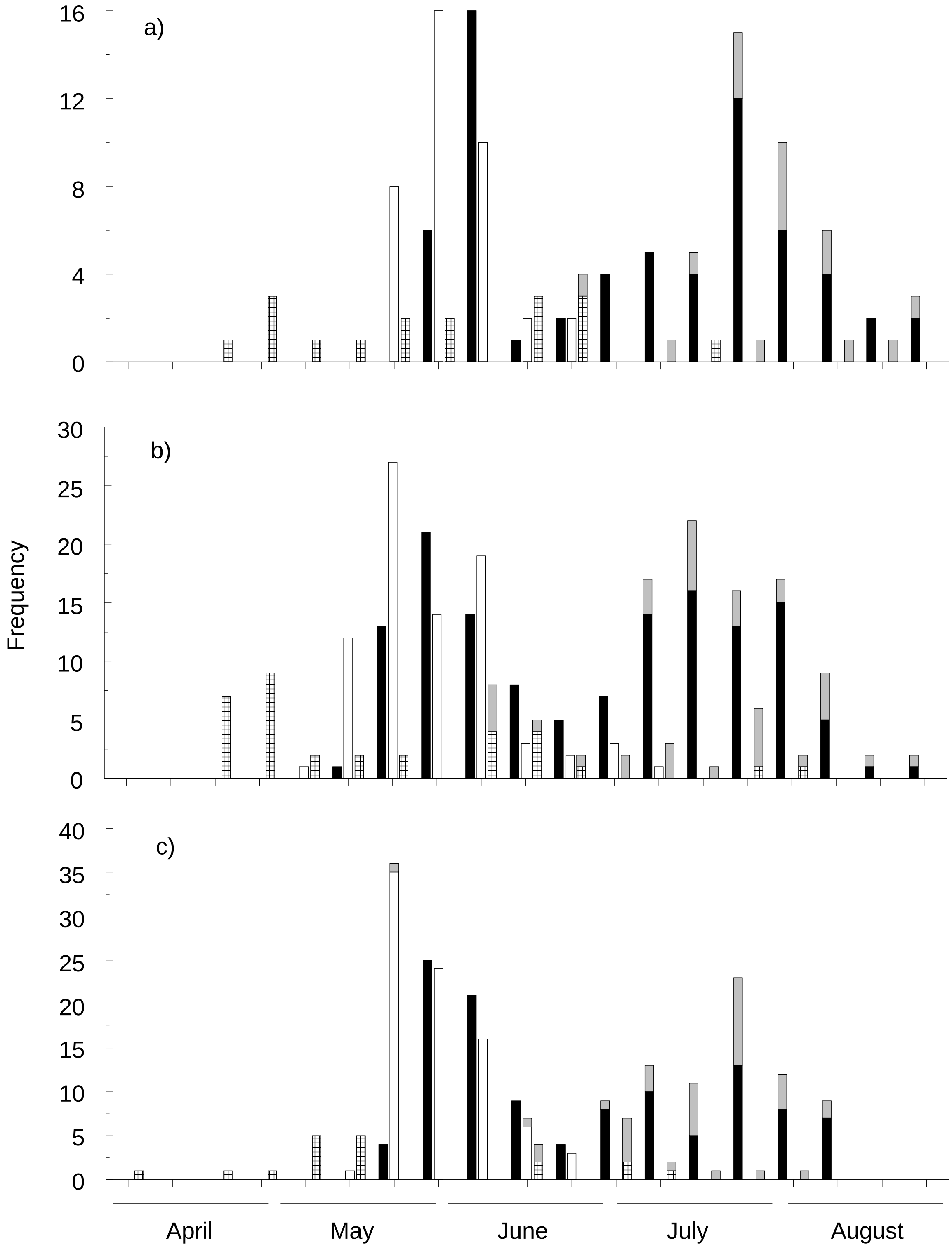
	2005		2006			2007			Overall
	R-1 to R-2	T-3 to T-6	R-1 to R-2	T-3 to T-6	S-7 and S-9[a]	R-1 to R-2	T-3 to T-6	S-7 and S-9	
House wren									
Initiated	24	75	36	110	27	36	86	33	427
Incubated	19 (79%)	60 (80%)	34 (94%)	95 (86%)	26 (96%)	35 (97%)	75 (87%)	31 (94%)	375 (88%)
Hatched	19 (79%)	60 (80%)	32 (89%)	87 (79%)	26 (96%)	34 (94%)	64 (74%)	29 (88%)	351 (82%)
Fledged[b]	15 (63%)	43 (57%)	25 (69%)	70 (64%)	18 (67%)	31 (86%)	56 (65%)	19 (58%)	277 (65%)
Predated	0 (0%)	11 (15%)[c]	7 (19%)	13 (12%)	3 (11%)[c]	2 (6%)	24 (28%)[d]	11 (33%)[d]	71 (17%)
Abandoned	4 (17%)	8 (11%)	0 (0%)	10 (9%)	3 (11%)	2 (6%)	5 (6%)	3 (9%)	35 (8%)
Unknown	3 (13%)	6 (8%)	1 (3%)	10 (9%)	1 (4%)	0 (0%)	1 (1%)	0 (0%)	22 (5%)
Other	0 (0%)	4 (5%)	2 (6%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	8 (2%)
Failed	2 (8%)	3 (4%)	1 (3%)	5 (5%)	2 (7%)	1 (3%)	0 (0%)	0 (0%)	14 (3%)
Tree swallow									
Initiated	24	29	26	38	31	25	48	24	245
Incubated	14 (58%)	24 (83%)	23 (88%)	34 (89%)	28 (90%)	23 (92%)	43 (90%)	23 (96%)	212 (87%)
Hatched	14 (58%)	23 (79%)	21 (81%)	30 (79%)	26 (84%)	21 (84%)	38 (79%)	23 (96%)	196 (80%)
Fledged	14 (58%)	18 (62%)	19 (73%)	26 (68%)	24 (77%)	18 (72%)	34 (71%)	23 (96%)	176 (72%)
Predated	2 (8%)	9 (31%)[e]	2 (8%)	7 (18%)	3 (10%)	4 (16%)	10 (21%)[c]	1 (4%)	38 (16%)
Abandoned	7 (29%)	0 (0%)	2 (8%)	3 (8%)	1 (3%)	2 (8%)	1 (2%)	0 (0%)	16 (7%)
Unknown	1 (4%)	0 (0%)	3 (12%)	1 (3%)	1 (3%)	0 (0%)	1 (2%)	0 (0%)	7 (3%)
Other	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)	1 (4%)	2 (4%)	0 (0%)	4 (2%)
Failed	0 (0%)	2 (7%)	0 (0%)	1 (3%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	4 (2%)
Eastern bluebird									
Initiated	12	18	15	40	5	14	18	0[f]	122
Incubated	10 (83%)	15 (83%)	15 (100%)	33 (83%)	4 (80%)	12 (86%)	17 (94%)		106 (87%)
Hatched	10 (83%)	15 (83%)	12 (80%)	29 (73%)	4 (80%)	10 (71%)	16 (89%)		96 (79%)
Fledged	7 (58%)	11 (61%)	10 (67%)	20 (50%)	2 (40%)	9 (64%)	16 (89%)		75 (61%)
Predated	1 (8%)	3 (17%)[c]	1 (7%)[c]	7 (18%)[c]	2 (40%)	3 (21%)	2 (11%)		19 (16%)
Abandoned	2 (17%)	2 (11%)	4 (27%)	5 (13%)	0 (0%)	0 (0%)	0 (0%)		13 (11%)
Unknown	0 (0%)	2 (11%)	0 (0%)	2 (5%)	0 (0%)	0 (0%)	0 (0%)		4 (3%)
Other	1 (8%)	0 (0%)	0 (0%)	6 (15%)	1 (20%)	2 (14%)	0 (0%)		10 (8%)
Failed	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		1 (1%)

[a] S-7 and S-9 were monitored in 2006 and 2007
[b] Fledged at least one nestling
[c] One nest that was preyed upon successfully fledged at least one nestling
[d] Two nests that were preyed upon successfully fledged at least one nestling
[e] Three nests that were preyed upon successfully fledged at least one nestling
[f] No clutches were initiated at S-7 and S-9 in 2007

House wrens and eastern bluebirds are known to nest multiple times during a breeding season, and it has been suggested that in parts of their range, tree swallows can successfully have two broods [41,42]. Adult females were captured and banded or recaptured at 91%, 98%, and 96% of clutches that hatched nestlings among all study sites for house wrens, tree swallows, and eastern bluebirds, respectively. No female tree swallows successfully raised two broods in one season, however two females nested again after their first nesting attempt was preyed upon. Multiple nesting attempts by adult females were more common in house wrens (18 to 29% by year) and eastern bluebirds (33 to 56% by year), and the proportion of multibrooded females per season increased yearly over the course of the study. One house wren female attempted three clutches in 2006 at S-7 with the first being successful and the second two failing. Two eastern bluebird females at T-3 and T-4 attempted three clutches in 2006 with two being successful and one failing for each female, and one eastern bluebird female at T-3 nested three times in both 2006 and 2007, fledging at least one nesting during each nesting attempt. Despite the high percentage of multiple nesting attempts by female house wrens and eastern bluebirds individual nesting attempts were still considered independent samples.

To account for some of the potential differences between first and subsequent nesting attempts as well as any temporal variation within a season nesting attempts were characterized as occurring EARLY or LATE during the nesting season. Species-specific mean dates of clutch incubation (23 June, 24 May, and 29 May for house wrens, tree swallows, and eastern bluebirds, respectively) were used to separate the nesting season into two parts and to limit the data set to include only those clutches that were completed

Figure 4.3. Frequency of clutch incubation initiations for house wren (black), tree swallow (open), and eastern bluebird (checked) clutches during 7-d windows for a) 2005, b) 2006, and c) 2007 for all study sites near Midland, Michigan, USA. Grey-topped bars indicate a known subsequent nesting attempt by a female during that season. Scale varies between years.



a)
b)
c)
16
12
8
4
0
30
25
20
15
10
5
0
40
35
30
25
20
15
10
5
0
Frequency
April
May
June
July
August

(Figure 4.3). Female house wrens and eastern bluebirds that attempted two clutches within a nesting season began incubating the first brood during the EARLY season 95% and 84% of the time, respectively. Female house wrens, tree swallows, and eastern bluebirds that only attempted one clutch within a nesting season began incubating during the EARLY season 47%, 62%, and 55% of the time, respectively. Additionally, an individual female's reproductive success both averaged by nesting season and for the whole study was investigated and is reported (Table 4.2).

Measured nest parameters were adjusted to account for eggs sampled for contaminant analyses. Percent of clutches that had eggs removed yearly for house wrens, tree swallows, and eastern bluebirds ranged from 6 to 25%, 17 to 32%, and 3 to 23%, respectively, with the least percent removed in 2007 for all species. Since fully developed nestlings were collected just prior to fledge, it was assumed that any nestlings collected would have successfully fledged provided the remaining portion of the nesting attempt was successful. Fledging success and productivity were not adjusted for sampled nestlings. Additionally, clutch size was not adjusted for eggs sampled because egg sampling occurred after clutch completion. Since 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. Subsequently, brood size (product of clutch size and hatching success) and number of fledglings (product of clutch size and productivity) were estimated based on adjusted nesting parameters.

Table 4.2. Mean overall measures of nesting success by study area for individually identified female house wrens, tree swallows, and eastern bluebirds breeding in the river floodplains near Midland, Michigan. Experimental units for individual seasons are unique females per year and for the overall study are unique females.

	Individual seasons						Overall study					
	R-1 to R-2		T-3 to T-6		S-7 and S-9[a]		R-1 to R-2		T-3 to T-6		S-7 and S-9	
	n	Mean ± SD (range)[b]	*n*	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	Mean ± SD (range)
House wren												
Nesting attempts	58	1.3 ± 0.46 (1–2)	155	1.2 ± 0.40 (1–2)	36	1.3 ± 0.51 (1–3)	52	1.4 ± 0.67 (1–4)	142	1.3 ± 0.57 (1–4)	34	1.4 ± 0.60 (1–3)
Eggs laid	58	7.8 ± 3.4 (4–15)	155	6.7 ± 2.6 (2–14)	36	7.8 ± 3.1 (4–16)	52	8.7 ± 4.8 (4–25)	142	7.3 ± 3.4 (2–26)	34	8.2 ± 3.8 (4–21)
Nestlings hatched	58	6.2 ± 3.4^{AB} (2–14)	155	5.2 ± 2.3^{B} (1–13)	36	6.5 ± 2.9^{A} (3–14)	52	6.9 ± 4.5 (2–23)	142	5.6 ± 3.0 (1–26)	34	6.9 ± 3.5 (3–18)
Nestlings fledged	58	5.5 ± 3.6 (0–14)	151	4.4 ± 2.4 (0–13)	36	5.1 ± 3.4 (0–13)	52	6.1 ± 4.6 (0–22)	139	4.8 ± 3.0 (0–25)	34	5.4 ± 4.0 (0–17)
Tree swallow												
Nesting attempts	60	1.0 ± 0.0 (1–1)	88	1.0 ± 0.15 (1–2)	49	1.0 ± 0.0 (1–1)	51	1.2 ± 0.42 (1–3)	72	1.3 ± 0.53 (1–3)	38	1.3 ± 0.46 (1–2)
Eggs laid	60	4.8 ± 1.2^{B} (1–7)	88	5.4 ± 1.4^{AB} (1–13)	49	5.6 ± 1.0^{A} (3–7)	51	5.5 ± 2.5^{B} (1–15)	72	6.6 ± 3.2^{AB} (1–20)	38	7.2 ± 2.9^{A} (3–14)
Nestlings hatched	60	3.9 ± 1.4^{B} (1–6)	88	3.8 ± 1.4^{B} (0–7)	49	4.8 ± 1.4^{A} (1–7)	51	4.5 ± 2.1^{B} (1–11)	72	4.7 ± 2.7^{B} (0–15)	38	6.2 ± 2.9^{A} (1–13)
Nestlings fledged	54	3.6 ± 1.5^{B} (0–6)	85	3.4 ± 1.6^{B} (0–7)	47	4.4 ± 1.6^{A} (1–7)	51	4.2 ± 2.3^{B} (0–11)	69	4.2 ± 2.8^{B} (0–13)	36	5.7 ± 2.8^{A} (1–13)
Eastern bluebird[c]												
Nesting attempts	21	1.5 ± 0.68 (1–3)	37	1.6 ± 0.59 (1–3)	2	(2–2)	18	1.7 ± 1.3 (1–6)	30	2.0 ± 1.2 (1–5)	2	(2–2)
Eggs laid	21	6.8 ± 3.6 (2–15)	37	7.3 ± 3.1 (2–16)	2	(8–8)	18	7.9 ± 6.6 (2–29)	30	9.0 ± 5.9 (2–24)	2	(8–8)
Nestlings hatched	21	4.9 ± 3.1 (0–13)	37	5.9 ± 2.5 (2–11)	2	(6–7)	18	5.7 ± 4.9 (0–20)	30	7.4 ± 4.4 (2–18)	2	(6–7)
Nestlings fledged	20	4.3 ± 2.4 (0–10)	35	4.5 ± 2.5 (0–10)	2	(2–4)	17	5.1 ± 3.8 (0–13)	28	5.7 ± 3.4 (0–13)	2	(2–4)
Male nestlings	19	2.3 ± 1.5 (0–6)	35	2.8 ± 1.5 (0–7)	2	(1–3)	16	2.6 ± 2.2 (0–8)	28	3.5 ± 2.1 (0–8)	2	(1–3)
Female nestlings	17	2.6 ± 2.2 (0–8)	31	2.3 ± 1.4 (1–5)	2	(1–1)	15	2.9 ± 2.9 (0–10)	25	2.8 ± 1.7 (1–7)	2	(1–1)

[a] S-7 and S-9 were monitored in 2006 and 2007

[b] Means with different uppercase letters were significantly different ($P<0.05$)

[c] S-7 and S-9 were not included in statistical comparisons for eastern bluebirds

Measures of nesting success for all clutches were included in comparisons up to the point that they were preyed upon, abandoned due to human interference or failed and thereafter removed from comparisons.

Measures of nesting success were not different for the species examined when compared among years, but were different if separated seasonally into EARLY and LATE nest initiation dates. Clutch size, predicted brood size, predicted number of fledglings, fledgling success, and productivity for house wrens and tree swallows were greater for EARLY nests as compared to LATE, while hatching success was similar (Table 4.3). Measures of nesting success for eastern bluebirds were similar regardless of the temporal resolution.

Mean overall hatching success, fledging success, and productivity for all species were variable among years with the ranges of 68 to 88%, 54 to 97%, and 48 to 82%, respectively (Table 4.3). Both EARLY and overall predicted brood sizes were greater for house wrens ($F=4.03$ $p=0.0199$, $F=5.02$ $p=0.0072$, respectively) and tree swallows ($F=5.60$ $p=0.0049$, $F=7.80$ $p=0.0006$, respectively) at Saginaw River SAs than at the Tittabawassee River SAs or for tree swallows at the RAs (Table 4.3). Fledging success for house wrens LATE ($F=5.41$ $p=0.0053$) was greater at reference areas despite greater brood sizes ($F=4.03$ $p=0.0199$) and predicted number of fledglings ($F=4.68$ $p=0.0108$) EARLY at Saginaw River SAs (Table 4.3). Clutch size and predicted number of fledglings were greater for tree swallows at Saginaw River SAs compared to the other study areas for both the EARLY ($F=8.55$ $p=0.0003$, $F=3.90$ $p=0.0232$, respectively) and overall ($F=5.79$ $p=0.0036$, $F=5.53$ $p=0.0047$, respectively) time periods (Table 4.3). Overall productivity for tree swallows was greater at Saginaw River SAs compared to

Table 4.3. Measures of nesting success for EARLY, LATE, and all nesting attempts for house wrens, tree swallows, and eastern bluebirds breeding in the river floodplains near Midland, Michigan during 2005-2007.

	EARLY nesting attempts						LATE nesting attempts						All nesting attempts					
	R-1 to R-2		T-3 to T-6		S-7 and S-9[a]		R-1 to R-2		T-3 to T-6		S-7 and S-9		R-1 to R-2		T-3 to T-6		S-7 and S-9	
	n	Mean (SD)[b]	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
House wren																		
Hatching Success[c]	38	0.81 (0.24)	93	0.76 (0.25)	23	0.83 (0.26)	44	0.80 (0.19)	113	0.78 (0.23)	30	0.81 (0.20)	82	0.81 (0.21)	206	0.77 (0.24)	53	0.82 (0.23)
Fledging Success[d]	35	0.93 (0.19)	84	0.93 (0.18)	20	0.97 (0.071)	42	0.81^{A} (0.34)	106	0.73^{A} (0.40)	25	0.54^{B} (0.42)	77	0.86^{A} (0.29)	190	0.82^{AB} (0.33)	45	0.73^{B} (0.38)
Productivity[e]	35	0.78 (0.25)	84	0.73 (0.23)	20	0.85 (0.21)	42	0.65 (0.32)	106	0.61 (0.37)	25	0.48 (0.40)	77	0.71 (0.29)	190	0.66 (0.32)	45	0.65 (0.37)
Clutch Size	38	6.4 (0.86)	93	6.0 (1.3)	23	6.3 (1.6)	45	5.7 (0.90)	113	5.3 (1.0)	30	5.6 (1.2)	83	6.0 (0.96)	206	5.6 (1.2)	53	5.9 (1.4)
Predicted Brood Size[f]	35	5.5^{AB} (1.6)	84	4.9^{B} (1.6)	20	5.8^{A} (1.5)	42	4.6 (1.3)	106	4.2 (1.4)	25	4.6 (1.5)	77	5.0^{AB} (1.5)	190	4.5^{B} (1.6)	45	5.1^{A} (1.6)
Predicted # fledglings[g]	35	5.1^{AB} (1.9)	84	4.5^{B} (1.8)	20	5.7^{A} (1.5)	42	3.8 (1.9)	106	3.2 (2.1)	25	2.8 (2.5)	77	4.4 (2.0)	190	3.8 (2.1)	45	4.1 (2.5)
Tree swallow																		
Hatching Success	34	0.82 (0.22)	49	0.78 (0.22)	30	0.87 (0.17)	20	0.80 (0.24)	37	0.73 (0.27)	19	0.84 (0.27)	54	0.81 (0.23)	86	0.76 (0.25)	49	0.86 (0.21)
Fledging Success	33	0.95 (0.18)	48	0.95 (0.14)	30	0.95 (0.14)	19	0.96 (0.11)	33	0.85 (0.30)	17	0.87 (0.22)	52	0.95 (0.16)	81	0.91 (0.22)	47	0.92 (0.17)
Productivity	33	0.80 (0.23)	48	0.75 (0.21)	30	0.82 (0.19)	19	0.78 (0.23)	33	0.63 (0.30)	17	0.79 (0.27)	52	0.80^{AB} (0.23)	81	0.70^{B} (0.26)	47	0.81^{A} (0.22)
Clutch Size	35	5.1^{B} (1.0)	51	5.4^{B} (0.94)	31	6.0^{A} (0.82)	21	4.8 (1.1)	38	4.9 (0.78)	19	4.9 (1.0)	56	5.0^{B} (1.1)	89	5.2^{B} (0.90)	50	5.6^{A} (1.0)
Predicted Brood Size	33	4.3^{B} (1.3)	48	4.3^{B} (1.2)	30	5.3^{A} (1.3)	19	4.0 (1.5)	33	3.6 (1.4)	17	4.5 (1.3)	52	4.2^{B} (1.4)	81	4.0^{B} (1.4)	47	5.0^{A} (1.4)
Predicted # fledglings	33	4.1^{AB} (1.5)	48	4.1^{B} (1.4)	30	5.0^{A} (1.4)	19	3.8 (1.4)	33	3.1 (1.6)	17	4.0 (1.7)	52	4.0^{AB} (1.5)	81	3.7^{B} (1.5)	47	4.6^{A} (1.6)
Eastern bluebird[h]																		
Hatching Success	15	0.72 (0.31)	23	0.78 (0.29)	1	1.0	16	0.68 (0.38)	29	0.88 (0.17)	1	0.67	31	0.70 (0.34)	52	0.84 (0.24)	2	0.83 (0.24)
Fledging Success	14	0.80 (0.33)	21	0.84 (0.25)	1	0.80	14	0.88 (0.29)	28	0.89 (0.24)	1	1.0	28	0.84 (0.31)	49	0.87 (0.24)	2	0.90 (0.14)
Productivity	14	0.61 (0.33)	21	0.72 (0.24)	1	0.80	14	0.65 (0.33)	28	0.79 (0.27)	1	0.67	28	0.63 (0.33)	49	0.76 (0.26)	2	0.73 (0.094)

Table 4.3 (Continued)

Clutch Size	16	4.8	27	4.8	2	5.0	17	4.4	30	4.4	2	3.0	33	4.5	57	4.6	4	4.0
		(0.86)		(0.64)				(1.0)		(0.81)				(0.94)		(0.75)		(1.2)
Predicted	14	3.7	21	4.0	1	5.0	14	3.6	28	3.9	1	2.0	28	3.6	49	4.0	2	3.5
Brood Size		(1.4)		(0.89)				(1.5)		(1.1)				(1.4)		(1.0)		(2.1)
Predicted #	14	2.9	21	3.4	1	4.0	14	3.1	28	3.6	1	2.0	28	3.0	49	3.5	2	3.0
fledglings		(1.7)		(1.2)				(1.6)		(1.4)				(1.6)		(1.3)		(1.4)

[a] S-7 and S-9 were monitored in 2006 and 2007

[b] Means with different uppercase letters were significantly different ($P<0.05$)

[c] Hatching success was adjusted for any eggs removed for contaminant analyses or broken by researchers

[d] Fledging success includes nestlings collected for contaminant analyses if the remainder of the clutch was successful

[e] Productivity is defined as the number of nestlings fledged per eggs laid

[f] Brood size was predicted based on clutch size and hatching success

[g] Predicted number of fledglings was defined as the product of clutch size and productivity

[h] S-7 and S-9 were not included in statistical comparisons for eastern bluebirds

Tittabawassee River SAs (F=3.96 p=0.0208), while RAs were intermediate (Table 4.3). Eastern bluebird measures of nesting success were similar between Tittabawassee River SAs and RAs; however Saginaw River SAs were not included in statistical comparisons due to low occupancy.

