This is to certify that the
dissertation entitled

AN ECOLOGICAL RISK ASSESSMENT OF FISH-EATING BIRDS EXPOSED TO POLYCHLORINATED DIBENZOFURANS AND DIBENZO-P-DIOXINS WITHIN THE TITTABAWASSEE RIVER FLOODPLAIN, MI, USA

presented by

RITA MARIE SESTON

has been accepted towards fulfillment
of the requirements for the

Doctoral degree in Zoology-Environmental Toxicology

John P. Giesy
Major Professor's Signature

May 2, 2010
Date

MSU is an Affirmative Action/Equal Opportunity Employer

AN ECOLOGICAL RISK ASSESSMENT OF FISH-EATING BIRDS EXPOSED TO POLYCHLORINATED DIBENZOFURANS AND DIBENZO-*P*-DIOXINS WITHIN THE TITTABAWASSEE RIVER FLOODPLAIN, MI, USA

by

Rita Marie Seston

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Zoology-Environmental Toxicology

2010

ABSTRACT

AN ECOLOGICAL RISK ASSESSMENT OF FISH-EATING BIRDS EXPOSED TO POLYCHLORINATED DIBENZOFURANS AND DIBENZO-*P*-DIOXINS WITHIN THE TITTABAWASSEE RIVER FLOODPLAIN, MI, USA

by

Rita Marie Seston

Concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) in the Tittabawassee River (TR) and associated floodplains downstream of Midland, MI, USA are greater than at upstream locations and regional background concentrations. Sediments and floodplain soils in downstream study areas (SAs) contain total concentrations of the seventeen 2,3,7,8-substituted PCDD/DF congeners (ΣPCDD/DFs) ranging from $1.0x10^2$ to $5.4x10^4$ ng/kg dry wt, respectively. In contrast, concentrations of ΣPCDD/DFs in sediments and soils from upstream reference areas were 10- to 20-fold less. 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 is the result of historical chemical production and associated waste management practices. Concerns about potential ecological impacts of the elevated concentrations of PCDD/DFs within the TR floodplain led to a site-specific multiple lines of evidence study was executed including dietary- and tissue-based exposures assessments and measurements of population health. Two fish-eating bird species that breed along the TR [great blue heron (*Ardea herodias*; GBH) and belted kingfisher (*Ceryle alcyon*; BKF)] 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. During the 2005-2007 breeding seasons, a total of 37 BKF nest chambers were excavated for sample collection and monitored for reproductive effort and success. For GBH, three breeding colonies located within the SA were monitored during the 2006 and 2007 breeding seasons. Nests within each colony were also accessed for sample collection. Concentrations of ΣPCDD/DF 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in both eggs and nestlings of BKF from the SA were 5- to 21-fold greater than in those from upstream reference areas (RAs). Concentrations of $TEQ_{WHO\text{-}Avian}$ blood plasma of adult GBH from the SA was 4- to 8-fold greater compared to those from the RA. Contaminant concentrations in GBH eggs and nestlings were similar among all studied breeding colonies. Predicted dietary exposures followed this same spatial trend in both species, being 150- to 190-fold greater along the TR compared to upstream RAs. Comparison of the predicted daily dietary dose to the TRV suggested there was the risk of adverse effects as a result of exposure to PCDD/DFs. This is in contrast to the conclusions drawn from both the tissue-based exposure and effects assessments and site-specific measures of individual and population health. This inconsistency is likely the result of the dietary exposure and effects assessments being more conservative, based on the greater number of assumptions that must be made and the greater uncertainty associated with the dosing methodology from which the TRV was derived. Therefore, the overall conclusion of the research presented herein is that the populations of BKF and GBH breeding along the TR are not at risk despite elevated concentrations of PCDD/DFs in the diet and tissues.

To my big sister – for blazing the trail

ACKNOWLEDGEMENTS

There are a countless number of people whose friendship and guidance have helped me make it through this degree program. Dr. John Giesy provided an environment which allowed me to grow professionally and personally through trial and error. All of my fellow colleagues in the Wildlife Toxicology Laboratory (Tim Fredricks, Dusty Tazelaar, Emily Koppel, Lori Williams, Will Folland, Mike Nadeau, Casey Bartrem, Dave Hamman, Patrick Bradley, Mick Kramer, Nozomi Ikeda, and many others) provided invaluable support in the pursuit research, recreational, and social objectives. I have the utmost gratitude toward the local landowners and parks in the area of the Tittabawassee River for allowing us to traipse around their property at all hours of the day and for sharing their local wisdom with us. Their kindness and openness was pivotal to the success of the research project. I truly appreciate my family for not giving up on me even though it seems I could never remember to call home to let everyone know what I was up to. Last, but certainly not least, thanks to Dr. Matt Zwiernik. His guidance, support, encouragement, and belief in me helped me to succeed in spite of myself. Because of him, I now believe I am capable of just about anything. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

ADD_{pot} – potential average daily dose

ARNT – AhR nuclear translocator

AhR – aryl hydrocarbon receptor

AHY – after hatch year

BKF – Belted kingfisher (*Ceryle alcyon*)

BW – body weight

°C – degrees centigrade

CI – confidence interval

CNC – Chippewa Nature Center

COC – chemicals of concern

COPEC – compound of potential environmental concern

CYP1A – cytochrome P4501A

d – day

DEQ – Department of Environmental Quality

DNA – deoxyribonucleic acid

DOW – The Dow Chemical Company

DDT – dichloro-diphenyl-trichloroethane

DDE – dichloro-diphenyl-dichloroethylene

DDXs – dichlorodiphenyltrichloroethane and related metabolites

DRE – dioxin-responsive element

dw – dry weight

ERA – ecological risk assessment

EROD –7-ethoxy-resorufn-*O*-deethylase

g – gram

GBH – Great blue heron (*Ardea herodias*)

GHO – Great horned owl (*Bubo virginianus*)

GLEMEDS – Great Lakes embryo, mortality, edema, and deformities syndrome

h – hour

HpCB – heptachlorinated biphenyl

HpCDD – heptachlorodibenzo-*p*-dioxin

HpCDF – heptachlorodibenzofuran

HQ – hazard quotient

HxCB – hexachlorinated biphenyl

HxCDD – hexachlorodibenzo-*p*-dioxin

HxCDF – hexachlorodibenzofuran

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

IR – intake rate

km - kilometer

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

µg - microgram

MSU-WTL – Michigan State University-Wildlife Toxicology Laboratory

ng – nanogram

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

OC – organochlorine pesticide

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

SC – scientific collection

SNWR – Shiawassee National Wildlife Refuge

SR – Saginaw River

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

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

Rita Marie Seston

Overview

The Tittabawassee River downstream of Midland, Michigan, USA, has been shown to contain elevated concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs). This contamination is a result of historical chemical production processes and waste management techniques performed at the Dow Chemical Company Mid-Michigan Plant located in Midland. Due to the persistence of these compounds of potential environmental concern (COPECs), resident wildlife species may be exposed to concentrations of PCDFs and PCDDs which could potentially affect their health, at either the individual or population level. To understand both exposure and associated effects, field studies were conducted over several years in support of a site-specific ecological risk assessment. This assessment utilizes a multiple lines evidence approach to reduce the uncertainty which is often inherent in the risk assessment process.

To most accurately assess an ecosystem as complex as the Tittabawassee River floodplain, the site-specific risk assessment employs many different receptor species, each with multiple lines of evidence utilized. Receptor species were chosen to be representative of the various feeding guilds that have the greatest potential for exposure to the COPECs. Due to the tendency of PCDD/DFs to bioaccumulate through trophic transfer, species located near the top of food webs were selected as the most appropriate receptors. With a primarily aquatic-based exposure pathway, the American mink (*Mustela vison*) was chosen as the top-tier mammalian receptor. Several passerine species were selected as intermediate avian receptor species of aquatic-, terrestrial-, and combined-based exposure pathways, the great horned owl (*Bubo virginianus*) was selected as the top-tier avian receptor of a primarily terrestrial-based exposure pathway,

and the great blue heron (*Ardea herodias*; GBH) and belted kingfisher (*Ceryle alcyon*; BKF) were selected as top-tier avian receptors of an aquatic-based exposure pathway. Dietary exposure and tissue-based exposure, combined with assessments of individual and population health of each receptor species, are the multiple lines of evidence to be employed in assessing the risk present to resident species of the Tittabawassee River floodplain. Integrating the data resulting from these multiple assessments provides a better understanding of the contaminants and their movement within the ecosystem. In turn, this reduces the uncertainty inherent in the risk assessment process and provides sound data for use in making decisions regarding the future of the site.

The focus of the research described in this dissertation is the movement of COPECs through an aquatic-based exposure pathway, monitoring population-health parameters, and assessing overall risk posed by PCDD/DFs to piscivorous avian species nesting in the Tittabawassee River floodplain. The GBH and BKF were chosen as the receptor species that could best meet this objective.

Site Description

Located in the east-central lower peninsula of Michigan, the Tittabawassee River (TR) is a tributary of the Saginaw River (SR), which eventually empties into Saginaw Bay and Lake Huron. The TR runs through The Dow Chemical Company (DOW) property, which is located on the southern edge of Midland and is the accepted source of the PCDD/DF contamination. The area henceforth referred to as the study area (SA) includes approximately 37 km of the TR (sites T-3 to T-6) and associated wetlands from DOW to the confluence of the TR and SR and 35 km of the SR (sites S-7 to S-9) until it

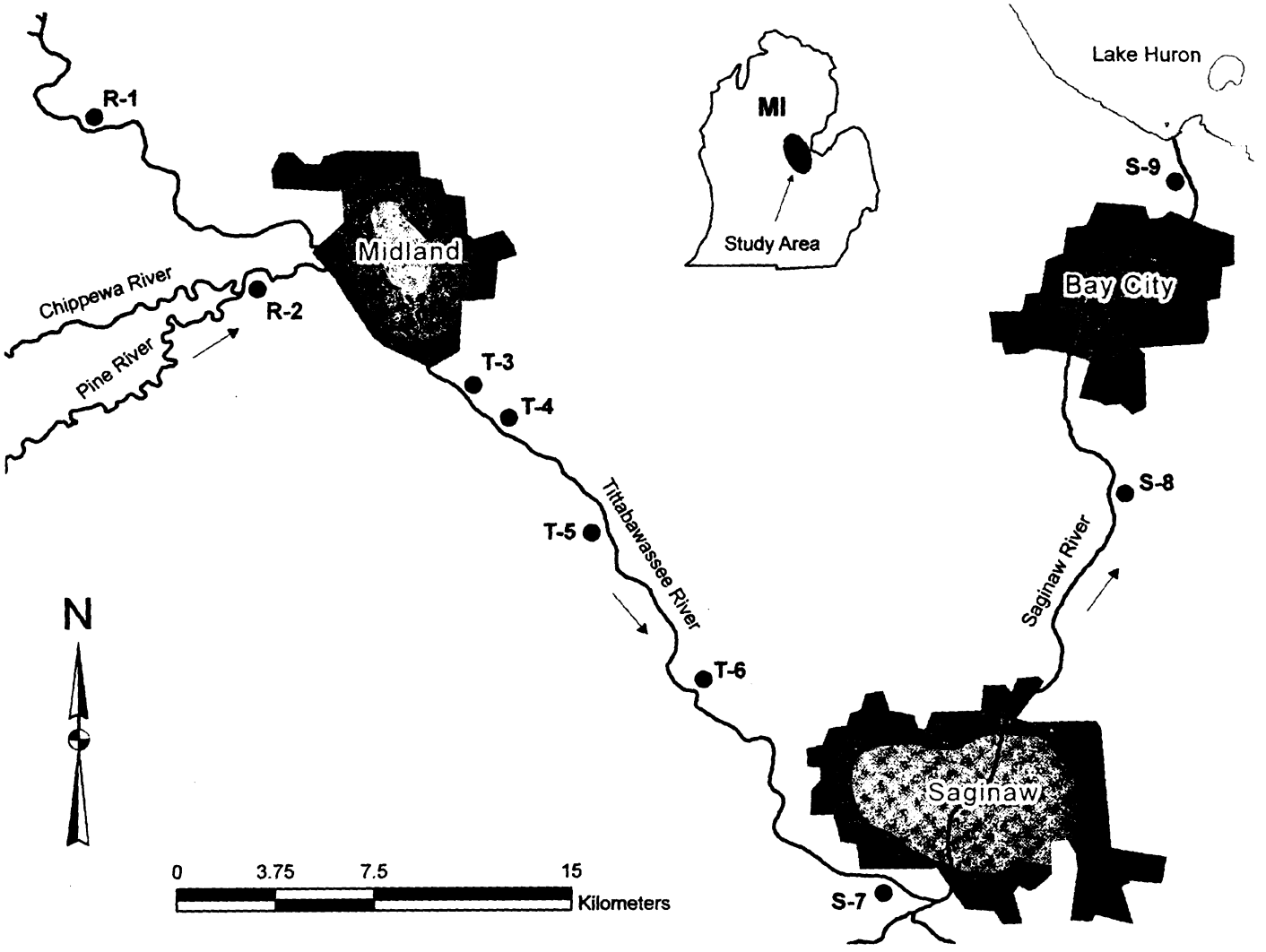
enters Saginaw Bay (Figure 1.1). Sampling sites located in the SA were chosen to characterize maximal exposure potential designated as "worst case scenario" locations based on a previous study that measured soil and sediment concentrations (Hilscherova *et al.* 2003) and availability of landowner access to sites. The reference area (RA) is composed of the TR upstream of Midland, together with the Pine and Chippewa Rivers, both of which are tributaries of the TR upstream of Midland. Sampling locations in the RA were on the upstream TR (R-1) and on the Pine River, just upstream of its confluence with the Chippewa River (R-2). Distinct sampling areas were assessed individually as well as grouped spatially based on river characteristics. Spatial groupings included reference (RA) R-1 and R-2, upper Tittabawassee River (UTR) T-3 and T-4, lower Tittabawassee River (LTR) T-5 to S-7, and SR S-8 and S-9. Components of each receptor species diet were collected from these sampling areas while the area of collection of the tissue of receptor species was determined by nest location.

Founded in 1897, the Midland plant is DOW's headquarters and was historically their primary chemical research and manufacturing facility. Initial operations at DOW were focused on mining brine and extracting bromine and chlorine to produce brominated and chlorinated compounds. Using an electrolytic process that generated chlorine from brine, bleach was Dow's dominant product until its production stopped in 1914, although the production of other chlorine-based products continued. Electrolytic processes using carbon electrodes were used at DOW until the 1980s. Although these processes have ceased, PCDD/DFs were likely released into the environment as unwanted by-products. Concentrations of PCDD/DFs in sediments and floodplain soils downstream of Midland were 10- to 20- fold greater than those upstream, yielding concentrations ranging from

102 to 53,600 pg/g dry weight (dw) (Hilscherova *et al.* 2003). Rivers in industrialized areas of the eastern United States, including the Housatonic River and the Passaic River, have sediment contamination of PCDD/DFs ranging from 160 to 5,400 pg/g dw (with one sample at 82,000 pg/g dw) and 370 to 24,000 pg/g dw, respectively (Eitzer 1993; Wenning *et al.* 1992). The Dutch River Rhine has PCDD/DFs concentrations ranging from 200 to 18,000 pg/g dw (Evers *et al.* 1988). Sediments collected from Masan Bay, Korea contained PCDD/DFs concentrations ranging from 122 to 16,729 pg/g dry wt (Kannan *et al.* 2007). In Tokyo Bay, Japan, sediment concentrations of PCDD/DFs range from 3,150 to 20,300 pg/g dry wt. (Sakurai *et al.* 2000). These studies indicate that PCDD/DFs concentrations found in the TR study area are elevated but remain comparable to those reported in a number of other industrialized locations.

In addition to PCDD/DFs, concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) were measured in the soils and sediments of the TR. Concentrations of total PCBs in sediments of the TR were less than 150 ng/g dry weight (Hilscherova *et al.* 2003; Michigan Department of Environmental Quality 2002). However, downstream of the TR, in the SR and Saginaw Bay, PCBs have been measured at relatively great concentrations (Froese *et al.* 1998; Giesy *et al.* 1997; Kannan *et al.* 2008; Ludwig *et al.* 1993). From approximately 1936 until the early 1970s, various brominated and chlorinated compounds were manufactured at the Michigan Chemical/Velsicol Corporation, and were subsequently released into the Pine River, a tributary of the Chippewa River and eventually the TR. Dichlorodiphenyltrichloroethane (DDT) was released into the Pine River environment due to activities at the Velsicol Chemical Company and consequently became a primary contaminant of concern in the

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). Direction of river flow is designated by arrows; suspected source of contamination is enclosed the dashed oval.


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

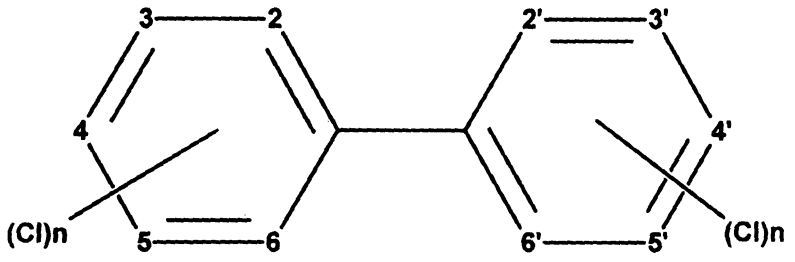
area as relatively great concentrations of DDT and its metabolites (DDXs) were measured in Pine River sediments and various fish species (Michigan Department of Environmental Quality 2000). Continued presence of DDXs within this system and the risk they may pose to wildlife residing in the TR SA led to their inclusion as COPECs. Of all identified COPECs, PCDD/DFs in the TR floodplain remain the primary concern due to their elevated concentrations in comparison to predicted toxic thresholds.

Contaminant Description

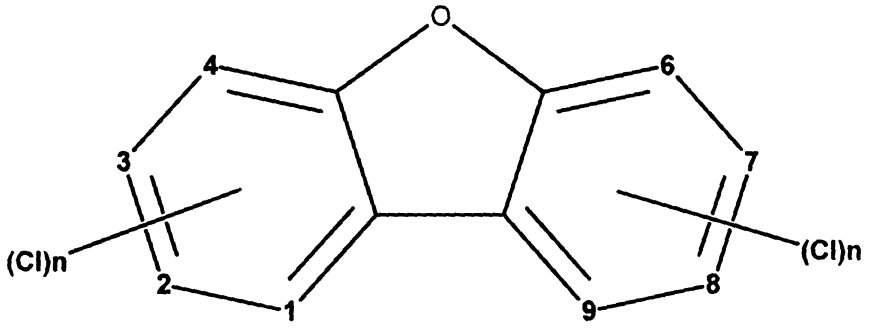
PCDD/DFs and PCBs are chemically classified as halogenated aromatic hydrocarbons. There are a total of 75 PCDD congeners and 135 PCDF congeners. PCDDs consist of two benzene rings with varying numbers and positions of chlorine atom substitutions, connected by two oxygen atoms. PCDFs are structurally very similar, differing in that the two benzene rings are joined by only one oxygen atom. PCB congeners consist of two benzene rings connected by a single C–C bond with varying numbers and positions of chlorine atom substitutions. Of 209 PCB congeners, 12 are coplanar congeners that 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 at the 2,3,7,8-positions exhibit the greatest toxicity, and are thus of the greatest interest (7 PCDD and 10 PCDF congeners). The congener thought to be the most potent and thus most widely studied of these compounds is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The structure of TCDD and related compounds are shown in Figure 1.2. This suite of compounds 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). In order to investigate the complex mixtures of these compounds which occur in the environment, the concept of toxic equivalency factors (TEFs) has been developed (van den Berg *et al.* 1998). Specific TEFs were developed for mammals, birds, and fish to account for differences in sensitivities between these taxa. Based on the assumption that all dioxin-like compounds exhibit their toxicity through the same mechanism of action, the toxicity of a mixture of these compounds should be additive. TEFs have been developed for the 17 2,3,7,8-substituted PCDD/DF congeners and 12 structurally related PCB congeners (Table 1.1). Using TEFs, concentrations of each congener can be converted to TCDD equivalents (TEQs) to assess the overall toxic potential of a mixture of compounds. Although this scheme is useful in gaining an understanding of the toxic potential of a mixture of these compounds, it is important that TEQs are not used to compare between soils/sediments and biota or movement through trophic levels.

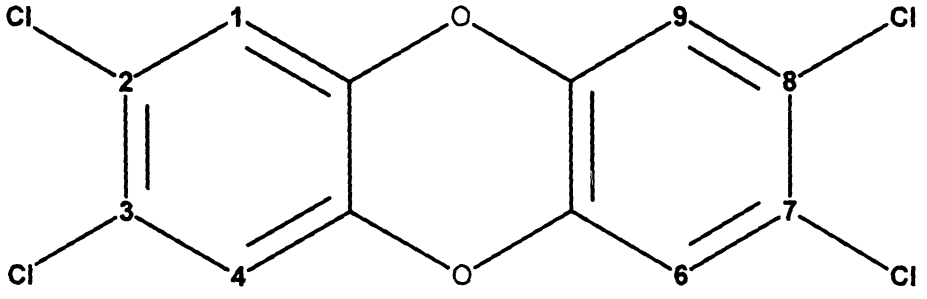
Although natural combustion and geological processes may result in trace quantities of PCDD/DFs, essentially all PCDD/DFs in the environment are unwanted byproducts from various anthropogenic activities (Czuczwa *et al.* 1984; Schecter *et al.* 1988). The formation of PCDD/DFs can occur during various combustion processes, including the incineration of municipal and industrial solid waste (Goovaerts *et al.* 2008; Lasagni *et al.* 2009; Lustenhouwer *et al.* 1980). Dioxin-like compounds are also formed through various processes at paper and pulp mills. For example, the use of elemental chlorine in the bleaching process and the use of wood contaminated with polychlorophenolic-based



biphenyl



dibenzofuran



TCDD

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.

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

wood preservatives leads to mill effluents which contain PCDFs and PCDDs (Bright *et al.* 1999; Clement *et al.* 1989; Swanson *et al.* 1988). PCDFs and PCDDs are inadvertently created during the production of chlorine and chlorinated compounds, such as chlorophenols (Hutzinger *et al.* 1985; Rappe *et al.* 1991). The persistent and lipophilic nature of PCDD/DFs leads to their accumulation in soils and sediments, and makes improper waste storage and the disruption of contaminated sites important sources of these compounds into the environment (USEPA 1994b). The predominant congeners found in the TR SA, TCDF and OCDD, suggest the contamination originated from the production of chlorophenol or chlorobenzene, general chlor-alkali processes, or associated waste products (Hilscherova *et al.* 2003; Kannan *et al.* 1998; Kannan *et al.* 2008).

Toxicology of Dioxin-Like Compounds

Since the discovery of its potency, TCDD has been an extensively studied compound. Dioxin-like compounds bind with high affinity to the aryl hydrocarbon receptor (AhR), a ligand-activated nuclear transcription factor (Safe 1986). Activation of this receptor-mediated pathway results in a diverse array of effects, including biochemical adaptive changes such as enzyme induction, developmental deformities, reproductive failure, hepato-toxicity, immuno-toxicity, carcinogenicity, wasting syndrome, and eventually death. TCDD is one of the most toxic of the dioxin-like compounds, as it binds with the greatest affinity to the AhR (Poland and Knutson 1982). Structurally related compounds bind the AhR with varying degrees of affinity and thus their toxicity varies (Safe 1986). Free AhR resides in the cytoplasm, but upon binding with a ligand, such as TCDD, the

AhR translocates to the nucleus. In the nucleus, AhR dimerizes with its DNA binding partner, the AhR nuclear translocator (ARNT). This heterodimer then binds specific DNA response elements, known as dioxin-responsive elements, leading to an increase in the transcription of certain genes, such as mammalian CYP1A1 and CYP1A2 and avian CYP1A4 and CYP1A5. However, the relationship between the induction of these genes and the toxicity of these compounds is not completely understood (Schmidt and Bradfield 1996).

The toxicity of dioxin-like compounds, particularly TCDD, has been well established in birds. Egg injection studies using the domestic chicken (*Gallus gallus*) have calculated 50% lethal dose (LD50) values that range between 122-297 pg/g wet wt TCDD (Allred and Strange 1977; Blankenship *et al.* 2003; Henshel *et al.* 1997; Powell *et al.* 1996; Verrett 1976). Developmental abnormalities observed after *in ovo* exposure to TCDD and other dioxin-like compounds in various avian species include edema of the head and neck, microphthalmia (reduced eye size), liver damage, and skeletal and beak deformities (Blankenship *et al.* 2003; Hoffman *et al.* 1998; Powell *et al.* 1996; Sanderson and Bellward 1995). Comparative egg injection studies have shown a large difference in species sensitivity to the toxic effects of dioxin-like compounds exists. Chickens have been shown to be up to 250-fold more sensitive than turkeys (*Meleagris gallopavo*), pheasants (*Phasianus colchicus*), ducks (mallard (*Anas platyrhynchos*) and goldeneye (*Bucephala clangula*), domestic geese (*Anser anser*), herring gulls (*Larus argentatus*), and black-headed gulls (*Larus ridibundus*) (Brunström 1988; Brunström and Lund 1988; Brunström and Reutergardh 1986). Other species studied include the American kestrel

(*Falco sparverius*) and common tern (*Sterna hirundo*), in which EROD activity was 800- and 1000-fold less, respectively, than in the chicken (Hoffman *et al.* 1998).

In addition to laboratory studies, the toxic effects of dioxin-like compounds have been observed in field studies of avian species. In the 1960s, population declines in colonial fish-eating birds of the Great Lakes were largely attributed to exposure to high levels of PCBs (Gilbertson *et al.* 1991). For instance, Ludwig *et al.* (1996) determined there was a relationship between TCDD-EQs and 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. A later study also observed decreased hatching success and increased incidence of nestling deformities in a colony of double-crested cormorants located in an area along Lake Michigan contaminated with PCBs when compared to a reference colony (Larson *et al.* 1996). Impaired reproductive success of Forster's terns (*Sterna forsteri*) was associated with a median egg TCDD concentration of 37 pg/g wet wt (Kubiak *et al.* 1989). The concentration of TCDD in eggs appeared to be related to the severity of reproductive failure observed in colonies of herring gulls around the Great Lakes, although a casual relationship could not be established (Gilbertson 1983). The symptoms observed were consistent with those of chick-edema disease in domestic chickens, which include edemas, hydropericardium, ascites, liver enlargement, porphyria, liver necrosis with fatty degeneration, and high rate of mortality, following exposure to AhR active compounds (Gilbertson 1983; Vos 1972; Vos and Koeman 1970). In wildlife exposed to these contaminants, this suite of symptoms has been labeled Great Lakes embryo, mortality, edema, and deformities syndrome (GLEMEDS) (Gilbertson *et al.* 1991). Common cormorants (*Phalacrocorax*

carbo) with hepatic total-TEQ concentrations ranging between 12-1900 pg/g wet wt exhibited a correlation between TEQs and CYP1A protein levels (Kubota *et al.* 2005). CYP1A protein and EROD induction in bald eagle (*Haliaeetus leucocephalus*) hatchlings were also correlated with TCDD, TCDF, and TEQs (Elliott *et al.* 1996).

Nesting colonies of GBH near paper and pulp mills in British Columbia have been monitored for exposure to the dioxin-like compounds present in mill effluents. In 1987, colony failure coincided with a 3-fold increase in TCDD concentrations (210 pg/g wet wt), but the authors determined the poor productivity was most likely a result of disturbance due to human or bald eagle activity and subsequent predation by crows (*Corvus caurinus*) and ravens (*Corvus corax*) (Elliott *et al.* 1989). Paired eggs were collected from nests within these same breeding colonies, contaminant concentrations were measured in one and the other was artificially incubated and allowed to hatch in a laboratory (Bellward *et al.* 1990; Hart *et al.* 1991; Sanderson *et al.* 1994). Hatching success was not different between eggs collected from control and contaminated colonies, but subcutaneous edema was observed in 4 of 12 hatchlings from the most contaminated colony (211 pg/g wet wt TCDD) (Hart *et al.* 1991). Comparisons between hatchlings and eggs from the same clutch revealed that TCDD concentrations regressed negatively with growth measures in hatchlings, including yolk-free body weight, tibia length, and organ weights, and positively with hepatic microsomal EROD activity in GBH hatchlings (Bellward *et al.* 1990; Hart *et al.* 1991). Continued monitoring of these colonies has shown a decrease in the severity of these effects as environmental concentrations of PCDDs and PCDFs have decreased due to process changes implemented by the pulp industry (Sanderson *et al.* 1994). Deformities in pipping embryos from other GBH

breeding colonies near paper and pulp mills were observed with TCDD concentrations in eggs ranging from 1.7-8.3 pg/g wet wt, but the overall reproductive success of the colonies was not affected (Thomas and Anthony 1999).

Dioxin-like compounds have also been found in herons from areas not associated with the paper and pulp industry. Skeletal deformities, namely multiple fractures of the tarsus and tibia and metacarpal bones, were present in grey heron (*Ardea cinerea*) nestlings in the United Kingdom with total TEQs in nestling adipose tissue ranging from 300-640 pg/g wet wt (Thompson *et al.* 2006), but no statistical relationship between contaminant concentrations and the frequency of these deformities was reported. Pipping embryos of black-crowned night-herons (*Nycticorax nycticorax*) with PCB-TEQs of approximately 290 pg/g wet wt had elevated EROD activity compared to those collected from associated reference areas, but no gross abnormalities were observed. Reproductive success, measured as clutch size and hatching success, of GBH was not adversely affected with concentrations of PCB-TEQs in nestling adipose tissue ranging from 13-100 pg/g lipid weight (lw) (Straub *et al.* 2007). Concentrations of PCB-TEQs (approximately 48 pg/g wet wt) and DF-TEQs (11 pg/g wet wt) in GBH eggs collected along the Mississippi River were too low to induce EROD activity (Custer *et al.* 1997). Although the TEQ scheme is helpful in summing the toxic potential of the PCDD, PCDF, and PCB congeners, comparisons between studies reporting TEQs must be done cautiously, as different studies may have reported different or incomplete congener lists and used different TEFs to arrive at their end values. The best attempt to reduce these discrepancies was done in the above study summaries by converting reported values with the TEFs reported by van den Berg (1998) when possible.

There is very limited information available on the presence and the potential effects of dioxin-like compounds in BKF. In China, eggs of a different species of kingfisher, the lesser pied kingfisher (*Ceryle rudis*), collected from an area of PCDD/DF contamination contained 1.6 pg/g wet wt of DF-TEQs (Fang *et al.* 2007). Total PCB concentrations in adult and juvenile BKF collected along the Sheboygan River in Wisconsin, another site of PCB contamination, had concentrations of total PCBs ranging from 65-220 μg/g (Heinz *et al.* 1984). Unfortunately, these studies only reported tissue residues and no data on potentially associated individual or population health effects were presented. Eggs of BKF collected from along the Hudson River, which is an area heavily contaminated with PCBs, contained concentrations of total PCBs ranging from 2 to 80 μg/g and total TEQs ranging from 100 to 5200 pg/g wet wt (geometric mean of 620 pg/g wet wt) (Custer *et al.* 2010). No relationship was observed between these contaminant concentrations and reproductive success.

Selection of Receptor Species

Selection of an appropriate species is a key element of effective ecological risk assessments (ERA), especially when site-specific field studies are to be employed. When selecting a species to serve as a receptor in a site-specific ecological risk assessment, the intensity of that species exposure to the COPEC(s) must be considered (USEPA 1994a). In general, PCDD/DFs in the environment are predominately associated with particulate matter, such as sediments, suspended material, and soils. Although the uptake of PCDD/DFs from contaminated soil into plant tissue is very limited (Hülster 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. The lipophilic nature and resistance to biological degradation of PCDD/DFs make these compounds likely to bioaccumulate up the food web. Consequently, species located at the top of the food chain are the most likely to experience the greatest exposure to PCDD/DFs through biomagnification. As an example, fish, ducks, and fish-eating birds that were part of a food web associated with sediments contaminated with PCDD/DFs had much greater concentrations of these compounds in their tissues than aquatic vegetation and benthic invertebrates collected in the same area (Wu *et al.* 2001). When selecting a receptor species to investigate bioaccumulative compounds, such as PCDD/DFs, it should be a species that is situated near the top of the food chain, representing the greatest dietary exposure potential on a body weight normalized basis.

Another important factor to consider during the selection of a receptor species is the relative sensitivity of a species to a contaminant (USEPA 1994a). It is not feasible to study all species present at a site, so only those which are sensitive to the COPECs should be considered for selection as a receptor. 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 PCDFs and PCDDs. Many reptiles and amphibians are also present on site, but studies indicate that they are also not particularly sensitive to the effects of PCDD/DFs (Jung and Walker 1997). Fish have been shown to have the necessary receptor to elicit the toxic effects of PCDD/DFs, but hepatic concentrations of the receptor appear to be lower in fish than in mammals (Hahn 1998). In mammals, toxicity of PCDD/DFs seems to vary significantly among species, but in general, mammals have shown moderate sensitivity to dioxin-like

compounds. American mink (*Mustela vison*), was included as the only mammalian receptor species in the TR ERA, as previous studies have shown it to be sensitive to the toxic effects of dioxin-like compounds (Aulerich *et al.* 1988; Beckett *et al.* 2008; Heaton *et al.* 1995; Hochstein *et al.* 1988; Tillitt *et al.* 1996). 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 susceptibility between species to these toxic effects has been observed, with the domestic chicken being 10- to 100- fold more sensitive to these effects (Brunström 1988; Hoffman *et al.* 1998; Kennedy *et al.* 1996). The observed differences in sensitivity to dioxin-like compounds among taxa may be attributable to varying concentrations of the AhR in certain tissues, differences in degradation potential, or species differences in the AhR construct and associated ligand binding affinity (Hahn 1998). Further work has been done to characterize differences in the AhR between avian species which exhibit different levels of sensitivity to AhR-active compounds (Head *et al.* 2008; Karchner *et al.* 2006). Briefly, avian species may be broadly categorized to three levels of sensitivity to AhR-active compounds based on key amino acid residues in the ligand binding domain of the AhR (Head *et al.* 2008). An ideal receptor species would have relatively great sensitivity to the COPECs so that it could be considered protective of other, potentially less sensitive, species on-site.

Site-Specific Receptor Species

The great blue heron and belted kingfisher were selected to investigate the movement of PCDD/DFs through an aquatic-based exposure pathway and the potential risk these

contaminants may pose to wildlife in the Tittabawassee River floodplain. In addition to each of these species being piscivorous birds that nest in the Tittabawassee River floodplain, each has other species-specific attributes which make it appropriate for use as a receptor species in the TR ERA.

GBH possess many of the characteristics that are desirable in a receptor species, and as such, are often selected as an ecological receptor of concern in risk assessments. GBH have a broad distribution across geographic regions and habitat types, residing in freshwater, estuarine, and marine habitats throughout North America (Butler 1992). GBH are a colonially-nesting species, with a rookery containing as many as 1300 breeding pairs recorded (Desgranges and Desrosiers 2006). With breeding pairs concentrated in one area, the colonies are more conspicuous to researchers than single-nesting species and allows for the assessment of population health rather than the outcome of a few nesting pairs. There are many closely related species for which GBH could serve as a surrogate species or that could be studied utilizing the described methods. Additionally, GBH are a charismatic species that is widely recognized by the general public, which would have an interest in preserving this species.

As a long-lived territorial species at the top of the aquatic food web, GBH have the potential to bioaccumulate local contaminants over a long period of time (Custer *et al.* 1991). Band recoveries have shown GBH may live to be at least 20 years old (Bayer 1981). GBH are year-round residents in areas of its range where foraging remains available during winter months, particularly in coastal areas (Butler 1997). The territorial nature of GBH leads to the active defense of distinct, identifiable foraging areas local to the breeding colony (Marion 1989; Peifer 1979), thus GBH exposure may have

better defined spatial boundaries as compared to other more opportunistically feeding piscivorous birds. Studies tracking adult GBH from rookeries to foraging areas have determined that adult GBH forage a mean distance between 3.1 km and 6.5 km from breeding colonies, although a distance as great as 34.1 km has been recorded (Dowd and Flake 1985; Peifer 1979; Thompson 1978). To directly characterize site-specific dietary exposure, the habit of GBH nestlings to regurgitate their stomach contents when under duress can be exploited by collecting the regurgitant to determine dietary composition and contaminant concentrations. Previous studies have detected local organochlorine contaminants in the tissues of GBH (Champoux *et al.* 2002; Custer *et al.* 1997; Elliott *et al.* 2001; Harris *et al.* 2003; Straub *et al.* 2007; Thomas and Anthony 1999). The benefits and potential drawbacks of using GBH as a receptor species have previously been outlined (Seston *et al.* 2009).

Another avian piscivore along the Tittabawassee River, the belted kingfisher, has species-specific attributes which make it another attractive receptor species for inclusion in the TR ERA. As a piscivorous species, the KF has a high trophic status and thus has great exposure potential to bioaccumulative COPECs. Additionally, BKF have a high rate of food intake, consuming nearly 50% of their body weight in food daily (Davis 1980). BKF forage primarily on the most available fish species present within its foraging range, but will also take crayfish, frogs, salamanders, lizards, small snakes, and insects when water conditions decrease fishing success (Davis 1982; Salyer and Lagler 1949; White 1938). During the breeding season, BKF defend a mean territory size of 1.03 km of river length (Davis 1982). Individuals may maintain their territories year-round, unless the water freezes over, restricting food resources (Hamas 1994). Due to

their limited foraging range in combination with the aggressive territorial behavior of BKF and the fact that nestlings are restricted to food brought to them by adults, contamination in nestlings can be related to prey from a defined reach of river proximal to the nest site.

The BKF has a widespread distribution, inhabiting diverse aquatic habitats throughout North America (Hamas 1994). Unlike the colonially-nesting GBH, BKF are solitary nesters, aggressively defending their breeding territory from conspecifics (Davis 1980). Physical characteristics of an ideal nest site include steep earthen banks with substrate comprised of approximately 75% sand, with clay and silt, and with little vegetation or other potential impediments to the excavation of the nest burrow (Brooks and Davis 1987). BKF construct a subterranean burrow, usually between 1-2 m in length, that terminates is a nest chamber. Burrows are generally located near the top of high banks as a means to deter predation and prevent flooding of the nest chamber (Davis 1980). Locating and securing suitable nesting habitat is the most important factor in the establishment of BKF breeding territories, and is often a limiting factor in overall abundance (Brooks and Davis 1987; Davis 1982). Where there is a lack of natural nesting sites but available food resources, BKF have been shown to utilize artificial nest sites created through anthropogenic activities, such as gravel pits and road cuts (Hamas 1974). As with the GBH, the widespread distribution and charismatic nature of the BKF draws the attention of the public to its preservation.

Research Objectives

Due to the presence of PCDD/DFs at elevated levels in the Tittabawassee River and associated floodplains, there is concern that resident wildlife species may be exposed to concentrations of these compounds which could potentially affect their health, at either the individual or population level. To understand both exposure and associated effects, field studies were conducted over several years in support of a site-specific ecological risk assessment. Many different receptor species were included in this site-specific assessment to most accurately assess the complex Tittabawassee River floodplain ecosystem. The focus of the research in the following dissertation is the movement of PCDFs and PCDDs through an aquatic-based exposure pathway, monitoring population-health parameters, and assessing overall risk posed by these contaminants to piscivorous avian species nesting in the Tittabawassee River floodplain. As top-tier representatives of an aquatic-based food web, the great blue heron and belted kingfisher were selected as the receptor species which could best meet this objective. Dietary exposure and tissue-based exposure, combined with assessments of population health of each receptor species, are the multiple lines of evidence to be employed in assessing the risk present to resident species of 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 means possible. To achieve that objective, all aspects of the study were performed following standard operation procedures (GBH adult handling 05/07-069-00; GBH nest monitoring 05/07-066-00; Protocol for belted kingfisher monitoring and tissue

collection 05/07-071-00; Field studies in support of TR ERA 03/04-042-00; Protocol for fish sampling 05/07-059-00) 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 SC1254 for GBH and BKF/SC permit for fish (Zwiernik)/SC permit for amphibians (Zwiernik); USFWS Migratory Bird Scientific Collection Permit MB1000062-0; and subpermitted under US Department of the Interior Federal Banding Permit 22926) are on file at Michigan State University-Wildlife Toxicology Laboratory are on file at MSU-WTL.

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 Company, Midland, Michigan to J.P. Giesy and M.J. Zwiernik of Michigan State University.

References

Allred, P. M., and Strange, J. R. (1977). The effects of 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on developing chicken embryos. *Archives of Environmental Contamination and Toxicology* **6**, 483-489.

Aulerich, R. J., Bursian, S. J., and Napolitano, A. C. (1988). Biological effects of epidermal growth factor and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on development parameters of neonatal mink. *Archives of Environmental Contamination and Toxicology* **17**, 27-31.

Barron, M. G., Galbraith, H., and Beltman, D. (1995). Comparative reproductive and developmental toxicology of PCBs in birds. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology and Toxicology* **112**(1), 1-14.

Bayer, R. D. (1981). Regional variation of great blue heron longevity. *Journal of Field Ornithology* **52**(3), 210-213.

Beckett, K., Yamini, B., and Bursian, S. (2008). The effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) on mink (*Mustela vison*) reproduction and kit survivability and growth. *Archives of Environmental Contamination and Toxicology* **54**(1), 123-129.

Bellward, G. D., Norstrom, R. J., Whitehead, P. E., Elliott, J. E., Bandiera, S. M., Dworschak, C., Chang, T., Forbes, S., Cadario, B., Hart, L. E., and Cheng, K. M. (1990). Comparison of polychlorinated dibenzodioxin levels with hepatic mixed-function oxidase induction in great blue herons. *Journal of Toxicology and Environmental Health* **30**, 33-52.

Blankenship, A. L., Hilscherova, K., Nie, M., Coady, K. K., Villalobos, S. A., Kannan, K., Powell, D. C., Bursian, S. J., and Giesy, J. P. (2003). Mechanisms of TCDD-induced abnormalities and embryo lethality in white leghorn chickens. *Comp Biochem. Physiol C. Toxicol. Pharmacol.* **136**(1), 47-62.

Bright, D. A., Cretney, W. J., Macdonald, R. W., Ikonomou, M. G., and Grundy, S. L. (1999). Differentiation of ploychlorinated dibenzo-*p*-dioxin and dibenzofuran sources in coastal British Columbia, Canada. *Environmental Toxicology and Chemistry* **18**(6), 1097-1108.

Brooks, R. P., and Davis, W. J. (1987). Habitat selection by breeding belted kingfishers (*Ceryle alcyon*). *American Midland Naturalist* **117**(1), 63-70.

Brunström, B. (1988). Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. *Poultry Science* **67**(1), 52-57.

Brunström, B., and Lund, J. (1988). Differences between chick and turkey embryos in sensitivity to 3,3',4,4'-tetrachloro-biphenyl and in concentration affinity of the hepatic receptor for 2,3,7,8-tetrachlorodibenzo-para-dioxin. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology* **91**(2), 507-512.

Brunström, B., and Reutergardh, L. (1986). Differences in sensitivity of some avian species to the embryotoxicity of a PCB, 3,3',4,4'-tetrachlorobiphenyl, injected into the eggs. *Environmental Pollution Series A-Ecological and Biological* **42**(1), 37-45.

Butler, R. W. (1992). Great blue heron. A. Poole, P. Stettenheim, & F. Gill (Eds.), *The Birds of North America*, No. 25. Philadelphia: The Academy of Natural Sciences; Washington, DC: The American Ornithologists's Union.

Butler, R. W. (1997). The Great Blue Heron: A Natural History and Ecology of a Seashore Sentinel, UBC Press, Vancouver, BC.

Champoux, L., Rodrigue, J., Desgranges, J., Trudeau, S., Hontela, A., Boily, M., and Spear, P. (2002). Assessment of contamination and biomarker responses in two species of herons on the St. Lawrence River. *Environmental Monitoring and Assessment* **79**, 193-215.

Clement, R. E., Tashiro, C., Suter, S., Reiner, E., and Hollinger, D. (1989). Chlorinated dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) in effluents and sludges from pulp and paper mills. *Chemosphere* **18**, 1189-1197.

Custer, T. W., Custer, C. M., and Gray, B. R. (2010). Polychlorinated biphenyls, dioxins, furans, and organochlorine pesticides in belted kingfisher eggs from the upper Hudson River basin, New York, USA. *Environmental Toxicology and Chemistry* **29**(1), 99-110.

Custer, T. W., Hines, R. K., Melancon, M. J., Hoffman, D. J., Wickliffe, J. K., Bickham, J. W., Martin, J. W., and Henshel, D. S. (1997). Contaminant concentrations and biomarker response in great blue heron eggs from 10 colonies on the upper Mississippi River, USA. *Environmental Toxicology and Chemistry* **16**(2), 260-271.

Custer, T. W., Rattner, B. A., Ohlendorf, H. F., and Melancon, M. J. (1991). Herons and egrets as proposed indicators of estuarine contamination in the United States. *Acta XX Congressus Internationalis Ornithologici* **20**, 2474-2479.

Czuczwa, J. M., McVeety, B. D., and Hites, R. A. (1984). Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in sediments from Siskiwit Lake, Isle Royale. *Science* **226**, 568-569.

Davis, W. J. The belted kingfisher, Megaceryle alcyon: its ecology and territoriality. 1980. Cincinnati, OH, University of Cincinnati.

Davis, W. J. (1982). Territory size in Megacerlye alcyon along a stream habitat. *The Auk* **99**, 353-362.

Desgranges, J., and Desrosiers, A. Breeding distribution and population trends of the great blue heron in Quebec, 1977-2001. Number 113. 2006. Canadian Wildlife Service. Occasional Paper.

Dowd, E. M., and Flake, L. D. (1985). Foraging habitats and movements of nesting great blue herons in a prairie river ecosystem, South Dakota. *Journal of Field Ornithology* **56**(4), 379-387.

Eisler, R. Dioxin hazards to fish, wildlife, and invertebrates: a synoptic review. 85(1.8). 1986. U.S. Fish and Wildlife Service Biological Report.

Eitzer, B. D. (1993). Comparison of point and nonpoint sources of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans to sediments of the Housatonic River. *Environmental Science and Technology* **27**(8), 1632-1637.

Elliott, J. E., Butler, R. W., Norstrom, R. J., and Whitehead, P. E. (1989). Environmental contaminants and reproductive success of great blue herons *Ardea herodias* in British Columbia, 1986-87. *Environmental Pollution* **59**, 91-114.

Elliott, J. E., Harris, M. L., Wilson, L. K., Whitehead, P. E., and Norstrom, R. J. (2001). Monitoring temporal and spatial trends in polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in eggs of great blue heron (*Ardea herodias*) on the coast of British Columbia, Canada, 1983-1998. *Ambio* **30**(7), 416-428.

Elliott, J. E., Norstrom, R. J., Lorenzen, A., Hart, L. E., Philibert, H., Kennedy, S. W., Stegeman, J. J., Bellward, G. D., and Cheng, K. M. (1996). Biological effects of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in bald eagle (*Haliaeetus leucocephalus*) chicks. *Environmental Toxicology and Chemistry* **15**(5), 782-793.

Evers, E. H. G., Ree, K. C. M., and Olie, K. (1988). Spatial variations and correlations in the distribution of PCDDs, PCDFs and related compounds in sediments from the river Rhine -- Western Europe. *Chemosphere* **17**(12), 2271-2288.

Fang, L., Zheng, M., Zhang, B., Gao, L., Liu, W., and Zhao, X. (2007). Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in eggs of eight avian species from Dongting Lake, China. *Chemosphere* **69**(3), 411-421.

Froese, K. L., Verbrugge, D. A., Ankley, G. T., Niemi, G. J., Larsen, C. P., and Giesy, J. P. (1998). Bioaccumulation of polychlorinated biphenyls from sediments to aquatic insects and tree swallow eggs and nestlings in Saginaw Bay, Michigan, USA. *Environmental Toxicology and Chemistry* **17**(3), 484-492.

Giesy, J. P., Jude, D. J., Tillitt, D. E., Gale, R. W., Meadows, J. C., Zajieck, J. L., Peterman, P. H., Verbruggee, D. A., Sanderson, J. T., Schwartz, T. R., and Tuchman, M. L. (1997). Polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in fishes from Saginaw Bay, Michigan. *Environmental Toxicology and Chemistry* **16**(4), 713-724.

Gilbertson, M. (1983). Etiology of chick edema disease in herring gulls in the lower Great Lakes. *Chemosphere* **12**(3), 357-370.

Gilbertson, M., Kubiak, T., Ludwig, J., and Fox, G. (1991). Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. *Journal of Toxicology and Environmental Health* **33**, 455-520.

Goovaerts, P., Trinh, H. T., Demond, A., Franzblau, A., Garabrant, D., Gillespie, B., Lepkowski, J., and Adriaens, P. (2008). Geostatistical modeling of the spatial distribution of soil dioxins in the vicinity of an incinerator. 1. Theory and application to Midland, Michigan. *Environmental Science & Technology* **42**(10), 3648-3654.

Hahn, M. E. (1998). The aryl hydrocarbon receptor: A comparative perspective. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* **121**(1-3), 23-53.

Hamas, M. J. (1974). Human incursion and nesting sites of the belted kingfisher. *The Auk* **91**(4), 835-836.

Hamas, M. J. (1994). Belted kingfisher (*Cerlye alcyon*). A. Poole, P. Stettenheim, & F. Gill (Eds.), *The Birds of North America*, No. 25. Philadelphia: The Academy of Natural Sciences; Washington, DC: The American Ornithologists's Union.

Harris, M. L., Elliott, J. E., Butler, R. W., and Wilson, L. K. (2003). Reproductive success and chlorinated hydrocarbon contamination of resident great blue herons (*Ardea herodias*) from coastal British Columbia, Canada, 1977 to 2000. *Environmental Pollution* **121**, 207-227.

Hart, L. E., Cheng, K. M., Whitehead, P. E., Shah, R. M., Lewis, R. J., Ruschkowski, S. R., Blair, R. W., Bennett, D. C., Bandiera, S. M., Norstrom, R. J., and Bellward, G. D. (1991). Dioxin contamination and growth and development in great blue heron embryos. *Journal of Toxicology and Environmental Health* **32**, 331-344.