Throughout the study the majority of adult females were individually identified at each nesting attempt. This enabled comparisons to be made on an individual per season basis as well as overall measures of nesting success by individual females for the study period. However, females that were unsuccessful in hatching clutches could not be banded (16% of house wrens; 6% of tree swallows; 7% of eastern bluebirds) which likely resulted in an underestimation of both seasonal as well as overall number of nesting or re-nesting females. Additionally, nesting attempts that were preyed upon, abandoned, or otherwise unsuccessful were included in a female's yearly and overall measures of nesting success. The majority of house wren (91%), tree swallow (80%), and eastern bluebird (82%) females bred during only one nesting season. However, several females bred during two or all three seasons for house wrens (19 and 1, respectively), tree swallows (30 and 3, respectively), and eastern bluebirds (8 and 1, respectively). Eastern bluebird females initiated up to six clutches with eight individuals nesting greater than two times, house wren females initiated up to four clutches with nine individuals nesting greater than two times, and tree swallows initiated up to three clutches with only four females nesting three times. Of all renesting females, only one tree swallow switched study areas between years (R-2 in 2005 and T-6 in 2006). Tree swallow females at Saginaw River SAs hatched and fledged more nestlings by season (F=9.29 p<0.0001, F=6.83 *p*=0.0014, respectively) and for the overall study period (F=6.28 *p*=0.0024, F=5.52 *p*=0.0049, respectively) than at the other study areas (Table 4.2). Female house wrens at the Saginaw River SAs hatched more nestlings by season (F=4.05 *p*=0.0186) than females at Tittabawassee River SAs, while reference areas were intermediate (Table 4.2). Measures of nesting success for eastern bluebirds were similar between RAs and Tittabawassee River SAs, with individual females fledging up to 13 nestlings over the study period (Table 4.2).

Nesting tree swallow and eastern bluebird females were identified as either second-year or after-second-year when captured. Overall, 19% of the breeding tree swallow females were classified as SY, while 57% of the breeding eastern bluebird females were classified as SY among all study areas. Clutch size (F=16.72 *p*<0.0001), brood size (F=9.69 *p*=0.0022), estimated number of fledglings (F=13.78 *p*=0.0003), fledging success (F=6.51 *p*=0.0116), and productivity (F=5.71 *p*=0.0180) were greater for tree swallow ASY females compared to SY females, while hatching success (F=2.37 *p*=0.1252) was similar. ASY female tree swallows had 13%, 20%, 25%, 9%, and 16% greater clutch sizes, brood sizes, estimated number of fledglings, fledging success, and productivity, respectively, than SY females. Only clutch size (F=5.79 *p*=0.0184) was 8% greater for ASY eastern bluebird females compared to SY females.

Of all nestlings that successfully fledged (1124 house wrens, 609 tree swallows, and 201 eastern bluebirds), three house wren (all female), six tree swallow (3 male and 3 female), and three eastern bluebird (1 male and 2 female) nestlings that hatched on-site returned to breed in a subsequent year. One eastern bluebird female nestling that hatched at T-3 returned to breed at T-4, but all others returned to their natal site. Two tree swallow nestlings (1 male and 1 female) were hatched at R-2 and bred at T-3, while one female nestling hatched at T-6 and bred at T-3. One female house wren nestling hatched at S-7 and returned to breed at T-5, while the other females returned to their natal sites.

During routine nest monitoring, nestlings were observed for gross morphological abnormalities as potential assessment endpoints for individual health. Two tree swallow nestlings from a clutch at R-1 had foot deformities. One had a club foot and the other was missing a foot. Interestingly, concentrations of PCDD/DFs were similar in tree swallow eggs at RAs and Tittabawassee and Saginaw River SAs [31]. The greatest concentrations of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ in eggs of tree swallows were observed at RAs, albeit the congener profiles were different. Congener profiles in tree swallow eggs at upstream RAs are composed of primarily PCDDs opposed to TCDF and 2,3,4,7,8-PeCDF at downstream SAs. No other deformities were observed for eastern bluebirds or house wrens among all study areas for the duration of the study.

The potential influence of weather on nest success was investigated by looking for correlations of weather and weather events to health related measurement endpoints. Daily minimum, mean, and maximum temperatures were averaged and rainfall was summed for individual nesting attempts from the date of clutch initiation through date of fledge. Hatching success, fledgling success, and productivity were individually correlated with the four weather parameters for the nest period and ranges of coefficients of determination (r^2) were 0.22 to 0.24, 0.14 to 0.23, and 0.13 to 0.24 for house wrens, tree swallows, and eastern bluebirds, respectively. Some correlations were significant but examining the data further revealed that the marginal correlations were spurious and not indicative of true trends in the measures of nesting success.

Eggs and nestlings

Mean egg masses by clutch were not different among study areas for house wrens ($F=1.89$ $p=0.1530$) or tree swallows ($F=1.15$ $p=0.3194$), but were greater at Tittabawassee River SAs compared to RAs for eastern bluebirds ($F=4.04$ $p=0.0207$). Mean (±SD) egg mass for 233 house wren clutches was 1.45±0.12 g (ranged 1.16 to 1.80), and was 1.84±0.14 g (ranged 1.49 to 2.25) for 121 tree swallow clutches. Mean (±SD) egg mass for eastern bluebird clutches at Tittabawassee River SAs was 3.12±0.29 g ($n=62$; ranged 2.37 to 3.77), at RAs it was 2.96±0.23 g ($n=34$; ranged 2.47 to 3.38), while at Saginaw River SAs it was 3.12±0.10 g ($n=4$; ranged 2.99 to 3.20). Saginaw River SAs were not included in the among-site comparisons for eastern bluebirds because of low box occupancy at those locations.

Mean nestling masses post-hatch by clutch were similar for house wrens among all study areas, but were different on some days for tree swallows and eastern bluebirds. Tree swallow nestlings 8-d post-hatch had greater masses at RAs and Tittabawassee River SAs compared to those at Saginaw River SAs ($F=5.52$ $p=0.0050$), while masses on the other days were similar among study areas. Eastern bluebird nestlings had greater masses 8 d ($F=14.60$ $p=0.0003$), 12 d ($F=13.58$ $p=0.0004$), and 14 d ($F=6.08$ $p=0.0161$) post-hatch at Tittabawassee River SAs compared to RAs. Mass gained per day was not different among SAs for house wrens ($F=0.79$ $p=0.4562$), tree swallows ($F=0.93$ $p=0.3965$), or eastern bluebirds ($F=3.05$ $p=0.0853$). Mean mass gained per day for all study areas was greatest in eastern bluebirds (1.87±0.31 g; $n=75$), least in house wrens (0.97±0.13 g; $n=197$), and intermediate in tree swallows (1.66±0.25 g; $n=122$). Growth rate constants (95% CIs) for house wren broods at RAs, Tittabawassee River SAs, and

Saginaw River SAs ranged from 0.40 to 0.52 (n=48), 0.40 to 0.49 (n=123), and 0.40 to 0.56 (n=40), respectively. Growth rate constants (95% CIs) for tree swallow broods at RAs, Tittabawassee River SAs, and Saginaw River SAs ranged from 0.47 to 0.58 (n=39), 0.47 to 0.58 (n=58), and 0.38 to 0.52 (n=37), respectively. Growth rate constants (95% CIs) for eastern bluebird broods at RAs and Tittabawassee River SAs ranged from 0.29 to 0.45 (n=27) and 0.39 to 0.50 (n=47), respectively. Comparisons were not made between tree swallow and house wren mean growth rate constants for locations due to the nearly complete overlap in 95% CIs. However, eastern bluebird growth rate constants (mean±SD) were similar between RAs (0.37±0.20) and Tittabawassee River SAs (0.44±0.19; t_{72}=1.5453, p=0.1267).

Discussion

Site-specific residues

Concurrent studies quantified the concentrations of PCDD/DFs and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) based on World Health Organization toxic equivalence factors [17] in dietary items, reconstituted diet, eggs, and nestlings of house wrens, tree swallows, and eastern bluebirds [31,32]. Oligocheata (non-depurated) and brachycera (diptera) contained the greatest mean concentrations of ΣPCDD/DFs of the major site-specific dietary items collected via food web sampling at downstream SAs, while dietary items at RAs were lesser. Accumulation of ΣPCDD/DFs and $TEQ_{WHO\text{-}Avian}$ from site-specific reconstituted food web-based dietary insect samples for nestlings at study areas (SAs) was 31- to 121-fold and 9- to 64-fold greater than at proximally located RAs, respectively. The sum of concentrations of PCDD/DFs in eggs of house wrens and eastern bluebirds from SAs were 4- to 19-fold greater compared to those from RAs, while eggs of tree swallows were similar among areas. Despite similar concentrations in eggs of tree swallows between study areas the congener profiles at downstream SAs were dominated by 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentadibenzofuran (2,3,4,7,8-PeCDF) opposed to primarily dioxin congeners at RAs. Maximum concentration of ΣPCDD/DF in eggs of house wrens, tree swallows, and eastern bluebirds were 7.2×10^3 ng/kg at T-4, 2.0×10^3 ng/kg at R-1, and 2.4×10^3 ng/kg at T-6, respectively. Additionally, concentrations of ΣPCDD/DFs in nestlings of all studied species at SAs were 4- to 49-fold greater compared to RAs. Maximum concentration of ΣPCDD/DFs in nestlings of house wrens, tree swallows, and eastern bluebirds occurred at T-6 and were 1.7×10^3 ng/kg, 7.3×10^3 ng/kg, and 2.1×10^3 ng/kg, respectively. Concentrations of $TEQ_{WHO\text{-}Avian}$ in both eggs and nestlings of all study species were positively correlated with concentrations of ΣPCDD/DFs [31].

Reproductive success

During the first breeding season after placement of new nest boxes, reproductive measures and occupancy can be variable until a substantial breeding population is established. Tree swallows monitored during the year after box placement on the Saginaw River floodplain in Michigan were limited by occupancy and variable reproductive performance [43]. In the current study, despite box placement on site one year prior to monitoring, occupancy for all species was least during the initial year. Prior to the initiation of this study only R-2 had a nest box trail. This site had an established nesting population of primarily tree swallows that was previously considered a local unexposed population for an assessment of exposure to PCBs on the Saginaw River [7]. Previous research on the Kalamazoo River in Michigan noted potential differences in box occupancy between newly and previously established nest box trails [12]. Additionally, nest box placement can affect occupancy rates, which is why boxes were deliberately placed at study sites to attempt to maximize occupancy by all three study species. Tree swallow adults defend adjacent nest boxes from conspecifics [44] but will often nest in close proximity to other species such as bluebirds [45-48]. Since house wrens, tree swallows, and eastern bluebirds are generally nest-site limited [49] establishing a stable nesting population for these species can occur quickly given appropriate habitat availability on site.

During the study, nests that were abandoned or preyed upon accounted for the majority of failed nesting attempts for all species. Nest abandonment and predation events accounted for approximately 25%, 23%, and 27% of house wren, tree swallow, and eastern bluebird clutches that failed during 2005 through 2007. Abandonment rates for all study species were similar to those previously reported for eastern bluebirds in an unexposed population in Minnesota [50]. Tree swallows breeding at PCB contaminated sites along the Hudson River had a greater incidence of abandoned clutches compared to those from upstream of the contamination [51]. However abandonment rates were similar between study areas in the current study, while the proportions of predated nests were variable between areas. Predation events only accounted for 17%, 16%, and 16% of initiated clutches for house wrens, tree swallows, and eastern bluebirds, respectively, but a large proportion (68%) of that percent occurred at Tittabawassee River SAs for all three studied species. Snakes predated 17% of eastern bluebird nests in Kentucky [52], while in the current study snake predations were only observed at S-7. The majority of predations at the other sites were caused by mammals and birds. House sparrows (*Passer domesticus*) and house wrens were the primary avian predators for tree swallow and eastern bluebird nests, while house wren nests were predated by primarily mammals. Predation rates were similar for studied species to house wren in Wyoming [53] and Ohio [54], and all currently studied species in Ontario [55]. However, nest parasitism was extremely rare in the current study, approximately 60% of house wren nests were parasitized by shiny cowbirds (*Molothrus bonariensis*) in Argentina with a mean of 1.7 eggs per nest [56].

Overall, the reproductive endpoints examined were successful for the species studied during 2005 through 2007, but there were some species and endpoint specific differences noted among study areas. For nesting success endpoints, measured values were within the species-specific ranges previously reported in the literature [12,39,53,57-62]. Nesting attempts were fairly evenly distributed around the species specific mean date of clutch initiation. By splitting the nesting season for each studied species into EARLY and LATE nesting attempts it was possible to separate the majority of multiple broods and investigate potential temporal reproductive differences in nest timing.

Fledging success for house wrens was lower for all study areas during the LATE period compared to the EARLY, however it decreased by 43% at Saginaw River SAs compared to only 12 and 20% at RAs and Tittabawassee River SAs, respectively. Similarly fledging success for house wrens in Wyoming was lesser for those breeding later in the season [63], while fledging success was temporally similar in Michigan [12]. It is unknown why broods at Saginaw River SAs had over two times greater reduction in

LATE fledging success compared to the other study areas. Despite the decreased fledging success for LATE nesting house wrens at Saginaw River SAs, the overall predicted brood size was greater and the overall predicted number of fledglings was similar to other study areas.

Tree swallow nesting success endpoints based on counts (clutch size, predicted brood size and predicted number of fledglings) were greater for EARLY and for overall nesting attempts at Saginaw River SAs compared to the other study areas. The inherent interrelatedness of these variables undoubtedly is the reason for the similar statistical trends. The difference is primarily driven by the reproductive output of the tree swallows breeding at S-9, and likely related to site-specific habitat or food availability differences between it and the other sites. Most boxes at S-9 are adjacent to the bank opposed to other study sites in which the boxes are separated from the bank by floodplain forest. However, the quantification of food availability was beyond the scope of this research, the relative abundance of Diptera and Hemiptera in close proximity to the nest boxes was possibly greater at this site. Sites in Ontario had a 7-fold difference in insect abundance which resulted in increased tree swallow productivity [64], and tree swallows breeding downstream of a pulp/paper mill also had increased reproductive output likely due to nutrient enrichment and a subsequent increase in insect abundance [65]. Additionally, increased food abundance early in the nesting season at study locations along a lakeshore compared to along a roadside resulted in greater tree swallow clutch sizes [66]. However, in the current study the habitat differences were less severe between study areas.

For all species studied hatching success was the only nesting success endpoint measured that was similar between EARLY and LATE nesting attempts. Hatching success is widely used as one of the most sensitive endpoints for avian exposure assessment [5,67-71] and it was expected that increased time spent on-site would lead to greater site-specific exposure to contaminants, which would result in decreased hatching success later in the season. However, like hatching success, egg concentrations for the species studied were similar throughout the breeding season [31], which was likely due to the fact that most passerine species ingest resources for egg production near the time of breeding [72,73].

For house wrens and tree swallows, clutch size, predicted brood size, predicted number of fledglings, fledging success, and productivity were greater for EARLY than for LATE nesting attempts, while for eastern bluebirds these endpoints were similar. Several studies have documented decreases in clutch size later in the breeding season [12,74-78]), which inherently should lead to lesser predicted brood sizes and predicted number of fledglings. Reduced reproductive success later in the breeding season has been previously reported for several species [39,76,79-81]. The specific cause(s) of the lesser reproductive success across study areas for these endpoints during LATE nesting attempts is beyond the scope of this project. However, since these endpoints generally decreased similarly across study areas it is unlikely due to greater exposure to contaminants at downstream study areas.

Site-specific exposures of passerine birds to dioxin-like compounds throughout the United States and Canada have reported mixed results as to potential reproductive effects. The majority of studies have reported no or variably significant reproductive effects

[8,12,23,51,62,71,82], while tree swallow hatching success was decreased on the primarily PCB contaminated Housatonic River in New York [67] and the TCDD contaminated Woonasquatucket River in Rhode Island [5]. However, studied species along the Tittabawassee River had similar ΣPCDD/DF or $TEQ_{WHO\text{-}Avian}$ concentrations in eggs [31] to the studies on the Housatonic and Woonasquatucket rivers, a similar decrease was not observed in hatching success.

Individual breeding birds were banded and subsequently identified at the majority of nesting attempts. It was therefore possible to calculate overall measures of nesting success for individual females by nesting season and for the complete study. By comparing reproductive parameters over a longer period than a single nesting season it is possible to get a broader scale depiction of population health between study areas. For example, nesting success from a 27 y study of eastern bluebirds reported 85% of nests fledged at least one nestling in 18 of 27 y (ranged 70 to 97%), but cautioned that in short-term studies higher than expected nesting success may be reported by chance alone [57]. Despite only monitoring sites for a 3 y period, eastern bluebird females laid a greater number of eggs, hatched more nestlings, and fledged more nestlings than western bluebirds (*Sialia mexicana*) monitored for 7 y in Oregon [83]. The estimates of overall nesting success for unique females in this study is likely an underestimate of the total reproductive output for adult females since undoubtedly some birds previously or subsequently bred on site. Tree swallows have been recorded breeding for up to nine to 12 seasons [84,85], eastern bluebirds have bred for five successive seasons [39], and a house wren was recorded that survived for 7 y [86]. However while these estimates are primarily high end survival estimates for individuals, they highlight the importance of cumulative lifetime reproductive monitoring. Additionally, for short-lived species the proportion of females that double brood can have substantial effects on the annual fecundity of a population [87]. For the current study, only house wrens and eastern bluebirds were successful in raising multiple broods in one season.

Few studies have quantified cumulative reproductive output of specific females. Despite only considering eastern bluebird pairs that fledged multiple broods in a season [39], the number of nestlings fledged per pair for a season was only slightly greater than that observed in the current study that considered all nesting attempts per season. Overall measures of nesting success for uniquely identified eastern bluebird females was statistically similar between study areas, despite a trend toward greater nesting success at Tittabawassee River SAs. Greater proportions of female eastern bluebird nestlings have been reported [88], with research on western bluebirds reporting a similar gender proportion [89]. In the current study gender proportions were variable among study areas, which was likely due to limited overall sample size compared to longer-term studies. It was estimated that house wrens breeding in Ohio need to lay approximately six eggs per season to ensure replacement of themselves and maximize their survivorship and lifetime reproduction [90]. House wrens in the current study laid more than six eggs per season at all study areas. Uniquely identified tree swallow females at Saginaw River SAs had a trend toward greater reproductive success by season and for the overall study compared to RAs or Tittabawassee River SAs. The trend is similar to that observed for early and overall nesting attempts for all tree swallow females discussed previously.

Survival until first reproductive season can have a large influence on population fitness in passerine birds. The post-fledging period for newly fledged passerines is considered a critical survival window that can limit first year survival and population recruitment. Subtle biochemical changes from exposures to contaminants in passerines can possibly affect survival especially for nestlings immediately post-fledging [18,29,91-94]. However, minimum radio transmitter mass has advanced enough in recent years to facilitate use on small passerines [95-99] it was beyond the scope of the current study to incorporate nestling survival monitoring with telemetry. Additionally, research on effects of transmitter attachment on survival of small passerines is relatively sparse and inconclusive [100-103]. Estimates of post-fledging survival probability from transmitter studies are variable, but were 0.37 over 20 d in lark buntings (*Calamospiza melanocorys*) [104] and 0.63 over 72 d in meadowlarks (*Sturnella magna*) [105]. Only 19% of hooded warblers (*Wilsonia citrina*) survived the 28 d post-fledging telemetry monitoring period with the highest mortality rates observed in the first 4 d after fledge [106]. Estimated survival rate to first breeding season for banded house sparrow fledglings was 30% [107], and weather based factors decreased juvenile survival in a European wren species during their first year to a greater extent than adult survival over a similar period [108]. During the current study 0.3 to 1.5% of banded nestlings were recaptured for the species studied. The slightly lower than expected return rates across all study sites are likely due both to a limited sampling duration and area. Monitoring both adult and nestling band returns for house wrens, tree swallows, and eastern bluebirds continued during the 2008 and 2009 breeding seasons, which should better quantify site-specific survival. Nestling dispersal distance from natal areas is generally greater than that of returning adults, and was estimated for house wren nestlings (607 to 674 m) and adults (67 to 134 m) in Illinois [109]. However, mean migration dispersal distances for tree swallow adults breeding in

New York were greater and ranged from 2.4 to 8.4 km for males and females, respectively [110]. In the current study most nestlings returned to their natal study site, while four nestlings returned to a different study site up to approximately 25 km away. However, only one tree swallow adult returned to breed at a different study site approximately 30 km from the previous years study site. Given these study-specific dispersal distances it is probable that survival for both nestlings and adults are underestimated due to offsite dispersal. However, off-site dispersal is likely similar between study areas since both adult and nesting tree swallow dispersal in Saskatchewan Canada was not influenced by previous reproductive success [111].

Increased incidences of deformity and embryo death were correlated with colonial waterbird exposure to planar halogenated compounds in the Great Lakes from 1986–1991 [112]. Lower bill deformities and subcutaneous edema were documented in a single clutch of wood duck (*Aix sponsa*) eggs exposed to PCDD/DFs in Arkansas [113]. Developmental abnormalities were also observed after *in ovo* exposure to TCDD and other dioxin-like compounds in various avian species and included edemas of the head and neck, liver damage, and skeletal and beak deformities [70,114,115], however similar abnormalities were not always present [69]. Several studies of sites contaminated with either PCBs or PCDD/DFs throughout the US on house wrens, tree swallows, and eastern bluebirds have not observed any developmental abnormalities [5,12,62,67]). In the current study, potential lower leg deformities were observed in one clutch of tree swallow nestlings at R-1. These two nestlings with abnormal legs survived through fledge, and it is unknown whether the potential deformities were correlated with site-specific contaminant exposures.

Weather variables were not correlated with measured reproductive endpoints for the current study but periods of extreme weather can affect reproduction and survival in passerine birds. Shifts in local weather conditions during critical reproductive periods can not only compromise reproductive success but also adult survival. House wrens nesting in the floodplain of the North Platte River in Wyoming during years of flooding had later clutch initiation dates and subsequently lower clutch sizes compared to areas without flooding [63]. Cold rainy periods, when altricial nestlings are still dependent on adults for both dietary requirements and thermoregulation, can result in brood or even adult losses [43,116-118]. Tree swallows breeding in wetlands exposed to compounds from oil sands tailings in Alberta, Canada experienced harsh weather and a subsequent nestling die-off of 48% at reference sites while mortality rates at reclaimed wetlands ranged from 59 to 100% [91]. At wetlands exposed to site-specific contaminants nestlings may be less able to withstand additional stressors that could decrease post-fledgling survival [91]. Large die-offs of tree swallow adults have been reported following early season cold weather periods, and subsequent decreases in nesting effort [119]. However, tree swallow adults were found dead in nest boxes during each year of the current study, the distribution is fairly universal across study sites and the extent of deaths is generally low.

Additionally, studies have monitored birds exposed to contaminants for molecular [5,92,120-126], immune [18,29,127-130], morphometric [5,131-136], and genetic [6,137,138] responses with mixed results. The predominant problem with the majority of these measurement endpoints is relating them to altered survival or reproductive performance in field studies, while similar measurement endpoints are useful in laboratory studies to determine dose-response relationships [139-142]. Variability inherent in field studies that is generally assumed to be similar between proximally located exposed and unexposed sites, such as habitat, weather, and genetic relatedness of adults combined with limited sample sizes can add enough uncertainty to mask trends for these response variables. As a result the current study focused on collecting data on population health level response variables opposed to individual based responses.

Eggs and nestlings

Differences in nestling condition or egg mass have been widely investigated for possible effects on nest productivity with mixed results [77,143,144]. Stresses such as exposure to dioxin-like compounds can alter energetics that can lead to decreased nestling growth rates [145]. Tree swallows exposed to oil sands mining wastes had lower masses compared to unexposed populations [91]. However trees swallow nestlings exposed to elevated concentrations of PCBs on the Hudson River in New York had similar or greater growth compared to upstream locations [51]. Tree swallow mean egg masses were greater at PCB contaminated sites along the Kalamazoo River in Michigan [62]. Eggs and nestlings from the current study are within the ranges presented for house wrens [12,146], tree swallows [8,62,79,147], and eastern bluebirds [12]. Despite slight differences for tree swallow nestling masses and eastern bluebird egg and nestling masses for some measurements, the overall trend was similar between study areas for egg masses, nesting masses, nestling mass gained/d, and growth rate constants.

Conclusions

Overall reproductive success was investigated for house wrens, tree swallows, and eastern bluebirds nesting in the river floodplains near Midland, Michigan due to greater concentrations of ΣPCDD/DFs in biota at downstream study areas. Despite $TEQ_{WHO\text{-}Avian}$ concentrations comparable to some of the most contaminated sites throughout North America, passerines breeding along the Tittabawassee and Saginaw rivers brooded successful nests. Hatching success, a measurement endpoint that has been shown in both field and laboratory studies to be negatively impacted by exposure to dioxin-like compounds, was similar among study areas and uncontaminated sites. Hatching success was also the only variable that was temporally consistent for all species, while the majority of other measurement endpoints quantified for house wrens and tree swallows were greater earlier in the breeding season. Tree swallows at S-9 had greater values for egg and nestling count variables compared to the other study areas, which could be due to habitat differences and/or greater invertebrate abundance at this location. In general, reproductive success was similar or greater at downstream study areas during 2005 to 2007. Both adult and nestling post-fledging survival to the subsequent breeding season were generally similar among study areas; however results may be limited by the short study duration and narrow study corridor based on a riverine system. Ongoing site-specific banding studies will expand this dataset. Additionally, recent decreases in radio transmitter size and increases in battery life have made it more feasible to conduct an in-depth nestling post-fledging survival study for potential effects of site-specific contaminants on fledgling recruitment and survival. Additional manuscripts will discuss implications of these results by incorporating data from tissue exposure [31] and dietary exposure [32] into aquatic [148] and terrestrial [149] passerine risk assessments.