Head, J. A., Hahn, M. E., and Kennedy, S. W. (2008). Key amino acids in the aryl hydrocarbon receptor predict dioxin sensitivity in avian species. *Environmental Science & Technology* **42**(19), 7535-7541.

Heaton, S. N., Bursian, S. J., Giesy, J. P., Tillitt, D. E., Render, J. A., Jones, P. D., Verbrugge, D. A., Kubiak, T. J., and Aulerich, R. J. (1995). Dietary exposure of mink to carp from Saginaw Bay, Michigan. 1. Effects on reproduction and survival and the potential risks to wild mink populations. *Archives of Environmental Contamination and Toxicology* **28**, 334-343.

Heinz, G. H., Swineford, D. M., and Katsma, D. E. (1984). High PCB residues in birds from the Sheboygan River, Wisconsin. *Environmental Monitoring and Assessment* **4**, 155-161.

Henshel, D. S., Hehn, B., Wagey, R., Vo, M., and Steeves, J. D. (1997). The relative sensitivity of chicken embryos to yolk- or air-cell-injected 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environmental Toxicology and Chemistry* **16**(4), 725-732.

Hilscherova, K., Kannan, K., Nakata, H., Hanari, N., Yamashita, N., Bradley, P. W., McCabe, J. M., Taylor, A. B., and Giesy, J. P. (2003). Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. *Environmental Science and Technology* **37**(3), 468-474.

Hochstein, J. R., Aulerich, R. J., and Bursian, S. J. (1988). Acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to mink. *Archives of Environmental Contamination and Toxicology* **17**, 33-37.

Hoffman, D. J., Melancon, M. J., Klein, P. N., Eisemann, J. D., and Spann, J. W. (1998). Comparative developmental toxicity of planar polychlorinated biphenyl congeners in chickens, American kestrels, and common terns. *Environmental Toxicology and Chemistry* **17**(4), 747-757.

Hülster, A., and Marschner, H. (1993). Transfer of PCDD/PCDF from contaminated soils to food and fodder crop plants. *Chemosphere* **27**, 439-446.

Hutzinger, O., Blumich, M. J., Berg, M. v. d., and Olie, K. (1985). Sources and fate of PCDDs and PCDFs: An overview. *Chemosphere* **14**, 581-600.

Jung, R. E., and Walker, M. K. (1997). Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on development of anuran amphibians. *Environmental Toxicology and Chemistry* **16**(2), 230-240.

Kannan, K., Watanabe, I., and Giesy, J. P. (1998). Congener profile of polychlorinated/brominated dibenzo-*p*-dioxins and dibenzofurans in soil and

sediments collected at a former chlor-alkali plant. *Toxicological and Environmental Chemistry* **67**, 135-146.

Kannan, K., Yun, S., Ostaszewski, A., McCabe, J., MacKenzie-Taylor, D., and Taylor, A. (2008). Dioxin-like toxicity in the Saginaw River watershed: Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in sediments and floodplain soils from the Saginaw and Shiawassee Rivers and Saginaw Bay, Michigan, USA. *Archives of Environmental Contamination and Toxicology* **54**(1), 9-19.

Kannan, N., Hee Hong, S., Shim, W. J., and Hyuk Yim, U. (2007). A congener-specific survey for polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) contamination in Masan Bay, Korea. *Chemosphere* **68**(9), 1613-1622.

Karchner, S. I., Franks, D. G., Kennedy, S. W., and Hahn, M. E. (2006). The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor. *PNAS* **103**(16), 6252-6257.

Kennedy, S. W., Lorenzen, A., Jones, S. P., Hahn, M. E., and Stegeman, J. J. (1996). Cytochrome P4501A induction in avian hepatocyte cultures: a promising approach for predicting the sensitivity of avian species to toxic effects of halogenated aromatic hydrocarbons. *Toxicology and Applied Pharmacology* **141**(1), 214-230.

Kubiak, T. J., Harris, H. J., Smith, L. M., Schwartz, T. R., Stalling, D. L., Trick, J. A., Sileo, L., Docherty, D., and Erdman, T. C. (1989). Microcontaminants and reproductive impairment of the Forster's tern in Green Bay, Lake Michigan-1983. *Archives of Environmental Contamination and Toxicology* **18**, 706-727.

Kubota, A., Iwata, H., Tanabe, S., and Tobata, S. (2005). Hepatic CYP1A induction by dioxin-like compounds, and congener-specific metabolism and sequestration in wild common cormorants from Lake Biwa, Japan. *Environmental Science and Technology* **39**(10), 3611-3619.

Larson, J. M., Karasov, W. H., Sileo, L., Stromborg, K. L., Hanbidge, B. A., Giesy, J. P., Jones, P. D., Tillitt, D. E., and Verbrugge, D. A. (1996). Reproductive success, developmental anomalies, and environmental contaminants in double-crested cormorants (*Phalacrocorax auritus*). *Environmental Toxicology and Chemistry* **15**(4), 553-559.

Lasagni, M., Collina, E., Grandesso, E., Piccinelli, E., and Pitea, D. (2009). Kinetics of carbon degradation and PCDD/PCDF formation on MSWI fly ash. *Chemosphere* **74**(3), 377-383.

Ludwig, J. P., Auman, H. J., Kurita, H., Ludwig, M. E., Campbell, L. M., Giesy, J. P., Tillitt, D. E., Jones, P., Yamashita, N., Tanabe, S., and Tatsukawa, R. (1993).

Caspian tern reproduction in the Saginaw Bay ecosystem following a 100-Year flood event. *Journal of Great Lakes Research* **19**(1), 96-108.

Ludwig, J. P., Kurita-Matsuba, H., Auman, H. J., Ludwig, M. E., Summer, C. L., Giesy, J. P., Tillitt, D. E., and Jones, P. D. (1996). Deformities, PCBs, and TCDD-equivalents in double-crested cormorants (*Phalacrocorax auritus*) and caspian terns (*Hydroprogne caspia*) of the upper Great Lakes 1986 - 1991: testing a cause-effect hypothesis. *Journal of Great Lakes Research* **22**(2), 172-197.

Lustenhouwer, J. W. A., Olie, K., and Hutzinger, O. (1980). Chlorinated dibenzo-*p*-dioxins and related compounds in incinerator effluents: A review of measurements and mechanisms of formation. *Chemosphere* **9**, 501-522.

Marion, L. (1989). Territorial feeding and colonial breeding are not mutually exclusive: the case of the grey heron (*Ardea cinerea*). *The Journal of Animal Ecology* **58**(2), 693-710.

Michigan Department of Environmental Quality. Biological and chemical monitoring of the Pine River Gratiot and Midland Counties May and September 1999. MI/DEQ/SWQ-00/024. 2000.

Michigan Department of Environmental Quality. Report of trace metal and organic residues in sediment from the Tittabawassee River watershed. 2002. Lansing, MI.

Peifer, R. W. (1979). Great blue herons foraging for small mammals. *The Wilson Bulletin* **91**(4), 630-631.

Poland, A., and Knutson, J. C. (1982). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annual Review of Pharmacology and Toxicology* **22**, 517-554.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Giesy, J. P., Stromborg, K. L., and Bursian, S. J. (1996). Effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) injected into the yolks of chicken (*Gallus domesticus*) eggs prior to incubation. *Archives of Environmental Contamination and Toxicology* **31**, 404-409.

Rappe, C., Kjeller, L.-O., Kulp, S.-E., de Wit, C., Hasselsten, I., and Palm, O. (1991). Levels, profiles, and pattern of PCDDs and PCDFs in samples related to the production and use of chlorine. *Chemosphere* **23**, 1629-1636.

Safe, S. H. (1986). Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Annual Review of Pharmacology and Toxicology* **26**, 371-399.

Sakurai, T., Kim, J.-G., Suzuki, N., Matsuo, T., Li, D.-Q., Yao, Y., Masunaga, S., and Nakanishi, J. (2000). Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in sediment, soil, fish, shellfish, and crab samples from Tokyo Bay area, Japan. *Chemosphere* **40**, 627-640.

Salyer, J. C., and Lagler, K. F. (1949). The eastern belted kingfisher, *Megaceryle alcyon alcyon* (Linnaeus), in relation to fish management. *Transactions of the American Fisheries Societies* **76**, 97-117.

Sanderson, J. T., Elliott, J. E., Norstrom, R. J., Whitehead, P. E., Hart, L. E., Cheng, K. M., and Bellward, G. D. (1994). Monitoring biological effects of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in great blue heron chicks (*Ardea herodias*) in British Columbia. *Journal of Toxicology and Environmental Health* **41**, 435-450.

Sanderson, J. T., and Bellward, G. D. (1995). Hepatic microsomal ethoxyresorufin-*O*-deethylase-inducing potency in ovo and cytosolic Ah receptor binding affinity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: comparison of four avian species. *Toxicology and Applied Pharmacology* **132**, 131-145.

Schecter, A., Dekin, A., Weerasinghe, N. C. A., Arghestani, S., and Gross, M. L. (1988). Sources of dioxins in the environment: A study of PCDDs and PCDFs in ancient, frozen Eskimo tissue. *Chemosphere* **17**(4), 627-631.

Schmidt, J. V., and Bradfield, C. A. (1996). Ah receptor signaling pathways. *Annual Review of Cell and Developmental Biology* **12**, 55-89.

Seston, R. M., Zwiernik, M., Fredricks, T., Coefield, S., Tazelaar, D., Hamman, D., Paulson, J., and Giesy, J. (2009). Utilizing the great blue heron (*Ardea herodias*) in ecological risk assessments of bioaccumulative contaminants. *Environmental Monitoring and Assessment* **157**(1), 199-210.

Straub, C. L., Maul, J. D., Halbrook, R. S., Spears, B., and Lydy, M. J. (2007). Trophic transfer of polychlorinated biphenyls in great blue heron (*Ardea herodias*) at Crab Orchard National Wildlife Refuge, Illinois, United States. *Archives of Environmental Contamination and Toxicology* **52**(4), 572-579.

Swanson, S., Rappe, C., and Malmström, J. (1988). Emissions of PCDDs and PCDFs from the pulp industry. *Chemosphere* **17**(4), 681-691.

Thomas, C. M., and Anthony, R. G. (1999). Environmental contaminants in the great blue herons (*Ardea herodias*) from the Lower Columbia and Willamette Rivers, Oregon and Washington, USA. *Environmental Toxicology and Chemistry* **18**(12), 2804-2816.

Thompson, D. H. (1978). Feeding areas of great blue herons and great egrets nesting within the floodplain of the upper Mississippi River. *Proceedings of the Colonial Waterbird Group* **2**, 202-213.

Thompson, H. M., Fernandes, A., Rose, M., White, S., and Blackburn, A. (2006). Possible chemical causes of skeletal deformities in grey heron nestlings (*Ardea cinerea*) in North Nottinghamshire, UK. *Chemosphere* **65**, 400-409.

Tillitt, D. E., Gale, R. W., Meadows, J. C., Zajieck, J. L., Peterman, P. H., Heaton, S. N., Jones, P. D., Bursian, S. J., Kubiak, T. J., Giesy, J. P., and Aulerich, R. J. (1996). Dietary exposure of mink to carp from Saginaw Bay. 3. Characterization of dietary exposure to planar halogenated hydrocarbons, dioxin equivalents, and biomagnification. *Environmental Science & Technology* **30**(1), 283-291.

US Environmental Protection Agency (USEPA). Field Studies for Ecological Risk Assessment. 540-F-94-014. 1994a. Washington, D.C., US Environmental Protection Agency.

US Environmental Protection Agency (USEPA). Health Assessment for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds. EPA/600/BP-92/001c. 1994b. Washington, D.C., Office of Research and Development.

van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., and Zacharewski, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* **106**(12), 775-792.

Verrett, M. J. Investigation of the toxic and teratogenic effects of halogenated dibenzo-*p*-dioxins and dibenzofurans in the developing chicken embryo. Memorandum Report. 1976. Washington, D.C., U.S. Food and Drug Administration.

Vos, J. G. (1972). Toxicology of PCBs for mammals and for birds. *Environ. Health Perspect.* **1**, 105-117.

Vos, J. G., and Koeman, J. H. (1970). Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. *Toxicology and Applied Pharmacology* **17**(656), 668.

Welsch-Pausch, K., McLachlan, M. S., and Umlauf, G. (1995). Determination of the principal pathways of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to *Lolium multiflorus* (Welsh ray grass). *Environmental Science & Technology* **29**(4), 1090-1098.

Wenning, R. J., Harris, M. A., Ungs, M. J., Paustenbach, D. J., and Bedbury, H. (1992). Chemometric comparisons of polychlorinated dibenzo-*p*-dioxin and dibenzofuran residues in surficial sediments from Newark Bay, New Jersey and other industrialized waterways. *Archives of Environmental Contamination and Toxicology* **22**, 397-413.

White, H. C. (1938). The feeding of kingfishers: food of nestlings and effect of water height. *Journal of the Fisheries Research Board of Canada* **4**(1), 48-52.

Wu, W. Z., Schramm, K.-W., and Kettrup, A. (2001). Bioaccumulation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in the foodweb of Ya-Er Lake area, China. *Water Research* **35**(5), 1141-1148.

CHAPTER 2

Utilizing the great blue heron (*Ardea herodias)* in ecological risk assessments of bioaccumulative contaminants

Rita M. Seston[1], Matthew J. Zwiernik[2], Timothy B. Fredricks[1], Sarah J. Coefield[1], Dustin L. Tazelaar[2], David W. Hamman[3], John D. Paulson[4], and John P. Giesy[1,5]

[1]Zoology Department, Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA

[2]Animal Science Department, Michigan State University, East Lansing, MI 48824, USA

[3]College of Veterinary Medicine, Center for Comparative Epidemiology, Michigan State University, East Lansing, MI 48824, USA

[4]USDA-APHIS, Wildlife Services, Bismarck, ND 58501 USA

[5]Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, S7J 5B3, Canada

Abstract

Selection of an appropriate species is a key element of effective ecological risk assessments (ERA), especially when site-specific field studies are to be employed. Great blue herons (GBH) possess several ideal characteristics of a receptor species for the assessment of bioaccumulative compounds in the environment, such as ease of study, high potential for exposure, widespread distribution, and territorial foraging behavior. Methodologies for assessing exposure and population health are described herein. As outlined, the collection of GBH eggs, GBH nestling blood, and adult GBH blood allows for the determination of contaminant concentrations in various GBH tissues, a top-down assessment, which can be done in conjunction with predicted dietary exposure, a bottom-up assessment, to support a multiple lines of evidence approach. Additionally, population parameters, such as productivity and survival, can also be measured to elucidate if the contaminant exposure may be causing population level effects. Over the course of two years, three GBH rookeries were monitored for productivity and nestling exposure. Nests were monitored from blinds and individually accessed at multiple time points to obtain measures of nestling health, band nestlings, and collect eggs and nestling plasma. Multiple nests could frequently be accessed by climbing one tree, resulting in minimal effort to obtain the necessary sample size. Additionally, 51 adult GBH, captured in their foraging areas, were banded, and provided a blood sample. With these samples, a statistical difference in tissue based exposure was identified between the reference and target area. Statistically significant differences were also identified between the upper and lower reaches of the target area, thereby identifying a range of doses geographically which could be correlated to specific measurement endpoints. The ability to identify a

dose response greatly increases the ability of the dataset to determine causation, a key goal of such studies. Overall, the use of the described methods allowed for the collection of a statistically sufficient and ecologically relevant dataset with reasonable effort and minimal impact on GBH.

Introduction

Selection of appropriate species is a key element to allow effective ecological risk assessments (ERA), especially when site-specific field studies are to be employed. Ideally, representative species used in assessments of bioaccumulative compounds should have an elevated exposure potential, a widespread distribution, and be territorial. Data collected using the selected species should ultimately provide insight into the health of the entire ecosystem of the study site. Piscivorous birds are frequently selected as receptors for evaluating aquatic systems because they can be sensitive to the effects of contaminants and have the potential to accumulate persistent, lipophylic contaminants through trophic-transfer. The great blue heron (*Ardea herodias*; GBH) possesses several characteristics that make it an appropriate species to use as a receptor in ERAs concerning bioaccumulative contaminants in aquatic environments. Here we describe a multiple lines of evidence approach to elucidating exposure of GBH to contaminants through the diet and measured concentrations in specific GBH tissues. As a case study of the methodology, we have investigated the exposure of GBH to polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) in the Tittabawassee River basin, Michigan, USA.

The Tittabawassee River study area includes approximately 37 km of the Tittabawassee River from the upstream boundary of the city limits of Midland, MI to the confluence of the Tittabawassee and Saginaw Rivers downstream of Green Point Island (Figure 2.1). Just above the upstream boundary of the study area is a low-head dam. Throughout the study area, the river is free flowing to the confluence with the Saginaw River and eventually the Saginaw Bay and Lake Huron. The study area was selected because soils and sediments were found to contain elevated concentrations of PCDFs and PCDDs. Soils and sediments collected from within the study area contained mean PCDF/PCDD concentrations ranging from $1.0x10^2$ to $5.4x10^4$ pg/g dw, which were 10- to 20- fold greater than those collected upstream in reference areas (Hilscherova *et al.*, 2003). The source of this contamination has been identified as The Dow Chemical Company (USEPA, 1986)

PCDFs and PCDDs occur in the environment as mixtures and due to their hydrophobic characteristics and resistance toward metabolism, they have great potential to be accumulated through the food web. The toxicological response of primary concern is mediated through the aryl hydrocarbon receptor (AhR) and effects 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 and fledging success in aquatic avian species (Gilbertson, 1983; Hoffman *et al.*, 1987; Ludwig *et al.*, 1993; van den Berg *et al.*, 1994).

Desirable, species-specific characteristics of the GBH led to its inclusion as a receptor species in an ERA concerning PCDFs and PCDDs along the Tittabawassee River and its

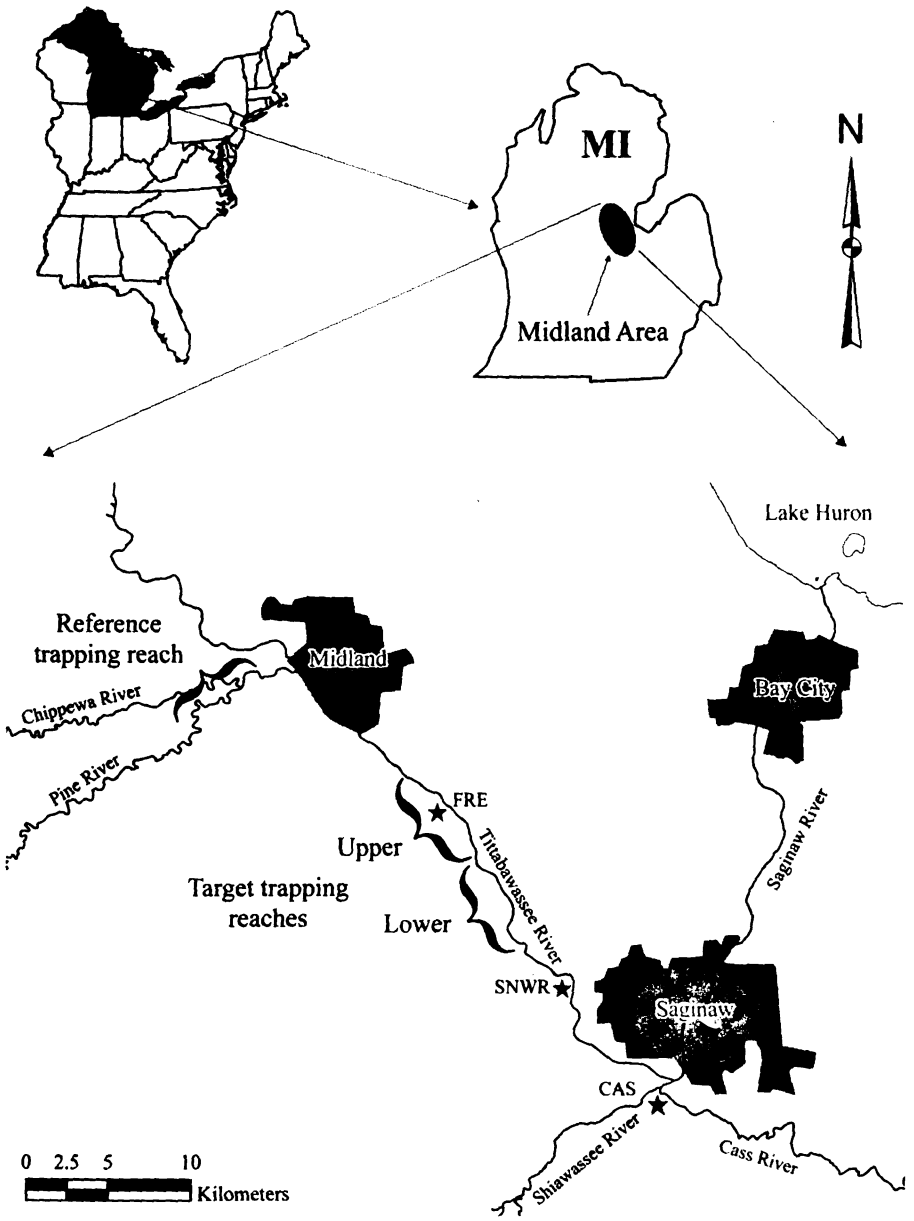


Figure 2.1. Location of great blue heron rookeries (FRE, SNWR, and CAS) and reaches within the Tittabawassee River study area, MI, USA where trapping occurred.

floodplain. The objective of this paper is to outline a series of methods and the associated effort necessary to effectively employ the GBH as a receptor in an ERA utilizing a multiple line of evidence approach.

Species Applicability

Species-specific attributes need to be considered when selecting a species for use as a receptor. GBH possess many of the characteristics that are desirable in a receptor species, and as such, are often selected as an ecological receptor of concern in risk assessments. GBH have a broad distribution across geographic regions and habitat types, residing in freshwater, estuarine, and marine habitats throughout North America (Butler, 1992). GBH are a colonially-nesting species, with a rookery containing as many as 1300 breeding pairs recorded (DesGranges and Desrosiers, 2006). With breeding pairs concentrated in one area, the colonies are more conspicuous to researchers than single-nesting species and allows for the assessment of population health rather than the outcome of a few nesting pairs. There are many closely related species for which GBH could serve as a surrogate species or that could be studied utilizing the described methods. Additionally, GBH are a charismatic species that is widely recognized by the general public, which would have an interest in preserving this species.

As a long-lived territorial species at the top of the aquatic food web, GBH have the potential to bioaccumulate local contaminants over a long period of time (Custer *et al.*, 1991). Band recoveries have shown GBH may live to be at least 20 years old (Bayer, 1981). GBH are year-round residents in areas of its range where foraging remains available during winter months, particularly in coastal areas (Butler, 1997). The

territorial nature of GBH leads to the active defense of distinct, identifiable foraging areas local to the breeding colony (Peifer, 1979; Marion, 1989), thus GBH exposure may have a greater spatial resolution as compared to other more opportunistically feeding piscivorous birds. Previous studies have detected local organochlorine contaminants in the tissues of GBH (Custer *et al.*, 1997; Thomas and Anthony, 1999; Elliott *et al.*, 2001; Champoux *et al.*, 2002; Harris *et al.*, 2003; Straub *et al.*, 2007).

Methods

Nest monitoring and fresh egg sampling

Great blue heron nests were monitored during the nesting season, which begins in mid- to late March and runs through mid-July, of 2006 and 2007. Colonies were visited several times over the breeding season to monitor reproductive success. Visits were coincident with estimated mean nesting, hatching, chick rearing, and fledgling periods and separated by a minimum of 1wk to minimize disturbance to breeding pairs. Calculation of events was based on a 2wk courtship/nesting period, 4wk incubation period, and a minimum of 8 wk from hatching to fledging (Harris *et al.*, 2003). For the second year of nest monitoring (2007), a helicopter with a stabilized zoom lens was employed to determine the number of eggs in each nest. Surveys were conducted from an altitude of 100 meters to minimize nest disturbance. By flying at this altitude, incubating GBH were not flushed from nests and were not visually disturbed by the helicopter. An entire rookery could be surveyed in approximately 15 min, capturing both video footage and still images. The contents of the nest could only be determined and counted for nests which the adults were not incubating at the time of survey, but the

number of active nests in the rookery could be determined. Hatching date was estimated by the presence of eggshells on the ground beneath nests and observing the act of herons presenting sticks to their mates (Moul *et al.*, 2001), along with hearing the nestlings calling in nests. Estimates of hatching and fledging success were then made when chicks were estimated to be 4 and 8 wk of age, respectively. At 4 wk of age the nestlings could be seen and counted in the nest and at 8 wk of age nestlings would perch on branches proximal to the nest. Observations were conducted from semi-permanent blinds erected in the nesting colony. A final visit to the colony was made each year once the leaves have fallen from the trees to make a count of the total number of nests.

Nests were located in Eastern cottonwood (*Populus deltoides*), silver maple (*Acer saccharinum*), or white ash (*Fraxinus americana*) trees at heights ranging between 15-25 m. Viable and nonviable eggs were collected from accessible nests in each nesting colony. Nest trees were selected based on the safety of access and the potential to reach multiple nests. Tree climbers accessed nests using tree-climbing spikes. Eggs were collected with a nylon stocking cup attached to the end of an extendable pole from the nesting tree or a neighboring tree that was in near enough proximity (Hines and Custer, 1995). A maximum of one viable egg was collected at random from each accessed nest that contained ≥ 2 eggs. In addition to viable eggs, all eggs which failed to hatch were collected for analysis of developmental stage and contaminant content. Eggs were weighed and measured, and then carefully transported to the laboratory in a crush-proof, water-proof container and kept at 4 °C until processing.

Capture and handling of nestling GBH

Blood was collected from nestlings when they were approximately four to five wk old based on methods previously described in (Henny and Meeker, 1981). At this age, nestlings were still limited to movement within the nest yet had sufficient mass to provide an adequate volume of plasma for residue analyses (McAloney, 1973). Some of the nestlings handled were older than the target age and proved to be more difficult to retrieve from and replace into the nest. An extendable pole with a retractable wire hoop was used to reach nestlings (Ketch-All Co., 4149 Santa Fe Rd. #2, San Luis Obispo, CA 93401). Individual nestlings were placed in a cloth bag and lowered to the ground from the nest. A 7-10 cm piece of closed-cell polyethylene foam tubing (7 cm OD X 3 cm ID Swim Noodle) was used to shield the potentially hazardous beak. The bill of the GBH was inserted into the tubing and a sock was pulled over both the tubing and the bird's head to cover the eyes of the captured bird and to keep the tubing in place. This combination reduced the chance of injury to personnel by covering the sharp beak, minimized visual stimulation resulting in a calming of the bird, while allowing it to breathe freely. Individuals were placed in the cloth bag to determine their body weight by a spring scale (Model 42500, Pesola AG, Switzerland). Lengths of exposed culmen and tarsus and masses were determined for each individual nestling. The age of each nestling was estimated using an equation relating age and culmen length from Quinney (1982). Individuals were fitted with USFWS bands on the tarsus and colored leg bands on the tibia (Simpson and Kelsall, 1978). Color leg bands were made from 49mm high x 66mm wide pieces of 2-ply plastic (1/16" Gravoglas 2-plex, matte-finish; Gravograph-New Hermes, Inc., Duluth, GA) wrapped around a wooden dowel to have a diameter

equal to that of the 7B USFWS leg bands (14mm), as described in Hayes and Barzen (2006).

Salvage nestlings were collected opportunistically following weather events or as a result of siblicide. Nestlings were examined for any gross external or internal abnormalities including liver, kidney, spleen, intestine, and gonad histology. Nestling stomach contents were analyzed to the lowest taxonomic identification possible to aid in the elucidation of a site-specific dietary composition. Contaminant concentrations were determined for liver, adipose, and skeletal muscle tissues of each individual nestling.

Capture and handling of adult GBH

Adult GBH were captured using modified foot hold traps set around feeding stations in predetermined GBH foraging areas. Foot hold traps were modified in a manner similar to that described by King *et al.* (1998). Briefly, the factory coil springs of Victor #3 Softcatch traps (Oneida Victor, Inc., Ltd., Euclid, OH) were replaced with weaker Victor #1.25 Softcatch coil springs. This modification lessened the initial impact of the padded jaws but still kept enough tension to hold the trapped bird's leg in place. The chain supplied with the trap was replaced with either a 15 cm or 30 cm length of elastic shock-cord attached with swivels on both ends to allow freedom of movement and minimization of injury to captured birds. Feeding stations were established in areas of the river with substrate ranging from sandy-silt to small pebbles, water depth of approximately 15 cm to 46 cm, and where GBH were observed foraging or tracks were present. The stations were placed in areas with little current to reduce stress on the bait fish, and free of debris to reduce the chance of injury to captured GBH. The feeding stations were open-top 46

cm L x 30 cm W x 41 cm H cages constructed of 1.25 cm galvanized hardware cloth on a frame of 0.60 cm hot-roll rod. Each station was fitted with 1.25 cm urethane pipe insulation along each of the long edges and anchored in the river by a 1.25 m piece of smooth rebar passed through two hoops on one corner of the cage. This design allowed the stations to float while remaining anchored, accommodating the fluctuating water levels of the river, and preventing the loss of the bait fish. The top-edge of the cage was fitted with 0.95 cm Tygon® tubing to protect the trapped bird from any potentially sharp edges. The feeding stations were stocked with forage fish collected from the immediate area. Fish were collected by seine net or backpack electro-fisher (Smith-Root LR-24, Smith-Root Inc., Vancouver, WA). Once GBHs were regularly foraging from the feeding station, approximately 40 modified traps were placed in a staggered configuration around the feeding station. The cord of each trap was outfitted with a clip, which attached the traps to a galvanized steel cord secured with stakes around the station. Loaded traps were set by placing firmly into the sediment to stabilize the trap, taking care not to bury the springs, pan, or pin. Feeding stations were monitored by personnel in a nearby blind (Doghouse blind, Ameristep Inc., Clio, MI) anytime the traps were set. Optimal blind location was on the bank opposite the feeding station, if the river could easily be crossed. This allowed for the largest field of view and minimized potential disturbance of GBH approaching the feeding station. If this was not possible, blinds were placed at least 30m away from the feeding station in as much cover that still allowed a clear view of the feeding station and shoreline. Trapping along this river system was limited to summer months, when the river's water levels were lower and more stable. Captured birds were approached with extreme caution as great blue herons are equipped

with strong, sharp beaks and are known to be aggressive (Butler, 1997). Personnel handling the birds were outfitted with appropriate protective gear including helmets fitted with face shields and thick woven clothing. Captured adult GBH were hooded in the same manner as nestlings.

Once safely immobilized, individuals were color marked using numbered color leg bands placed on the tibia. Color leg bands used on the adults were identical to those used on nestlings with the addition of unique numerical codes of 14 mm numbers spaced 11 mm apart in 3 vertical rows to increase visibility on the banded bird. Color marking was done to enable identification of captured adults from a distance. Measurements of other physical attributes such as the length of the exposed culmen, wing chord, and tarsus and mass were also recorded. Each individual was also fitted with a U.S. Fish & Wildlife Service band on the tarsus.

Blood plasma sampling

Blood from nestling and adult GBH was drawn from the brachialis vein using needles affixed to sterile syringes pre-rinsed with sodium heparin solution. 22-gauge needles (Becton Dickinson, Franklin Lakes, NJ) were used for nestlings while smaller 25-gauge needles were used for adults due to smaller vein size. To determine the maximum volume of blood that could be collected, the following set of equations were utilized; 7% body weight = total blood volume, 10% total blood volume = acceptable sample volume. Blood collection was most effectively performed with three people, one to hold the head, legs, and body of the GBH still, one keeping the wing outstretched and steady, and one to perform the blood draw. The blood sample was then transferred to a heparinized

Vacutainer™ (Becton Dickinson, Franklin Lakes, NJ) for transport back to the field laboratory. Each Vacutainer™ was labeled with the band number, trapping station ID, GPS coordinates of trapping station, date, and collector's initials. Whole blood samples were centrifuged and the plasma (supernatant) was decanted. Both plasma and packed cell volume were stored at -20 °C until analysis. Red blood cells were saved for future sexing of individuals.

Collection of prey items

Site-specific GBH dietary items, including forage fish, amphibians, and crayfish, were collected and analyzed for contaminant concentrations (Alexander, 1977). Collection of the dietary items occurred at 6 sampling locations, 2 in the reference area and 4 approximately equally spaced throughout the 27 km target area. The sampling scheme maximized information on dietary exposure including geographically associated contaminant variability and trends.

Sample processing and analytical techniques

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. Contents were then homogenized in a chemically cleaned Omni-mixer, lyophilized, and stored in clean jars until analysis (I-CHEM brand, Rockwood, TN). Tissues collected from salvage nestlings were also homogenized using a chemically cleaned Omni-mixer. All samples were analyzed for concentrations of the seventeen 2,3,7,8-substituted PCDF/D congeners, in addition to a subset of egg and tissue samples also being analyzed for PCB

and DDXs. Analyses were conducted in accordance with EPA Method 8290 with minor modifications (USEPA, 1998). In summary, biotic matrices were homogenized with anhydrous sodium sulfate and Soxhlet extracted for 16 hr using 400 mL toluene. The extraction solvent was transferred to hexane and the extract was concentrated to 10 mL. Before extraction known amounts of ^{13}C-labeled PCDF/Ds were added as internal standards to the sample. Extracts were initially purified by treatment with concentrated sulfuric acid. The extract was then passed through a multilayer silica gel column containing silica gel and sulfuric acid silica gel and eluted with 150 mL of 10% dichloromethane in hexane. The extract is then passed through a carbon column packed with 1 g of activated carbon-impregnated silica gel. The first fraction, eluted with 100 ml hexane, was kept for PCB analysis. The second fraction, eluted with 200 mL of toluene, contained the 2,3,7,8-substituted PCDF/Ds. PCDF/Ds 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.). Generally, the mass spectrometer was operated at an EI energy of 60 eV and an ion current of 600 μA. PCDD/DF congeners were monitored by single ion monitoring (SIM) at the two most intensive ions at the molecular ion cluster. Concentrations of certain PCDF/D congeners, particularly TCDD and TCDF congeners were confirmed by using a DB-17 (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 surrogate

spikes, blanks, and duplicates. Soxhlet extractions and chemical analyses were conducted at AsureQuality Limited, Lower Hutt, New Zealand.

Results

Rookery

Three active GBH rookeries were located within the study area. The Freeland rookery (FRE) was established in 2001, and contained 44 nests in 2006 and 46 nests in 2007. The Shiawassee National Wildlife Refuge rookery (SNWR), established in 1999, contained 161 nests in 2006, but nest occupancy has drastically decreased after the recent establishment of predatory avian species, including bald eagles (*Haliaeetus leucocephalus*), great horned owls (*Bubo virginianus*), and red-tailed hawks (*Buteo jamaicensis*), within the rookery. A second rookery located on the Shiawassee National Wildlife Refuge near the confluence of the Cass and Shiawassee Rivers (CAS), contains approximately 35 nests. CAS was established in 1989 but has only been occupied periodically. No rookeries were located within the reference area. From each rookery, a target sample size of eight fresh eggs was collected, each from separate nests in the rookery. This collection of tissue from 24 different nests required climbing only 7 different trees, with up to 4 nests being accessible in one tree. Fresh egg sampling often involved incubation disturbance within the rookery so it was only done when temperatures were greater than 15 °C. Time required for egg sampling was on average 0.66 h/egg or 2.25 h/tree, with sampling efficiency increasing as climbers became more experienced with the technique. Nestling banding and blood plasma collection occurred at all rookeries. At FRE, 12 nests were accessed for nestling banding and blood plasma

collection, for a total of 20 nestling blood plasma samples. Four nests at SNWR produced 8 nestling blood plasma samples. Fourteen nestling blood samples were collected from 7 separate nests at CAS. Time required for nestling blood plasma collection averaged 0.90 h/sample or 1.63 h/nest. Two salvage nestlings were collected from 1 nest at FRE, 7 salvage nestlings were collected from 3 separate nests at SNWR, and 2 salvage nestlings from 2 separate nests at CAS. A summary of collected tissues is outlined in Table 2.1.

Adult trapping

Twelve GBH trapping stations were established, 3 located in the reference area and 9 in the target study area. By employing the described methods, there were 62 capture events, which included the capture of 51 GBH, 9 recaptures, and 2 escapes. All GBH recaptures occurred at their original trapping station, with one exception where the trapping stations were less than 500 m apart. Once recaptures occurred at any given feeding station, that station was moved to a new foraging territory to target new GBH. One GBH was recaptured two consecutive years at the same feeding station. As many as 5 individuals were trapped at one feeding station over the course of a field season. Of the 51 GBH captured, 15 were in the reference area and 36 in the target area (Table 2.1). On average, a GBH adult plasma sample was obtained for every 15.26 h of active trapping, which was conducted by a two-person team. Including recaptures and escapes, on average one GBH was captured for every 12.56 h of active trapping. These figures do not include time spent maintaining the stock of fish in the feeding stations. Normalized to the number of trapping hours, the most successful time of day to conduct trapping was

Table 2.1. Description of sampling effort and summary of great blue heron tissues collected through utilizing described methodologies. Note that there was no rookery located in the reference area to sample. Necessary adult plasma sample size in reference area obtained after 2006 field season.

Year	Reference	Target			
	Adult Plasma	*Adult Plasma*	*Egg Collection**	*Nestling Plasma**	*Nestling Tissue**
2005	5	15			
2006	10	9	6 (6)	13 (8)	9 (4)
2007		12	19 (18)	29 (15)	2 (2)
Total	15	36	25 (24)	42 (23)	11 (6)

* values in parentheses indicate the total number of nests samples collected from (*n*)

from 0600 to 1000 (Figure 2.2), with a trapping success rate of approximately 0.12 GBH/h. Average time from capture to release of GBH was 60 min. Injuries associated with adult trapping and handling were low and no injuries were sustained to adult GBH that would be expected to impact survival. A damaged or torn leg scale was noted for 8 of the 62 birds trapped and a broken phalange (non-hallux) was noted for a single bird.

Sampling effectivity

Power analyses were conducted using total TEQs (pg/mL) in the blood plasma of sampled adults from the 2005 field season (Table 2.2). These analyses revealed that a significant difference could be discerned between the reference and target area with a Type I (α) error rate of 0.05 and a Type II (β) error rate of 0.20 with as few as 4 samples from each area. Additionally, the target area could be divided into an upper and lower reach with significant differences discernable at the same errors rates with 14 samples collected from each area. Analytical results from the other matrices were not available to run site-specific power analyses.

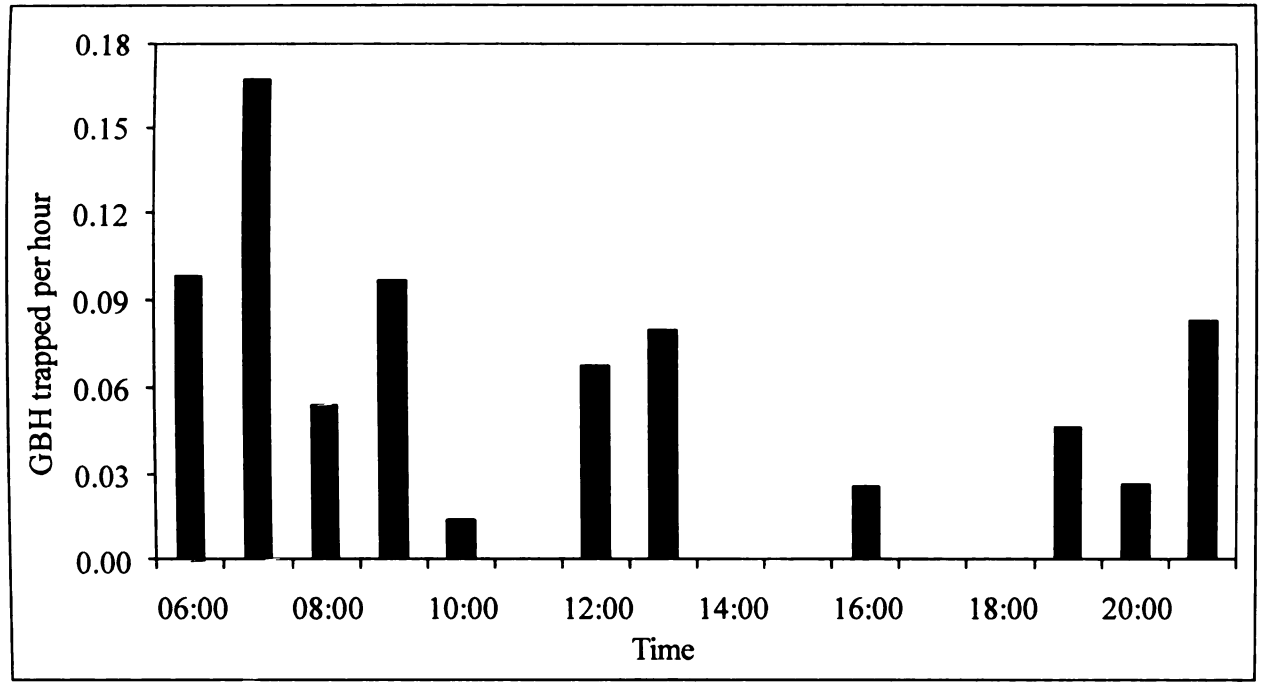


Figure 2.2. Number of great blue herons trapped in the Tittabawassee River study area between 2005 and 2007 using the bait station and foot-hold trapping method. Number of GBH normalized to the number of trapping hours.

Table 2.2. Total $TEQ_{WHO\text{-}Avian}$ (pg/mL) in adult GBH blood plasma from Tittabawassee River study area collected during 2005 field season.

	Mean	St. Dev.	Min	Max
Reference (n=5)	1.8	0.75	1.2	3.0
Upper Target (n=10)	8.4	5.5	2.2	17
Lower Target (n=5)	12	5.5	8.5	20

Discussion

Selection of a receptor species is a key element to an effective ERA, especially when site-specific field studies are to be employed. As a long-lived species near the top of the aquatic food web, GBH have the potential to be highly exposed to contaminants for many years. Residing in freshwater, estuarine, and marine habitats throughout North America, GBH have the potential to be utilized in ERAs in many different locales. The territorial foraging behavior of GBH leads to the active defense of distinct and identifiable foraging areas. Additionally, GBH have many closely related species which share some of these desirable attributes and could be studied using these same methods. All of these characteristics make the GBH a model receptor species for the assessment of bioaccumulative compounds in an aquatic food web, and led to their inclusion in the ERA conducted for the Tittabawassee River floodplain.

Although all of the aforementioned characteristics are important to have in a receptor species, they become irrelevant if the species is too difficult to study and acquire the necessary samples. Since GBH are a colonial nesting species, the discovery of one rookery results in the location of tens to hundreds of breeding pairs. This allows a multitude of nests to be monitored simultaneously for reproductive success and nestling dietary composition, and an assessment of population health rather than the health of a few individuals. In the studied rookeries, multiple nests could often be sampled for eggs or nestling handling by climbing one tree, reducing the effort needed to obtain the necessary sample size. In addition to comparing population health parameters from study areas to appropriate reference areas, comparisons may also be made with other studies

which report productivity that are available in the literature (Pratt, 1970; Thomas and Anthony, 1999; Harris *et al.*, 2003; Witt, 2006). Conversely, GBH characteristics of nesting at great heights in small diameter and sometimes dead trees can make both ground-based observations and physical nest access challenging. For the rookeries monitored here, ground blind observations were supplemented with observations from rotary wing aircraft to assess clutch size. However physical nest access was limited to trees that were safely climbable. Date, time of flight, and nonrandom limitations to physical nest access may add bias to measurements and should be noted for within and across study comparisons.

The collection of nestling blood plasma and eggs from the same nest allows for the possible derivation of a plasma-to-egg ratio, which would eliminate the need for destructive egg sampling. GBH are a migratory species, so it is possible that contaminants transferred to the egg from the female were accumulated elsewhere (Henny, 1986); however, eggs collected from rookeries in the study area exhibited low variation in total TEQs and congener profiles within and among rookeries, suggesting this was not an important factor at this site. Nestlings are considered to be more representative of local contamination as they are confined to the nest and rely on the food brought to them by adults (Olsson *et al.*, 2000; Neigh *et al.*, 2006). Studies tracking adult GBH from rookeries to foraging areas have determined that adult GBH forage a mean distance between 3.1 km and 6.5 km from breeding colonies, although a distance as great as 34.1 km has been recorded (Thompson, 1978; Peifer, 1979; Dowd and Flake, 1985). To further characterize site-specific dietary exposure, the habit of GBH nestlings to regurgitate their stomach contents when under duress can be exploited by collecting the

regurgitant to determine dietary composition and contaminant concentrations. Additionally, the described methods facilitate the collection of plasma samples from multiple nestlings within a single nest to determine intra-brood variation. This dataset combined with eggs from the same nest can generate plasma-to-egg ratios, which can be a useful tool; however, they must only be used when both eggs and plasma are representative of local contamination. These ratios are especially desirable when dealing with endangered or threatened species when avoiding any destructive sampling is of great importance (Strause, *et al.*, 2007).

The territorial foraging of GBH facilitates the establishment of feeding stations in multiple foraging territories in the area of interest to capture different individuals with a low rate of recapture. Throughout the three field seasons during which trapping of adult GBH was performed, the feeding station and foot-hold trap method proved to be very effective, as demonstrated by the fifty different individuals that were captured, banded, and provided a blood plasma sample. As many as five individuals were trapped at one feeding station over the course of one field season. Along a river system in South Dakota, Dowd and Flake (1985) determined that radio-tagged GBH would return to the same general areas of the river, but other GBH were also observed using the same areas. Additionally, fledgling GBH did not seem to display aggressive territorialism over foraging areas and were often seen foraging in flocks. In the closely related grey heron (*Ardea cinerea*), other foragers would visit actively defended territories in the absence of the territory owner (Marion, 1989). One individual GBH was recaptured two consecutive years at the same feeding station, suggesting territories may be maintained over multiple years. No banded birds were recaptured at feeding stations other than where they were

initially trapped, except where the feeding stations were less than 500 m apart, again reinforcing the territoriality of GBH foraging. Additionally, the recapture of GBH, sometimes multiple times in the same day, suggests that either this trapping method was not traumatic or injurious as compared to the desire for easy prey. The level of effort involved in adult trapping could potentially be lessened by focusing trapping effort during certain times of the day, during the nesting season, or in areas of group foraging. When normalized to the number of trapping hours, the period from 0600 to 1000 had the highest trapping success rate, at approximately 0.12 GBH/hr. In the present study, trapping was focused in solitary feeding areas in an attempt to quantify site fidelity and to minimize the effects of foraging disturbance on additional birds.

The temporal consistency of both data access and exposure potential for GBH adds potential flexibility in study design and sampling efforts. For instance, we used power analysis of first year data to identify spatially explicit boundaries for which statistically significant differences could potentially be identified at a reasonable level of effort. This adds value to the study by providing for a real-time cost benefit analysis and the most efficient allocation of resources.

The methodologies employed in this study provided multiple ways of estimating exposure, including dietary exposure and tissue-based exposure assessments. Although an exact site-specific dietary composition was not calculated, a combination of literature-based diets and observations of site-specific GBH foraging led to the collection of forage fish, crayfish, and amphibians as the primary diet components. Analysis of these items allowed for the calculation of estimated average daily intake and resulting HQs for dietary exposure. Determination of concentrations of PCDDs/Fs in egg, nestling tissues,

and nestling and adult plasma allowed for the determination of HQs based on tissue concentrations. Comparisons can then be made between the HQs derived from the varying approaches to determine the accuracy of predicting exposure through the diet and the importance of collecting receptor tissues.

Acknowledgements

The authors would like to thank all staff and students of the Michigan State University – Wildlife Toxicology Laboratory field crew; namely Michael W. Nadeau, Will R. Folland and Stephanie C. Plautz for their tree-climbing abilities, along with Emily M. Koppel, Jeremy N. Moore, Bretton J. Joldersma, Megan L. Barker, Joost van Dam, Lori E. Williams, and Casey L. Bartrem. We gratefully acknowledge Mikes Fales for his design and fabrication of specialized equipment which was pivotal to the success of this research. Additionally, we would like to recognize Patrick W. Bradley, Michael J. Kramer, and Nozomi Ikeda for their assistance in the laboratory, James Dastyck and Steven Kahl of the United States Fish and Wildlife Service – Shiawassee National Wildlife Refuge, for their assistance and access to the refuge, the Saginaw County Parks and Recreation Commission for access to Imerman Park, and the Tittabawassee Township Park Rangers for access to Tittabawassee Township Park and Freeland Festival Park. We would also like to acknowledge the greater than 50 cooperating landowners throughout the study area who have granted us access to their property, making our research possible. 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 means possible. To achieve that objective, all aspects of the study were performed following standard operation procedures (GBH adult handling 03/05-036-00; GBH nest monitoring 05/07-066-00; Field studies in support of TR ERA 03/04-042-00; Protocol for fish sampling 03/04-043-00) 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 SC1254/SC permit for fish (Zwiernik)/SC permit for amphibians (Zwiernik) and USFWS Migratory Bird Scientific Collection Permit MB1000062-0) are on file at MSU-WTL.

References

Alexander, G. R. (1977). Food of vertebrate predators on trout waters in north central lower Michigan. *Michigan Academician*, **10**, 181-195.

Bayer, R. D. (1981). Regional variation of great blue heron longevity. *Journal of Field Ornithology*, **52**, 210-213.

Butler, R. W. (1992). Great blue heron. A. Poole, P. Stettenheim, & F. Gill (Eds.), *The Birds of North America*, No. 25. Philadelphia: The Academy of Natural Sciences; Washington, DC: The American Ornithologists's Union.

Butler, R. W. (1997). *The Great Blue Heron: A Natural History and Ecology of a Seashore Sentinel.* (Vancouver, BC:UBC Press)

Champoux, L., Rodrigue, J., DesGranges, J., Trudeau, S., Hontela, A., Boily, M., & Spear, P. (2002). Assessment of contamination and biomarker response in two species of herons on the St. Lawerence River. *Environmental Monitoring and Assessment*, **79**, 193-215.