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CHAPTER 5

Multiple lines of evidence in a risk assessment of tree swallows exposed to dioxin-like compounds associated with the Tittabawassee River near Midland, Michigan, USA

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Abstract

Concentrations of dioxin-like compounds, primarily polychlorinated dibenzofurans (PCDFs), 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, which were compared with upstream reference areas (RAs), selected toxicity reference values (TRVs), and the results from other similarly contaminated field sites. The current 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 measured during the 2005 to 2007 breeding seasons. A hazard assessment based on estimated dietary exposures suggested that tree swallow populations would be severely affected at both the Tittabawassee and Saginaw river SAs. However, when exposures were measured in eggs and compared to appropriate TRVs, little effect on populations was predicted, despite uncertainties associated with potential polychlorinated biphenyl (PCB) co-contamination in eggs among SAs. Hatching success was weakly negatively correlated with concentrations of $TEQs_{WHO\text{-}Avian}$ in individual eggs, however among study areas it was similar (76–86%) and total clutch failures were rare. Other measures of the condition of the populations indicated no difference between the Tittabawassee and Saginaw river SAs and RAs.

Keywords: *Tachycineta bicolor*; tissue exposure; dietary exposure; productivity; furans; dioxins

Introduction

Tittabawassee River sediments and floodplain soils downstream of Midland, Michigan (USA) are contaminated with polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs). 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], which contributes to the uniqueness of the site relative to other sites that are generally contaminated with polychlorinated biphenyls (PCBs) or PCDDs. Contributions of PCDF to the congener profile are similar to 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 [6,7]. The lipophilic nature and slow degradation rates of these compounds [8] when sheltered from ultraviolet solar radiation, combined with consistent inundation of the floodplain, led to the 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 elevated concentrations of PCDFs, PCDDs, and PCBs in fish collected downstream of Midland. Wild game consumption advisories were issued in 2004 based on elevated concentrations in deer and turkey. A 2003 report to the Michigan Department of Environmental Quality (MIDEQ) 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 [9]. 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 [10]. In particular, AhR-mediated compounds have been shown to decrease hatching success, adult responsiveness and immune function, and increase enzyme induction of birds [11–16]. Recent findings provide evidence of the molecular basis for variation in avian species sensitivity to dioxin-like compounds [17,18].

Species that are at the top of the food web are generally considered the most likely to experience greater exposure to dioxin-like compounds [19–26]. However, high trophic status species often 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 with a completely site-specific foraging range has potential for greater exposures to site-specific contaminants than higher trophic status species.

Tree swallows (*Tachycineta bicolor*) were selected to determine the extent and distribution of chemical exposure through the aquatic food chain and associated risk downstream of Midland. Tree swallows eat primarily emergent aquatic invertebrates [27–29] and have been shown to have exposure links to contaminated sediments [30–35]. They readily occupy nest boxes when provided, and forage in close proximity to their nest while breeding [36,37]. Additionally, 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 U.S.A., is commonly encountered and generally nest in close proximity to conspecifics [38]. 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 tree swallows allowed for better experimental control and eliminated time-intensive nest searching.

Numerous studies have monitored tree swallows for exposure to and/or effects of polychlorinated biphenyls (PCBs) across North America [30,31,33,35,39–50]. However, the exposure and potential effects of elevated exposures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on tree swallows has been limited to a study along the Woonasquatucket River in Rhode Island [51] and no studies have directly assessed effects at sites where exposures were primarily to PCDFs.

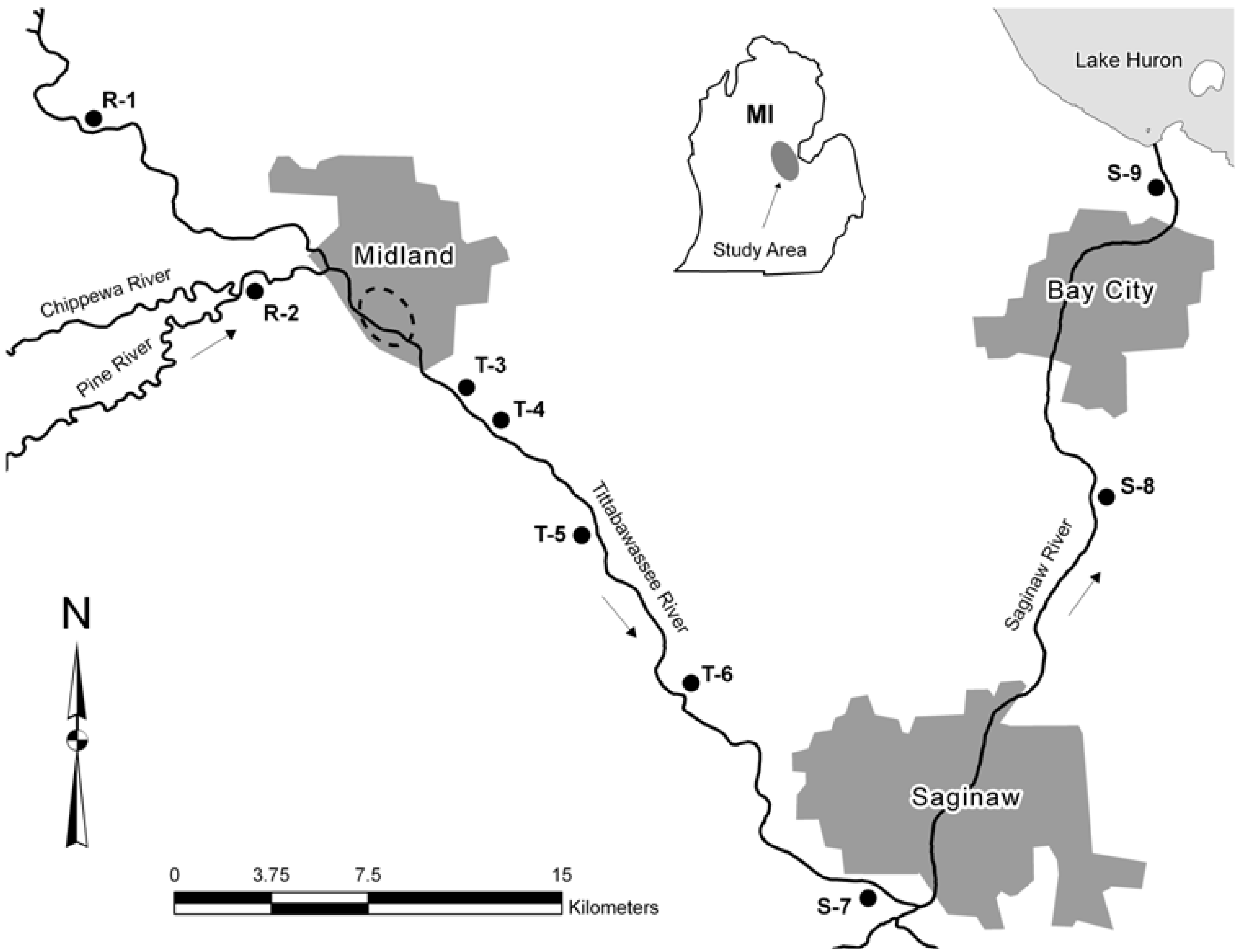
The primary objective of this study was to use a multiple lines of evidence approach [52] to evaluate the potential for adverse effects on tree swallows breeding downstream of Midland, Michigan, where exposure to dioxin-like compounds is primarily to PCDFs. Site-specific measures of exposure included measured concentrations of PCDD/DFs in eggs, nestlings, and diet. Dietary concentrations were quantified by both direct analyses of bolus samples as well as by diet reconstruction based on site-specific concentrations in invertebrate orders and dietary composition by order determined by percent mass from bolus samples. In addition, reproductive success and nestling growth were measured. Potential for adverse effects was evaluated by comparing the concentrations of TCDD equivalents ($TEQ_{WHO\text{-}Avian}$) based on World Health Organization (WHO) TCDD equivalency factors for birds ($TEF_{WHO\text{-}Avian}$) [10] in the diet and tissues of tree swallows to available toxicity reference values (TRVs). Predicted hazard quotient values based on TRVs were compared to site-specific measures of population condition. Additionally, comparisons were made between these results and similar field-based measures of exposure, productivity, and nestling growth. Using a hazard assessment approach combined with site-specific multiple-lines of evidence can strengthen confidence, minimize uncertainty, and broaden the applicability of risk assessment outcomes.

Methods

Site description

The study was conducted on the Tittabawassee, Chippewa, and Saginaw rivers, in the vicinity of Midland, Michigan (Figure 5.1). 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 putative sources of PCDD/DFs (Hilscherova et al. 2003) on the Tittabawassee (R-1) and Chippewa (R-2) rivers (Figure 5.1). Study areas (SAs) downstream of the putative sources of PCDD/DFs include approximately 72 km of free flowing river from the upstream boundary defined as the low-head dam near Midland, Michigan through the confluence of the Tittabawassee and Saginaw rivers to where the Saginaw River enters Saginaw Bay in Lake Huron. SAs 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 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

Figure 5.1. 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 S-9) were monitored from 2005–2007. Direction of river flow is designated by arrows; suspected source of contamination is enclosed the dashed oval.


R-1
R-2
Chippewa River
Pine River
Midland
T-3
T-4
T-5
T-6
Tittabawassee River
MI
Study Area
Lake Huron
S-9
Bay City
S-8
Saginaw River
Saginaw
S-7
N
0
3.75
7.5
15
Kilometers

continuous foraging area of between 1 and 3 km of river. S-8 was an exception and was only used for sediment and dietary food web sampling. No studies of birds were conducted at this location.

Nest box monitoring

Standard passerine nest boxes with wire mesh predator guards around the entrance hole and mounted to a greased metal post were used to facilitate monitoring of nesting activity and collection of samples [53]. 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 subsequent to placement of nest boxes and continued through 2007 at all sites. Individual nest boxes were placed at study sites to maximize occupancy of several passerine species [54] 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 [53,55]. In general, boxes were monitored twice a week for occupancy beginning in early April. Boxes were monitored daily after clutch initiation through incubation and subsequently near the expected hatch or fledge day for each species. Masses of eggs were determined on the date laid, and masses of nestlings were measured 4 times over the brood rearing period. Eggs for use in residue quantification were collected after clutch completion and prior to the fifth day of incubation. Therefore, clutch size was not adjusted for egg sampling. However, hatching success, fledging success, and productivity measurements were calculated based on an adjusted clutch size since the fertility and hatchability of the collected egg was unknown at collection. Additionally, brood size and number of fledglings were predicted based on the adjusted hatching success and productivity, respectively. A maximum of one nestling per nesting attempt was collected from randomly selected boxes for residue quantification 14-d post-hatch. Since fully developed nestlings were collected just prior to fledge, it was assumed that any nestlings collected would have successfully fledged provided the remaining portion of the nesting attempt was successful. Therefore, fledging success and productivity were not adjusted for sampled nestlings. This compromise in the experimental design was used so that the most accurate, clutch-specific estimates of concentrations of PCDD/DFs could be made.

Nestlings and adults were banded with US Fish and Wildlife Service aluminum leg bands throughout the study. Adults were actively trapped by researchers at the nest box during each nesting attempt. During routine handling birds were monitored for gross external morphological abnormalities.

Dietary exposure

Detailed site descriptions and protocols for collecting and handling samples of representative invertebrate orders collected on-site and dietary bolus samples collected from nestlings have been previously presented [56]. 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 × 30 m grids proximal to the river bank, one for sampling of terrestrial invertebrates and one for collection of benthic and emergent aquatic invertebrates. Sites in the SA were selected based on maximizing the potential for collecting food items with the greatest contaminant concentrations for a given nest box trail given the available soil and sediment data. Sampling methods were designed to target aquatic emergent insects, benthic invertebrates, and terrestrial invertebrates in order to collect the necessary biomass for residues analyses and to obtain a representative sample of available dietary items at each site. Invertebrates were categorized taxonomically to the order level for each life stage collected during each sampling period per site. Samples were then homogenized and stored at -20 ºC until extraction.

Dietary food items were collected as bolus samples from nestlings using a black electrical cable-tie fitted at the base of their neck [57]. Samples were collected from nestlings between the ages of 4- and 12-d post-hatch, approximately 1 h after ligature application and nests were not sampled on consecutive days. Invertebrates in each bolus sample were classified to taxonomic order and the total number and mass of each order was recorded for each sample. 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. Additionally, bolus samples were recombined for residue analyses based on clutch from which each sample was collected and combined with other proximally and temporally located boxes to obtain the necessary biomass for residue quantification.

Dietary exposures of adults were estimated using the U.S. Environmental Protection Agency (USEPA) Wildlife Exposure Factors Handbook (WEFH) equations for passerine birds [58]. USEPA WEFH equation 3-4 was used to calculate food intake rate based on site-, species- and age-specific body masses. Potential average daily dose (ADD_{pot}; ng $TEQ_{WHO\text{-}Avian}$/kg body weight/d) was calculated using equation 4-3 [58] assuming that

100% of the foraging range was within the associated study area. Dietary concentrations in food items were estimated using two methods: 1) food web-based diet: multiplying study-specific dietary compositions for major (>1% by mass) prey items by respective area-specific (R-1 to R-2; T-3 to T-6; S-7 to S-9) average, minimum and maximum concentrations of $TEQs_{WHO\text{-}Avian}$ in associated prey items, and 2) bolus-based diet; area-specific average, minimum and maximum concentrations from actual bolus samples collected from nestlings. Minimum and maximum concentrations were chosen to describe the range of possible invertebrate concentrations found on site, which the authors expected to include the worst-case scenario for dietary exposure. Dietary exposure estimates apply only to the nesting period as foraging habits and range are likely more variable outside the nesting period.

Chemical analyses

Concentrations of seventeen 2,3,7,8-substituted PCDD/DF congeners were quantified in all samples whereas concentrations of polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDT) and related metabolites were measured in a subset of egg samples. Congener residues were quantified in accordance with EPA Method 8290/1668A with minor modifications [59]. A more detailed description of methods and the measured concentrations have been previously reported [55,56]. Briefly, samples were homogenized with anhydrous sodium sulfate, spiked with known amounts of ^{13}C-labeled analytes (as internal standards), and Soxhlet extracted. Ten percent of the extract was removed for lipid content determination. Sample purification included the following: treatment with concentrated sulfuric acid, silica gel, sulfuric acid silica gel, acidic alumina and carbon column chromatography. Components were analyzed using high-resolution gas chromatography/high-resolution mass spectroscopy, a Hewlett-Packard 6890 GC (Agilent Technologies, Wilmington, DE) connected to a MicroMass® high-resolution mass spectrometer (Waters Corporation, Milford, MA). Chemical analyses included pertinent quality assurance practices, including matrix spikes, blanks, and duplicates.

Toxicity reference values

Selection of appropriate toxicity reference values is an essential step in the risk assessment process. TRVs 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 [51] 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 hazard quotients (HQs) and subsequent assessment of risk. In this study, dietary exposure- and egg exposure-based TRVs were used to evaluate the potential adverse effects of site-specific contamination on tree swallows.

Laboratory-based dosing studies incorporating PCDD/DF dietary exposure-based effects assessments are lacking for passerines and limited in general for avian species. A study that dosed adult hen ring-necked pheasants (*Phasianus colchicus*) with intraperitoneal injections of TCDD for a 10 wk exposure period was selected as the dietary exposure-based TRV for the study described herein [12]. The major limitation of the Nosek et al. [12] study was that hens were exposed to TCDD via 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. Additionally, while this study was not conducted on a passerine species, galliforms are generally considered to have greater sensitivity to dioxin-like compound exposures [14,60–62] and recent findings provide evidence suggesting a molecular basis to this variation [17,18] with ring-necked pheasants being similar to the passerines studied (SW Kennedy *personal communication*). The dietary-based TRVs were determined by converting the weekly exposure at which adverse effects on fertility and hatching success were determined (1000 ng TCDD/kg/wk) to a LOAEC for daily exposure of 140 ng TCDD/kg/d. 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) (Table 5.1).

Ring-necked pheasant egg-injection studies [12,13,63] with TCDD were selected as the most applicable for deriving egg-based TRVs in the current study. The three studies that dosed ring-necked pheasant hens or eggs were combined to determine a geometric mean NOAEC of 710 ng/kg ww and LOAEC of 7,940 ng/kg ww as egg exposure-based TRVs [64]. Other egg-injection studies that dosed bobwhite quail (*Colinus virginianus*)

Table 5.1. Toxicity reference values (TRVs) for total TEQs$_{WHO\text{-}Avian}$[a] concentrations selected for comparison to tree swallows exposed to PCDD/DFs in the river systems downstream of Midland, Michigan, USA during 2005–2007.

	NOAEC	LOAEC	Reference
Dietary exposure-based[b]	14	140	[12]
Egg exposure-based[c]	710	7,940	[64][d]

[a] TEQs$_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] ng/kg/d ww
[c] ng/kg ww
[d] Calculated from studies by Nosek et al. [12,13,63]

[65] and double-crested cormorant (*Phalacrocorax auritus*) [15,62] 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 [44,51] 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 tree swallows breeding in the river floodplains downstream of Midland was assessed through a multiple lines of evidence approach [52], which incorporated both dietary-based and tissue-based exposure estimates in addition to monitoring site-specific productivity. Potential effects of dietary- and tissue-based exposures were assessed by calculating hazard quotients. Concentrations of ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ in eggs and dietary estimates [potential average daily dose (ADD_{pot})] were divided by egg exposure- or dietary exposure-based NOAEC or LOAEC TRVs

(Table 5.1), respectively. Hazard quotients were determined based on the upper 95% confidence level (UCL) for geometric means at individual study locations for egg exposures and 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. HQs for dietary exposure were calculated based on $TEQs_{WHO\text{-}Avian}$ 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 and compared to relevant literature-based field studies. Incorporation of both dietary- and tissue-based assessment endpoints has been shown to greatly reduce uncertainty in risk assessments of persistent organic pollutants [66].

Statistical analyses

Individual nesting attempts were considered the experimental unit for statistical comparisons. Egg-based exposure comparisons were made between sampling locations [55]. 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 [56]. In depth descriptions of productivity measures and associated statistical analyses have been previously reported [53].

Total concentrations of the 17 individual 2,3,7,8-substituted PCDD/DF congeners are reported as the sum of all congeners (ng/kg ww). For individual congeners for which concentrations were less than the limit of quantification, a proxy value of half the sample method detection limit was assigned. Concentrations of $TEQ_{WHO\text{-}Avian}$ (ng/kg ww) were calculated for PCDD/DFs by summing the product of the concentration of each congener, multiplied by its avian $TEF_{WHO\text{-}Avian}$ [10]. Total concentrations of 12 non- and mono-*ortho*-substituted PCB congeners are reported as the sum of these congeners (ΣPCBs) for a subset of egg samples that were screened for co-contaminants. Additionally, dichloro-diphenyl-trichloroethane (2',4' and 4',4' isomers) and dichloro-diphenyl-dichloroethylene (4',4') are reported as the sum of the *o*,*p*- and *p*,*p*-isomers (DDT metabolites) for the same subset of samples as for PCBs.

Statistical analyses were performed using SAS® software (Release 9.1; SAS Institute Inc., Cary, NC, USA). Prior to the use of parametric statistical procedures, normality was evaluated using the Shapiro–Wilks test and the assumption of homogeneity of variance was evaluated using Levene's test. Since the $TEQ_{WHO\text{-}Avian}$ 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 $TEQs_{WHO\text{-}Avian}$ concentrations at each study location a probabilistic modeling approach was used to portray the distributions. The mean and standard deviation of transformed egg values were used to generate a sample of 10,000 random egg values based on a lognormal distribution. This probabilistic model is presented as cumulative frequency distributions based on

ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ concentrations. The association between concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ and hatching success was evaluated with Pearson's correlation coefficients for nesting attempts in which both data were collected. Statistical significance was considered at $P<0.05$.

Results

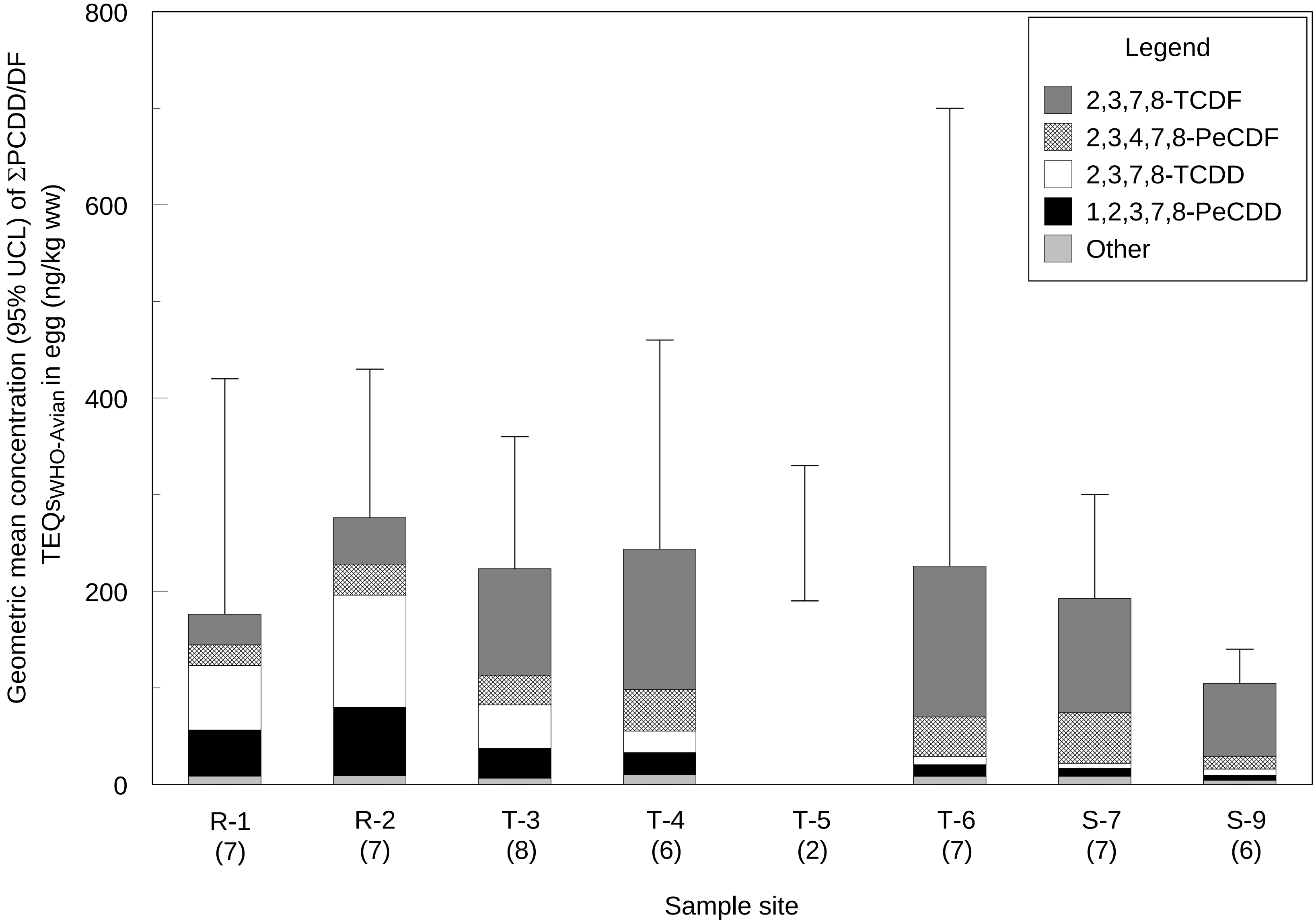
Site-specific endpoints

Among all study sites, 245 tree swallow 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, which had few tree swallow nesting attempts due to box placement constraints from farming practices. Additionally, concentrations of ΣPCDD/DF were quantified in eggs (n=50) and nestlings (n=45) tissues collected from individual tree swallow nesting attempts. Samples of boluses were collected throughout the nesting season from 96 tree swallow nesting attempts to determine site-specific foraging patterns and to determine bolus-based dietary exposure to PCDD/DFs.

Tissue residues

Concentrations of PCDD/DFs and $TEQ_{WHO\text{-}Avian}$ were quantified in tree swallow eggs and nestlings collected on-site [55]. Geometric mean concentrations of $TEQs_{WHO\text{-}Avian}$ in eggs of tree swallows were similar among study locations (Figure 5.2). However, patterns of relative concentrations of congeners in eggs from more downstream SAs averaged 49–72% for 2,3,4,7,8-pentadibenzofuran (2,3,4,7,8-PeCDF) and 13–27% for

Figure 5.2. Geometric mean concentrations of ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ in tree swallow eggs collected during 2005–2007 from the river floodplains near Midland, Michigan, USA. Error bars show the 95% upper confidence level (UCL); reference areas (R-1 and R-2); Tittabawassee River study areas (T-3 to T-6); Saginaw River study areas (S-7 to S-9); range reported for T-5 where n=2; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; Other = sum of remaining 13 2,3,7,8-substituted PCDD/DF congeners.