Custer, T. W., Rattner, B. A., Ohlendorf, H. F., & Melancon, M. J. (1991). Herons and egrets as proposed indicators of estuarine contamination in the United States. *Acta XX Congressus Internationalis Ornithologici*, **20**, 2474-2479

Custer, T. W., Hines, R. W., Melancon, M. J., Hoffman, D. J., Wickliffe, J. K., Bickham, J. W., Martin, J. W., Henshel, D. S. (1997). Contaminant concentrations and biomarker response in great blue heron eggs from 10 colonies on the upper Mississippi River, USA. *Environmental Toxicology and Chemistry*, **16**, 260-271.

DesGranges, J. & Desrosiers, A. (2006). Breeding distribution and population trends of the great blue heron in Quebec, 1977-2001. Canadian Wildlife Service (CWS) Occasional Paper No. 113. Environment Canada, Ottawa, Ontario, Canada.

Dowd, E. M., & Flake, L. D. (1985). Foraging habitats and movements of nesting great blue herons in a prairie river ecosystem, South Dakota. *Journal of Field Ornithology*, **56**, 379-387.

Elliott, J. E., Harris, M. L., Wilson, L. K., Whitehead, P. E., & Norstrom, R. J. (2001). Monitoring temporal and spatial trends in polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in eggs of great blue heron (*Ardea herodias*) on the coast of British Columbia, Canada, 1983-1998. *Ambio*, **30**, 416-428

Gilbertson, M. (1983). Etiology of chick edema disease in herring gulls in the lower Great Lakes. *Chemosphere*, **12**, 357-370.

Harris, M. L., Elliott, J. E., Butler, R. W., & Wilson, L. K. (2003). Reproductive success and chlorinated hydrocarbon contamination of resident great blue herons (*Ardea herodias*) from coastal British Columbia, Canada, 1977 to 2000. *Environmental Pollution*, **121**, 207-227.

Hayes, M. A. & Barzen, J. A. 2006. Dynamics of breeding and non-breeding sandhill cranes in south-central Wisconsin. *The Passenger Pigeon*, **68**, 345-352

Henny, C. J. & Blus, L. J. (1986). Radiotelemetry locates wintering grounds of DDE-contaminated black-crowned night-herons. *Wildlife Society Bulletin*, **14**, 236-241.

Henny, C. J. & Meeker, D. L. (1981). An evaluation of blood plasma for monitoring DDE in birds of prey. *Environmental Pollution (Series A)*, **25**, 291-304.

Hilscherova, K., Kannan, K., Nakata, H., Hanari, N., Yamashita, N., Bradley, P. W., McCabe, J. M., & Giesy, J. P. (2003). Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. *Environmental Science & Technology*, **37**, 468-474

Hines, R. K. & Custer, T. W. (1995). Evaluation of extendable pole-net to collect heron eggs in the canopy of tall trees. *Colonial Waterbirds*, **18**, 120-122

Hoffman, D. J., Rattner, B. A., Sileo, L., Docherty, D. E., & Kubiak, T. J. (1987). Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. *Environmental Research*, **42**, 176-184.

King, D. T., Paulson, J. D., Leblanc, D. J., & Bruce, K. (1998). Two capture techniques for American white pelicans and great blue herons. *Colonial Waterbirds*, **21**, 258-260

Ludwig, J. P., Auman, H. J., Kurita, H., Ludwig, M. E., Campbell, L. M., Giesy, J. P., Tillitt, D. E., Jones, P., Yamashita, N., Tanabe, S., & Tatsukawa, R. (1993). Caspian tern reproduction in the Saginaw Bay ecosystem following a 100-year flood event. *Journal of Great Lakes Research*, **19**, 96-108

Marion, L. (1989). Territorial feeding and colonial breeding are not mutually exclusive: the case of the grey heron (*Ardea cinerea*). *The Journal of Animal Ecology*, **58**, 693-710

McAloney, K. (1973). The breeding biology of the great blue heron on Tobacco Island, Nova Scotia. *Canadian Field-Naturalist*, **87**, 137-140

Moul, I. E., Vennesland, R. G., Harris, M. L., & Butler, R. W. (2001). Standardizing and interpreting nesting records for great blue herons in British Columbia. Canadian

Wildlife Service (CWS) Progress Note No. 217. CWS, Pacific and Yukon Region, Vancouver, BC, Canada. Retrieved from http://www.cws-scf.ec.gc.ca/publications/notes/217/cont_e.pdf

Neigh, A. M., Zwiernik, M. J., Bradley, P. W., Kay, D. P., Park, C. S., Jones, P. D., Newsted, J. L., Blankenship, A. L., & Giesy, J. P. (2006). Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund Site, MI, USA. *Environmental Toxicology and Chemistry*, **25**, 428-437.

Olsson, A., Ceder, K., Bergman, Å., & Helander, B. (2000). Nestling blood of the white-tailed sea eagle (*Haliaeetus albicilla*) as an indicator of territorial exposure to organohalogen compounds - an evaluation. *Environmental Science & Technology*, **34**, 2733-2740.

Peifer, R. W. (1979). Great blue herons foraging for small mammals. *The Wilson Bulletin*, **91**, 630-631.

Pratt, H. M. (1970). Breeding biology of great blue herons and common egrets in central California. *The Condor*, **72**, 407-416

Quinney, T. E. (1982). Growth, diet, and mortality of nestling great blue herons. *Wilson Bulletin*, **94**, 571-577

Simpson, K. & Kelsall, J. P. (1978). Capture and banding of adult great blue herons at Pender Harbour, British Columbia. *Proceedings Colonial Waterbird Group*, **3**, 71-78

Straub, C. L., Maul, J. D., Halbrook, R. S., Spears, B., & Lydy, M. J. (2007). Trophic transfer of polychlorinated biphenyls in great blue heron (*Ardea herodias*) at Crab Orchard National Wildlife Refuge, Illinois, United States. *Archives of Environmental Contamination & Toxicology*, **52**, 572-579

Strause, K. D., Zwiernik, M. J., Im, S. H., Newsted, J. L., Kay, D. P., Bradley, P. W., Blankenship, A. L., Williams, L. L., Giesy, J. P. (2007). Plasma to egg conversion factor for evaluating polychlorinated biphenyl and DDT exposures in great horned owls and bald eagles. *Environmental Toxicology and Chemistry*, **26**, 1399-1409.

Thomas, C. M. & Anthony, R. G. (1999). Environmental contaminants in the great blue herons (*Ardea herodias*) from the Lower Columbia and Willamette Rivers, Oregon and Washington, USA. *Environmental Toxicology & Chemistry*, **18**, 2804-2816

Thompson, D. H. (1978). Feeding areas of great blue herons and great egrets nesting within the floodplain of the upper Mississippi River. *Proceedings of the Colonial Waterbird Group*, **2**, 202-213.

U.S. Environmental Protection Agency (USEPA) (1986) Michigan Dioxin Studies. Dow Chemical Wastewater Characterization Study. Tittabawassee River Sediments and Native Fish. EPA-905/4-88-003. U.S. Environmental Protection Agency, Region V, Chicago, IL.

U.S. Environmental Protection Agency (USEPA). (1998). Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurnas (PCDFs) by high-resolution gas chromatography/high-resolution mas spectrometry (HRGC/HRMS). Revision 1. Method 8290A. SW-846. U.S. Environmental Protection Agency, Washington, DC.

van den Berg, M., Craane, B., Sinnige, T., van Mourik, S., Dirksen, S., Boudewijn, T., van der Gaag, M., Lutke-Schipholy, I. J., Spenkelink, B., & Brouwer, A. (1994). Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (*Phalacrocorax carbo*) after in ovo exposure. *Environmental Toxicology & Chemistry*, **13**, 803-816

van den Berg, M., Birnbaum, L., Bosveld, A., Brunstrom, B., Cook, P., Feeley, P., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T. et al. (1998). Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife. *Environmental Health Perspectives*, **106**, 775-792

Witt, J. W. (2006). Great blue heron productivity at Mason Neck National Wildlife Refuge in northern Virginia, and the potential impacts of weather during a 13-year interval. *Waterbirds*, **29**, 345-349

CHAPTER 3

Dietary exposure of great blue heron (*Ardea herodias)* to PCDD/DFs in the Tittabawassee River floodplain, MI, USA

Rita M. Seston[1], Timothy B. Fredricks[1], Dustin L. Tazelaar[2], Sarah J. Coefield[1], Patrick W. Bradley[2], Shaun A. Roark[3], John L. Newsted[3], Denise P. Kay[3], Matthew J. Zwiernik[2], and John P. Giesy[1,4,5]

[1]Zoology Department, Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA

[2]Animal Science Department, Michigan State University, East Lansing, MI 48824, USA

[3]ENTRIX, Inc., Okemos, MI, USA

[4]Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, S7J 5B3, Canada

[5]Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, SAR, China

Abstract

Concentrations of dioxin-like compounds, primarily polychlorinated dibenzofurans (PCDFs), in soils and sediments of the Tittabawassee River (TR) and associated floodplains downstream of Midland, Michigan (USA) were greater than upstream sites and prompted a site-specific risk assessment of great blue herons (GBH). Dietary exposure of GBH to PCDFs and polychlorinated dibenzo-*p*-dioxins (PCDDs) was evaluated based on site-specific concentrations of residues in prey items. Concentrations of ΣPCDD/DFs and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in prey items collected from the TR were consistently greater than those collected from associated reference areas (RAs) and further downstream in the Saginaw River (SR). The average daily dose (ADD_{pot}) of ΣPCDD/DFs to GBH was 45- to 54-fold greater along the TR and 12-fold greater along the SR when compared to the RA. ΣPCDD/DFs were normalized to $TEQ_{WHO\text{-}Avian}$, and fold differences in the ADD_{pot} increased, being 150- to 190-fold greater along the TR and 36-fold greater along the SR than they were in the RA. Greater fold changes in the ADD_{pot} based on $TEQ_{WHO\text{-}Avian}$ between the RA and the TR and SR was due to prey items from the latter reaches having a greater relative toxic potency of ΣPCDD/DFs, primarily from greater amounts of 2,3,7,8-tetrachlorodibenzofuran but also 2,3,4,7,8-pentachlorodibenzofuran. Potential for adverse population-level effects from site-specific contaminant exposures were evaluated via comparison to selected toxicity reference values. The prediction of minimal to no risk of adverse population-level effects resultant from the assessment of site-specific dietary exposure of GBH to ΣPCDD/DFs along the TR and SR is consistent with site-specific assessments of tissue-based exposures as well as population condition.

Introduction

A screening-level risk assessment pertaining to dioxin-like compounds in the Tittabawassee River (TR) floodplain predicted the great blue heron (*Ardea herodias;* GBH) to have one of the greatest potential exposures to dioxin-like compounds of avian species nesting along the TR (Galbraith Environmental Sciences LLC. 2003). That preliminary assessment was based on minimal information and had to make a number of assumptions. In the study described herein, additional data was collected that allowed a refined estimate of dietary exposure of GBH to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs) along the TR. Measured site-specific dietary exposure was then compared to toxicity reference values (TRVs) to evaluate the potential for adverse effects of PCDD/DFs on GBH that forage within the TR floodplain.

As a result of historical chemical production and management of associated wastes, the Tittabawassee and Saginaw Rivers downstream of Midland, MI, USA contain elevated concentrations of PCDD/DFs (USEPA 1986). Sediments and associated floodplain soils collected from downstream study areas (SA) were found to contain total concentrations of the 2,3,7,8-substituted PCDD/DFs (ΣPCDD/DFs) ranging from $1.0x10^2$ to $5.4x10^4$ ng/kg dw. Mean concentrations of ΣPCDD/PCDF in sediments and floodplain soils in the reference area (RA) upstream of Midland were 10- to 20-fold less (Hilscherova *et al.* 2003). The persistence of these compounds in the environment, combined with their toxicity and potential to bioaccumulate, has led to concern over the potential exposures of wildlife species foraging in the Tittabawassee and Saginaw River floodplains.

PCDD/DFs often occur in the environment as complex mixtures, and may also be in the presence of structurally related polychlorinated biphenyls (PCBs). Due to the lipophilic characteristics of these compounds and their resistance to degradation in the environment (Mandal 2005) they have the potential to be accumulated through the food web. The critical responses of these compounds are mediated through the aryl hydrocarbon receptor (AhR) and include enzyme induction, teratogenicity, immunotoxicity, and adverse effects on reproduction, development, and endocrine functions (Allred and Strange 1977; Brunström and Andersson 1988; Powell *et al.* 1996a; Verrett 1976). In particular, AhR-mediated compounds have been shown to decrease hatching success and fledging success in aquatic avian species (Gilbertson 1983; Hoffman *et al.* 1987; van den Berg *et al.* 1994a). The sensitivities of a number of species to AhR-mediated effects have been determined in laboratory studies or inferred from observations of populations exposed in the wild. Sensitivities have been shown to vary among species (Brunström 1988). For example, the domestic chicken (*Gallus gallus*), which is considered to be the most sensitive avian species, is over 1000-times more sensitive to embryo-lethal effects than is the mallard (*Anas platyrhynchos*) (Head *et al.* 2008). Recent research has suggested the ligand binding domain (LBD) of the AhR in avian species is highly conserved and can be classified by a few amino acid sequences. The specific configuration of the LBD directly affects the binding affinity of ligand (dioxin-like compounds), thereby influencing the organisms response and sensitivity to toxic effects. (Head *et al.* 2008; Karchner *et al.* 2006). This confirms the importance of species-specific exposure and responses among taxa.

The primary objective of this study was to describe the dietary exposure of GBH to PCDD/DFs in the Tittabawassee River basin and predict the risk this exposure poses to GBH breeding on-site. To characterize PCDD/DF exposure, concentrations of ΣPCDD/DF, ΣPCB, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents ($TEQ_{WHO\text{-}Avian}$) based on World Health Organization 2,3,7,8-TCDD equivalency factors for birds ($TEF_{WHO\text{-}Avian}$) (van den Berg *et al.* 1998) were measured in dietary items collected from reference and study areas. Concentrations of PCDD/DFs and the patterns of their relative congener concentrations in dietary items were evaluated for spatial trends. To estimate the potential for adverse effects of the measured dietary exposure, predicted average daily doses (ADD_{pot}) were compared to TRVs. To facilitate a multiple lines of evidence approach (Fairbrother 2003) to determine the risk present to GBH along the Tittabawassee River floodplain the results of the dietary exposure assessment were compared to previously conducted assessments of GBH tissue exposure and population condition (Seston *et al.* 2010a). Integration of multiple lines of evidence can help reduce the uncertainty inherent in the risk assessment process (Leonards *et al.* 2008).

Methods

Site description

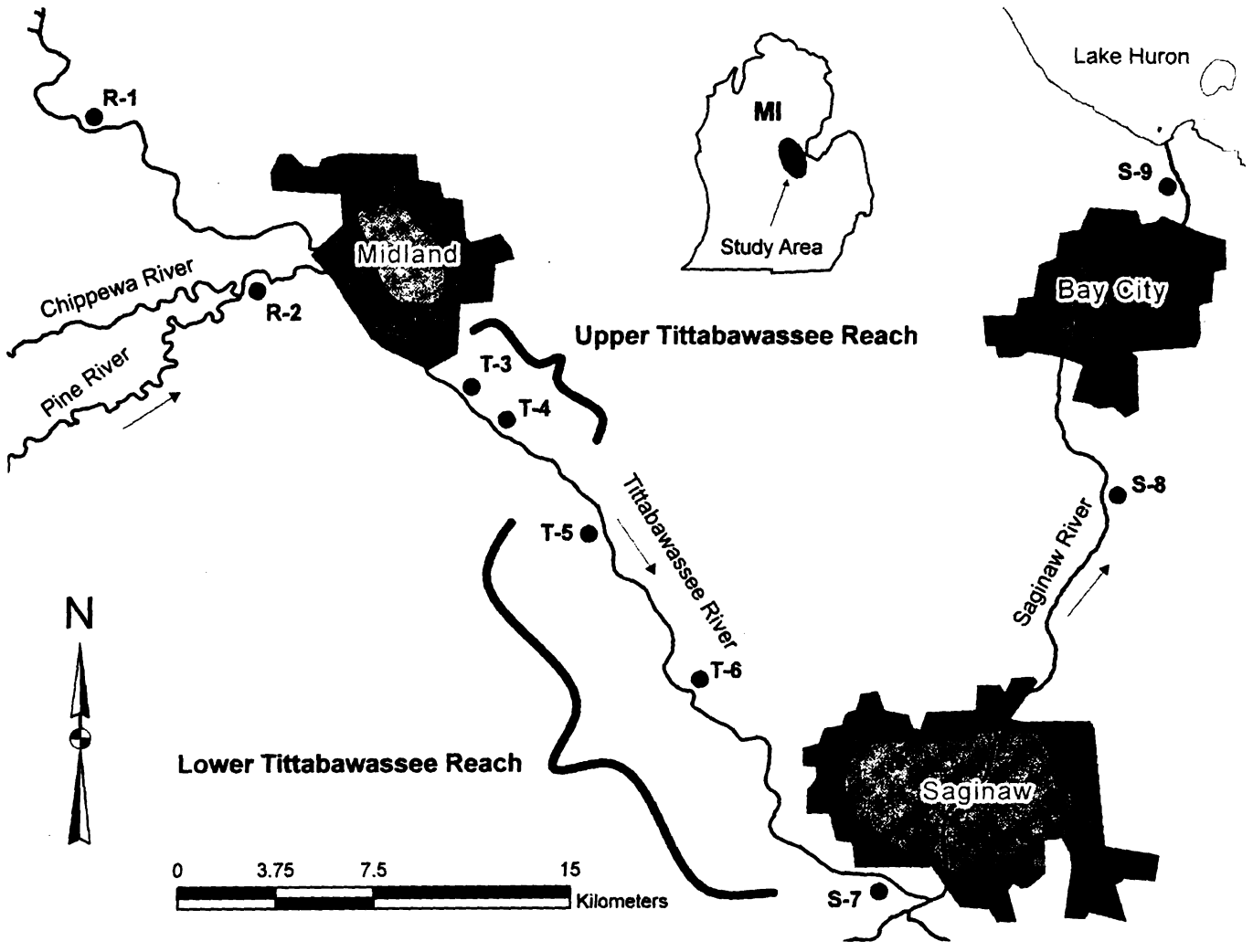
The assessment was conducted in the vicinity of the city of Midland, located in the east-central lower peninsula of Michigan (USA). The Tittabawassee River (TR) is a tributary of the Saginaw River (SR), which eventually empties into Saginaw Bay of Lake Huron. The TR runs through The Dow Chemical Company (DOW) property, which is located on the southern edge of Midland and is the accepted source of the PCDD/DF

contamination (USEPA 1986). The area henceforth referred to as the study area (SA) included approximately 37 km of the TR (sites T-3 to T-6) and associated wetlands from DOW to the confluence of the TR and SR. Additionally, the SA included 35 km of the SR (sites S-7 to S-9) downstream of the confluence to where it enters Saginaw Bay (Figure 1). Sampling sites located in the SA were chosen to characterize maximal exposure potential designated as potential "worst case scenario" locations based on a previous study that measured soil and sediment concentrations (Hilscherova *et al.* 2003) and availability of landowner access to sites. The reference area (RA) was composed of the TR upstream of Midland, together with the Pine and Chippewa Rivers, both of which are tributaries of the TR upstream of Midland. Sampling locations in the RA were on the upstream TR (R-1) and on the Pine River, just upstream of its confluence with the Chippewa River (R-2). Distinct sampling areas were assessed individually as well as grouped spatially based on river characteristics. Spatial groupings included reference (RA) R-1 and R-2, upper Tittabawassee River (UTR) T-3 and T-4, lower Tittabawassee River (LTR) T-5 to S-7, and Saginaw River (SR) S-8 and S-9.

Receptor species selection

Selection of an appropriate study species is a key element of effective ecological risk assessments (ERAs), especially when site-specific field studies are to be employed (USEPA 1994). Piscivorous birds are frequently selected as receptors for evaluating aquatic systems because they can be sensitive to the effects of contaminants and have the potential to accumulate persistent, lipophilic contaminants through trophic-transfer. The GBH possesses several characteristics that make it a suitable species to use as a receptor

Figure 3.1. Sampling locations for dietary components along the Chippewa, Tittabawassee, and Saginaw river floodplains, Michigan, USA. Reference area (R-1 and R-2; RA); Upper Tittabawassee River (T-3 and T-4; UTR); Lower Tittabawassee River (T-5, T-6, and S-7; LTR); and Saginaw River (S-8 and S-9; SR).


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

in ERAs concerning bioaccumulative contaminants in aquatic environments (Seston *et al.* 2009). As a long-lived, territorial, apex predator in the aquatic food web, the GBH has great potential to accumulate local contaminants over a long period of time, especially in areas of its range where foraging is available over the winter months, allowing them to be year-round residents (Butler 1997; Custer *et al.* 1991). GBH have a broad distribution across geographic regions and habitat types, residing in freshwater, estuarine, and marine habitats throughout North America (Butler 1992), which makes them a potential receptor for sites of aquatic-based contamination at many different localities (Champoux *et al.* 2002; Custer *et al.* 1997; Elliott *et al.* 2001; Harris *et al.* 2003; Straub *et al.* 2007; Thomas and Anthony 1999). The territorial nature of GBH leads to the active defense of distinct, identifiable foraging areas within 3.2 km to 6.5 km of the breeding colony (Marion 1989; Peifer 1979). Therefore, the spatial boundaries of exposure of GBH to persistent and bioaccumulative compounds can be better defined than may be possible with other more opportunistically feeding piscivorous birds.

Collection of prey items

Prey items of GBH, including forage fish, crayfish, and amphibians, were collected and concentrations of PCDD/DFs (n=188) and PCBs (n=20) determined. Dietary items were collected from nine sampling locations (Figure 3.1). The sampling scheme maximized information on dietary exposure including geographically associated contaminant variability and trends. Forage fish of a size class consumed by GBH (≤25 cm in length) were collected by either electro-fishing or seine netting. Three species of amphibian common to the area, the wood frog (*Rana sylvatica*), leopard frog

(*Rana pipiens*), and green frog (*Rana clamitans*) were collected. Individual frogs were captured by hand or dip net. Crayfish were collected with a seine net or modified minnow traps. Forage fish were analyzed as composite samples, whereas individual amphibians and crayfish were analyzed separately unless they needed to be combined to obtain sufficient sample mass.

Dietary exposure calculations

Exposure of GBH to PCDD/DF via the diet was estimated by use of information provided in the U.S. Environmental Protection Agency (USEPA) Wildlife Exposure Factors Handbook (WEFH; USEPA 1993). Major factors influencing dietary exposure included body mass (BW), daily food intake rate [FIR; g wet wt food/g body weight (BW)/d], dietary concentrations (C), and proportion of foraging time spent on-site (AUF). A mean body mass of 2.3 kg was determined by (Henning *et al.* 1999) after an extensive review of data available in the literature. Using the equation developed to determine FIR for wading birds (Kushlan 1978) the USEPA WEFH reports a body-weight normalized FIR of 0.18 kg food (wet wt) $\cdot$kg $bw^{-1} \cdot d^{-1}$ for GBH. The ADD_{pot}, expressed as ng/kg bw/d was calculated using equation 4-3 from the WEFH (USEPA 1993). Incidental sediment ingestion was also included in the ADD_{pot} using equation 4-23 (USEPA 1993).

GBH have a relatively large foraging range, which can result in site-nesting GBH spending a portion of their time foraging outside of the SA (Dowd and Flake 1985; Peifer 1979; Thompson 1978). In addition, GBH are a migratory species in areas of its range where foraging is not available during winter months due to ice cover (Butler 1992). To examine the effects of foraging range and site-use, ADD_{pot} was calculated by use of three

different area use factors, including 25%, 75%, 100% on-site foraging. The off-site portion of the diet was estimated based on contaminant concentrations in prey collected from the RA. Contaminant concentrations in RA prey items were significantly less than those in prey items collected from the study area and were assumed to be representative of non-point source exposures.

The relative proportion of each type of prey to the GBH diet was determined through a combination of site-specific observations and data reported in the literature. Observations of GBH foraging, in combination with prey remains and stomach contents revealed the site-specific diet to contain primarily fish in addition to amphibians and crayfish. However, a small sample size precluded the determination of the relative contribution of each prey item taxa to the dietary composition. A previous study of GBH in Michigan reported the diet to be composed of 94 to 98% fish (Alexander 1977). Thus, the diet selected here to investigate dietary exposure comprised 96% forage fish, 2% amphibians, and 2% crayfish. Reach-specific concentrations of ΣPCDD/DFs in prey items were multiplied by their relative contribution to the dietary composition. Exposure was estimated using the geometric mean and associated 95% confidence interval of concentrations of residues in prey items from each reach (RA, UTR, LTR, and SR) (Table 3.2). PCBs were not measured in all frogs or crayfish, thus it was not possible to directly calculate total $TEQ_{WHO\text{-}Avian}$ exposure associated with PCDD/DFs and PCBs for all samples. Where both PCDD/DF and PCB data was available, concentrations of total $TEQs_{WHO\text{-}Avian}$ in frogs and crayfish were less than proximally collected fish. To estimate a conservative exposure of GBH to total $TEQs_{WHO\text{-}Avian}$ a dietary composition of 100% fish was utilized.