Geometric mean concentration (95% UCL) of ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ in egg (ng/kg ww)
0
200
400
600
800
Legend
2,3,7,8-TCDF
2,3,4,7,8-PeCDF
2,3,7,8-TCDD
1,2,3,7,8-PeCDD
Other
R-1 (7)
R-2 (7)
T-3 (8)
T-4 (6)
T-5 (2)
T-6 (7)
S-7 (7)
S-9 (6)
Sample site

2,3,7,8-tetrachlorodibenzofuran (TCDF) as opposed to 38–42% for TCDD and 26–27% for 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) at the RAs. Maximum concentration of $TEQs_{WHO\text{-}Avian}$ in eggs of tree swallows among all study locations was 730 ng/kg at R-1. Co-contaminants were screened in 3 egg samples. Concentrations of the DDT complex were at background levels (ranged 0.14–0.72 mg/kg ww), while ΣPCB $TEQs_{WHO\text{-}Avian}$ were 62 ng/kg ww at R-2, 520 ng/kg ww at T-4 and 160 ng/kg ww at T-6 that corresponded to 36%, 47% and 23% of the total dioxin-like $TEQs_{WHO\text{-}Avian}$ for those samples, respectively. In nestlings, concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ at the Tittabawassee and Saginaw river SAs were 3- to 34-fold greater than those in nestlings from RAs. Maximum concentration of $TEQs_{WHO\text{-}Avian}$ in nestling tree swallows occurred at T-6 and was 6000 ng/kg ww. The relative potency of the exposure mixture was quite consistent in both eggs and nestlings for tree swallows, as concentrations of $TEQ_{WHO\text{-}Avian}$ were positively correlated with concentrations of ΣPCDD/DFs. Profiles of relative concentrations of congeners were similar in eggs and nestlings at Tittabawassee and Saginaw river SAs.

Dietary exposures

Concentrations of PCDD/DFs and $TEQ_{WHO\text{-}Avian}$ were quantified in site-specific food webs by making measurements in collected invertebrates from all study areas and bolus samples collected from tree swallow nestlings at RAs and Tittabawassee River SAs [56]. Site-specific dietary composition was determined by quantifying the mass of individual invertebrate orders to the overall dietary mass from bolus samples. Potential average daily dose (ADD_{pot}; ng/kg body weight/d) based on $TEQ_{WHO\text{-}Avian}$ 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 tree swallows, while food web-based dietary exposure estimates were 11-fold greater at Saginaw River SAs (Table 5.2).

Table 5.2. Potential average (range) $TEQ_{WHO\text{-}Avian}$ [a] daily dose (ADD_{pot}; ng/kg body weight/d) 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.

	R-1 and R-2 [b]	T-3 to T-6	S-7 to S-9
Bolus	4.9 (1.4–8.8) [c,d]	200 (24–800)	– [e]
Food web	6.1 (1.3–13)	250 (34–630)	70 (40–120)

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values

[b] R-1 and R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area

[c] Values were rounded and represent only two significant figures

[d] Food ingestion rate was calculated from equations in The Wildlife Exposure Factors Handbook [58]

[e] Residue analyses were not conducted on bolus collected invertebrates at S-7 and S-9

Nesting success

Reproductive parameters including clutch size, egg mass, hatching success, predicted brood size, nestling growth, fledging success, predicted number of fledglings and productivity were monitored for tree swallows breeding in the river floodplains near Midland, Michigan [53]. Of all initiated clutches 73% successfully fledged at least one nestling. In general, tree swallows had greater measures of reproductive success at Saginaw River SAs compared to Tittabawassee River SAs, while 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, while productivity at Tittabawassee River SAs was 70% compared to 80–81% at the other study areas. Overall hatching success at RAs, Tittabawassee River SAs, and Saginaw River SAs were 81%, 76%, and 86% at RAs, respectively, and were not statistically different between areas. Since adult females were captured and uniquely identified during nesting attempts it was possible to determine overall nesting success per female for the duration of the study. Number of nesting attempts per female was similar between study areas, while eggs laid, nestlings hatched and nestlings fledged were generally greater at Saginaw River SAs compared to RAs and Tittabawassee River SAs. Total nestlings fledged per female from 2005 to 2007 averaged (range) 4.2 (0–11; *n*=51), 4.2 (0–13; *n*=69) and 5.7 (0–13; *n*=36) for RAs, Tittabawassee River SAs and Saginaw River SAs, respectively. Nestling growth rate constants and mass gained per day were similar among study areas [53].

Hazard assessment

Statistically significant, negative correlations were observed between hatching success and concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ in tree swallow eggs for clutches where both data endpoints were measured. Overall hatching success averages ranged from 76–86% among study areas, but hatching success for individual eggs was negatively correlated with $TEQs_{WHO\text{-}Avian}$ (R=-0.42975, p=0.0063, n=39; Figure 5.3).

Predicted probabilistic distributions of expected cumulative percent frequencies of ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ concentrations in eggs were compared to selected TRVs. Predicted distributions of $TEQ_{WHO\text{-}Avian}$ concentrations in tree swallow eggs were greater

Figure 5.3. Correlation plot of percent hatching success and ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ in tree swallow eggs for nesting attempts with data collected for both variables from the river floodplains near Midland, Michigan during 2005–2007. R- and p-values and sample size indicated; 1=R-1; 2=R-2; 3=T-3; 4=T-4; 5=T-5; 6=T-6; 7=S-7; 9=S-9.

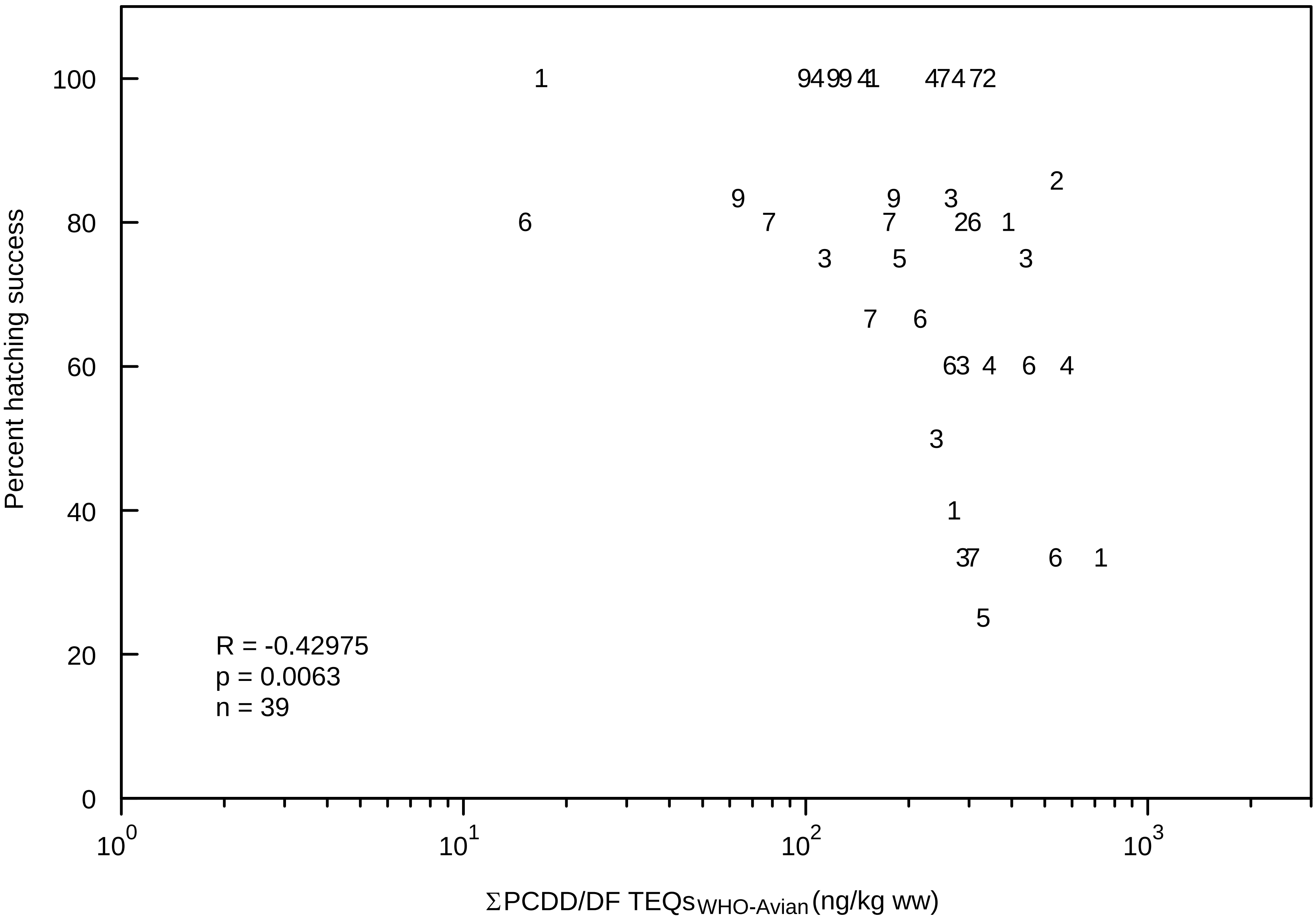
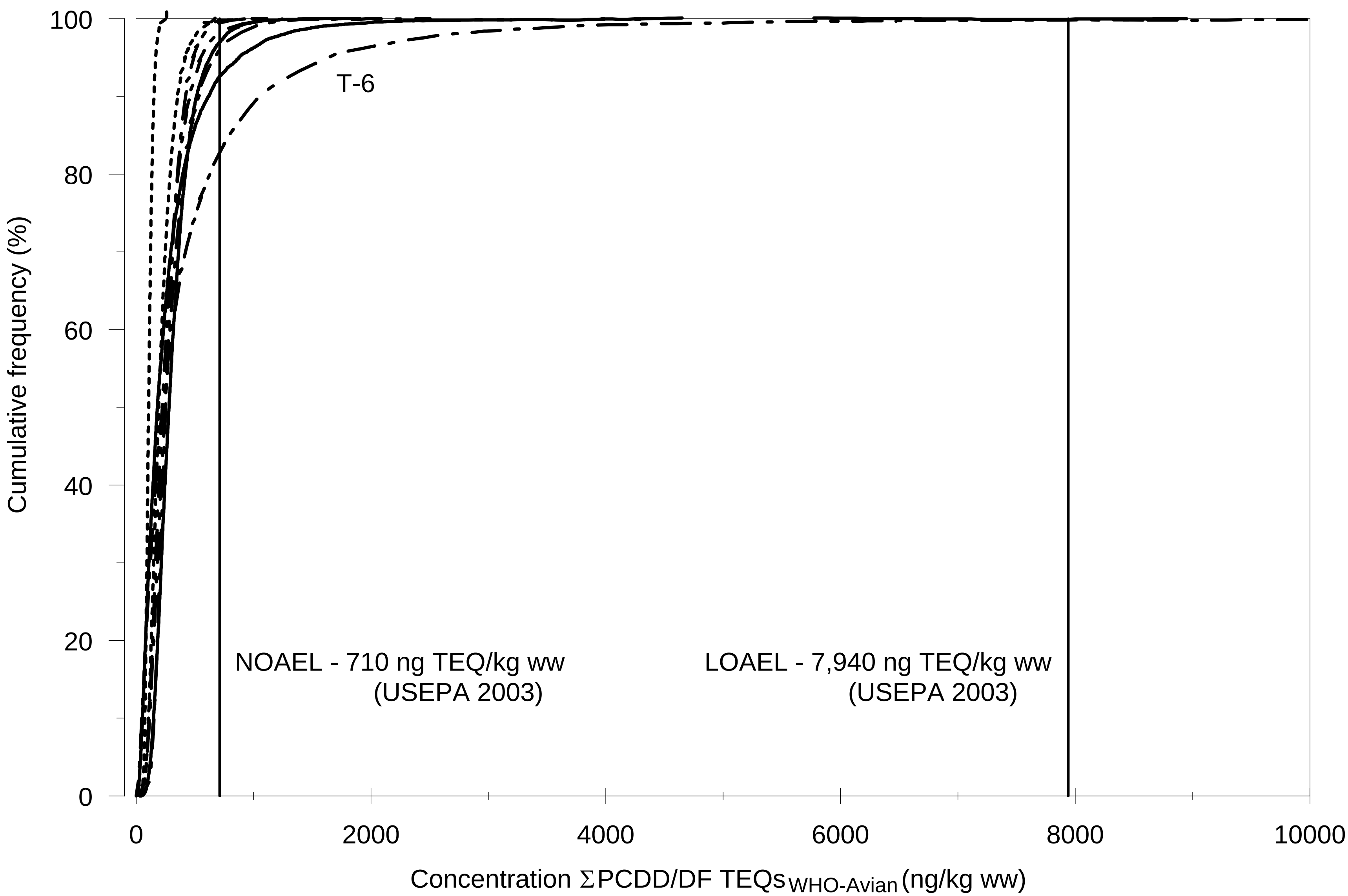

Percent hatching success
0
20
40
60
80
100
10^0
10^1
10^2
10^3
ΣPCDD/DF TEQs WHO-Avian (ng/kg ww)
R = -0.42975
p = 0.0063
n = 39

Figure 5.4. Modeled probabilistic distribution of expected cumulative percent frequencies for tree swallow egg $TEQ_{WHO\text{-}Avian}$ concentrations ng/kg ww from site-specific eggs collected in the river floodplains around Midland, Michigan in 2005–2007. 10,000 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; NOAEC and LOAEC indicated by vertical bars.



Cumulative frequency (%)
100
80
60
40
20
0
0
2000
4000
6000
8000
10000
T-6
NOAEL - 710 ng TEQ/kg ww
(USEPA 2003)
LOAEL - 7,940 ng TEQ/kg ww
(USEPA 2003)
Concentration ΣPCDD/DF TEQs WHO-Avian (ng/kg ww)

than the NOAEC (710 ng/kg ww; USEPA 2003) for all sites other than S-9 (Figure 5.4). R-1 and T-6 had 7.4% and 17% of the predicted distribution greater than the NOAEC, respectively, while 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 frequency was greater than the LOAEC (7,940 ng/kg ww; USEPA 2003).

Upper 95% confidence level (geometric mean) concentrations of ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ in tree swallow 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 0.2 among all study sites. The greatest HQ determined was less than 1.0 based on NOAEC at T-6 (Figure 5.5).

Dietary exposures based on minimum measured concentrations of ΣPCDD/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 ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ at Tittabawassee River SAs were greater than the LOAEC TRV, while Saginaw River SAs HQs were less than 1.0 (Figure 5.6). Both food web- and bolus-based estimates of dietary exposure were less than dietary-based LOAEC and NOAEC TRVs at RAs. The maximum bolus-based hazard quotient at Tittabawassee River SAs was 57 based on the NOAEC, while based on the LOAEC the HQ was approximately 6 (Figure 5.6). The maximum and minimum food web-based dietary exposure HQs based on the NOAEC at Saginaw River SAs were 9 and 3 for the range of measured concentrations of ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$, respectively (Figure 5.6).

Figure 5.5. Hazard quotients (HQ) for the effects of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ for tree swallow eggs collected in 2005–2007 in the river floodplains near Midland, Michigan, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). 95% confidence intervals (LCL/UCL) based on the geometric mean concentrations are presented; range presented for T-5 where n=2; reference areas (R-1 and R-2); Tittabawassee River study areas (T-3 to T-6); Saginaw River study areas (S-7 and S-9).

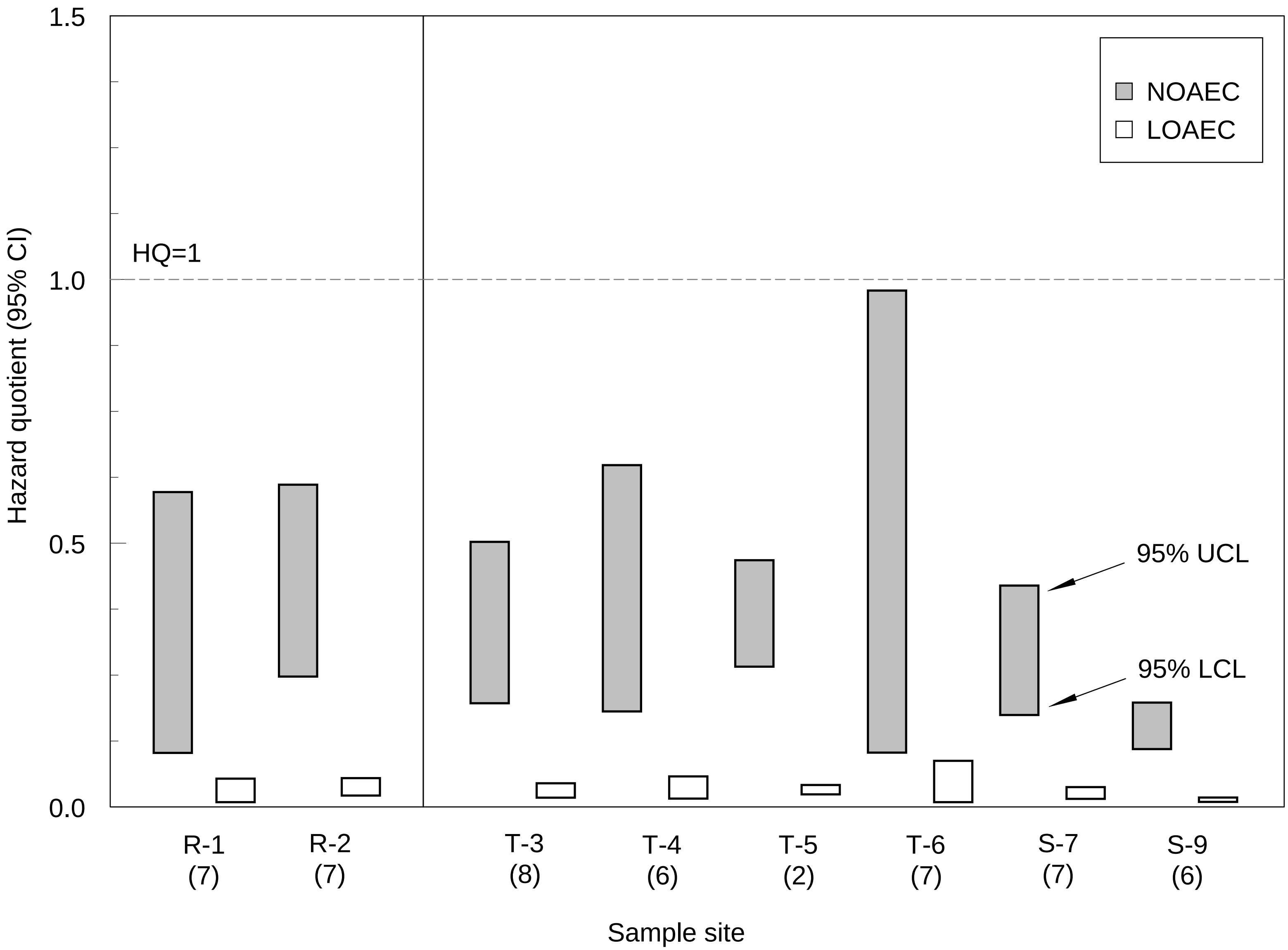
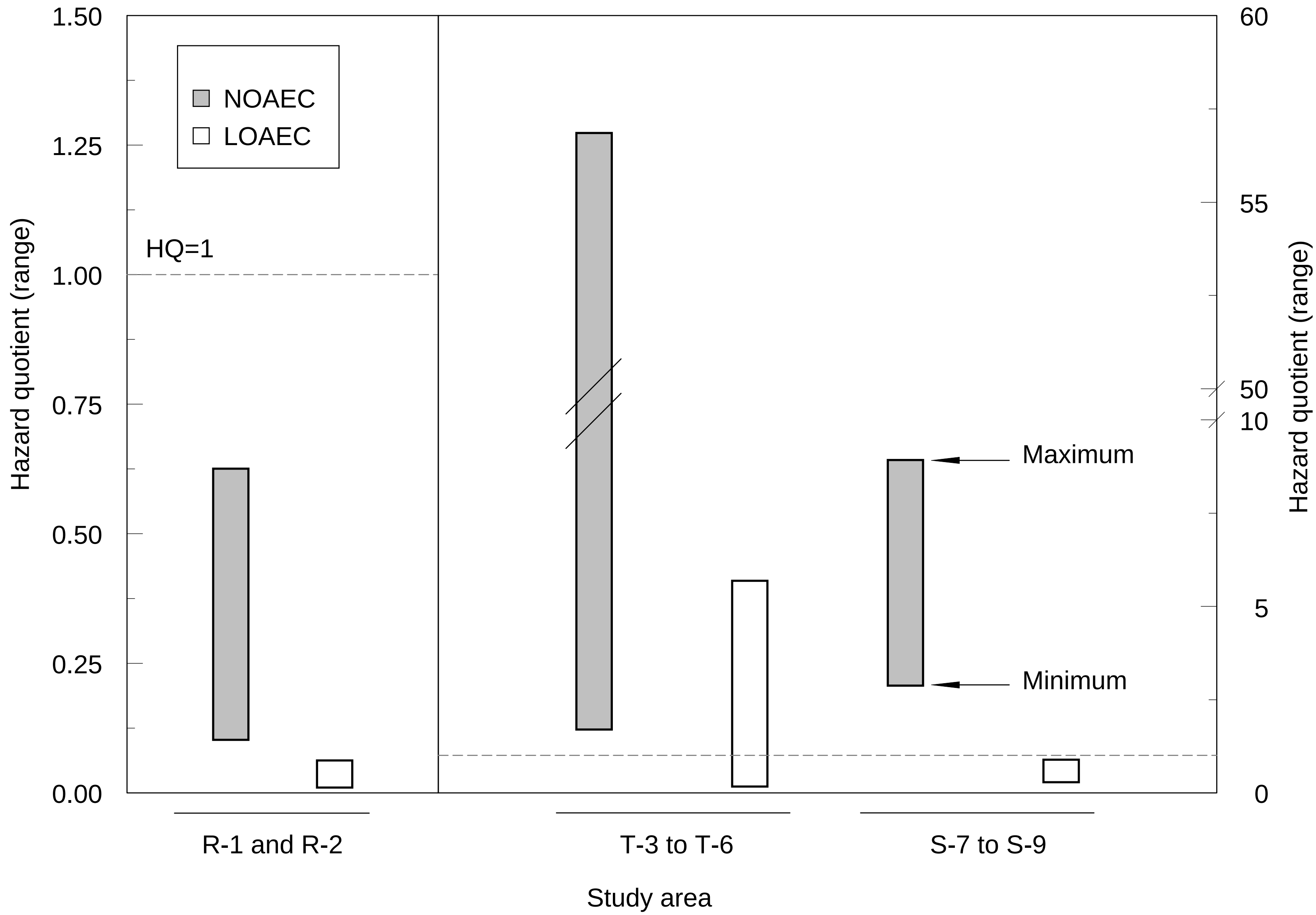

Hazard quotient (95% CI)
1.5
1.0
0.5
0.0
HQ=1
NOAEC
LOAEC
95% UCL
95% LCL
R-1 (7)
R-2 (7)
T-3 (8)
T-4 (6)
T-5 (2)
T-6 (7)
S-7 (7)
S-9 (6)
Sample site

Figure 5.6. Hazard quotients (HQ) for the effects of potential ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ daily dietary dose calculated from site-specific bolus-based (R1 to T-6) and food web-based (S-7 to S-9) dietary exposure for adult tree swallows collected in 2005–2007 from the river floodplains near Midland, Michigan, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). HQs based on measured concentration ranges are presented; left y-axis for reference areas (R-1 and R-2); right y-axis (note 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 since no bolus samples were collected from those sites.



NOAEC
LOAEC
HQ=1
Maximum
Minimum
1.50
1.25
1.00
0.75
0.50
0.25
0.00
60
55
50
10
5
0
Hazard quotient (range)
Hazard quotient (range)
R-1 and R-2
T-3 to T-6
S-7 to S-9
Study area

Discussion

The tree swallow is a relevant study species for the assessment of risk in aquatic environments due to ease of study, high 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 concern over the sensitivity of the species to dioxin-like compounds [44,48,51,67–69].

Recent research has determined potential molecular differences in sensitivities of birds to dioxin-like compounds [17,18]. Specifically, the difference between species-specific sensitivities to dioxin-like compounds is potentially tied to amino acid substitution differences in the aryl hydrocarbon receptor (AhR) ligand binding domain (LBD) between species (SW Kennedy *personal communication*). Based on these findings, the tree swallow 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. Continued research into the species- and congener-specific differences in sensitivities to dioxin-like compounds in birds is necessary. By combining multiple lines of evidence for tree swallows 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 tree swallows as receptors at contaminated sites, the greatest limiting factor for developing accurate assessments of risk for birds exposed to dioxin-like compounds is still a lack of comprehensive studies designed to determine thresholds for effects. The domestic chicken (*Gallus domesticus*) has been widely studied and is considered to be the most sensitive bird species to the effects of dioxin-like compounds [14,60,61,70–72]. After considering a number of criteria the TRVs used herein were based on studies of the ring-necked pheasant [12,13,63], and were less conservative compared to TRVs based on chicken exposures. For the dietary exposure-based HQs, the TRVs based on intraperitoneal injections of TCDD in hen ring-necked pheasants [12] would be expected to over estimate 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 [20,73–77]. Recently, it has been determined that the relative sensitivity of birds to the effects of AhR-active compounds can be predicted from the structure of the LBD of the AhR [17,18]. Thus, the most appropriate TRV for the tree swallow was deemed to be based on the Nosek et al. [12] dietary exposure. 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 tree swallow.

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 into the likelihood of site-specific effects. For all study locations, hazard quotients were less than 1.0 based on reported 95% UCLs for $TEQ_{WHO\text{-}Avian}$ concentrations in tree swallow eggs using the ring-necked pheasant NOAEC (Figure 5.5). However, TRVs based on an eastern bluebird egg injection study [16] were less conservative than the pheasant study (NOAEC 1,000 ng TCDD/kg ww and LOAEC 10,000 ng TCDD/kg ww) and if used would result in HQs that range from 0.14 to 0.7 for all study locations. HQs greater than 1.0 indicate that exposures exceed the threshold for adverse effects, suggesting that there is the potential 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 $TEQ_{WHO\text{-}Avian}$ in eggs at these sites the percent of the frequency distribution greater than the NOAEL ranged up to 17% (Figures 4). However, less than 1% of the frequency distribution for concentrations of $TEQ_{WHO\text{-}Avian}$ in tree swallow eggs was greater than the LOAEC among all study locations. Based on 3 samples that were screened for ΣPCB $TEQs_{WHO\text{-}Avian}$ in the current study, it was determined that the addition of ΣPCB $TEQs_{WHO\text{-}Avian}$ to the ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ could contribute up to a 2-fold increase in predicted HQs. At most locations this increase would bring the egg-based HQs up to approximately 1.0, while at T-6 the HQs would be approximately 2.0. For all three samples, the proportion of the $TEQs_{WHO\text{-}Avian}$ contributed by PCBs were dominated by congeners 77 > 126 > 81 > 105, with the remaining eight congeners making up less than 2% of the profile. Additional sampling would provide a better understanding of the potential contributions of PCBs to the egg-based hazard assessment for tree swallows along the Tittabawassee River. 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 tree swallows exists among study locations.

Hazard quotient values based on concentrations of $TEQ_{WHO\text{-}Avian}$ contributed by PCDD/DFs in bolus samples from nestling tree swallows, were greater than or equal to 1.0 at Tittabawassee and Saginaw River SAs based on the minimum value of $TEQ_{WHO\text{-}Avian}$ concentrations and the NOAEC. The HQs based on the maximum value of $TEQ_{WHO\text{-}Avian}$ concentrations and LOAEC also exceeded 1.0 at Tittabawassee River SAs, while RAs and Saginaw River SAs were less than 1.0 (Figure 5.6). Food web-based dietary exposure HQs (data not presented) were similar to bolus-based HQs at Tittabawassee River SAs [56]. Bolus-based dietary exposures were selected since they represented actual invertebrates collected on-site by tree swallows and included the greatest potential exposure estimates due to a greater range of values. Since 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. Maximum HQs based on NOAEC and LOAEC values were 57 and 6 at Tittabawassee River SAs, respectively, while the NOAEC value was 9 at Saginaw River SAs (Figure 5.6).

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 ww at unexposed and exposed sites, respectively [51]. If these data are converted to a daily dietary dose based on site- and species-specific ingestion rates calculated from data collected in the current study, tree swallow exposure at the contaminated sites along the Woonasquatucket River would range from 61 to 190 ng

TEQ/kg BW/d. In that study, hatching success was negatively impacted 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 ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ in the current study was 800 ng/kg BW/d, which is 4-fold 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 (Table 5.2). However, egg-based exposure differences in the current study indicate that adult tree swallow foraging areas during egg production may include off site areas that would explain the lesser egg exposures [55] compared to those from the Woonasquatucket River study despite greater dietary-based exposure estimates from the brood rearing period.

Additional uncertainty exists because dietary-based samples were not screened for co-contaminants, of which PCBs are of greatest concern considering egg-based residues analyses. The actual effect threshold for individuals is likely between the established no- and lowest-effect TRV values. Based on the range of exposures to TEQs$_{WHO\text{-}Avian}$ for tree swallows at study areas downstream of Midland and available dietary-based TRVs, the potential exists for population-level effects on hatching success but the likelihood is small given the conservative nature of the assessment.

Multiple lines of evidence and population-level effects

Predicted effects on productivity based on tissue- and dietary-based exposure estimates were compared with measured nesting success for tree swallows in a site-specific multiple lines of evidence assessment of hazard [52,78–81]. Exposure and productivity were directly measured to minimize uncertainties associated with predicting the potential for adverse effects based solely on concentrations in abiotic matrices [66,82]. Productivity endpoints for tree swallows were quantified at sites studied near Midland, Michigan, which minimized potential uncertainties associated with reproductive performance on site. However, uncertainties associated with PCB exposures and selected dietary-based TRVs complicate the site-specific hazard assessment. Additionally, while egg-based $TEQs_{WHO\text{-}Avian}$ at RAs were similar to SAs, the congener profile at RAs was dominated by dioxins opposed to furans at SAs (Figure 5.2). Co-contaminants, specifically PCBs, need to be further researched on-site in tissue samples to better understand their site-specific distribution. For terrestrial-foraging passerines PCBs contributed less than 8% to the total concentrations of $TEQ_{WHO\text{-}Avian}$ in eggs [55] opposed to 23 to 47% for aquatic-foraging tree swallows. One possibility for the discrepancy between dietary- and tissue-based HQs is that the dietary-based HQs are likely more conservative due to the NOAEC and LOAEC based on intraperitoneal injections that likely resulted in an overestimation of both [12] as opposed to more realistic exposure routes such as dietary gavages or spiked diets. Interestingly, dietary-based exposures to ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ on the Tittabawassee River were similar to or greater than those of tree swallows on the Woonasquatucket River [51]. Therefore, comparisons were made with the egg exposure based LD50 threshold for hatching success reported as 1,700 ng TCDD/kg ww in that study. Using the threshold for a decrease in hatching success from the Woonasquatucket River study, and comparing it to the modeled distribution of $TEQs_{WHO\text{-}Avian}$ for tree swallows at T-6, a 50% decrease would have been expected for approximately 5% of the population at that site as suggested by the egg-based exposure HQs (Figure 5.4). At all other sites near Midland, MI a 50% decrease in hatching success would only have been expected for less than 1% of the population. Hatching success for tree swallows at Tittabawassee River SAs (76%) or Saginaw River SAs (86%) were not significantly less than at RAs (81%) [53], but hatching success was negatively correlated with concentrations in eggs for individual clutches (Figure 5.3). Although statistically significant, the coefficient of determination (r^2=0.185) was weak. In addition, the comparison was possibly limited by the lack of a true unexposed population of tree swallows, in which one would expect lesser $TEQs_{WHO-Avian}$ and greater hatching success. Hatching problems at sites along the Woonasquatucket River were associated with total clutch losses as opposed to a reduction in hatching success [51], while decreased hatching success along the primarily PCB contaminated Housatonic River was variable and only significant for certain years of the data collected [44]. Complete clutch loss did occur in tree swallows breeding near Midland, Michigan, but was limited to 2% of all clutches that were incubated [53]. Additionally, average hatching success was similar among all study areas near Midland, Michigan to other study sites in North America [30,83–85].

Despite dietary- and tissue-based exposures for tree swallows in the current study that were comparable to populations exposed to dioxin-like compounds [44,51] combined with elevated dietary-based HQs at study areas downstream of Midland, overall productivity through fledging appeared to be unaffected. For the Woonasquatucket River [51] $TEQ_{WHO-Avian}$ exposures were primarily from TCDD (>89% of total $TEQ_{WHO-Avian}$), while for the Housatonic River [44] exposures were primarily from PCBs (86% of total $TEQ_{WHO\text{-}Avian}$) as compared to the current study where exposure of tree swallows was primarily from TCDF and secondarily from 2,3,4,7,8-PeCDF. Potential differences in the distribution and metabolism of specific congeners by birds [74,76,86] or differences in species-specific sensitivities to dioxin-like compounds [17,18] could account for the potential differences between some literature based thresholds and the lack of observed effects.

Additional information pertaining to post-fledge survival and recruitment of recently fledged nestlings may offer additional insight into population health and sustainability. However, due to the relatively short duration of this portion of the study, and inherently low recruitment and site fidelity of yearling passerines [87–91] few nestling band returns were reported [53]. However, a longer-term band return monitoring study is ongoing and may provide additional information on long-term survival and recruitment of exposed versus unexposed birds.

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Animal Use

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

Scientific Collection Permit MB102552-1, and subpermitted under US Department of the Interior Federal Banding Permit 22926) are on file at MSU-ATL.

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CHAPTER 6

Multiple lines of evidence risk assessment of terrestrial passerines exposed to PCDFs and PCDDs in the Tittabawassee River floodplain, Midland, Michigan, USA

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Abstract

A site-specific multiple lines of evidence hazard assessment was conducted for house wrens (*Troglodytes aedon*) and eastern bluebirds (*Sialia sialis*) along the Tittabawassee River downstream of Midland, Michigan (USA), where concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) in floodplain soils and sediments are greater compared to upstream areas and some of the greatest anywhere in the world. As a result, the potential for adverse population-level effects were evaluated for two terrestrial foraging passerine birds residing both upstream and downstream of the putative source. Lines of evidence included dietary- and tissue-based exposures and population productivity measurements for house wrens and eastern bluebirds for the breeding seasons of 2005 through 2007. A hazard assessment based on estimated dietary exposures suggested that both populations residing in the downstream floodplain would be severely affected. However, egg contaminant burdens were compared to appropriate toxicity reference values (TRVs) and predicted a low probability of population-level effects. Similarly, when the reproductive success of the breeding populations was measured no effects were observed. The most probable cause of the apparent dichotomy between the dietary- and tissue-based exposure assessments was that the dietary-based TRVs were overly conservative based on intraperitoneal injections in the ring-necked pheasant.

Keywords: house wren; eastern bluebird; reproductive success; dioxins; furans; hazard quotient

Introduction

Polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) present in the floodplain soils and sediments downstream of Midland (Hilscherova *et al.* 2003) are likely associated with the historical production of industrial organic chemicals and on-site storage and disposal of by-products, prior to the establishment of modern waste management protocols (Amendola and Barna 1986). The site-specific hydrology of the Tittabawassee River combined with the lipophilic nature and slow degradation rates of dioxin-like compounds (Mandal 2005) resulted in the presence of historical contamination (ATS 2007; ATS 2009) in both the aquatic and terrestrial food webs downstream of Midland. The Tittabawassee River system receives drainage inputs from approximately 5,426 km^2 of land surface, composed primarily of woodlands, agricultural lands, and urban areas. Water levels fluctuate naturally throughout the year. Increased flow due to spring thaw combined with the breakup of ice sheets along the river creates conditions that favor bank scouring and mobilization of sediments and floodplain soils. Annual floods suspend particulates which are deposited within the Tittabawassee River floodplain soils downstream of Midland, Michigan (USA).

Total concentrations of PCDD/DFs (ΣPCDD/DFs) collected from floodplain soils and sediments along the Tittabawassee River ranged from $1.0x10^2$ to $5.4x10^4$ ng/kg dw, while mean ΣPCDD/PCDF concentrations in soils and sediments in the reference area (RA) upstream of Midland were 10- to 20-fold less (Hilscherova *et al.* 2003). Floodplain soils downstream of the putative sources have concentrations of ΣPCDD/PCDF which are 6- to 10-fold greater than the proximal river sediments. The Tittabawassee River is one of three rivers that flow into the Saginaw River which is a larger slower moving river that is less flashy, with a wider channel and more urban surroundings than the Tittabawassee River. It is generally contained within its banks, limiting the dynamics with the floodplain soils that occur on the Tittabawassee River. For example, concentrations of ΣPCDD/DFs in soils and river sediments collected along the Saginaw River downstream of the confluence with the Tittabawassee River ranged from 2.3×10^{0} to 2.1×10^{3} ng/kg dw and 5.7×10^{1} to 4.7×10^{4} ng/kg dw, respectively (Kannan *et al.* 2008). In contrast to the Tittabawassee River, the floodplain soils along downstream reaches of the Saginaw River have approximately 10-fold lesser ΣPCDD/DF concentrations than river sediments.

Receptor species selection is an essential step in the risk assessment process. The nature of contamination within the Tittabawassee and Saginaw rivers is variable and as such required that receptor species be selected to account for these differences. While tree swallows (*Tachycineta bicolor*) have proven to be a sufficient study species for many contaminated sites, their aquatic-based diet (McCarty 1997; McCarty and Winkler 1999; Mengelkoch *et al.* 2004) would not account for the greater ΣPCDD/DF concentrations in the floodplain soils along the Tittabawassee River. Therefore, the current study focused on the terrestrial-based assessment of risk to house wrens (*Troglodytes aedon*) and eastern bluebirds (*Sialia sialis*) while concurrent studies utilized tree swallows for an aquatic-based assessment. Site-specific exposures and related effects assessments for a variety of terrestrial passerine species have been conducted (Ankley *et al.* 1993; Bishop *et al.* 1995; Custer *et al.* 2001; Henning *et al.* 2003; Arenal *et al.* 2004; van den Steen *et al.* 2006; van den Steen *et al.* 2007), but more commonly, tree swallows have been selected as target species in assessments of risk in aquatic-based studies (Shaw 1983;

DeWeese *et al.* 1985; Beaver 1992; Ankley *et al.* 1993; Bishop *et al.* 1995; Froese *et al.* 1998; Custer *et al.* 1998; Secord *et al.* 1999; Custer *et al.* 2000; Harris and Elliott 2000; Custer *et al.* 2002; Custer *et al.* 2003; Echols *et al.* 2004; Custer *et al.* 2005; Smits *et al.* 2005; Neigh *et al.* 2006b; Spears *et al.* 2008). However, house wrens and eastern bluebirds have recently been selected as receptors at terrestrially contaminated study sites (Thiel *et al.* 1988; Burgess *et al.* 1999; Custer *et al.* 2001; Mayne *et al.* 2004; Neigh *et al.* 2006a).

House wrens and eastern bluebirds were selected to determine the extent and distribution of exposure to ΣPCDD/DFs through the terrestrial food chain and associated risk downstream of Midland. These two species have an almost ubiquitous distribution both locally and throughout the U.S., are relatively common, and are often multi-brooded per season. Both are obligate cavity nesters and readily occupy a provided nest box that allows for better experimental control and eliminates time-intensive nest searching. Additionally, house wrens and eastern bluebirds are resistant to disturbance and have limited foraging range while nesting, so egg and nestling tissue residue concentrations are generally indicative of local exposure.

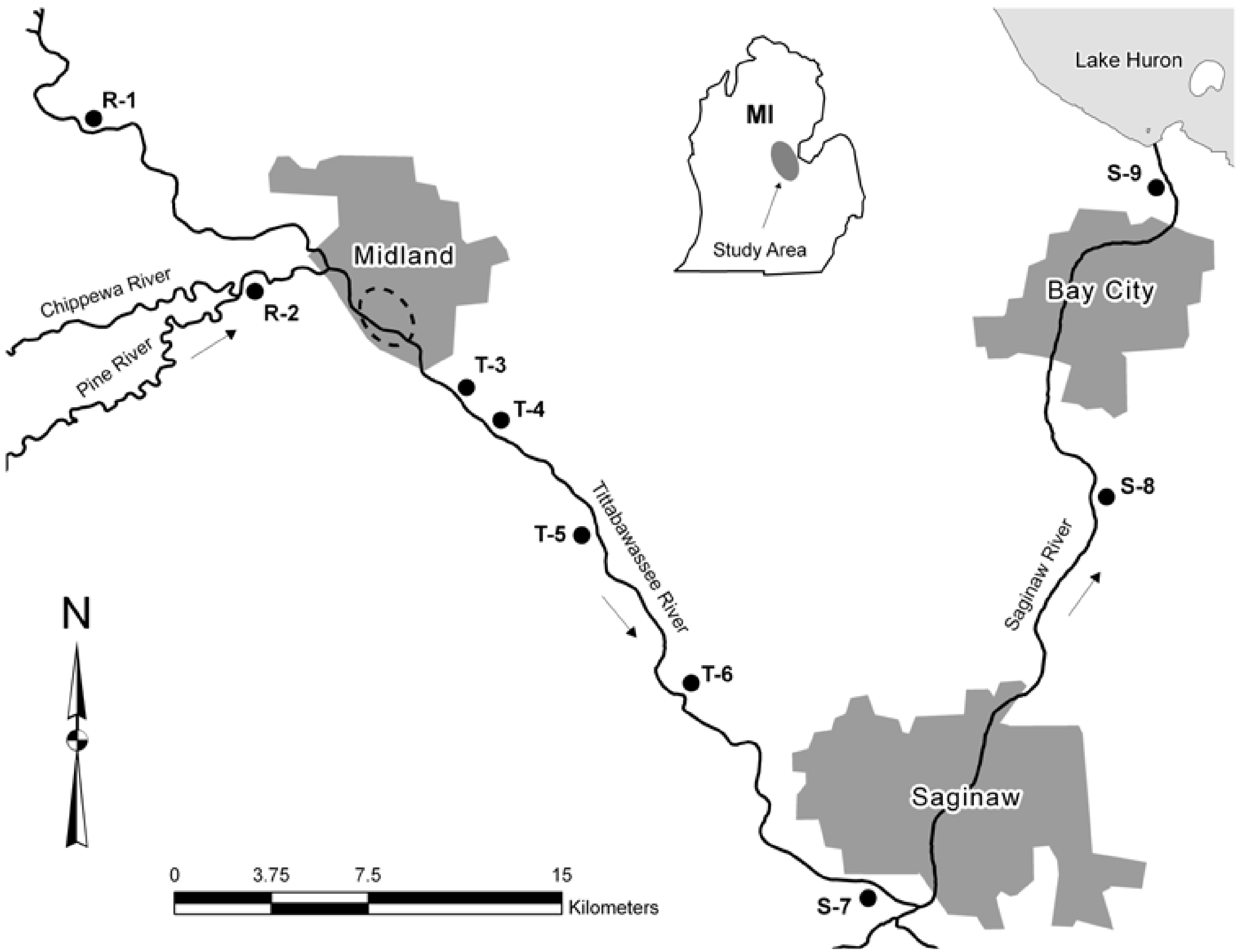
The primary objective of this study was to evaluate the potential for adverse effects on house wrens and eastern bluebirds breeding in the river floodplains downstream of Midland, Michigan using a multiple lines of evidence approach (Fairbrother 2003). Site-specific measures of exposure included concentrations of PCDD/DFs in eggs and nestlings, as well as in the diet that was studied by measuring concentrations in invertebrates collected from the site and in bolus samples. Species-specific dietary compositions were determined from bolus samples and then sufficient masses of invertebrates were collected so that concentrations of PCDD/DFs could be measured and used in the calculation of weighted average dietary exposure concentrations. In addition population level reproductive endpoints were measured. Potential for adverse effects was evaluated by comparing the concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents ($TEQ_{WHO\text{-}Avian}$) based on World Health Organization (WHO) TCDD equivalency factors for birds ($TEF_{WHO\text{-}Avian}$) (van den Berg *et al.* 1998) in the diet and tissues of house wrens and eastern bluebirds to available toxicity reference values (TRVs). Predicted hazard quotients based on TRVs were compared to site-specific measures of population condition. Additionally, comparisons were made between these results and similar field-based measures of exposure and productivity. Using a hazard assessment approach combined with site-specific multiple-lines of evidence for two species strengthens confidence, minimizes uncertainty, and broadens the applicability of risk assessment outcomes.

Methods

Site description

The study was conducted on the Tittabawassee, Chippewa, and Saginaw rivers, in the vicinity of Midland, Michigan (Figure 6.1). Nest boxes were placed and all samples were collected from within the 100-year floodplain of the individual rivers. Two reference areas were located upstream of the putative sources of PCDD/DFs (Hilscherova *et al.* 2003) on the Tittabawassee (R-1) and Chippewa (R-2) rivers (Figure 6.1). Study areas (SAs) downstream of the putative sources of PCDD/DFs include approximately 72 km of free flowing river from the upstream boundary defined as the low-head dam near

Figure 6.1. 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 S-9) were monitored from 2005–2007. Direction of river flow is designated by arrows; suspected source of contamination is enclosed the dashed oval.


R-1
R-2
Chippewa River
Pine River
Midland
T-3
T-4
T-5
T-6
Tittabawassee River
MI
Study Area
Lake Huron
S-9
Bay City
S-8
Saginaw River
Saginaw
S-7
N
0
3.75
7.5
15
Kilometers

Midland, Michigan through the confluence of the Tittabawassee and Saginaw rivers to where the Saginaw River enters Saginaw Bay in Lake Huron. The SAs along Tittabawassee River downstream of Midland included four sites (T-3 to T-6) approximately equally spaced, and three sites (S-7 to S-9) located at the initiation, median, and terminus of the Saginaw River. The seven SAs (T-3 to S-9) were selected for the Tittabawassee and Saginaw rivers, respectively, based on the necessity to discern spatial trends, ability to gain access privileges, and maximal receptor exposure potential based on floodplain width and measured soil and sediment concentrations (Hilscherova *et al.* 2003). Nest box trails at each study site contained between 30 and 60 nest boxes and spanned a continuous foraging area of between 1 and 3 km of river. S-8 was an exception and was only used for sediment and dietary food web sampling. No studies of birds were conducted at this location.

Nest box monitoring

Standard passerine nest boxes with wire mesh predator guards around the entrance hole and mounted to a greased metal post were used to facilitate monitoring of nesting activity and collection of samples (Fredricks *et al.* 2009b). 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 subsequent to placement of nest boxes and continued through 2007 at all sites. Individual nest boxes were placed at study sites to maximize occupancy of several passerine species (Horn *et al.* 1996) with relatively equal proportions of boxes placed in species-specific micro-habitats for each species studied.

Previous reports provide more detailed descriptions of study-specific nest monitoring and sample collection protocols used in the current study (Fredricks *et al.* 2009b; Fredricks *et al.* 2009c). In general, boxes were monitored twice a week for occupancy beginning in early April. Boxes were monitored daily after clutch initiation through incubation and subsequently near the expected hatch or fledge day for each species. Masses of eggs were determined on the date laid, and masses of nestlings were measured 4 times over the brood rearing period. Eggs for use in residue quantification were collected after clutch completion and prior to the fifth day of incubation. Therefore, clutch size was not adjusted for egg sampling. However, hatching success, fledging success, and productivity measurements were calculated based on an adjusted clutch size since the fertility and hatchability of the collected egg was unknown at collection. Additionally, brood size and number of fledglings were predicted based on the adjusted hatching success and productivity, respectively. A maximum of one nestling per nesting attempt was collected from randomly selected boxes for residue quantification, 10-d post-hatch for house wrens or 14-d post-hatch for eastern bluebirds. Since fully developed nestlings were collected just prior to fledge, it was assumed that any nestlings collected would have successfully fledged provided the remaining portion of the nesting attempt was successful. Therefore, fledging success and productivity were not adjusted for sampled nestlings. This compromise in the experimental design was used so that the most accurate, clutch-specific estimates of concentrations of PCDD/DFs could be made.

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

Dietary exposure

Detailed site descriptions and protocols for collecting and handling samples of representative invertebrate orders collected on-site and dietary bolus samples collected from nestlings have been previously described (Fredricks *et al.* 2009a). 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 × 30 m grids proximal to the river bank, one for sampling of terrestrial invertebrates and one for collection of benthic and emergent aquatic invertebrates. Sites in the SA were selected based on maximizing the potential for collecting food items with the greatest contaminant concentrations for a given nest box trail given the available soil and sediment data. Sampling methods were designed to target aquatic emergent insects, benthic invertebrates, and terrestrial invertebrates in order to collect the necessary biomass for residues analyses and to obtain a representative sample of available dietary items at each site. Invertebrates were categorized taxonomically to the order level for each life stage collected during each sampling period per site. Samples were then homogenized and stored at -20 ºC until extraction.

Dietary food items were collected as bolus samples from nestling house wrens and eastern bluebirds using a black electrical cable-tie fitted at the base of their neck (Mellott and Woods 1993). Samples were collected from nestlings between the ages of 3- and 9-d post-hatch for house wrens and 4- and 12-d post-hatch for eastern bluebirds. Bolus samples were collected from nestlings approximately 1 h after ligature application. Nests were not sampled on consecutive days. Invertebrates in each bolus sample were classified to order and the total number and mass of each order was recorded for each sample. The site-specific diet for both species was determined based on the relative proportion of the total mass represented by each invertebrate order identified in the bolus samples. Additionally, bolus samples were recombined for residue analyses based on clutch from which each sample was collected and combined with other proximally and temporally located boxes to obtain the necessary biomass for residue quantification.

Dietary exposures of adults and nestlings were estimated using the U.S. Environmental Protection Agency (USEPA) Wildlife Exposure Factors Handbook (WEFH) equations for passerine birds (USEPA 1993). USEPA WEFH equation 3-4 was used to calculate food intake rate based on site-, species-, and age-specific body masses. Potential average daily dose (ADD_{pot}; ng $TEQ_{WHO\text{-}Avian}$/kg body weight/d) was calculated using equation 4-3 (USEPA 1993) assuming that 100% of the foraging range for each species was within the associated study area. Dietary concentrations in food items were estimated individually for house wrens and eastern bluebirds using two methods: 1) food web-based diet: multiplying study-specific dietary compositions for major (>1% by mass) prey items by respective area-specific (R-1 to R-2; T-3 to T-6; S-7 to S-9) average, minimum, and maximum concentrations of $TEQs_{WHO\text{-}Avian}$ in associated prey items for each study species, and 2) bolus-based diet; area-specific average, minimum, and maximum concentrations from actual bolus samples collected from nestlings of each species studied. Minimum and maximum concentrations were chosen to describe the range of possible invertebrate concentrations found on site, which the authors expected to include the worst-case scenario for dietary exposure. Dietary exposure estimates apply only to the nesting period for both adults and nestlings because foraging habits and range are likely more variable outside the nesting period.