Sample processing and analytical techniques

Concentrations of seventeen 2,3,7,8-substituted PCDD/DF congeners were measured in all samples while concentrations of the twelve dioxin-like PCBs were determined in a subset of samples. Tissues were homogenized in a chemically cleaned Omni-mixer and stored in clean jars until analysis (I-CHEM brand, Rockwood, TN). PCDD/DFs and PCBs were quantified in accordance with EPA Method 8290/1668A with minor modifications (USEPA 1998). In summary, biotic matrices were homogenized with anhydrous sodium sulfate and Soxhlet extracted for 16 hr with toluene. The extraction solvent was transferred to hexane and the extract was concentrated to 10 mL. Before extraction known amounts of ^{13}C-labeled analytes were added as internal standards to the sample. Extracts were initially purified by treatment with concentrated sulfuric acid. The extract was then passed through a multilayer silica gel column containing silica gel and sulfuric acid silica gel and eluted with 10% dichloromethane in hexane. The extract was then passed through a carbon column packed with activated carbon-impregnated silica gel. The first fraction, eluted with hexane, was kept for PCB analysis. The second fraction, eluted with toluene, contained the 2,3,7,8-substituted PCDD/DFs. PCDD/DFs were analyzed via HRGC-HRMS, using 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. PCDD/DF congeners

were monitored by single ion monitoring (SIM) at the two most intensive ions of the molecular ion cluster. Concentrations of certain PCDD/DF congeners, particularly TCDD and TCDF congeners were confirmed by using a DB-17 (60 m x 0.25 mm i.d., 0.25 μm film thickness) column (Agilent Technologies, Wilmington, DE). Losses of congeners during extraction were corrected based on recoveries of ^{13}C-labeled 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. Soxhlet extractions and instrumental analyses were conducted at AsureQuality Ltd, Lower Hutt, New Zealand.

Statistical analyses

Residues in dietary items were reported several different ways. Total concentrations of the seventeen 2,3,7,8-substituted PCDD/DF congeners are reported as the sum of all congeners (ΣPCDD/DFs) as well as $TEQ_{WHO\text{-}Avian}$ (ng/kg wet weight (wet wt)). Individual congeners that were less than the limit of quantification were assigned a value of half the sample method detection limit on a per sample basis. Total concentrations of twelve non- and mono-*ortho*-substituted PCB congeners are reported as the sum of these congeners (ng/kg wet wt) (ΣPCBs) for a subset of samples. Concentrations of $TEQ_{WHO\text{-}}$

$_{Avian}$ (ng/kg wet wt) 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). The term $DF\text{-}TEQ_{WHO\text{-}Avian}$ refers to summation of the TEQs of individual PCDD and PCDF congeners while the term $PCB\text{-}TEQ_{WHO\text{-}Avian}$ refers to the TEQ from PCBs. Total $TEQs_{WHO\text{-}Avian}$ refers to the summation of both $DF\text{-}TEQs_{WHO\text{-}Avian}$ and $PCB\text{-}TEQs_{WHO\text{-}Avian}$.

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) before statistical analyses. PROC GLM was used to make comparisons for three or more locations. When significant differences among locations were indicated, the Tukey-Kramer test was used to make comparisons between individual locations. PROC TTEST was used to make comparisons between two groups. Differences were considered to be statistically significant at $p<0.05$.

Selection of toxicity reference values

Literature-based no observed adverse effect concentrations (NOAECs) and lowest observed adverse effect concentrations (LOAECs) were used for calculation of hazard quotients (HQs) and subsequent assessment of risk. In this study, dietary exposure-based TRVs based on the same or similar compounds were identified from the literature and compared to predicted site-specific dietary exposure of GBH. Resulting HQs are

presented as a range bounded by the LOAEC associated HQs at the low end and the NOAEC associated HQs at the high end. It should be noted that the NOAEC and LOAEC associated HQs are a function of the experimental design (dosing regime) and the actual threshold concentration at which effects would be expected to occur lies somewhere within the described range.

Laboratory studies of effects from dietary exposure to PCDD/DFs are limited for avian species. The TRV selected for use in this assessment was derived from a study which dosed adult hen ring-necked pheasants (*Phasianus colchicus*) with TCDD through intraperitoneal (IP) injection (Nosek *et al.* 1992). 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. Adverse effects were not observed at the next lesser dose, which was determined to be the NOAEC for dietary exposure (14 ng TCDD/kg/d).

Results

ΣPCDD/DF and ΣPCB concentrations

Concentrations of ΣPCDD/DFs in each type of prey, including frogs, crayfish, and forage fish, exhibited consistent spatial trends that differed slightly from the trend observed in sediment. In prey items, concentrations of ΣPCDD/DFs were least in RA, greater in UTR, greatest in LTR, and intermediate in the SR (Table 3.1). Mean concentrations of ΣPCDD/DFs were 9-, 18-, and 1- fold greater in frogs, 28-, 72-, and 10-fold greater in crayfish, and 47-, 58-, and 13-fold greater in forage fish collected from the UTR, LTR, and SR than those from the RA, respectively. Mean concentrations of

Table 3.1. $TEQ_{WHO\text{-}Avian}$[a] in prey items of great blue herons collected during 2004-2006 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[b] (ng/kg wet wt) are given as the geometric mean and sample size in parentheses (n) over the 95% confidence interval and range (min-max).

	Reach			
	Reference Area	Upper Tittabawassee	Lower Tittabawassee	Saginaw River
Frog				
ΣPCDD/DF	5.5 (29) A	49 (51) B	100 (55) C	6.7 (12) A
	4.3-6.9	33-73	78-140	4.7-9.4
	(1.8-21)	(4.4-920)	(17-3300)	(3.3-26)
ΣPCB	N/A	N/A	1300 (4)	N/A
			880-2000	
			(940-1700)	
DF-$TEQ_{WHO\text{-}Avian}$	1.0 (29) A	20 (51) B	56 (55) C	2.9 (12) A
	0.81–1.3	13–31	42–76	1.9–4.5
	(0.29–3.4)	(1.1–460)	(9.1–1900)	(1.5–14)
PCB-$TEQ_{WHO\text{-}Avian}$	N/A[c]	N/A	1.5 (4)	N/A
			0.86–2.5	
			(1.1–2.0)	
Crayfish				
ΣPCDD/DF	5.0 (5) A	140 (7) B	360 (8) C	50 (8) D
	1.8–13	83–240	200–650	34–72
	(2.3–12)	(86–340)	(140–1300)	(28–110)
ΣPCB	N/A	–[e] (2)	– (1)	N/A
		–	–	
		(2200-2200)	(2100)	
DF-$TEQ_{WHO\text{-}Avian}$	0.91 (5) A	55 (7) B	160 (8) C	27 (8) B
	0.29–2.8	24–130	110–250	18–41
	(0.34–2.9)	(12–190)	(75–420)	(13–61)
PCB-$TEQ_{WHO\text{-}Avian}$	N/A	– (2)	– (1)	N/A
		–	–	
		(3.3-4.3)	(5.3)	
Forage Fish				
ΣPCDD/DF	– (2)	– (2)	260 (5) A	59 (4) B
	–	–	95–730	26–130
	(4.1–5.1)	(200–220)	(83–610)	(37–210)
ΣPCB	– (2)	– (2)	18000 (5) A	25000 (4) A
	–	–	4600–72000	10000–62000

Table 3.1 (cont'd)

	(880–1000)	(9300–15000)	(7100–110000)	(15000–47000)
DF-$TEQ_{WHO\text{-}Avian}$	– (2)	– (2)	180 (5) A	33 (4) B
	–	–	66–480	20–56
	(0.90–0.91)	(130–170)	(74–440)	(25–53)
PCB-$TEQ_{WHO\text{-}Avian}$	– (2)	– (2)	31 (5) A	55 (4) A
	–	–	10–92	12–250
	(1.3–1.8)	(30–39)	(13–100)	(24–150)

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

[b] Values have been rounded and represent only two significant figures

[c] N/A = no samples collected from this location

[d] Means identified with the same uppercase letter are not significantly different among reach at the p=0.05 level using Tukey-Kramer means separation test.

[e] – geometric mean and confidence intervals not calculated for sites with fewer than 3 samples. These sites were not included in reach comparisons.

ΣPCDD/DFs in sediments were least in the RA (31 ng/kg, dw), greatest in the UTR (1400 ng/kg, dw), and then decreased downstream in the LTR (960 ng/kg, dw) and the SR (350 ng/kg, dw). Consistent with the aforementioned spatial trends, concentrations of ΣPCDD/DFs were statistically significantly greater in dietary components collected from reaches of the Tittabawassee River compared to the RA. Similarly, concentrations of ΣPCDD/DFs in dietary items from the SR were also greater that their RA collected counterparts, but the differences varied in their significance (Table 3.1). Concentrations of ΣPCDD/DFs were significantly greater in sediment and crayfish collected from the SR compared to those from the RA (p=0.0066 and p=0.0101). In frogs, concentrations of were not significantly different between the RA and SR (p=0.9526).

Trends in concentrations of ΣPCDD/DF were also observed among prey item types. In general, concentrations of ΣPCDD/DF were least in frogs and similar between crayfish and forage fish. Mean ΣPCDD/DF concentrations in frogs were 3- to 4-fold less than in crayfish and forage fish collected from the UTR and LTR and 7- to 9-fold less than in crayfish and forage fish from the SR reach. Concentrations of ΣPCDD/DF were not different among prey items in the RA (Table 3.1).

Spatial trends also occurred in the relative contribution of PCDDs, PCDFs, and individual congeners to ΣPCDD/DF in dietary components. Sediment from the RA had a greater percent contribution of dioxins to ΣPCDD/DF than those collected from the UTR, LTR, and SR reaches (80% compared to 56%, 43%, and 42%, respectively). A similar pattern was observed in prey of GBH, with RA frogs, crayfish, and forage fish having 67%, 61%, and 74%, of their ΣPCDD/DF contributed by dioxins, respectively. In the RA, octachlorodibenzo-*p*-dioxin (OCDD) was consistently the greatest proportion of

ΣPCDD/DF in all dietary components. Furans contributed between 60% to 85% of the ΣPCDD/DF in prey items collected from TR reaches, and 54% to 74% to those collected from the SR. 2,3,7,8-tetrachlorodibenzofuran (TCDF) is the predominant congener contributing to ΣPCDD/DF in prey items (23% to 56%) collected from the UTR, LTR, and SR. These patterns remained constant among the different dietary components (Figure 3.2).

Concentrations of ΣPCBs in prey items followed a different spatial trend than that of ΣPCDD/DFs, being greatest in the SR. Inadequate sample size precluded statistical comparisons among reaches for most prey item taxa. Concentrations of ΣPCBs in forage fish collected from the SR reach were greater than those from the LTR, although the difference was not significant (p=0.4812). PCB congeners 105 and 118 were the predominant congeners present.

DF-TEQ$_{WHO\text{-}Avian}$ and PCB-TEQ$_{WHO\text{-}Avian}$

Concentrations of DF-TEQ$_{WHO\text{-}Avian}$ in prey of GBH were least in the RA, greater in UTR, greatest in LTR, and intermediate in the SR (Table 3.1). This was the same trend observed for concentrations of ΣPCDD/DFs from which they were calculated. Mean concentrations of DF-TEQ$_{WHO\text{-}Avian}$ were 20-, 56-, and 3-fold greater in frogs, 60-, 180-, and 30-fold greater in crayfish, and 170-, 200-, and 36-fold greater in forage fish collected from the UTR, LTR, and SR when compared to the RA, respectively. In sediment, mean concentrations of DF-TEQ$_{WHO\text{-}Avian}$ were 130-, 240-, and 84-fold greater at UTR, LTR, and SR reaches than the RA, respectively. Concentrations of DF-TEQs$_{WHO\text{-}Avian}$ in prey items from the UTR and LTR were statistically greater than those


SEDIMENT
FROGS

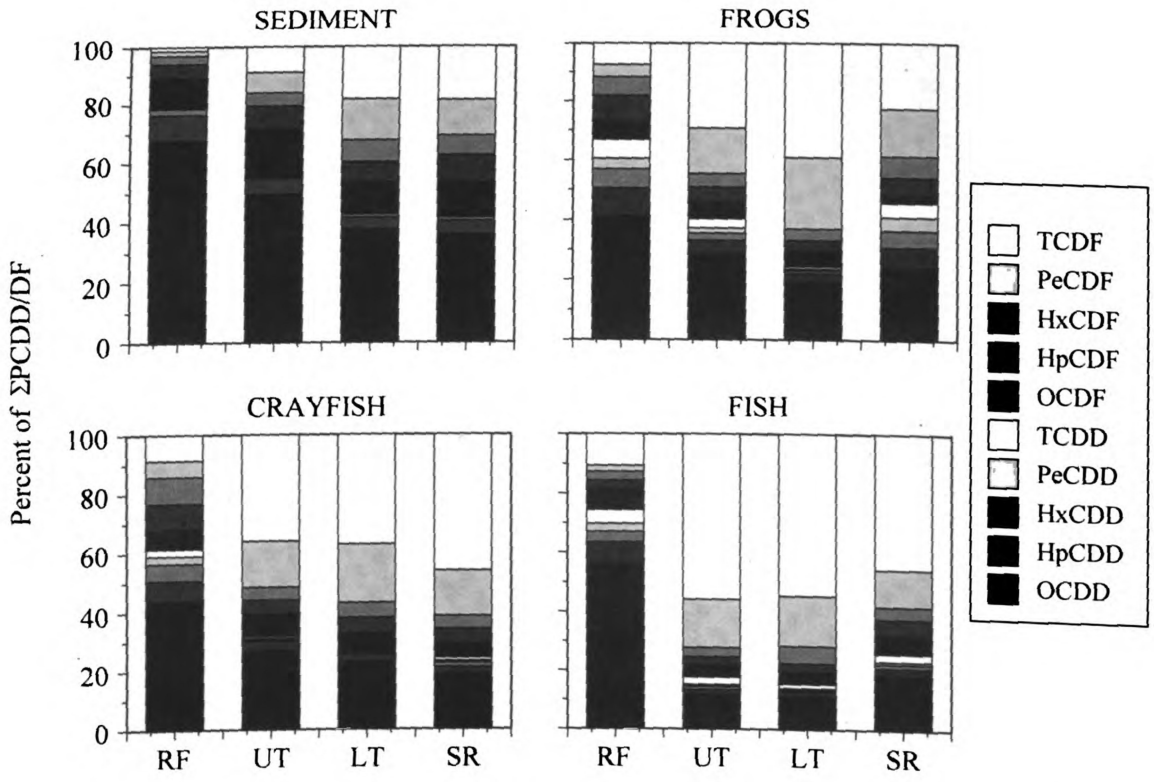


Figure 3.2. Percent mean contribution of individual 2,3,7,8-substituted congeners to ΣPCDD/DF in dietary items collected from reference (RF), Upper Tittabawassee (UT), Lower Tittabawassee (LT), and Saginaw River (SR) reaches.

collected from RA, which was consistent with concentrations of ΣPCDD/DF. Statistical comparisons of the concentrations of DF-$TEQ_{WHO\text{-}Avian}$ in prey from SR to those from RA were also similar to those based on ΣPCDD/DFs, except for crayfish from UTR which were not statistically different from those collected from the SR (p=0.1322).

The relative contribution of compound class and individual congeners to DF-$TEQ_{WHO\text{-}Avian}$ in dietary components also exhibited a spatial trend throughout the study area. Reference area sediment had a greater contribution of dioxins to DF-$TEQ_{WHO\text{-}Avian}$ than those collected from the UTR, LTR, and SR reaches (38% compared to 3.7%, 2.6%, and 3.4%, respectively). Similarly, prey items collected in the RA had a greater contribution of dioxins to DF-$TEQ_{WHO\text{-}Avian}$, primarily from 2,3,7,8-TCDD (13% to 31%) and 1,2,3,7,8-PeCDD (13% to 18%), than those from UTR, LTR, and SR reaches. Furans contributed between 86% to 99% of the DF-$TEQ_{WHO\text{-}Avian}$ in prey items collected from the Tittabawassee River reaches, and 78% to 97% to those collected from the SR. 2,3,7,8-TCDF and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) are the predominant congeners contributing to DF-$TEQ_{WHO\text{-}Avian}$ in prey items (51% to 82% and 12% to 25%, respectively) collected from UTR, LTR, and SR.

Spatial trends are also seen in the relative contribution of DF-$TEQ_{WHO\text{-}Avian}$ and PCB-$TEQ_{WHO\text{-}Avian}$ to total $TEQ_{WHO\text{-}Avian}$ in prey items. In the RA, PCBs accounted for a majority of the total $TEQ_{WHO\text{-}Avian}$ (63%) in fish, compared to fish collected from the Tittabawassee River which have a majority of their total $TEQ_{WHO\text{-}Avian}$ attributed to DF-$TEQ_{WHO\text{-}Avian}$ (81% and 85% for the UTR and LTR, respectively). Total concentrations of $TEQs_{WHO\text{-}Avian}$ were dominated by PCB-$TEQs_{WHO\text{-}Avian}$ in fish collected from the

Saginaw River (61% PCB- $TEQ_{WHO\text{-}Avian}$). PCB-$TEQs_{WHO\text{-}Avian}$ were dominated by congeners PCB-126, PCB-77, andPCB-81 in all reaches.

Dietary exposure

ADD_{pot} for GBH was consistently greatest within reaches of the Tittabawassee River when compared to either the RA or SR, regardless of whether it was based on ΣPCDD/DF, DF-$TEQ_{WHO\text{-}Avian}$, or total $TEQ_{WHO\text{-}Avian}$ (Table 3.2). The ΣPCDD/DF ADD_{pot} to resident GBH was 45- to 54-fold greater along the Tittabawassee River and 12-fold greater along the SR when compared to the RA. When based on DF-$TEQ_{WHO\text{-}Avian}$ fold differences in ADD_{pot} were greater, being 150- to 190-fold greater along the Tittabawassee River and 36-fold great along the SR when compared to the RA. The ADD_{pot} of total $TEQ_{WHO\text{-}Avian}$ based on a 100% fish diet was 75- to 86-fold greater along the Tittabawassee River and 39-fold greater along the SR when compared to the RA.

Dietary-exposure risk characterization

Estimates of dietary exposure based on 100% site use and geometric means of measured concentrations of DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ at Tittabawassee River reaches were greater than the diet-based NOAEC TRV, which resulted in HQs that were slightly greater than 1.0 (Figure 3.3). Maximum calculated HQs along the Tittabawassee River, based on the most conservative parameters of 100% site use and upper 95% upper confidence limit (95% UCL) of measured DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$, were between 1.0 and 10. When based on the more

Table 3.2. Predicted daily dietary dose of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ [a] (ng/kg body weight/d [b, c]) for adult great blue herons breeding during 2004-2006 within the Chippewa, Tittabawassee, and Saginaw river floodplains, Midland, Michigan, USA, based on the geometric mean (95% confidence interval) of site-specific dietary items.

Study Area	ΣPCDD/DFs	DF-$TEQ_{WHO\text{-}Avian}$ [a]	Total $TEQ_{WHO\text{-}Avian}$ [a, d]
Reference [e]			
100% On-site	0.97 (0.79–1.3)	0.17 (0.16–0.18)	0.44 (0.40–0.49)
Upper Tittabawassee River [f, g, h]			
100% On-site	44 (39–52)	26 (23–31)	33 (29–38)
75% On-site	33 (29–39)	20 (17–23)	25 (22–28)
25% On-site	12 (11–14)	6.7 (5.9–7.9)	8.5 (7.5–9.8)
Lower Tittabawassee River [i]			
100% On-site	52 (19–140)	33 (12–87)	38 (14–100)
75% On-site	39 (14–110)	24 (9.3–65)	29 (11–77)
25% On-site	14 (5.3–37)	8.3 (3.2–22)	9.8 (3.8–26)
Saginaw River			
100% On-site	12 (4.8–45)	6.2 (3.5–13)	17 (5.4–51)
75% On-site	9.5 (3.9–35)	4.7 (2.7–9.7)	13 (4.1–38)
25% On-site	3.8 (1.8–12)	1.7 (1.0–3.4)	4.5 (1.6–13)

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

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

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

[d] Total $TEQ_{WHO\text{-}Avian}$ based on a diet of 100% fish due to lack of PCB data in frogs and crayfish

[e] Two fish composite samples collected from Reference reach. Range represents daily dietary dose based on minimum-maximum of fish concentrations.

[f] Off-site diet assumed to be equal to reference area diet.

[g] Upper Tittabawassee River reach includes sites T-3 and T-4

[h] Two fish composite samples collected from Upper Tittabawassee River reach. Range represents daily dietary dose based on minimum-maximum of fish concentrations.

[i] Lower Tittabawassee includes sites T-5, T-6, and S-7

Figure 3.3. Range of hazard quotients for dietary-based exposure of great blue herons to DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ along the Chippewa, Tittabawassee, and Saginaw river floodplains based on assumption of 100% (A) and 75% (B) site-use.

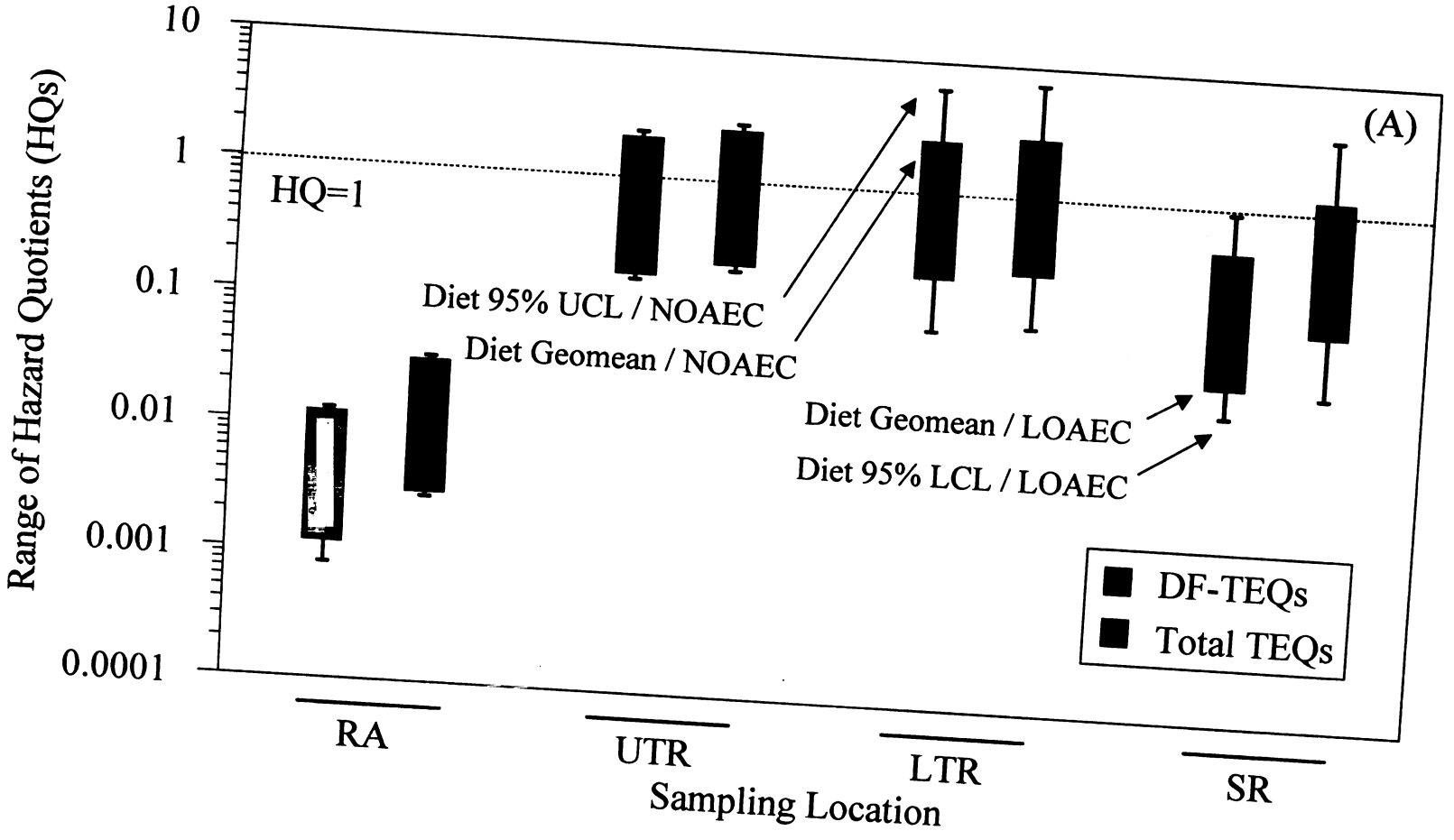
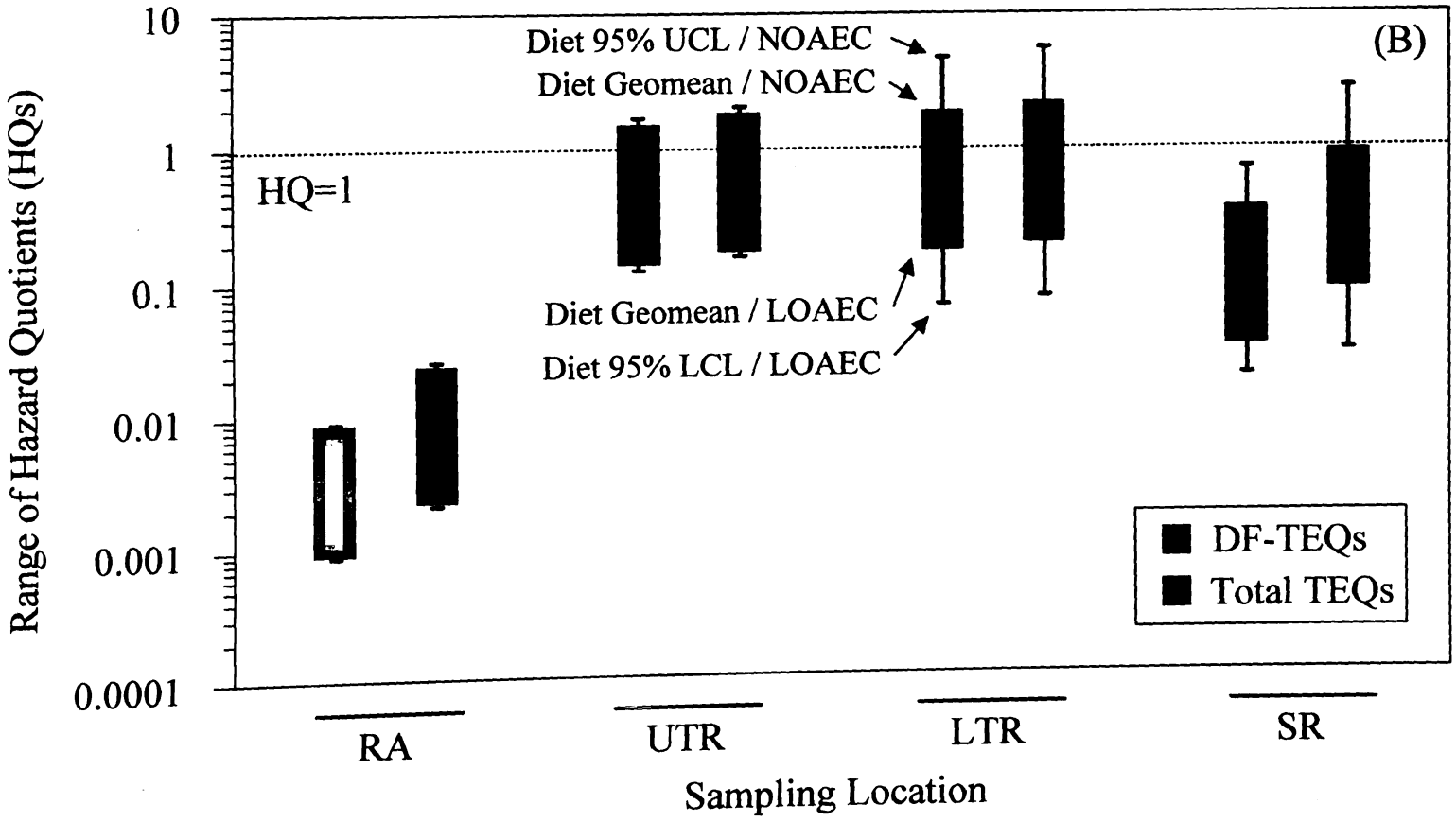