Chemical analyses

Concentrations of seventeen 2,3,7,8-substituted PCDD/DF congeners were quantified in all samples whereas concentrations of polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDT) and related metabolites were measured in a subset of egg samples. Congener residues were quantified in accordance with EPA Method 8290/1668A with minor modifications (USEPA 1998). A more detailed description of methods and the measured concentrations have been previously reported (Fredricks *et al.* 2009a; Fredricks *et al.* 2009b). Briefly, samples were homogenized with anhydrous sodium sulfate, spiked with known amounts of ^{13}C-labeled analytes (as internal standards), and Soxhlet extracted. Ten percent of the extract was removed for lipid content determination. Sample purification included the following: treatment with concentrated sulfuric acid, silica gel, sulfuric acid silica gel, acidic alumina and carbon column chromatography. Components were analyzed using high-resolution gas chromatography/high-resolution mass spectroscopy, a Hewlett-Packard 6890 GC (Agilent Technologies, Wilmington, DE) connected to a MicroMass® high-resolution mass spectrometer (Waters Corporation, Milford, MA). Chemical analyses included pertinent quality assurance practices, including matrix spikes, blanks, and duplicates.

Toxicity reference values

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

Laboratory-based dosing studies incorporating PCDD/DF dietary exposure-based effects assessments are lacking for passerines and limited in general for avian species. A study that dosed adult hen ring-necked pheasants (*Phasianus colchicus*) with intraperitoneal injections of TCDD for a 10 wk exposure period was selected as the dietary exposure-based TRV for this study (Nosek *et al.* 1992a). The major limitation of this study was that hens were exposed to TCDD via injections versus a true dietary based exposure. However dosing exposure efficiency through injections should be greater than that of gut transfer thus providing a slightly more conservative TRV. Although this study was not conducted on a passerine species, galliforms are generally considered to have greater sensitivity to dioxin-like compound exposures (Brunström and Reutergardh 1986; Brunström 1988; Powell *et al.* 1996; Powell *et al.* 1997a). In addition, recent evidence suggests a molecular basis for variation in avian species sensitivity to dioxin-like compounds (Karchner *et al.* 2006; Head *et al.* 2008) with ring-necked pheasants having similar sensitivity to the passerines studied (SW Kennedy *personal communication*). The dietary-based TRVs were determined by converting the weekly exposure at which adverse effects on fertility and hatching success were determined (1000 ng TCDD/kg/wk) to a LOAEC for daily exposure of 140 ng TCDD/kg/d (Table 6.1). The dosing regime was based on orders of magnitude differences and adverse effects were not present at the

Table 6.1. Toxicity reference values (TRVs) for total $TEQs_{WHO\text{-}Avian}$[a] concentrations selected for comparison to terrestrial passerines exposed to PCDD/DFs in the river systems downstream of Midland, Michigan, USA during 2005–2007.

Species	NOAEC	LOAEC	Reference
House wren			
Dietary exposure-based[b]	14	140	Nosek *et al.* 1992a
Egg exposure-based[c]	710	7,940	USEPA 2003[d]
Eastern bluebird			
Dietary exposure-based[b]	14	140	Nosek *et al.* 1992a
Egg exposure-based[c]	1,000	10,000	Thiel *et al.* 1988

[a] $TEQs_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values
[b] ng/kg/d ww
[c] ng/kg ww
[d] calculated from studies by Nosek *et al.* 1992a, Nosek *et al.* 1992b and Nosek *et al.* 1993

next lowest dose, which was determined to be the NOAEC for dietary exposure (14 ng TCDD/kg/d).

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

A more conservative egg exposure-based TRV was selected for house wren because differences in species-specific sensitivity between eastern bluebirds and house wrens was unknown. Three studies (Nosek *et al.* 1992a; Nosek *et al.* 1992b; Nosek *et al.* 1993) that dosed ring-necked pheasant hens or eggs were combined to determine a geometric mean NOAEC of 710 ng/kg ww and LOAEC of 7,940 ng/kg ww as egg exposure-based TRVs for house wrens (USEPA 2003).

Additional egg-injection studies that were evaluated but not selected for deriving TRVs included a bobwhite quail (*Colinus virginianus*) (McMurry and Dickerson 2001) and double-crested cormorant (*Phalacrocorax auritus*) (Powell *et al.* 1997b; Powell *et al.*

1998) studies. Reasons for not selecting them included limited sample size, failure to establish a dose-response relationship, and/or poor hatchability of non- or vehicle-injected controls.

Hazard assessment

Overall hazard of PCDD/DFs to house wrens and eastern bluebirds breeding in the river floodplains downstream of Midland was assessed through a multiple lines of evidence approach (Fairbrother 2003) that incorporated both dietary- and egg tissue-based exposure estimates in addition to measures of site-specific reproductive success. Potential effects of dietary- and tissue-based exposures were assessed by calculating hazard quotients (HQ) for each species. Concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ (ng/kg ww) in eggs and dietary estimates [potential average daily dose (ADD_{pot}; ng/kg/d)] were divided by egg exposure- or dietary exposure-based NOAEC or LOAEC TRVs (Table 6.1), respectively.

Hazard quotients for egg exposures were determined based on the upper 95% confidence level (UCL) of the geometric mean egg tissue residue concentrations at each study location. Hazard quotients for dietary exposures were based on ranges at RAs, Tittabawassee River SAs, and Saginaw River SAs divided be the selected TRV, respectively. Ranges were used for dietary exposure estimates due to limited sample sizes at most study locations. Further, samples of invertebrates from the food web were composites of all individuals of an order collected per location per sampling period, which provide an accurate estimate of the central tendency of the concentration estimates, but limit the information about variability within each order at a location. HQs for dietary exposure were calculated based on $TEQs_{WHO-Avian}$ in bolus-based dietary exposure estimates at reference and Tittabawassee River SAs, and on food web-based dietary exposure estimates at Saginaw River SAs. 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 evaluated for ecologically relevant endpoints at site-specific downstream and upstream study areas, and compared to relevant literature-based field studies. Incorporation of both dietary- and tissue-based assessment endpoints has been shown to greatly reduce uncertainty in risk assessments of persistent organic pollutants (Leonards et al. 2008).

Statistical analyses

Individual nesting attempts were considered the experimental unit for statistical comparisons. Egg-based exposure comparisons were made between sampling locations (Fredricks *et al.* 2009b). Samples from individual locations were combined by study area for comparisons of bolus- and food web-based dietary concentrations due to limited biomass collected at each location (Fredricks *et al.* 2009a). In-depth descriptions of productivity measures and associated statistical analyses have been previously reported (Fredricks *et al.* 2009c).

Total concentrations of the 17 individual 2,3,7,8-substituted PCDD/DF congeners are reported as the sum of all congeners (ng/kg ww). For individual congeners that were less than the limit of quantification a proxy value of half the sample method detection limit was assigned. Concentrations of $TEQ_{WHO-Avian}$ (ng/kg ww) were calculated for

PCDD/DFs by summing the product of the concentration of each congener, multiplied by its avian $TEF_{WHO\text{-}Avian}$ (van den Berg *et al.* 1998). Total concentrations of twelve non- and mono-*ortho*-substituted PCB congeners are reported as the sum of these congeners (ΣPCBs) for a subset of egg samples that were screened for co-contaminants. Additionally, dichloro-diphenyl-trichloroethane (2',4' and 4',4' isomers) and dichloro-diphenyl-dichloroethylene (4',4') are reported as the sum of the *o,p* and *p,p* isomers (DDT metabolites) for the same subset of samples as for PCBs.

Statistical analyses were performed using SAS® software (Release 9.1; SAS Institute Inc., Cary, NC, USA). Prior to the use of parametric statistical procedures, normality was evaluated using the Shapiro–Wilks test and the assumption of homogeneity of variance was evaluated using Levene's test. For concentration data that were not normally distributed, the data were transformed using the natural log (ln) of (x + 1). To better understand the potential distributions of the $TEQ_{WHO\text{-}Avian}$ concentrations at each study location a probabilistic modeling approach was used to portray the distributions. Probabilistic models were developed as cumulative frequency distributions based on ΣPCDD/DF $TEQ_{WHO\text{-}Avian}$ concentrations in eggs. The mean and standard deviation of transformed egg values were used to generate a sample of 10,000 random egg values based on a lognormal distribution. The association between concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ and hatching success by species was evaluated with Pearson's correlation coefficients for nesting attempts in which both data were collected. Statistical significance was considered at $P<0.05$.

Results

Site-specific endpoints

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

Tissue residues

Concentrations of PCDD/DFs and $TEQ_{WHO\text{-}Avian}$ were quantified in eggs and nestlings of house wrens and eastern bluebirds collected on-site (Fredricks *et al.* 2009b). Geometric mean concentrations of $TEQs_{WHO\text{-}Avian}$ in eggs of house wrens and eastern bluebirds from Tittabawassee River SAs were 5- to 91-fold greater than those from RAs (Figures 6.2 and 6.3), while concentrations in eggs collected from the Saginaw River SAs were intermediate. Patterns of relative concentrations of congeners in eggs from more downstream SAs were dominated primarily by 2,3,4,7,8-pentadibenzofuran (2,3,4,7,8-PeCDF) and to a lesser extent 2,3,7,8-tetrachlorodibenzofuran (TCDF) opposed to primarily dioxin congeners at RAs. Maximum concentration of $TEQs_{WHO\text{-}Avian}$ in eggs of house wrens and eastern bluebirds were 2300 ng/kg at T-3 and 1000 ng/kg at T-6,

Figure 6.2. Geometric mean concentrations of ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ in house wren eggs collected during 2005-2007 from the river floodplains near Midland, Michigan, USA. Error bars show the 95% upper confidence level (UCL); Reference areas (R-1 and R-2); Tittabawassee River study areas (T-3 to T-6); and Saginaw River study areas (S-7 to S-9).

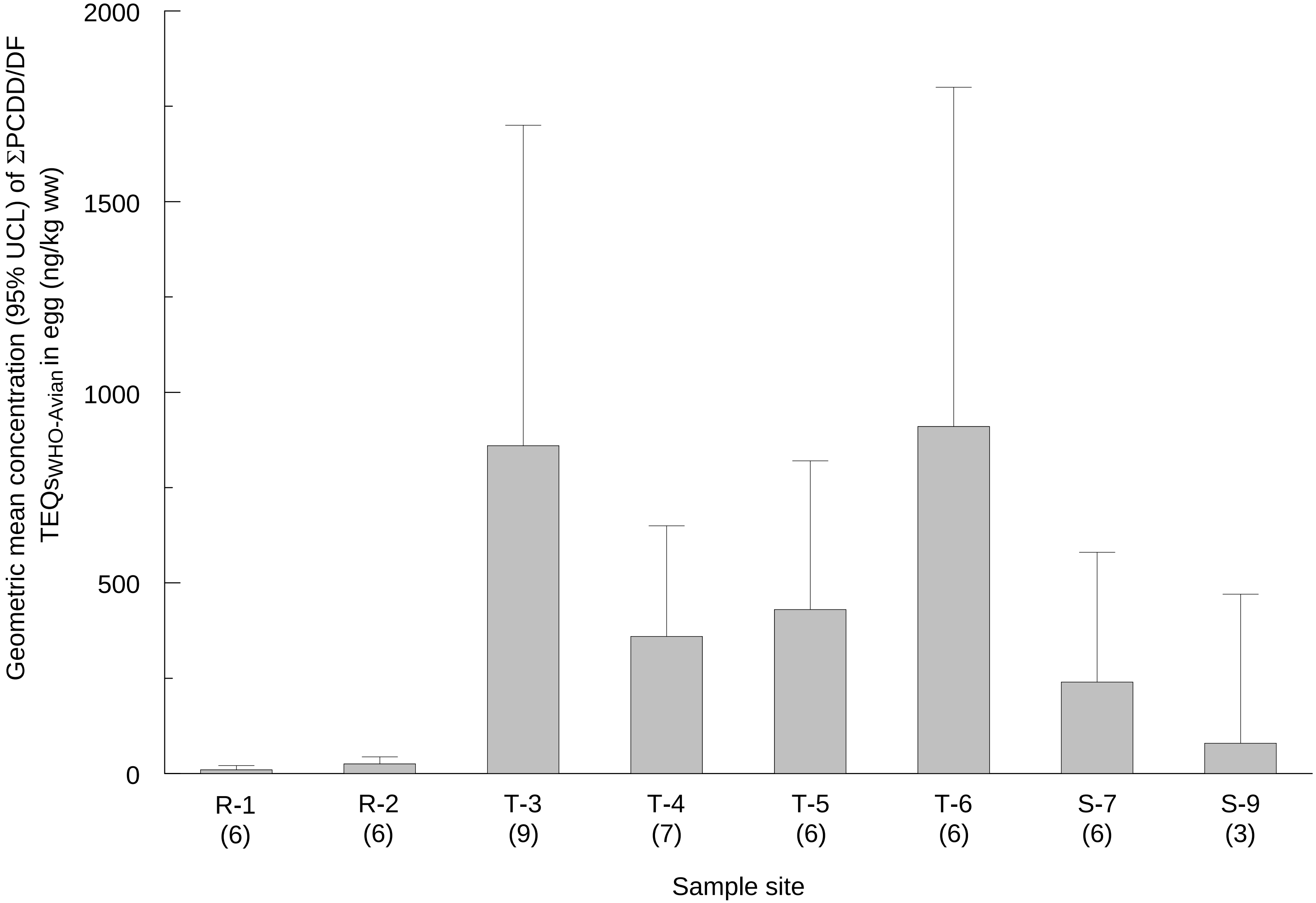
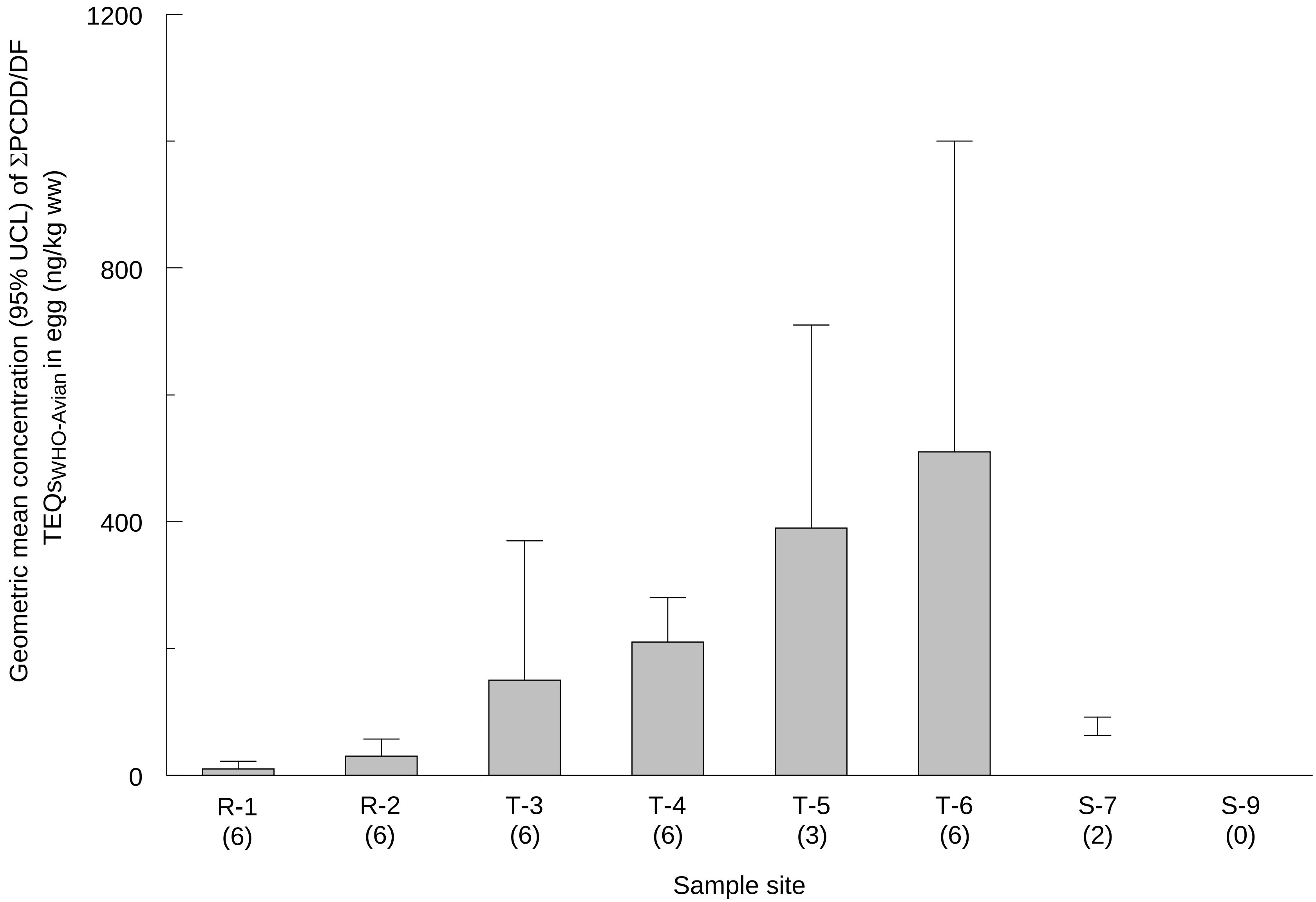

Geometric mean concentration (95% UCL) of ΣPCDD/DF
TEQ$_{WHO\text{-}Avian}$ in egg (ng/kg ww)
2000
1500
1000
500
0
R-1
(6)
R-2
(6)
T-3
(9)
T-4
(7)
T-5
(6)
T-6
(6)
S-7
(6)
S-9
(3)
Sample site

Figure 6.3. Geometric mean concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ in eastern bluebird eggs collected during 2005-2007 from the river floodplains near Midland, Michigan, USA. Error bars show the 95% upper confidence level (UCL); Reference areas (R-1 and R-2); Tittabawassee River study areas (T-3 to T-6); and Saginaw River study areas (S-7 to S-9); range presented for S-7 where n=2.



Geometric mean concentration (95% UCL) of ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ in egg (ng/kg ww)
1200
800
400
0
R-1 (6)
R-2 (6)
T-3 (6)
T-4 (6)
T-5 (3)
T-6 (6)
S-7 (2)
S-9 (0)
Sample site

respectively. Co-contaminants in eggs, including DDT and metabolites, and PCBs, were not significantly greater than established background concentrations for both species studied. Additionally, concentrations of ΣPCDD/DFs in nestlings of both species at SAs were 8- to 50-fold greater than those in nestlings from RAs. Maximum concentration of $TEQs_{WHO\text{-}Avian}$ in nestlings of house wrens and eastern bluebirds occurred at T-6 and were 1200 ng/kg and 1400 ng/kg, respectively. The relative potency of the exposure mixture was reasonably consistent in both eggs and nestlings of all studied species, as concentrations of $TEQ_{WHO\text{-}Avian}$ were positively correlated with concentrations of ΣPCDD/DFs. Nestling-based congener profiles were similar to egg-based profiles for both species studied and among study areas.

Dietary exposures

Concentrations of PCDD/DFs and $TEQ_{WHO\text{-}Avian}$ were quantified in site-specific food web invertebrates and bolus samples collected from both house wren and eastern bluebird nestlings (Fredricks *et al.* 2009a). Site-specific dietary composition was determined by quantifying the mass of individual invertebrate orders to the overall dietary mass from bolus samples. Potential average daily dose (ADD_{pot}; ng/kg body weight/d) based on $TEQ_{WHO\text{-}Avian}$ concentrations in bolus-based and food web-based dietary exposure estimates were 136- and 45-fold and 125- and 70-fold greater at Tittabawassee River SAs than at RAs for adult house wrens and eastern bluebirds, respectively, while food web-based dietary exposure estimates were intermediate at Saginaw River SAs (Table 6.2).

Table 6.2. Potential average (range) $TEQ_{WHO\text{-}Avian}$[a] daily dose (ADD_{pot}; ng/kg body weight/d) calculated from site-specific bolus-based and food web-based dietary exposure for adult house wrens and eastern bluebirds breeding during 2004–2006 within the river floodplains near Midland, Michigan, USA.

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

[a] $TEQ_{WHO\text{-}Avian}$ were calculated based on the 1998 avian WHO TEF values

[b] R-1 to R-2 = Tittabawassee and Chippewa rivers reference area; T-3 to T-6 = Tittabawassee River study area; S-7 to S-9 = Saginaw River study area

[c] Values were rounded and represent only two significant figures

[d] Food ingestion rate was calculated from equations in The Wildlife Exposure Factors Handbook (USEPA 1993)

[e] Residue analyses were not conducted on bolus collected invertebrates at S-7 and S-9

Reproductive success

Reproductive parameters including clutch size, egg mass, hatching success, predicted brood size, nestling growth, fledging success, predicted number of fledglings, and productivity were monitored for house wrens and eastern bluebirds breeding in the river floodplains (Fredricks *et al.* 2009c). Of all initiated clutches, 66% and 64% successfully fledged at least one nestling for house wrens and eastern bluebirds, respectively. In general, reproductive parameters for passerine species studied were similar or greater at downstream SAs compared to upstream RAs among all study years. However, house wren fledging success was greater at RAs (86%) compared to Saginaw River SAs (73%), while Tittabawassee River SAs (82%) were intermediate. However predicted brood size was greater at Saginaw River SAs (5.1 nestlings/brood) compared to Tittabawassee River SAs (4.5 nestlings/brood), while RAs (5.0 nestlings/brood) were intermediate. Since adult females were captured and uniquely identified during nesting attempts it was possible to determine overall nesting success per female for the duration of the study. Total nestlings fledged per female from 2005 to 2007 was similar among study areas and averaged (range) 5.2 (0–25) and 5.4 (0–13) for house wrens and eastern bluebirds, respectively. Nestling growth rate constants and mass gained per day were similar among study areas for both species studied (Fredricks *et al.* 2009c).

Hazard assessment

Hatching success was not negatively correlated with concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ in either house wren or eastern bluebird eggs for clutches with both data points measured. House wren eggs from RAs had lesser $TEQs_{WHO\text{-}Avian}$ but similar hatching success compared to downstream SAs, which resulted in a slightly negative correlation coefficient (R=-0.14526, p=0.3587, n=42; Figure 6.4) that was not significant. Overall mean hatching success for eastern bluebirds at RAs (70%) was not significantly less than Tittabawassee River SAs (84%) however the trend resulted in a significant positive correlation with $TEQ_{WHO\text{-}Avian}$ concentrations (R=0.47213, p=0.0198, n=24; Figure 6.5).

Predicted probabilistic distributions of expected cumulative percent frequencies based on concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ in eggs of house wren and eastern bluebirds were compared to selected TRVs. Predicted distributions of concentrations in

Figure 6.4. Correlation plot of percent hatching success and ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ in house wren eggs for nesting attempts with data collected for both variables from the river floodplains near Midland, Michigan during 2005–2007. R- and *p*-values and sample size indicated; 1=R-1; 2=R-2; 3=T-3; 4=T-4; 5=T-5; 6=T-6; 7=S-7; 9=S-9.

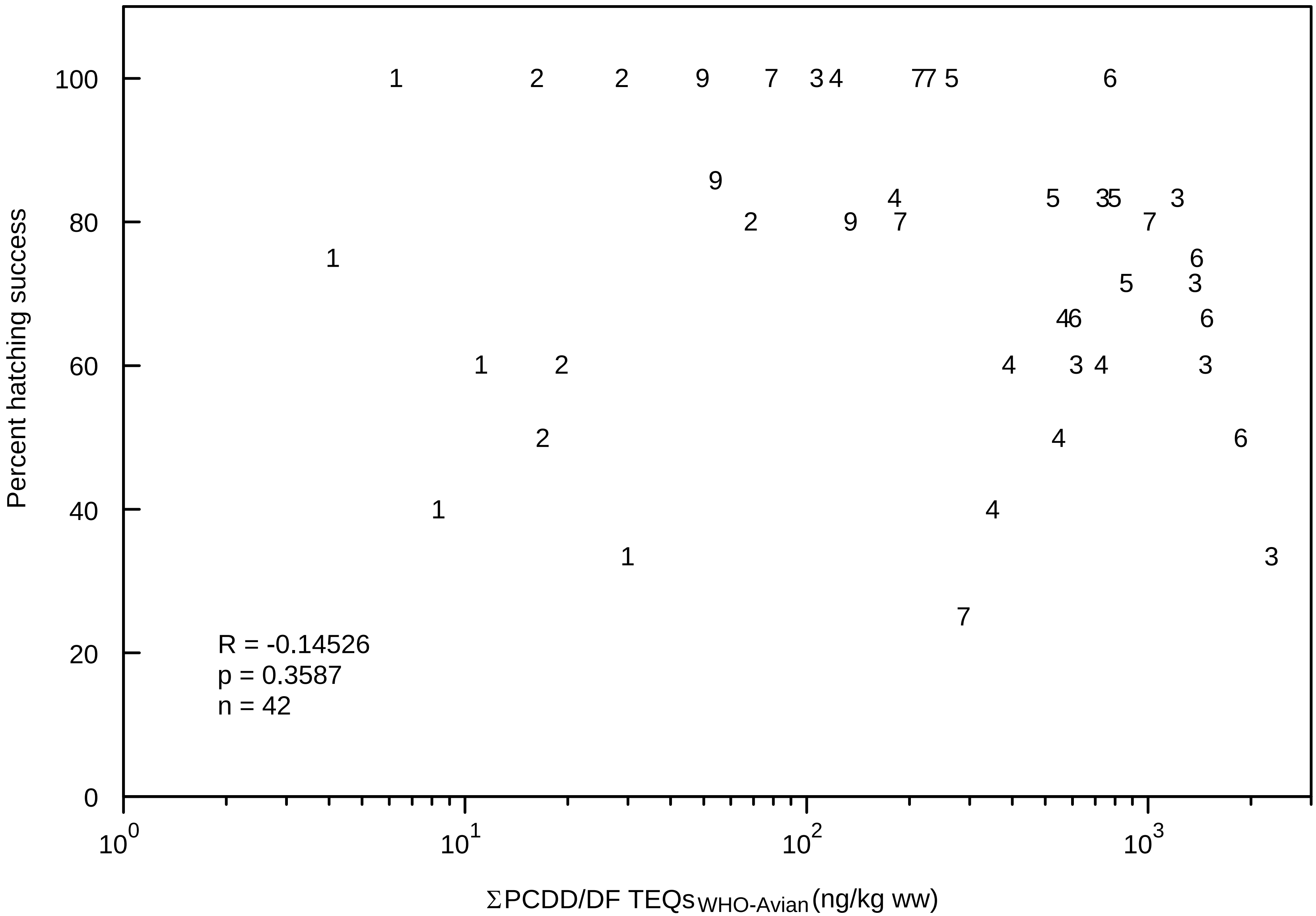
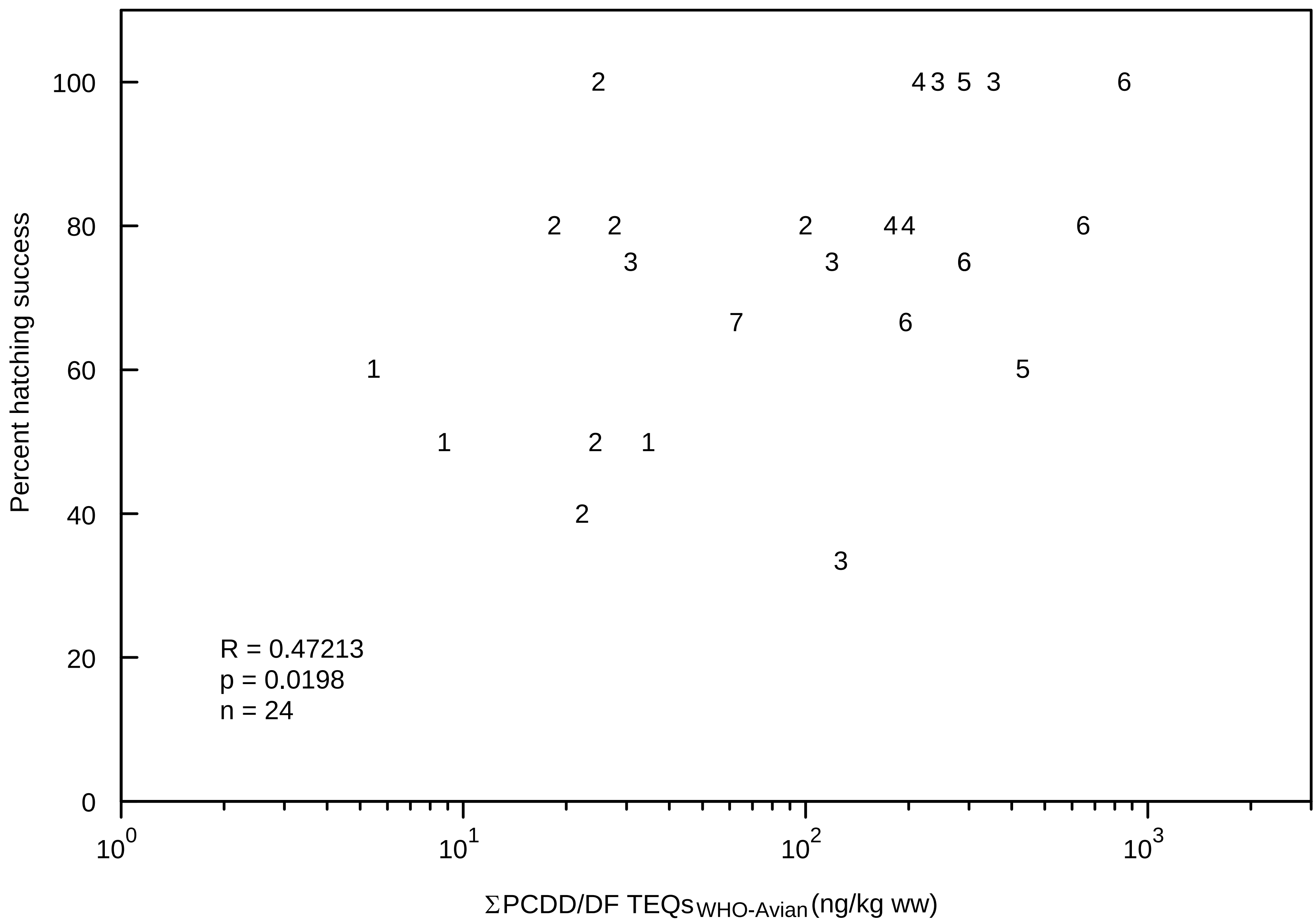

Percent hatching success
ΣPCDD/DF TEQs WHO-Avian (ng/kg ww)
R = -0.14526
p = 0.3587
n = 42

Figure 6.5. Correlation plot of percent hatching success and ΣPCDD/DF TEQs$_{\text{WHO-Avian}}$ in eastern bluebird eggs for nesting attempts with data collected for both variables from the river floodplains near Midland, Michigan during 2005–2007. R- and *p*-values and sample size indicated; 1=R-1; 2=R-2; 3=T-3; 4=T-4; 5=T-5; 6=T-6; 7=S-7.

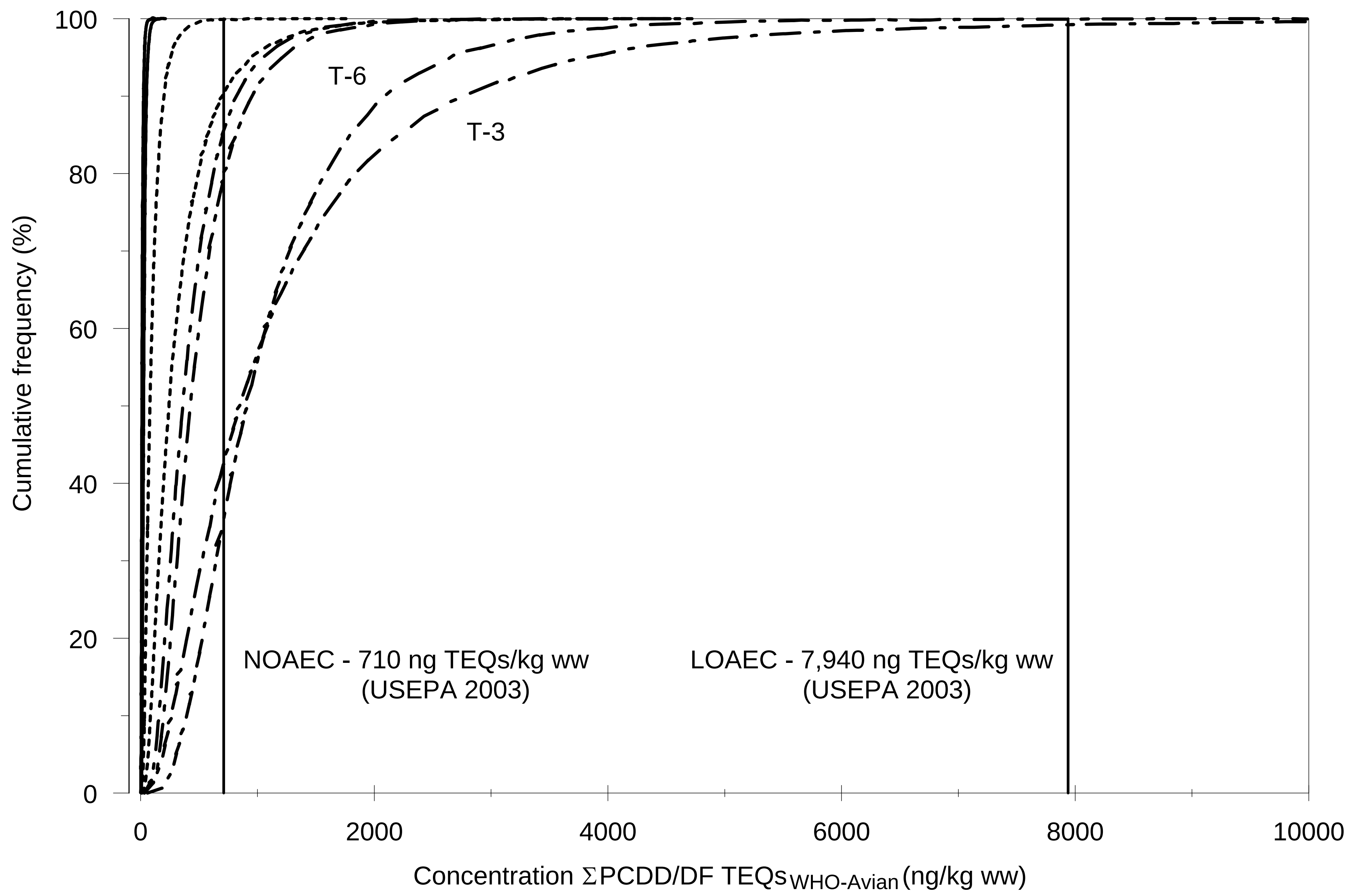

Percent hatching success
100
80
60
40
20
0
R = 0.47213
p = 0.0198
n = 24
10^0
10^1
10^2
10^3
ΣPCDD/DF TEQs$_{WHO-Avian}$ (ng/kg ww)

house wren eggs were greater than the NOAEC (710 ng/kg ww; (USEPA 2003)) for all sites other than RAs and S-9 (Figure 6.6). Sites T-3 and T-6 had 58% and 65% of the predicted distribution greater than the NOAEC, while S-9, T-4, and T-5 had 10%, 15%, and 21% of the frequency distribution greater than the NOAEC, respectively. Based on the predicted distributions at all study sites, less than 1% of the cumulative frequency was greater than the LOAEC (7,940 ng/kg ww; (USEPA 2003)). Predicted distributions of concentrations in eastern bluebird eggs were greater than the NOAEC (1,000 ng/kg ww; (Thiel *et al.* 1988)) at Tittabawassee River SAs, while RAs and Saginaw River SAs were not (Figure 6.7). Sites T-3 and T-6 had 1% and 15% of the predicted distribution greater than the NOAEC, while no study sites were greater than the LOAEC (10,000 ng/kg ww; (Thiel *et al.* 1988)).

Upper 95% confidence level (UCL; geometric mean) concentrations of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ in house wren and eastern bluebird eggs among all study sites were not greater than the species-specific egg-based LOAEC TRVs. Resulting HQs based on LOAECs for both species studied were less than 0.2 among all study sites. Tittabawassee River SAs T-6, T-3, and T-5 had HQs greater than one for house wren eggs based on the 95% UCL and NOAEC, but at all other sites HQs were less than 1.0 (Figure 6.8). Hazard quotients for eastern bluebird eggs based on the 95% UCL and NOAEC TRV were less than 0.7 for all sites except T-6 at which the HQ=1.0 (Figure 6.9).

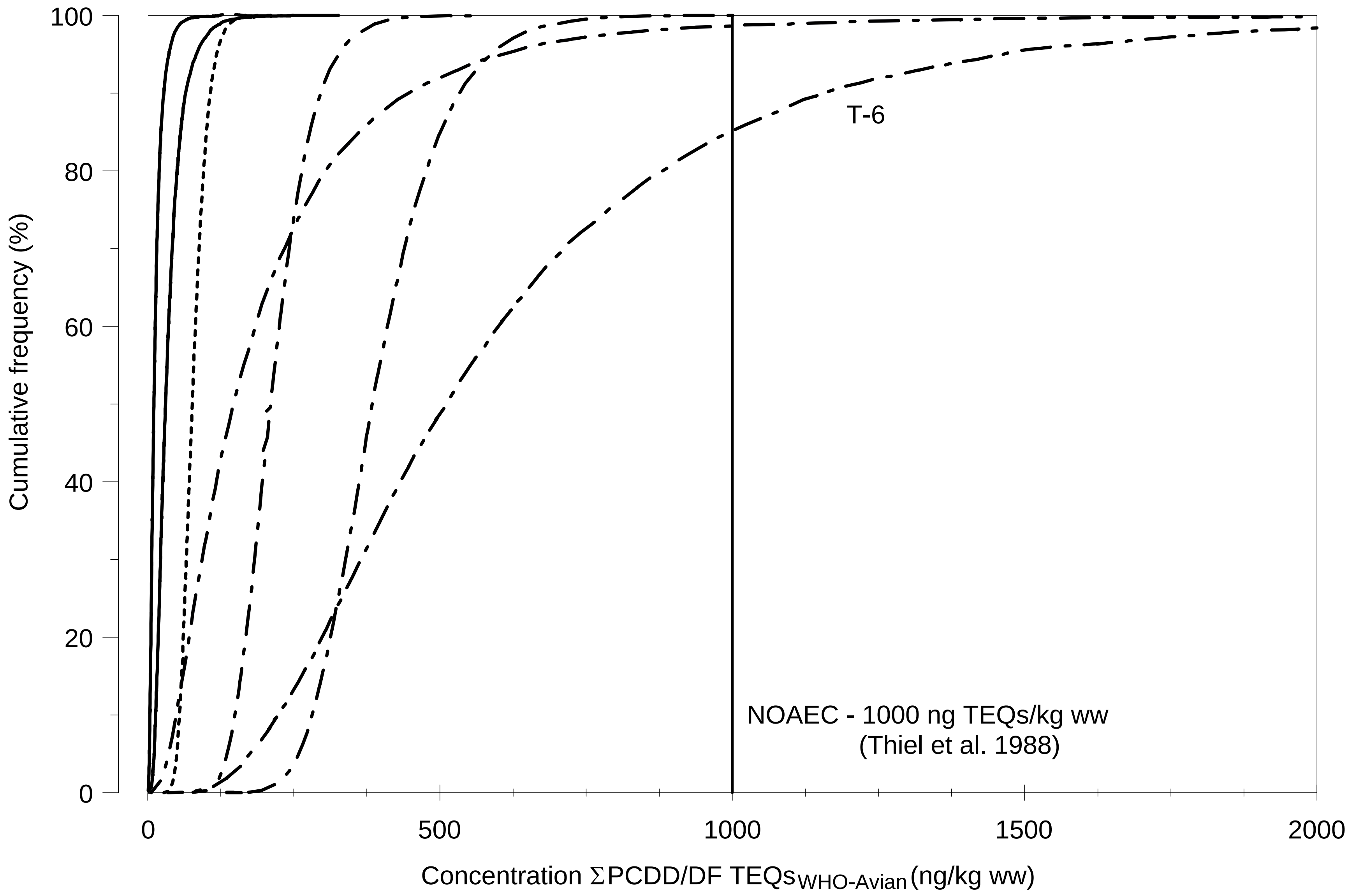
Dietary exposure-based on maximum measured ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ concentrations at Tittabawassee and Saginaw River SAs were greater than the diet-based NOAEC TRV for both species studied whether food web- or bolus-based estimates of dietary exposure were used at Tittabawassee River SAs. Dietary exposure-based

Figure 6.6. Modeled probabilistic distribution of expected cumulative percent frequencies for house wren egg $TEQ_{WHO-Avian}$ concentrations ng/kg ww in site-specific eggs collected from the river floodplains near Midland, Michigan in 2005-2007. 10,000 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 to show R-1 and R-2; NOAEC and LOAEC indicated by vertical bars.



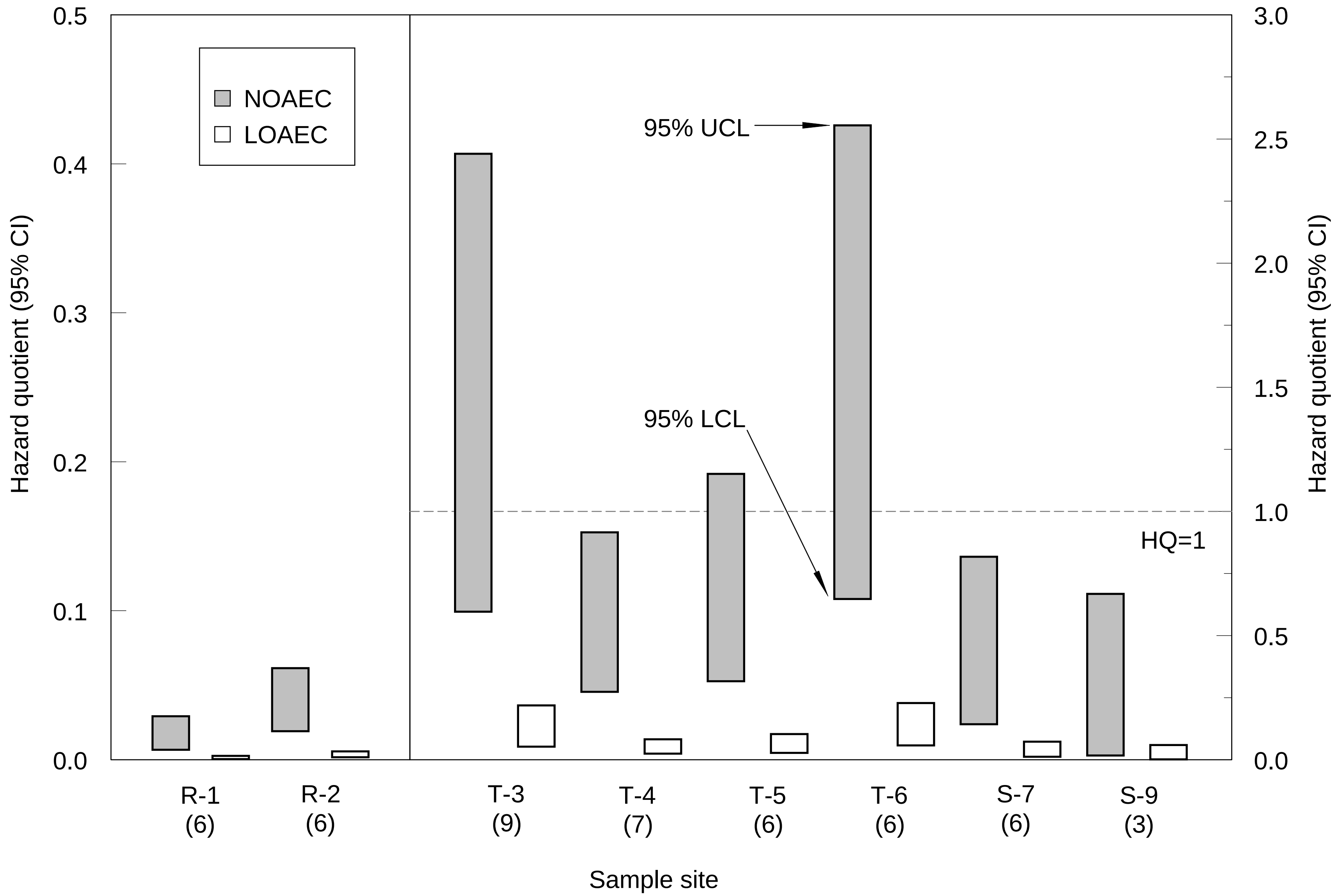
100
80
60
40
20
0
Cumulative frequency (%)
T-6
T-3
NOAEC - 710 ng TEQs/kg ww
(USEPA 2003)
LOAEC - 7,940 ng TEQs/kg ww
(USEPA 2003)
0
2000
4000
6000
8000
10000
Concentration ΣPCDD/DF TEQs WHO-Avian (ng/kg ww)

Figure 6.7. Modeled probabilistic distribution of expected cumulative percent frequencies for eastern bluebird egg $TEQ_{WHO\text{-}Avian}$ concentrations ng/kg ww in site-specific eggs collected from the river floodplains near Midland, Michigan in 2005-2007. 10,000 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 indicated by a dotted line; Y-axis offset to show R-1 and R-2; NOAEC indicated by a vertical bar; LOAEC (not indicated) is 10,000 ng TEQs/kg ww (Thiel et al. 1988).



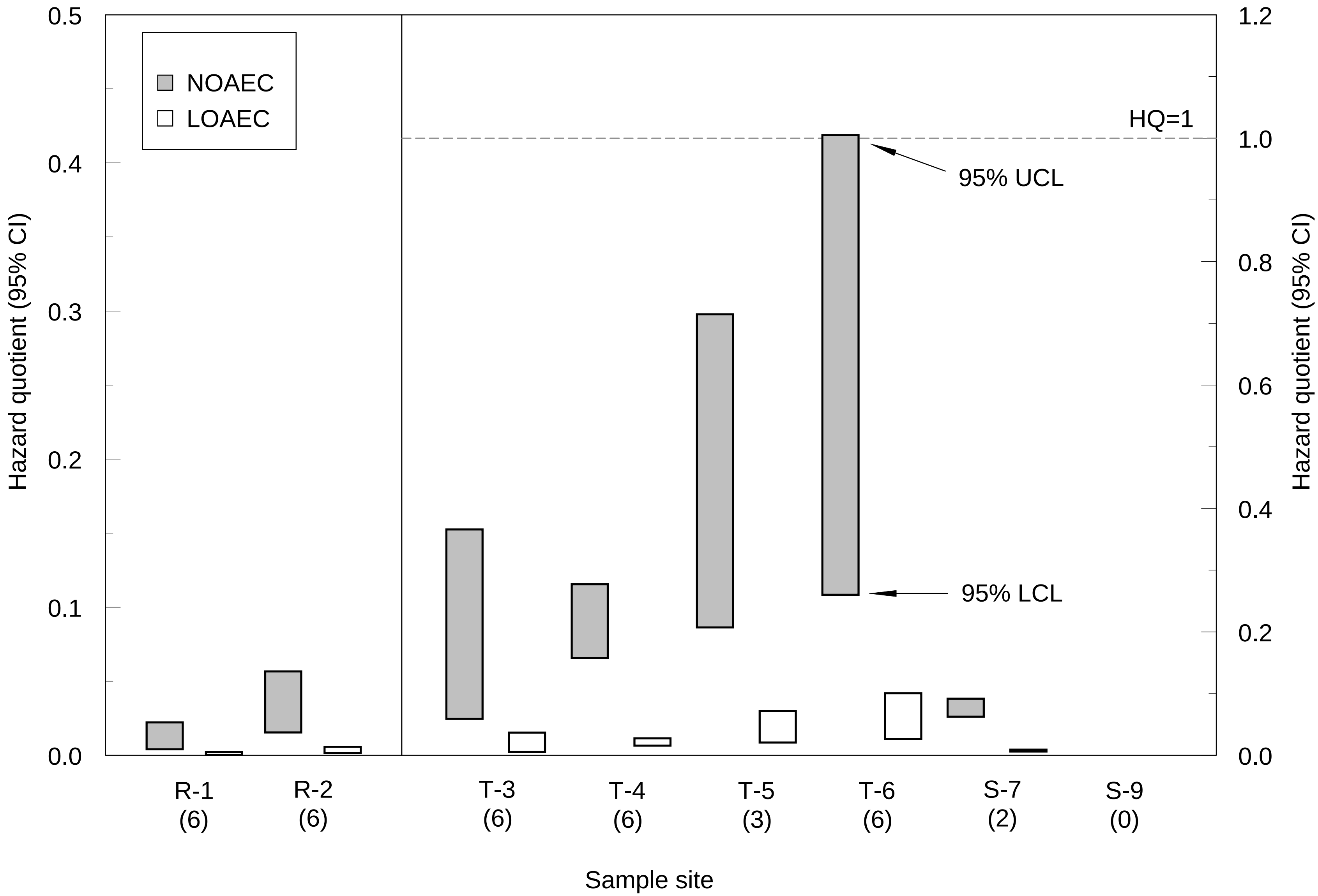
100
80
60
40
20
0
Cumulative frequency (%)
0
500
1000
1500
2000
Concentration ΣPCDD/DF TEQs$_{WHO-Avian}$ (ng/kg ww)
T-6
NOAEC - 1000 ng TEQs/kg ww
(Thiel et al. 1988)

Figure 6.8. Hazard quotients (HQ) for the effects of ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ for house wren eggs collected in 2005–2007 from the river floodplains near Midland, Michigan, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). 95% confidence intervals (LCL/UCL) based on the geometric mean concentrations are presented; Left y-axis for reference areas (R-1 and R-2); Right y-axis for Tittabawassee River study areas (T-3 to T-6) and Saginaw River study areas (S-7 to S-9).