10
1
0.1
0.01
0.001
0.0001
Range of Hazard Quotients (HQs)
HQ=1
(A)
Diet 95% UCL / NOAEC
Diet Geomean / NOAEC
Diet Geomean / LOAEC
Diet 95% LCL / LOAEC
DF-TEQs
Total TEQs
RA
UTR
LTR
SR
Sampling Location

Figure 3.3 (cont'd).



realistic estimate of 75% site use, dietary exposure-based estimates of HQs based on the geometric means of measured DF- $TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ at Tittabawassee River reaches were still greater than the diet-based NOAEC TRV, which resulted in HQs slightly greater than 1.0. Dietary exposure-based estimates based on the 95% UCL of measured concentrations of DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ compared to the diet-based LOAEC TRV never resulted in HQs greater than 1.0, regardless of percent site use or reach.

Discussion

PCDD/DFs and PCBs in GBH prey

The observed trends in concentrations of PCDD/DFs in prey items of GBH were consistent with those observed for dietary items and tissues of other receptor species studied within the SA (Coefield *et al.* 2010; Fredricks *et al.* 2010b; Fredricks *et al.* 2010d; Seston *et al.* 2010b). The pattern observed is likely the result of the downstream movement of this historical contamination from the upstream source. The presence of this trend in predicted GBH dietary exposure is a result of the large proportion of the GBH diet comprising small fish that tend to be young and are more intimately connected to local sediment contamination. As such, GBH foraging in downstream reaches of the TR would be consuming prey items exposed to sediment containing greater concentrations of PCDD/DFs. The direct connection of concentrations of PCDD/DFs in sediment and GBH exposure has previously been demonstrated. A rapid decrease in the concentration of PCDD/DFs in GBH eggs was observed directly following changes in

technologies employed at local pulp mills which decreased PCDD/DF releases (Elliott *et al.* 2001).

The observation that concentrations of PCBs were greater in forage fish from the SR compared to those from the TR is consistent with historical PCB contamination in areas downstream of the TR, including the Saginaw River and Saginaw Bay (Kannan *et al.* 2008) as well as some lesser sources upstream of the RA. Anadromous fishes from Saginaw Bay can move up into the TR, but there is a dam which may impede the movement of these fish into the RA. Furthermore, comparisons between fish and the more spatially confined frogs and crayfish (Hazlett *et al.* 1974; Martof 1953), found that fish generally contained greater concentrations of PCBs than frogs or crayfish collected from within the same reach. This also suggests that fish may be acquiring PCBs from areas other than the TR. Additionally, there may be incidental PCB inputs from the urbanized Midland, MI area.

Dietary exposure

Predictions of dietary exposure integrate measured contaminant body burdens of prey randomly selected at the correct proportions based on designated dietary composition. Concentrations of PCDD/DFs among species were generally spatially consistent while the relative contribution of contaminant compound class and individual congeners were not. The ADD_{pot} of total $TEQ_{WHO\text{-}Avian}$ was 13-21% greater than the ADD_{pot} of DF-$TEQ_{WHO\text{-}Avian}$ along the reaches of the TR, but was 61% and 64% greater in the RA and SR, respectively. The relative proportion of DF-$TEQ_{WHO\text{-}Avian}$ to ΣPCDD/DF for ADD_{pot} of GBH was 18% in the RA, while in the SA they ranged from 59-62% and 52%

for reaches along the TR and SR, respectively. Greater percentages observed along the TR and SR are due to prey items from those reaches having greater relative TCDD potency of ΣPCDD/DF, primarily from 2,3,7,8-TCDF and secondarily from 2,3,4,7,8-PeCDF.

Exposure of GBH through their diet was consistent with spatial trends of concentration of contaminants in sediments and prey items. However, the predicted $ADDs_{pot}$ were greater than would be expected based on published congener-specific biomagnification factors and site-specific concentrations in GBH tissues (Braune and Norstrom 1989; Kubiak *et al.* 1989; Seston *et al.* 2010a; Thomas and Anthony 1999). One plausible explanation based on site-specific observations of GBH foraging and published foraging characteristics is that GBH nesting along the TR forage off-site for a portion of their diet. Adult GBH forage a mean distance between 3.2 km and 6.5 km from rookeries during the breeding season, with a distance as great as 34 km recorded (Dowd and Flake 1985; Peifer 1979; Thompson 1978). The large foraging range of GBH leads to a potential for spending at least a portion of their time foraging off-site, especially early in the breeding season when water levels and turbidity are less than optimal for foraging in the river channel. Therefore, assuming 100% site-use is likely overestimating percentage of time resident GBH are foraging within the TR floodplain. As such, the dietary exposure estimates based on 75% site-use presented are likely more realistic.

The relative contribution of individual congeners was similar between site-specific prey items and GBH tissues. 2,3,7,8-TCDF was the predominant congener present in prey items sampled from the SA, with 2,3,4,7,8-PeCDF also having a significant

presence. In GBH tissues, the overall congener profile was similar to that observed in prey items, although there was a change in the predominant congener from 2,3,7,8-TCDF to 2,3,4,7,8-PeCDF. This pattern was also observed in other species foraging in the SA, including great horned owls, belted kingfishers, and several passerine species (Coefield *et al.* 2010; Fredricks *et al.* 2010a; Fredricks *et al.* 2010c; Seston *et al.* 2010b). Field studies have reported negligible bioaccumulation of 2,3,7,8-TCDF from prey by Forster's terns and herring gulls (Braune and Norstrom 1989; Kubiak *et al.* 1989). This may be due to preferential metabolism of 2,3,7,8-TCDF. A number of studies have reported similar clearance by various avian species exposed to mixtures of AhR-active compounds (Elliott *et al.* 1996; Kubota *et al.* 2005; Senthil Kumar *et al.* 2002). Data on avian toxicokinetics from controlled laboratory studies are limited, however, mammalian studies have shown the rate of metabolism of 2,3,7,8-TCDF to be elevated with increased concentrations of dioxin-like compounds and the subsequent induction of cytochrome P450 1A1 and/or 1A2, while 2,3,4,7,8-PeCDF is preferentially sequestered in the liver (van den Berg *et al.* 1994b; Zwiernik *et al.* 2008). This difference in toxicokinetics is a likely explanation for the observed difference in the relative contribution of the two furan congeners from diet to tissue.

Dietary-exposure risk characterization

Dietary exposures of GBH along the TR are not expected to result in adverse population-level effects. Maximum calculated HQs along the Tittabawassee River, based on the most conservative parameters of 100% site use and 95% UCL of measured DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$, remained between 1.0 and 10. Estimates of

dietary exposure based on the 95% UCL of measured concentrations of DF-$TEQs_{WHO\text{-}Avian}$ or total $TEQs_{WHO\text{-}Avian}$ in prey were never greater than the TRV based on the LOAEC, regardless of site use factor or reach. The actual threshold concentration at which effects would be expected to occur lies somewhere within the range delimited by the NOAEC and LOAEC, which are a function of the experimental design of the study from which they were derived. Since only a small portion of the range for GBH dietary exposure exceeds an HQ of 1.0, it is possible that none of the dietary exposures predicted here exceed the actual effect threshold concentration. However, forage fish, which were the primary component of the GBH diet, were composites of all individuals captured per location per sampling period. This provided an accurate estimate of the central tendency of concentration estimates in forage fish but limited characterization of the variability of concentrations among all forage fish at each sampling location. Thus, the upper end estimates of dietary exposure presented here, and the risk associated with that exposure, may be an underestimate of dietary exposure.

Uncertainty assessment

Concern associated with site-specific adverse effects can be assessed by using the HQ approach or a more probabilistic approach when data permits; however both of these processes are influenced by the selection of TRVs. The selection of TRVs often introduces significant uncertainty to hazard or risk assessments, especially in cases where comprehensive site-specific data sets describe exposure with great certainty. HQs greater than 1.0 are indicative of exposures that exceed the threshold for adverse effects and that there is the potential for effects to occur. Such assessments are commonly conservative

when extrapolating from individuals to populations, as they are based on assumptions of maximal exposure and do not account for compensatory mechanisms associated with wild populations (Fairbrother 2001). Conversely, the limited number of endpoints generally measured in laboratory studies may not capture an effect that could have implications at the population-level. Thus, there is uncertainty inherent in the HQ approach, meaning that HQ values greater than 1.0 do not necessarily translate into population-level or ecologically relevant adverse effects (Blankenship *et al.* 2008). Probabilistic exposure assessment might more accurately describe the exposure distribution; however the assessment of risk is only as accurate as the certainty associated with applicability of the TRV to the population-level dose response curve.

Laboratory studies of effects from dietary exposure to PCDD/DFs are limited for avian species. The TRV selected for use in this assessment was derived from a study which dosed adult hen ring-necked pheasants (*Phasianus colchicus*) with TCDD through intraperitoneal (IP) injection (Nosek *et al.* 1992). There are a few factors that make the use of this TRV conservative in determining the potential hazard of these compounds to GBH. Firstly, in Nosek *et al.* (1992) the hens were exposed to TCDD via IP injection versus a true dietary-based exposure, potentially resulting in greater exposure efficiency than that of gut transfer. Dietary exposure also allows for an increased potential for metabolism and excretion prior to reaching target tissues. Additionally, this study was not conducted on a piscivorous species, but on a member of Galliformes, which are generally considered to have greater sensitivity to dioxin-like compound exposures (Brunström 1988; Brunström and Reutergardh 1986; Powell *et al.* 1996b; Powell *et al.* 1997). Variability in sensitivity among species has been attributed to differences in the

amino acid sequence of the ligand binding domain of the AhR (Head *et al.* 2008; Karchner *et al.* 2006) with ring-necked pheasants having the construct which classifies them as moderately sensitive whereas the GBH construct classifies them as insensitive. Induction of 7-ethoxyresorufin-*O*-deethylase (EROD) activity was 10-fold greater following exposure to TCDD in the ring-necked pheasant than in the double-crested cormorant, a piscivorous species with the same AhR construct as the GBH. Furthermore, the dosing was done with only a single congener (TCDD), whereas the GBH being assessed are exposed to a complex mixture of dioxin-like compounds. Although previously assumed to be equitable in toxicity when normalized using $TEFs_{WHO\text{-}Avian}$, findings from recent studies investigating the relative potencies of individual PCDD/DF congeners in avian species suggest that this may not hold true in every exposure scenario (Hervé *et al.* 2010a; Hervé *et al.* 2010b).

Multiple lines of evidence

To increase the certainty of the conclusions drawn from the risk assessment of GBH along the TR floodplain, exposure of GBH to PCDD/DFs was assessed using both a "bottom-up" and "top-down" approach (Leonards *et al.* 2008). The "bottom-up" approach is exemplified by the dietary exposure approach presented here. The "top-down" approach was done by assessing the potential for effects based on site-specific concentrations of PCDD/DFs in eggs and blood plasma of GBH nesting along the TR (Seston *et al.*, 2010a). Estimates of potential for effects based on these two measures of exposure were compared to one another and also to a site-specific assessment of GBH population condition. Trends in concentrations of PCDD/DFs and the relative

contribution of individual congeners were consistent between the "bottom-up" and "top-down" assessments conducted for GBH nesting along the TR. The results of all three approaches in this multiple lines of evidence approach were consistent. Based on comparisons to TRVs from the literature, each line of evidence based on different estimates of exposure suggests that GBH foraging and breeding along the TR floodplain are at minimal to no risk of experiencing adverse population-level effects as a result of their exposure to PCDD/DFs. This was supported by the site-specific assessment of GBH population condition (Seston *et al.* 2010a).

Conclusions

The findings of this study predict that GBH foraging along the TR are more greatly exposed to PCDD/DFs than those in associated reference areas. This prediction is consistent with spatial trends observed in concentrations of PCDD/DFs in site-specific GBH tissues. However, concentrations of PCDD/DFs in eggs of GBH collected from rookeries within the TR floodplain were lesser than what would be expected given the dietary exposure predicted here. This is likely a result of GBH foraging outside of the assessment area. Direct foraging by GBH within the river corridor was established through visual observations and trapping of GBH. However, GBH can fly substantial distances from their rookery and adverse water conditions associated with frequent spring episodic rain events would be expected to preclude foraging within the river. Field researchers noted an absence of GBH foraging associated with adverse foraging conditions; however these observations were limited to the river corridor. Therefore, it is likely that during these times GBH were foraging in areas unassociated with the

assessment area. Site-specific visual observations and published BMFs suggest that the dietary exposure predictions presented here are likely an overestimate of their actual exposure. Despite this potential overestimation, the predicted level of exposure of GBH to PCDD/DFs has a low probability of causing adverse population level effects, based on TRVs available in the literature. This conclusion is supported by findings of site-specific assessments of GBH tissue exposure and population condition.

Acknowledgements

The authors would like to thank all staff and students of the Michigan State University – Wildlife Toxicology Laboratory field crew; namely M. Nadeau, W. Folland and D. Hamman, along with E. Koppel, J. Moore, L. Williams, and C. Bartrem. We gratefully acknowledge M. Fales for his design and fabrication of specialized equipment which was pivotal to the success of this research. Additionally, we would like to recognize M. Kramer and N. Ikeda for their assistance in the laboratory. The following people were key to the success of the project, J. Dastyck and S. Kahl of the USFWS – Shiawassee National Wildlife Refuge for their assistance and access to the refuge; Saginaw County Parks and Recreation Commission for access to Imerman Park; Tittabawassee Township Park Rangers for access to Tittabawassee Township Park and Freeland Festival Park; and Michael Bishop of Alma College for guidance as our Master Bander. We would also like to acknowledge the greater than 50 cooperating landowners throughout the study area who have granted us access to their property, making our research possible. Funding was provided through an unrestricted grant from The Dow

Chemical Company, Midland, Michigan to J. Giesy and M. Zwiernik of Michigan State University.

Animal Use

All aspects of this study that involved the use of animals were conducted using the most humane means possible. To achieve that objective, all aspects of the study were performed following standard operation procedures (GBH adult handling 03/05-036-00; GBH nest monitoring 05/07-066-00; Field studies in support of TR ERA 03/04-042-00; Protocol for fish sampling 03/04-043-00) 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 SC1254 for GBH/SC permit for fish (Zwiernik)/SC permit for amphibians (Zwiernik); USFWS Migratory Bird Scientific Collection Permit MB1000062-0; and subpermitted under US Department of the Interior Federal Banding Permit 22926) are on file at MSU-WTL.

Su

Ta
20
Ch

Supplemental Information

Table 3.3. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in sediment collected during 2003-2006 from the Chippewa/Pine, Tittabawassee, and Saginaw Rivers, Midland, MI, USA. Values[a] (ng/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	RA *n*=16	UTR *n*=23	LTR *n*=19	SR *n*=8
2378-TCDF	0.80±1.0	260±490	630±960	290±450
	0.039–3.4	10–2100	1.8–3500	2.5–1300
	4ND			
23478-PeCDF	0.43±0.42	87±150	250±460	130±210
	0.073–1.3	3.1–560	0.31–1900	0.57–610
	3ND			
12378-PeCDF	0.56±0.55	110±170	350±750	180±290
	0.083–1.8	3.4–570	0.43–3300	0.81–860
	7ND			
234678-HxCDF	0.51±0.25	6.9±8.9	22±40	23±18
	0.14–0.82	0.36–28	0.69–160	6.9–48
	11ND		2ND	4ND
123789-HxCDF		1.9±2.3	5.2±9.4	21±24
		0.21–7.3	0.24–37	2.2–48
	16ND	5ND	4ND	5ND
123678-HxCDF	0.70±0.50	16±22	58±120	53±65
	0.14–1.5	1.7–130	1.6–520	0.34–170
	9ND		2ND	2ND
123478-HxCDF	1.3±1.4	87±120	260±560	150±240
	0.15–4.5	4.8–390	0.26–2400	0.54–710
	5ND		1ND	2ND
1234789-HpCDF	0.62±0.25	17±28	19±33	38±21
	0.23–40	1.7–130	1.2–140	18–67
	10ND		2ND	4ND
1234678-HpCDF	4.3±6.6	210±410	120±110	370±450
	0.16–22	19–1900	0.31–480	0.38–1200
12346789-OCDF	9.2±13	730±2100	230±180	520±510
	0.22–40	25–10000	22–620	0.39–1200
	1ND		2ND	1ND

Table 3.3 (cont'd)

2378-TCDD	0.26±0.16	2.0±1.9	1.5±0.91	6.9±7.2
	0.086–0.52	0.26–7.5	0.15–3.2	0.079–18
	7ND	1ND		1ND
12378-PeCDD	0.28±0.16	110±170	1.3±0.68	9.7±4.2
	0.099–0.50	3.4–170	0.33–2.8	4.5–13
	8ND		2ND	4ND
123789-HxCDD	0.60±0.38	3.2±3.9	1.9±1.2	11±4.8
	0.13–1.1	0.48–16	0.44–4.9	5.4–15
	9ND		2ND	4ND
123678-HxCDD	0.82±0.69	10±20	4.4±3.0	26±10
	0.16–1.8	0.81–96	0.95–13	15–37
	8ND		2ND	4ND
123478-HxCDD	0.35±0.13	1.3±1.4	0.85±0.53	3.2±1.3
	0.13–0.43	0.19–5.2	0.21–1.9	1.2–4.1
	11ND		4ND	4ND
1234678-HpCDD	6.8±10	200±500	64±51	140±160
	0.34–30	11–2400	0.35–190	0.30–350
12346789-OCDD	64±97	1900±4800	630±500	1300±1400
	2.0–280	92–23000	3.0–1800	1.7–3100
Sum PCDD/DF	88±130	3600±8200	2600±3400	3100±3700
	2.9–390	280–40000	6.4–14000	9.7–9700

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 3.4. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in frogs collected during 2005-2006 from the within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (ng/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	RA *n*=29	UTR *n*=51	LTR *n*=55	SR *n*=12
2378-TCDF	0.44±0.47 0.076–2.2 2ND	42±68 0.34–370	86±190 5.1–1400	1.6±0.93 0.70–3.5
23478-PeCDF	0.20±0.10 0.081–0.49 9ND	11±14 0.21–69	30±64 1.9–450	1.3±2.7 0.33–9.7
12378-PeCDF	0.19±0.079 0.10–0.28 24ND	8.0±13 0.080–75	26±100 1.3–770	0.33±0.16 0.17–0.60
234678-HxCDF	0.13–0.18 27ND	0.65±0.61 0.15–2.8 24ND	1.7±5.9 0.11–37 16ND 5.8	0.57 11ND
123789-HxCDF	29ND	51ND 1.0±1.5	54ND 2.5±11	12ND
123678-HxCDF	0.11–0.16 27ND	0.11–7.4 14ND	0.087–76 4ND	0.14–1.1 10ND
123478-HxCDF	0.20±0.088 0.092–0.34 20ND	3.5±6.7 0.16–33 8ND	10±50 0.27–370	0.89±1.2 0.27–3.0 7ND
1234789-HpCDF	29ND 0.49±0.29 0.16–0.92 24ND	1.2±0.93 0.20–2.6 42ND 6.3±10 0.23–42 10ND	1.9±3.8 0.23–14 43ND 5.6±8.3 0.29–50	12ND 0.37±0.29 0.19–1.3
1234678-HpCDF	0.72±0.35 0.38–1.2 21ND	10±18 0.22–72 10ND	9.9±15 0.21–67 2ND	0.30 11ND
12346789-OCDF	0.43±0.28 0.12–1.1	1.3±1.1 0.27–7.4	0.96±0.77 0.20–4.1	0.35±0.13 0.20–0.58
2378-TCDD				

Table 3.4 (cont'd)

12378-PeCDD	4ND 0.25±0.13 0.12–0.55	0.66±0.44 0.20–2.7	1ND 0.57±0.32 0.18–1.6	1ND 0.37±0.21 0.18–0.91
123789-HxCDD	9ND 0.18	1ND 0.30±0.15 0.13–0.70	3ND 0.29±0.081 0.17–0.41	1ND
123678-HxCDD	28ND 0.30±0.12 0.16–0.43	33ND 0.74±0.61 0.14–2.6	47ND 0.72±0.48 0.23–2.2	12ND 0.37±0.33 0.13–1.1
123478-HxCDD	24ND	5ND 0.30±0.12 0.079–0.59	13ND 0.26±0.077 0.19–0.42	4ND 0.54
1234678-HpCDD	29ND 0.68±0.57 0.22–2.8	23ND 4.0±5.8 0.29–28	43ND 4.0±4.2 0.45–22	11ND 0.53±0.39 0.20–1.7
12346789-OCDD	1ND 3.2±3.4 0.57–13	31±56 1.3–250	31±40 2.6–1220	1.5±0.51 0.67–2.3
Sum PCDD/DF	5.4±4.8 0.96–19	120±180 3.5–920	210±450 17–3300	7.2±6.1 2.8–25

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 3.5. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in crayfish collected during 2005-2006 from the within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (ng/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	RA *n*=5	UTR *n*=7	LTR *n*=8	SR *n*=8
2378-TCDF	0.55±0.44 0.19–1.3	60±53 7.4–160	140±74 59–300	25±13 10–51
23478-PeCDF	0.28±0.23 0.051–0.59 1ND	12±9.1 2.6–26	35±27 12–96	3.7±1.8 0.33–9.7
12378-PeCDF	0.031–0.45 3ND	16±14 2.2–39 1ND	44±29 16–110	4.4±2.5 1.9–8.2
234678-HxCDF	0.018–0.18 3ND	0.43±0.26 0.20–0.77 3ND	1.7±1.6 0.44–4.9 1ND	
123789-HxCDF	5ND	0.064–0.12 5ND	0.48±0.38 0.19–0.91 5ND	8ND
123678-HxCDF	0.019–0.41 3ND	0.97±0.46 0.47–1.7	3.9±3.8 1.2–13	0.50±0.24 0.25–0.72 5ND
123478-HxCDF	0.56±0.45 0.048–0.89 2ND	5.2±2.6 1.9–8.7	19±17 5.3–57	1.3±0.64 0.76–2.5
1234789-HpCDF	5ND	0.71±0.52 0.21–1.4 3ND	1.7±1.2 0.64–3.6 3ND	8ND
1234678-HpCDF	0.47±0.32 0.11–0.68 2ND	7.8±9.3 2.3–28	26±25 3.3–64	2.8±2.3 0.77–7.1
12346789-OCDF	0.36±0.20 0.17–0.57 1ND	13±16 1.8–48	31±31 3.5–87	2.7±2.0 0.73–6.6
2378-TCDD	0.26±0.18 0.056–0.37	0.89±0.19 0.54–1.1	1.3±0.34 0.84–1.8	0.91±0.65 0.35–2.1