NOAEC
LOAEC
95% UCL
95% LCL
HQ=1
Hazard quotient (95% CI)
0.0
0.1
0.2
0.3
0.4
0.5
Hazard quotient (95% CI)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
R-1 (6)
R-2 (6)
T-3 (9)
T-4 (7)
T-5 (6)
T-6 (6)
S-7 (6)
S-9 (3)
Sample site

Figure 6.9. Hazard quotients (HQ) for the effects of ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ for eastern bluebird eggs collected in 2005–2007 from the river floodplains near Midland, Michigan, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). 95% confidence intervals (LCL/UCL) based on the geometric mean concentrations are presented; range presented for S-7 where *n*=2; Left y-axis for reference areas (R-1 and R-2); Right y-axis for Tittabawassee River study areas (T-3 to T-6) and Saginaw River study areas (S-7 to S-9).



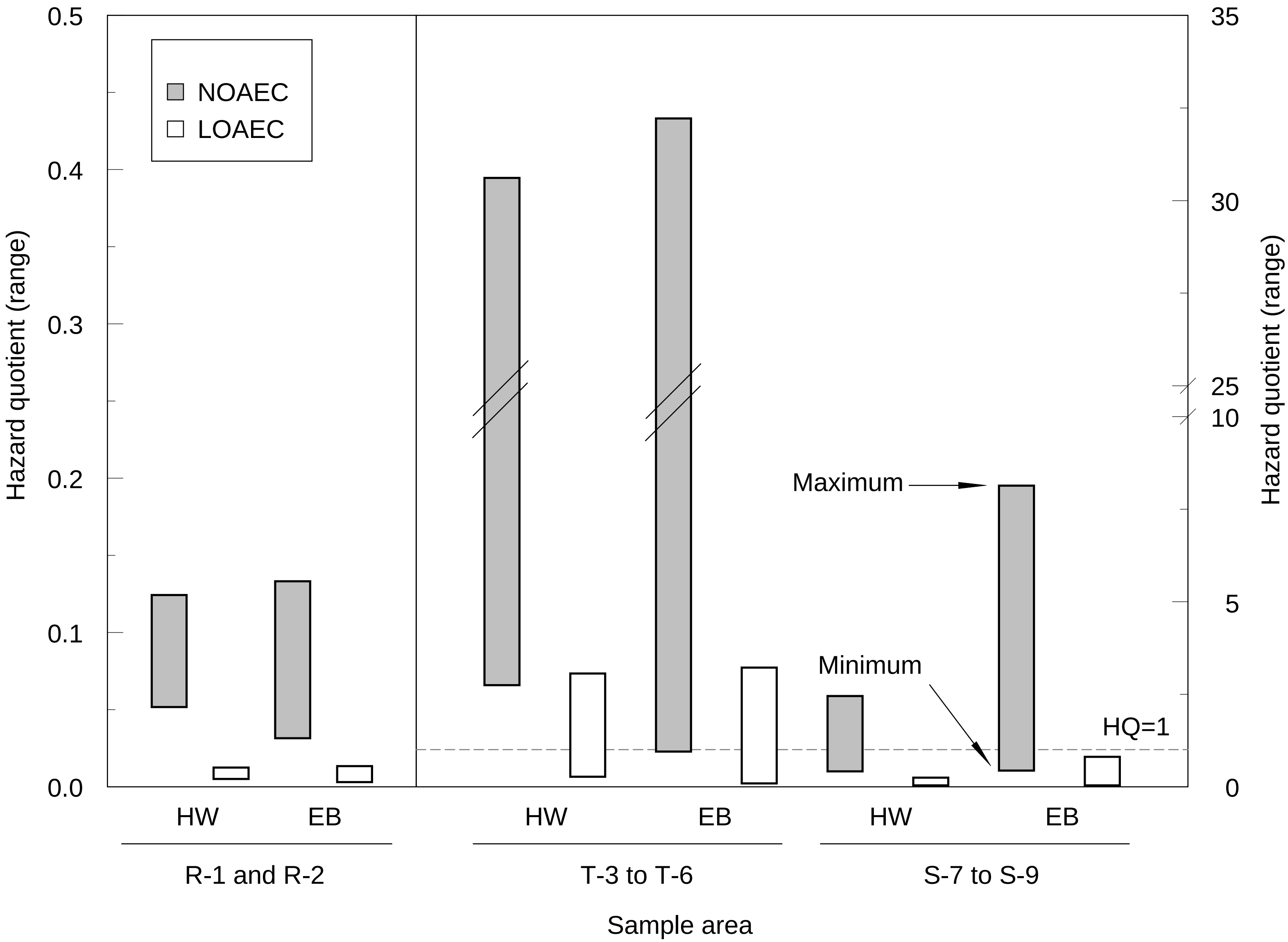
NOAEC
LOAEC
HQ=1
95% UCL
95% LCL
Hazard quotient (95% CI)
Hazard quotient (95% CI)
0.0
0.1
0.2
0.3
0.4
0.5
0.0
0.2
0.4
0.6
0.8
1.0
1.2
R-1 (6)
R-2 (6)
T-3 (6)
T-4 (6)
T-5 (3)
T-6 (6)
S-7 (2)
S-9 (0)
Sample site

estimates of minimum measured concentrations for both house wrens and eastern bluebirds at Tittabawassee River SAs were greater than LOAEC TRV, while Saginaw River SAs were lesser. Both food web- and bolus-based estimates of dietary exposure were less than associated LOAEC and NOAEC TRVs at RAs. Maximum bolus-based hazard quotients at Tittabawassee River SAs were greater than 30 for house wrens and eastern bluebirds based on the NOAEC, while based on the LOAEC values were greater than 3 (Figure 6.10). Maximum food web-based dietary exposure hazard quotients at Saginaw River SAs for house wrens and eastern bluebirds were 2 and 8, respectively (Figure 6.10).

Discussion

Overall, house wrens and eastern bluebirds were shown to be well suited to evaluate terrestrial-based contaminant exposures. House wren general population abundance, wide distributions, and lenient habitat requirements permitted collection of more than adequate measures of reproductive success and population health measurements. Challenges for house wren use included small nestling mass (10-d nestlings averaged approximately 10g) and egg mass (averaged approximately 1.4g) that may result in the need to pool samples to meet analytical detection limit requirements depending on the site and the analyte. Related dietary sampling of boluses for house wrens can also be limited by collection masses due to smaller invertebrates being fed to nestlings. Alternatively, eastern bluebirds nestlings and eggs are larger (14-d nestlings averaged approximately 28g and eggs averaged approximately 3.1 g) as are dietary items, but populations are smaller and habitat requirements are more stringent. Therefore, adequate

Figure 6.10. Hazard quotients (HQ) for the effects of potential ΣPCDD/DF TEQs$_{WHO\text{-}Avian}$ daily dietary dose calculated from site-specific bolus-based (R1 to T-6) and food web-based (S-7 to S-9) dietary exposure for adult house wren and eastern bluebird collected in 2005–2007 from the river floodplains near Midland, Michigan, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). HQs based on measured concentration ranges are presented; Left y-axis for reference areas (R-1 and R-2); Right y-axis for Tittabawassee River study areas (T-3 to T-6) and Saginaw River study areas (S-7 to S-9); food web-based dietary exposure is presented for S-7 to S-9 since no bolus samples were collected from those sites.



NOAEC
LOAEC
Hazard quotient (range)
0.5
0.4
0.3
0.2
0.1
0.0
35
30
25
10
5
0
Maximum
Minimum
HQ=1
HW
EB
HW
EB
HW
EB
R-1 and R-2
T-3 to T-6
S-7 to S-9
Sample area
Hazard quotient (range)

sample masses are available but often reproductive success and population health measures can be limited by low box occupancy. By combining multiple lines of evidence for these two passerine species, a balanced assessment of risk for the site of terrestrial-based contamination near Midland, Michigan was possible.

Toxicity reference values

Currently, the greatest limiting factor for developing accurate assessments of risk for birds exposed to dioxin-like compounds is a lack of comprehensive studies designed to determine thresholds for effects in ecologically relevant species. The domestic chicken (*Gallus domesticus*) has been widely studied and is considered to be the most sensitive bird species to the effects of dioxin-like compounds (Brunström and Reutergardh 1986; Brunström 1988; Powell *et al.* 1996; Henshel *et al.* 1997; Brunström and Halldin 1998; Blankenship *et al.* 2003). Considering a number of data usability criterion, the TRVs used herein were based on studies of the ring-necked pheasant and eastern bluebird rather than the more conservative chicken effects data. Thus, despite limited sample sizes, potential confounding factors based on field-incubated eggs, and potential congener-specific differences, the TRVs based on eastern bluebird egg injections (Thiel *et al.* 1988) are the best available for eastern bluebird egg exposure and hatching success due to species-similarity considerations. For dietary exposure-based TRVs the intraperitoneal injections of TCDD in hen ring-necked pheasants (Nosek *et al.* 1992a) likely overestimates effects thresholds for the passerine species studied here. A major limitation of this TRV is that the exposure route is not a true dietary dose, which does not take into account sequestration, metabolism, excretion, and bioavailability of the contaminants when bound to dietary items (Norstrom *et al.* 1976; Braune and Norstrom 1989; Elliott *et al.* 1996; Drouillard *et al.* 2001; Kubota *et al.* 2006; Wan *et al.* 2006). This limitation combined with recent findings that provide evidence suggesting a molecular basis for variation in avian species-specific sensitivities to dioxin-like compounds (Karchner *et al.* 2006; Head *et al.* 2008) should generate renewed scientific interest in conducting necessary chronic avian dietary-exposure studies on wildlife species. The differences between species-specific sensitivities to dioxin-like compounds are potentially tied to amino acid substitution differences in the aryl hydrocarbon receptor (AhR) ligand binding domain (LBD) between species (Kennedy *personal communication*). Based on these findings, the house wren and eastern bluebird AhR LBD were classified as species with moderate sensitivities to dioxin-like compounds, identical to the tree swallow, American robin (*Turdus migratorius*), and house sparrow (*Passer domesticus*) and closely related to the ring-necked pheasant.

Assessment of hazard

Assessing the potential for adverse effects by use of a HQ approach that is based on the most appropriate TRVs available for the species studied can provide information into the likelihood of site-specific effects. Hazard quotients greater than 1.0 were reported for 95% UCLs in house wren eggs based on the NOAEC at T-3, T-5, and T-6, while the 95% UCL in eastern bluebird eggs at T-6 equaled 1 (Figures 6.7 and 6.8). HQs greater than 1.0 are indicative of exposures that exceed the threshold for adverse effects and suggest there is the potential for adverse effects to occur. Compared to the predicted distributions of concentrations of $TEQ_{WHO\text{-}Avian}$ in eggs at these sites, the percent of the frequency distribution above the NOAEL ranged 21 to 65% for house wrens and was 15% for eastern bluebirds (Figures 6.5 and 6.6). However, less than 1% of the frequency distribution for concentrations of $TEQ_{WHO\text{-}Avian}$ in house wren eggs was greater than the LOAEC, and 0% of the predicted distribution was greater for eastern bluebirds. Importantly, the actual effect threshold for individuals is likely between the established no- and lowest-effect TRV values. Based on conservatively selected egg-based TRVs (likely based on a species with greater sensitivity) and 95% UCL exposures the potential for effects on individual house wrens at Tittabawassee River SAs is minimal, and effects on eastern bluebirds are not expected.

Hazard quotient values based on concentrations of $TEQ_{WHO\text{-}Avian}$ in bolus for both house wrens and eastern bluebirds had similar trends and were greater than or equal to 1.0 at Tittabawassee River SAs based on the minimum value of $TEQ_{WHO\text{-}Avian}$ concentrations and NOAEC. The HQs based on the maximum value of $TEQ_{WHO\text{-}Avian}$ concentrations and LOAEC also exceeded 1.0 for both species at that study area (Figure 6.10). Food web-based dietary exposure HQs (data not presented) were approximately 3-fold less than bolus-based HQs at Tittabawassee River SAs. Bolus-based dietary exposures were selected since they represented actual invertebrates collected on-site by the species studied and included the greatest potential exposure estimates. Since bolus-based exposures were not available for Saginaw River SAs food web-based dietary exposures were used to determine HQs, which were slightly less than those at Tittabawassee River SAs based on food web-based exposures. Dietary exposures measured in tree swallow nestlings exposed to primarily TCDD on the Woonasquatucket River in Massachusetts that ranged from 0.87 to 6.6 and from 72 to 230 ng TEQ/kg ww at unexposed and exposed sites, respectively (Custer *et al.* 2005). Converted to a daily dietary dose based on site- and species-specific ingestion rates calculated from data collected in the current study, house wren and eastern bluebird exposure would range from 66 to 209 and from 57 to 179 ng TEQ/kg BW/d, respectively. In the Woonasquatucket River study, hatching success was negatively impacted at exposed sites, and although beyond the scope of their conclusions it is likely that adult dietary exposure prior to breeding was similar to nestling exposures. Therefore, similar effects on hatching success could be expected at the comparable exposures measured the Tittabawassee River SAs (Table 6.2). Based on the range of exposures to $TEQs_{WHO\text{-}Avian}$ for house wrens and eastern bluebirds at study areas downstream of Midland and available dietary-based TRVs the potential exists for effects on hatching success but its likelihood is small given the conservative nature of the assessment.

Multiple lines of evidence and population-level effects

Predicted effects on productivity based on tissue- and dietary-based exposure estimates were compared with measured productivity of the terrestrial passerines studied to provide a site-specific multiple lines of evidence assessment of hazard (Menzie *et al.* 1996; Fairbrother 2003; Hull and Swanson 2006; Neigh *et al.* 2006a; Barnthouse *et al.* 2009). Exposure and productivity were directly measured to minimize potential uncertainties associated with predicting the potential for adverse effects based solely on concentrations in abiotic matrices (Chapman *et al.* 2002; Leonards *et al.* 2008). Measurement endpoints for house wrens and eastern bluebirds were well quantified at sites studied near Midland, Michigan, which minimized potential uncertainties associated with exposure potential and reproductive performance on-site. Uncertainty in selected dietary-based TRVs was primarily due to the use of intraperitoneal injections opposed to dietary gavages or spiked diets, which likely resulted in an overestimation of both NOAEC and LOAEC values. Since dietary exposures to ΣPCDD/DF $TEQs_{WHO\text{-}Avian}$ on the Tittabawassee River were similar to those based on tree swallows on the Woonasquatucket River (Custer *et al.* 2005) and due to the lack of field studies on house wrens and eastern bluebirds exposed to PCDD/DFs, comparisons were made with the egg exposure based LD50 threshold for hatching success reported as 1,700 ng TCDD/kg ww in that study. The threshold for a decrease in hatching success based on the modeled distribution of $TEQs_{WHO\text{-}Avian}$ for house wrens at T-3 and T-5 would have been exceeded for approximately 20-25% of the population, while for eastern bluebirds at T-6 less than 5% would have been affected (Figures 6.5 and 6.6). However, hatching success for house wrens at Tittabawassee River SAs (77%) was not significantly less than at RAs (81%) (Fredricks *et al.* 2009c), and was not correlated with concentrations in eggs for individual clutches (Figure 6.3). However statistical power to discern differences between measures of productivity for eastern bluebirds on-site were possibly limited by occupancy, reproductive parameters among study areas were similar to those reported for uncontaminated sites (Pinkowski 1979; Bauldry *et al.* 1995).

Despite dietary- and tissue-based exposures for both house wrens and eastern bluebirds that were comparable to tree swallows exposed to primarily TCDD at similarly contaminated sites (Custer *et al.* 2005) and elevated HQs at study areas downstream of Midland, overall productivity through fledging appeared to be unaffected. For the Woonasquatucket River, $TEQ_{WHO\text{-}Avian}$ exposures were primarily from TCDD (Custer *et* *al.* 2005) as compared to primarily 2,3,4,7,8-PeCDF and TCDF in terrestrial passerines tissue- and dietary-based exposures in the current study. Potential differences in the distribution and metabolism of specific congeners by birds (Norstrom *et al.* 1976; Norstrom *et al.* 1986; Elliott *et al.* 1996) or differences in species-specific sensitivities to dioxin-like compounds (Karchner *et al.* 2006; Head *et al.* 2008) could also account for potential differences between some literature based thresholds and the lack of effects observed.

Additional information pertaining to post-fledge survival and recruitment of recently fledged nestlings may offer additional insight into population health and sustainability. However, due to the relatively short duration of this portion of the study and inherently low recruitment and site fidelity of yearling passerines (Summers-Smith 1956; Adams *et al.* 2001; Robinson *et al.* 2007; Wells *et al.* 2007; Rush and Stutchbury 2008; Fredricks *et al.* 2009c) a comprehensive band monitoring data set of extended duration (2005–2010) for the birds described herein will be presented upon completion in subsequent publications.

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Animal Use

All aspects of the study that involved the use of animals were conducted in the most humane way possible. To achieve that objective, all aspects of the study design were performed following standard operating procedures (Protocol for Monitoring and Collection of Box-Nesting Passerine Birds 03/04-045-00; Field studies in support of Tittabawassee River Ecological Risk Assessment 03/04-042-00) approved by Michigan State University's Institutional Animal Care and Use Committee (IACUC). All of the necessary state and federal approvals and permits (Michigan Department of Natural

Resources Scientific Collection Permit SC1252, US Fish and Wildlife Migratory Bird Scientific Collection Permit MB102552-1, and subpermitted under US Department of the Interior Federal Banding Permit 22926) are on file at MSU-ATL.

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CHAPTER 7

Conclusions

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This dissertation details some of the first reported exposures of passerine species to elevated concentrations of primarily polychlorinated dibenzofurans and potential effects on associated reproductive endpoints. Overall, house wrens, tree swallows, and eastern bluebirds breeding along the river floodplains near Midland, Michigan (USA) during 2005 to 2007 successfully reproduced despite elevated dietary- and tissue-based exposures. A site-specific multiple lines of evidence approach to hazard assessment including dietary- and tissue-based exposures combined with reproductive productivity measures was used to minimize uncertainty in the assessment conclusions. Nearly 300 nest boxes were monitored daily during the breeding seasons at two reference areas (RAs), four Tittabawassee River study areas (SAs), and two Saginaw River SAs. Occupancy for all species studied in 2005 was less than in subsequent years, and species-specific occupancy was greatest in house wrens, least in eastern bluebirds, and intermediate for tree swallows. Eastern bluebirds were the only species studied for which the data reported were potentially limited by occupancy at a few locations. Despite the lesser occupancy at some locations, eastern bluebirds at downstream study areas had greater reproductive success despite elevated exposures when compared to RAs.

Tissue-based exposures were greater at SAs for eggs and nestlings of all species studied compared to RAs, with the exception that tree swallow eggs were similar among locations. However, relative percent congener profiles for all species were composed of primarily 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) at SAs while RAs were composed of dioxin congeners including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The best explanation for the elevated concentrations of dioxins at upstream RAs in tree swallows is that after arriving on-site prior to breeding the foraging ranges of females include a proximally located contaminated site. Several facts support this conclusion: tree swallow females are considered income breeders which means the majority of the resources used for egg development are acquired during egg laying, foraging ranges of tree swallows feeding nestlings are reduced in area compared to other times of the year, and nestling and dietary congener profiles and concentrations are similar to the other two species at RAs. Otherwise, concentrations of ΣPCDD/DFs were greatest in most other sample types at T-6 (near Imerman Park) along the Tittabawassee River. This is most likely caused by the river dynamics at that location resulting in a large depositional area during yearly high-water events. There were also species-specific exposure differences for both quantity and congener profile. Based on dietary items the congener profiles at SAs were dominated by TCDF and 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin for both aquatic- and terrestrial-based diets. Tree swallow egg and nestling tissue residues at downstream SAs were dominated by TCDF as expected from dietary-based exposures, however house wren and eastern bluebird tissues residues were dominated by 2,3,4,7,8-PeCDF. This is likely due to both species- and congener-specific differences in metabolism, sequestration, and elimination although little research has been done on birds and these congeners. Additionally, based on a limited sample of three tree swallow eggs that were screened for co-contaminants, elevated concentrations of polychlorinated biphenyls were detected in both RAs and SAs. This additional exposure would need to be more extensively studied to fully understand the exposures of tree swallows breeding on site to PCBs.

Site-specific dietary compositions were determined by collecting bolus samples from nestlings of each species studied. Dietary exposures were estimated for all three species studied using both bolus samples as well as site-specific composite samples of invertebrate orders. Since estimated exposure ranges were similar between the two methods for each species, I recommend the use of bolus samples in future dietary exposure assessments for amenable species. Analytical analyses of site-specific bolus samples provide results from actual dietary samples collected by foraging adults from the study areas. This eliminates uncertainties with estimating site- or species-specific dietary compositions, foraging ranges of adults, or defining appropriate invertebrate sampling areas. Although bolus sampling requires more time spent training technicians compared to the field collection of invertebrate samples, the time saved by eliminating the need to sort composite invertebrate samples to taxonomic orders for analyses is by far greater.

By monitoring a diverse array of productivity endpoints for the three passerines studied over several breeding seasons it was possible to further refine those that are most likely to be informative of potential effects of exposures to dioxin-like compounds. The most time intensive endpoints monitored were adult nest attentiveness and massing nestlings. Monitoring the number of feeding trips by adults over a given period of time is confounded by many other factors (such as human disturbance, time of day, weather, age of nestlings, adult age/experience etc.) and is unquestionably tied to nest success, fledging success, and nestling growth. Measuring nestling masses at multiple time points during development again is tied to nest success and fledging success. However, greater mass at fledge has not conclusively been correlated with potentially greater post-fledging survival or fitness. Therefore future research should not include nest monitoring for adult attentiveness or nesting growth parameters, due to either the time-intensive nature of the measurements, inherent variability surrounding the endpoints, or lack of potential contaminant dosing studies for effects monitoring. Although, measuring egg masses on the date laid can provide a true egg mass and prevent having to use correction factors to "back-calculate" egg mass for eggs collected later in incubation, abandoned, or addled. The incorporation of a banding program for nestlings and adults provides necessary information on site-use, fidelity, renesting or multiple nesting attempts, and addresses questions concerning potential "source" versus "sink" population dynamics at a contaminated site. Trapping adults at each nesting attempt, although time and labor intensive is the only method to determine site-specific survival and fidelity for exposed populations. Basic measures of clutch size, hatching success, brood size, fledging success, number of fledglings, productivity, and nest success which are fairly easy to monitor, provide essential information, and are easily incorporated into a monitoring program. The addition of a monitoring program to better determine post-fledging survival of nestlings using radio-telemetry, although beyond the scope of this research would help quantify potential effects of site-specific contamination on survival, and is recommended.

Potential for site-specific adverse effects from dietary- and tissue-based exposures for both aquatic and terrestrial species-specific assessments were based on established toxicity reference values (TRVs). Eastern bluebird and tree swallow egg-based exposures were at or below the no observed adverse effect concentration (NOAEC) threshold at all study areas. Even with the uncertainties associated with potential PCB exposures for tree swallows the hazard potential for egg-based exposures was still near the NOAEC. House wren egg-based exposures were above the NOAEC threshold at several Tittabawassee River SAs and based on probabilistic modeling greater than half of the eggs at those sites would exceed the NOAEC, while less than 1% would exceed the lowest observed adverse effect concentration (LOAEC). Dietary exposures for all three species were greater than both the NOAEC and LOAEC TRVs at both Tittabawassee and Saginaw River SAs indicating an elevated potential for adverse effects. Unfortunately, the best available TRVs for passerines are based on intraperitoneal injections which likely overestimate exposures compared to dietary spiking or gavage dosing. This combined with comparisons of egg- and dietary-based exposure assessments with site-specific productivity measurements resulted in the overall conclusion that passerine populations breeding in the river floodplains downstream of Midland, Michigan are not at risk despite elevated concentrations of PCDFs in their diet and tissues.

Future laboratory-based studies should focus on incorporating congener specific egg- and dietary-based exposures to PCDD/DFs with recent advances in the molecular determinations of species sensitivities to AhR mediated effects to establish updated TRVs based on ecologically relevant endpoints in wildlife species. Future field-based studies should incorporate potential long-term survival and post-fledging survival of nestlings to better quantify potential effects of contaminant exposures. The potential for adverse effects from additional stresses such as migration, molt, or winter weather could potentially contribute to significant effects on survival that were not completely quantified in the current 3-year study. Additionally, continued monitoring of the Tittabawassee and Saginaw River floodplains downstream of Midland, Michigan for potentially elevated exposures to PCBs is suggested because of their similar modes of action and remaining uncertainties associated with the species-specific exposure differences reported. Similar to most species that inhabit the floodplain environments downstream of Midland, Michigan, breeding habitat and available nesting sites are likely the most critical aspects of a sustainable nesting population opposed to contaminant exposures. Any remediation action suggested or taken along this stretch of river should primarily consider the habitat based effects of those actions, since based on my research few if any significant population-level or individual-based effects based on contaminant exposures were observed or predicted.