Table 3.5 (cont'd)

12378-PeCDD	2ND 0.23±0.20 0.030–0.44	0.49±0.15 0.30–0.65	0.86±0.38 0.44–1.6	2ND 0.49±0.19 0.25–0.71
123789-HxCDD	2ND	0.25±0.12 0.15–0.39	0.19–1.6	4ND
123678-HxCDD	5ND	4ND 0.59±0.34 0.24–1.1	6ND 1.4±1.3 0.22–3.7	8ND 0.36
123478-HxCDD	5ND	3ND 0.14±0.049 0.11–0.20	2ND 0.14–0.90	7ND
1234678-HpCDD	5ND 0.42±0.32 0.18–0.83	4ND 5.0±5.8 1.4–18	6ND 15±19 1.9–58	8ND 1.4±0.86 0.52–2.9
12346789-OCDD	2.8±2.1 0.88–5.4	44±54 8.1–170	140±170 14–520	10±6.8 2.7–22
Sum PCDD/DF	5.4±4.6 1.5–11	160±100 83–340	450±380 140–1300	52±27 27–110

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 3.6. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in forage fish composites collected during 2004-2007 from the within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (ng/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	RA *n*=2	UTR *n*=2	LTR *n*=5	SR *n*=4
2378-TCDF			170±110	27±8.4
	0.47–0.49	99–140	53–300	21–40
23478-PeCDF			35±30	4.5±2.6
	0.058–0.077	19–22	5.9–78	2.1–8.0
12378-PeCDF			31±30	4.0±3.0
		14–14	4.3–78	2.0–8.3
	2ND			
234678-HxCDF			2.9±2.6	0.18±0.21
		0.82–0.91	0.35–6.3	0.0080–0.42
	2ND		1ND	1ND
123789-HxCDF				
			0.38–0.65	
	2ND	2ND	3ND	4ND
123678-HxCDF	0.055		4.5±5.2	0.55±0.53
		1.5–1.5	0.21–13	0.15–1.3
	1ND			
123478-HxCDF			19±24	2.0±2.0
		3.1–5.2	0.84–58	0.54–4.9
	2ND			
1234789-HpCDF			1.3±1.1	0.19±0.15
		0.50–0.54	0.26–2.6	0.063–0.13
	2ND		1ND	1ND
1234678-HpCDF			8.2±4.5	3.7±2.6
	0.072–0.092	5.1–5.5	2.8–13	1.3–7.1
12346789-OCDF			12±8.1	4.5±3.9
	0.29–0.35	6.5–11	3.0–25	1.3–9.8
2378-TCDD			4.6±2.6	1.7±0.96
	0.20–0.23	4.7–6.0	1.4–10	0.94–3.0

Table 3.6 (cont'd)

12378-PeCDD			1.2±0.65	0.52±0.26
	0.095–0.13	1.2–1.6	0.33–1.9	0.28–0.84
123789-HxCDD			0.33	
	2ND	0.12–0.49		0.063–0.84
123678-HxCDD			4ND	2ND
	2ND	0.88–1.5	1.0±0.49 0.39–1.6	0.55±0.31 0.21–0.85
123478-HxCDD			1ND	
	2ND	0.52–1.0	1.2±0.19 0.99–1.4	0.040
1234678-HpCDD			2ND	3ND
	0.33–0.34	3.6–4.1	5.0±3.2 1.4–10	1.9±1.2 0.80–3.5
12346789-OCDD			31±14	13±11
	2.1–3.0	20–28	8.3–44	4.2–28
Sum PCDD/DF			330±220	64±36
	3.8–4.6	200–220	81–610	36–120

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 3.7. Concentrations of twelve dioxin-like polychlorinated biphenyl congeners in forage fish composites collected during 2004-2007 from the within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (µg/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	RA *n*=2	UTR *n*=2	LTR *n*=5	SR *n*=4
PCB 77			0.28±0.16	1.0±0.95
	0.012–0.016	0.36–0.47	0.12–0.51	0.22–2.2
PCB 81			0.17±0.20	0.18±0.12
	0.0042–0.0059	0.084–0.11	0.043–0.52	0.084–0.33
PCB 126			0.95±0.13	0.061±0.032
	0.0026–0.0033	0.031–0.042	0.017–0.33	0.033–0.099
PCB 169			0.0089±0.010	
			0.0026–0.021	
	2ND	2ND	2ND	4ND
PCB 105			7.5±10	6.0±3.1
	0.17–0.22	1.8–3.3	1.6–26	3.3–9.8
PCB 114			0.61±0.85	0.55±0.34
	0.012–0.016	0.14–0.27	0.12–2.1	0.26–0.95
PCB 118			20±26	18±9.7
	0.52–0.62	5.7–9.0	4.3–66	9.0–29
PCB 123			0.56±0.73	0.43±0.26
	0.020–0.022	0.18–0.29	0.097–1.9	0.21–0.74
PCB 156			2.1±3.0	1.3±0.46
	0.069–0.077	0.56–0.84	0.45–7.5	0.89–1.9
PCB 157			0.45±0.63	0.27±0.085
	0.015–0.017	0.14–0.19	0.098–1.6	0.19–0.37
PCB 167			0.94±1.3	0.65±0.21
	0.040–0.040	0.34–0.41	0.23–3.2	0.48–0.91
PCB 189			0.22±0.33	0.13±0.055
	0.0074–0.0087	0.59–0.88	0.049–0.80	0.079–0.19
Sum PCB			33±44	28±15
	0.88–1.0	9.3–15	7.1–110	15–47

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

Table 3.8. Concentrations of twelve dioxin-like polychlorinated biphenyl congeners in frogs and crayfish collected during 2004-2007 from the within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (µg/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	Frog LTR n=4	Crayfish UTR n=2	Crayfish LTR n=1
PCB 77	0.011±0.0055		0.086
	0.004–0.016	0.050–0.063	
PCB 81	0.0039±0.0013		0.0035
	0.0024–0.0054	0.0027–0.0031	
PCB 126	0.0057±0.0014		0.0057
	0.0037–0.0072	0.0040–0.0073	
PCB 169	0.0048±0.0017		0.0040
	0.0030–0.0070	0.0050–0.0080	
PCB 105	0.14±0.080		0.43
	0.050–0.24	0.27–0.58	
PCB 114	4ND	2ND	1ND
PCB 118	0.81±0.38		0.92
	0.40–1.1	1.0–1.4	
PCB 123	4ND	2ND	1ND
PCB 156	0.29±0.089	0.86	0.44
	0.20–0.41	1ND	
PCB 157	4ND	2ND	1ND
PCB 167	0.14±0.090	0.16	0.20
	0.033–0.20	1ND	
PCB 189	4ND	2ND	1ND
Sum PCB	1.4±0.34		2.1
	0.94–1.7	2.2–2.2	

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

References

Alexander, G. R. (1977). Food of vertebrate predators on trout waters in north central lower Michigan. *Michigan Academician* **10**, 181-195.

Allred, P. M., and Strange, J. R. (1977). The effects of 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on developing chicken embryos. *Archives of Environmental Contamination and Toxicology* **6**, 483-489.

Blankenship, A. L., Kay, D. P., Zwiernik, M. J., Holem, R. R., Newsted, J. L., Hecker, M., and Giesy, J. P. (2008). Toxicity reference values for mink exposed to 2,3,7,8-tetrachlodibenzo-*p*-dioxin (TCDD) equivalents (TEQs). *Ecotoxicology and Environmental Safety* **69**(3), 325-349.

Braune, B. M., and Norstrom, R. J. (1989). Dynamics of organochlorine compounds in herring gulls: III. Tissue distribution and bioaccumulation in Lake Ontario gulls. *Environmental Toxicology and Chemistry* **8**(10), 957-968.

Brunström, B. (1988). Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. *Poultry Science* **67**(1), 52-57.

Brunström, B., and Andersson, L. (1988). Toxicity and 7-ethoxyresorufin O-deethylase-inducing potency of coplanar polychlorinated-biphenyls (PCBs) in chick-embryos. *Archives of Toxicology* **62**(4), 263-266.

Brunström, B., and Reutergardh, L. (1986). Differences in sensitivity of some avian species to the embryotoxicity of a PCB, 3,3',4,4'-tetrachlorobiphenyl, injected into the eggs. *Environmental Pollution Series A-Ecological and Biological* **42**(1), 37-45.

Butler, R. W. (1992). Great blue heron. A. Poole, P. Stettenheim, & F. Gill (Eds.), *The Birds of North America*, No. 25. Philadelphia: The Academy of Natural Sciences; Washington, DC: The American Ornithologists's Union.

Butler, R. W. (1997). The Great Blue Heron: A Natural History and Ecology of a Seashore Sentinel, UBC Press, Vancouver, BC.

Champoux, L., Rodrigue, J., Desgranges, J., Trudeau, S., Hontela, A., Boily, M., and Spear, P. (2002). Assessment of contamination and biomarker responses in two species of herons on the St. Lawrence River. *Environmental Monitoring and Assessment* **79**, 193-215.

Coefield, S. J., Zwiernik, M. J., Fredricks, T. B., Seston, R. M., Nadeau, M. W., Tazelaar, D. L., Kay, D. P., Newsted, J. L., and Giesy, J. P. (2010). Ecological risk assessment of great horned owls (*Bubo virginianus*) exposed to PCDDs/PCDFs in the Tittabawassee River floodplain in Midland, Michigan, USA. *Environmental Toxicology and Chemistry* (in press).

Custer, T. W., Hines, R. K., Melancon, M. J., Hoffman, D. J., Wickliffe, J. K., Bickham, J. W., Martin, J. W., and Henshel, D. S. (1997). Contaminant concentrations and biomarker response in great blue heron eggs from 10 colonies on the upper Mississippi River, USA. *Environmental Toxicology and Chemistry* **16**(2), 260-271.

Custer, T. W., Rattner, B. A., Ohlendorf, H. F., and Melancon, M. J. (1991). Herons and egrets as proposed indicators of estuarine contamination in the United States. *Acta XX Congressus Internationalis Ornithologici* **20**, 2474-2479.

Dowd, E. M., and Flake, L. D. (1985). Foraging habitats and movements of nesting great blue herons in a prairie river ecosystem, South Dakota. *Journal of Field Ornithology* **56**(4), 379-387.

Elliott, J. E., Harris, M. L., Wilson, L. K., Whitehead, P. E., and Norstrom, R. J. (2001). Monitoring temporal and spatial trends in polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in eggs of great blue heron (*Ardea herodias*) on the coast of British Columbia, Canada, 1983-1998. *Ambio* **30**(7), 416-428.

Elliott, J. E., Wilson, L. K., Langelier, K. W., and Norstrom, R. J. (1996). Bald eagle morality and chlorinated hydrocarbon contaminants in livers from British Columbia, Canada, 1989-1994. *Environmental Pollution* **94**(1), 9-18.

Fairbrother, A. (2003). Lines of evidence in wildlife risk assessments. *Human and Ecological Risk Assessment* **9**, 1475-1491.

Fairbrother, A. (2001). Putting the impacts of environmental contamination into perspective. In *Ecotoxicology of Wild Mammals* (R.F.Shore and B.A.Rattner, Eds.), pp. 671-689. John Wiley & Sons Ltd., West Sussex, England.

Fredricks, T. B., Giesy, J. P., Coefield, S. J., Seston, R. M., Haswell, M. M., Tazelaar, D. L., Bradley, P. W., Moore, J. N., Roark, S. A., and Zwiernik, M. J. (2010a). Dietary exposure of three passerine species to PCDD/DFs from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. *Environmental Monitoring and Assessment*: available online DOI: 110.1007/s10661-010-1319-5.

Fredricks, T. B., Giesy, J. P., Coefield, S. J., Seston, R. M., Tazelaar, D. L., Roark, S. A., Kay, D. P., Newsted, J. L., and Zwiernik, M. J. (2010b). Multiple lines of evidence risk assessment of terrestrial passerines exposed to PCDFs and PCDDs in the Tittabawassee River floodplain, Midland, Michigan, USA. *Human and Ecological Risk Assessment* (in press).

Fredricks, T. B., Zwiernik, M. J., Seston, R. M., Coefield, S. J., Plautz, S. C., Tazelaar, D. L., Shotwell, M. S., Bradley, P. W., Kay, D. P., and Giesy, J. P. (2010c).

Passerine exposure to primarily PCDFs and PCDDs in the river floodplains near Midland, Michigan, USA. *Archives of Environmental Contamination and Toxicology* **58**, 1048-1064.

Fredricks, T. B., Zwiernik, M. J., Seston, R. M., Coefield, S. J., Tazelaar, D. L., Roark, S. A., Kay, D. P., Newsted, J. L., and Giesy, J. P. (2010d). Multiple lines of evidence risk assessment of tree swallows exposed to PCDFs and PCDDs in the Tittabawassee River floodplain, Midland, Michigan, USA. *Environmental Toxicology and Chemistry* (in review).

Galbraith Environmental Sciences LLC. Tittabawassee River Aquatic Ecological Risk Assessment. 2003. Newfane, Vermont.

Gilbertson, M. (1983). Etiology of chick edema disease in herring gulls in the lower Great Lakes. *Chemosphere* **12**(3), 357-370.

Harris, M. L., Elliott, J. E., Butler, R. W., and Wilson, L. K. (2003). Reproductive success and chlorinated hydrocarbon contamination of resident great blue herons (*Ardea herodias*) from coastal British Columbia, Canada, 1977 to 2000. *Environmental Pollution* **121**, 207-227.

Hazlett, B., Rittschof, D., and Rubenstein, D. (1974). Behavioral biology of the crayfish *Orconectes virilis* I. Home range. *American Midland Naturalist* **92**(2), 301-319.

Head, J. A., Hahn, M. E., and Kennedy, S. W. (2008). Key amino acids in the aryl hydrocarbon receptor predict dioxin sensitivity in avian species. *Environmental Science & Technology* **42**(19), 7535-7541.

Henning, M. H., Shear Weinberg, N. M., Wilson, N. D., and Iannuzzi, T. J. (1999). Distribution for key exposure factors controlling the uptake of xenobiotic chemicals by great blue herons (*Ardea herodias*) through ingestion of fish. *Human and Ecological Risk Assessment* **5**(1), 125-144.

Hervé, J. C., Crump, D., Jones, S. P., Mundy, L. J., Giesy, J. P., Zwiernik, M. J., Bursian, S. J., Jones, P. D., Wiseman, S. B., Wan, Y., and Kennedy, S. W. (2010a). Cytochrome P4501A induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and two chlorinated dibenzofurans in primary hepatocyte cultures of three avian species. *Toxicological Sciences* **113**(2), 380-391.

Hervé, J. C., Crump, D., Mclaren, K., Giesy, J. P., Zwiernik, M. J., Bursian, S. J., and Kennedy, S. W. (2010b). Relative potencies of dioxin-like chemicals in primary cultures of herring gull hepatocytes: 2,3,4,7,8-PeCDF is a more potent CYP1A inducer than 2,3,7,8-TCDD. *Environmental Toxicology and Chemistry*; available online 10.1002/etc.255.

Hilscherova, K., Kannan, K., Nakata, H., Hanari, N., Yamashita, N., Bradley, P. W., McCabe, J. M., Taylor, A. B., and Giesy, J. P. (2003). Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. *Environmental Science and Technology* **37**(3), 468-474.

Hoffman, D. J., Rattner, B. A., Sileo, L., Docherty, D. E., and Kubiak, T. J. (1987). Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. *Environmental Research* **42**, 176-184.

Kannan, K., Yun, S., Ostaszewski, A., McCabe, J., MacKenzie-Taylor, D., and Taylor, A. (2008). Dioxin-like toxicity in the Saginaw River watershed: Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in sediments and floodplain soils from the Saginaw and Shiawassee Rivers and Saginaw Bay, Michigan, USA. *Archives of Environmental Contamination and Toxicology* **54**(1), 9-19.

Karchner, S. I., Franks, D. G., Kennedy, S. W., and Hahn, M. E. (2006). The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor. *PNAS* **103**(16), 6252-6257.

Kubiak, T. J., Harris, H. J., Smith, L. M., Schwartz, T. R., Stalling, D. L., Trick, J. A., Sileo, L., Docherty, D., and Erdman, T. C. (1989). Microcontaminants and reproductive impairment of the Forster's tern in Green Bay, Lake Michigan-1983. *Archives of Environmental Contamination and Toxicology* **18**, 706-727.

Kubota, A., Iwata, H., Tanabe, S., and Tobata, S. (2005). Hepatic CYP1A induction by dioxin-like compounds, and congener-specific metabolism and sequestration in wild common cormorants from Lake Biwa, Japan. *Environmental Science and Technology* **39**(10), 3611-3619.

Kushlan, J. A. Feeding ecology of wading birds. Sprunt, A., IV, Ogden, J. C., and Winckler, S. Research Report No. 7, 249-296. 1978. National Audubon Society. Wading Birds.

Leonards, P. E. G., van Hattum, B., and Leslie, H. (2008). Assessing the risks of persistent organic pollutants to top predators: A review of approaches. *Integrated Environmental Assessment and Management* **4**(4), 386-398.

Mandal, P. K. (2005). Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology: Part B* **175**, 221-230.

Marion, L. (1989). Territorial feeding and colonial breeding are not mutually exclusive: the case of the grey heron (*Ardea cinerea*). *The Journal of Animal Ecology* **58**(2), 693-710.

Martof, B. (1953). Home range and movements of the green frog, *Rana clamitans*. *Ecology* **34**(3), 529-543.

Nosek, J. A., Craven, S. R., Sullivan, J. P., Hurley, S. S., and Peterson, R. E. (1992). Toxicity and reproductive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in ring-necked pheasant hens. *Journal of Toxicology and Environmental Health* **35**, 187-198.

Peifer, R. W. (1979). Great blue herons foraging for small mammals. *The Wilson Bulletin* **91**(4), 630-631.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Giesy, J. P., Stromborg, K. L., and Bursian, S. J. (1996a). Effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) injected into the yolks of chicken (*Gallus domesticus*) eggs prior to incubation. *Archives of Environmental Contamination and Toxicology* **31**, 404-409.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Stromborg, K. L., Kubiak, T. J., Giesy, J. P., and Bursian, S. J. (1997). Organochlorine contaminants in double-crested cormorants from Green Bay, Wisconsin: II. Effects of an extract derived from cormorant eggs on the chicken embryo. *Archives of Environmental Contamination and Toxicology* **32**, 316-322.

Powell, D. C., Aulerich, R. J., Stromborg, K. L., and Bursian, S. J. (1996b). Effects of 3,3,',4,4',-tetrachlorobiphenyl, 2,3,3',4,4'-pentachlorobiphenyl, and 3,3',4,4',5-pentachlorobiphenyl on the developing chicken embryo when injected prior to incubation. *Journal of Toxicology and Environmental Health* **49**, 319-338.

Senthil Kumar, K., Kannan, K., Giesy, J. P., and Masunaga, S. (2002). Distribution and elimination of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, and p,p'-DDE in tissues of bald eagles from the upper peninsula of Michigan. *Environmental Science & Technology* **36**(13), 2789-2796.

Seston, R. M., Fredricks, T. B., Tazelaar, D. L., Coefield, S. J., Bradley, P. W., Newsted, J. L., Roark, S. A., Kay, D. P., Giesy, J. P., and Zwiernik, M. J. (2010a). Tissue-based risk assessment of great blue heron (*Ardea herodias*) exposed to PCDD/DFs in the Tittabawassee River floodplain, MI, USA. *Environmental Toxicology and Chemistry* (in press).

Seston, R. M., Giesy, J. P., Fredricks, T. B., Tazelaar, D. L., Coefield, S. J., Bradley, P. W., Roark, S. A., Newsted, J. L., Kay, D. P., and Zwiernik, M. J. (2010b). Multiple lines of evidence risk assessment of belted kingfisher exposed to PCDFs and PCDDs in Tittabawassee River floodplain, Midland, MI, USA. *Environmental Toxicology and Chemistry* (in review).

Seston, R. M., Zwiernik, M., Fredricks, T., Coefield, S., Tazelaar, D., Hamman, D., Paulson, J., and Giesy, J. (2009). Utilizing the great blue heron (*Ardea herodias*) in ecological risk assessments of bioaccumulative contaminants. *Environmental Monitoring and Assessment* **157**(1), 199-210.

Straub, C. L., Maul, J. D., Halbrook, R. S., Spears, B., and Lydy, M. J. (2007). Trophic transfer of polychlorinated biphenyls in great blue heron (*Ardea herodias*) at Crab Orchard National Wildlife Refuge, Illinois, United States. *Archives of Environmental Contamination and Toxicology* **52**(4), 572-579.

Thomas, C. M., and Anthony, R. G. (1999). Environmental contaminants in the great blue herons (*Ardea herodias*) from the Lower Columbia and Willamette Rivers, Oregon and Washington, USA. *Environmental Toxicology and Chemistry* **18**(12), 2804-2816.

Thompson, D. H. (1978). Feeding areas of great blue herons and great egrets nesting within the floodplain of the upper Mississippi River. *Proceedings of the Colonial Waterbird Group* **2**, 202-213.

US Environmental Protection Agency (USEPA). Michigan Dioxin Studies. Dow Chemical Wastewater Characterization Study. Tittabawassee River Sediments and Native Fish. EPA-905/4-88-003. 1986. U.S. Environmental Protection Agency, Region V, Westlake, OH.

US Environmental Protection Agency (USEPA). Wildlife Exposure Factors Handbook. EPA/600/R-93/187. 1993. Washington, DC 20460, U.S. Environmental Protection Agency.

US Environmental Protection Agency (USEPA). Field Studies for Ecological Risk Assessment. 540-F-94-014. 1994. Washington, D.C., US Environmental Protection Agency.

US Environmental Protection Agency (USEPA). Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Revision 1. Method 8290A. SW-846. 1998. Washington, DC, US Environmental Protection Agency.

van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., and Zacharewski, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* **106**(12), 775-792.

van den Berg, M., Craane, B. L. H. J., Sinnige, T., van Mourik, S., Dirksen, S., Boudewijn, T., van der Gaag, M., Lutke-Schipholt, I. J., Spenkelink, B., and Brouwer, A. (1994a). Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (*Phalacrocorax carbo*) after in ovo exposure. *Environmental Toxicology and Chemistry* **13**(5), 803-816.

van den Berg, M., De Jongh, J., Poiger, H., and Olson, J. R. (1994b). The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Critical Reviews in Toxicology* **24**(1), 1-74.

Verrett, M. J. Investigation of the toxic and teratogenic effects of halogenated dibenzo-*p*-dioxins and dibenzofurans in the developing chicken embryo. Memorandum Report. 1976. Washington, D.C., U.S. Food and Drug Administration.

Zwiernik, M. J., Bursian, S. J., Aylward, L. L., Kay, D. P., Moore, J., Rowlands, C., Woodburn, K., Shotwell, M., Khim, J. S., Giesy, J. P., and Budinsky, R. A. (2008). Toxicokinetics of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF in mink (*Mustela vison*) at ecologically relevant exposures. *Toxicological Sciences* **105**(1), 33-43.

CHAPTER 4

Tissue-based risk assessment of great blue heron (*Ardea herodias)* exposed to PCDD/DFs in the Tittabawassee River floodplain, MI, USA

Rita M. Seston[1], Timothy B. Fredricks[1], Dustin L. Tazelaar[2], Sarah J. Coefield[1], Patrick W. Bradley[2], John L. Newsted[3], Denise P. Kay[3], Scott D. Fitzgerald[4], John P. Giesy[1,5], and Matthew J. Zwiernik[2]

[1]Zoology Department, Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA

[2]Animal Science Department, Michigan State University, East Lansing, MI 48824, USA

[3]ENTRIX, Inc., Okemos, MI 48864, USA

[4]Department of Pathobiology and Diagnostic Investigation, Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI 48824, USA

[5]Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, S7J 5B3, Canada

Abstract

Concentrations of dioxin-like compounds, primarily polychlorinated dibenzofurans (PCDFs), in soils and sediments of the Tittabawassee River (TR) and associated floodplains downstream of Midland, Michigan (USA) were greater than upstream sites and prompted a site-specific risk assessment of great blue herons (GBH). Tissue exposure of PCDFs and polychlorinated dibenzo-*p*-dioxins (PCDDs) was assessed in multiple GBH tissue types, including blood plasma of adults, eggs, as well as blood plasma, adipose, liver, and muscle of nestlings. Adult GBH exposure was associated with foraging area and age class, with concentrations of PCDD/DFs being greater in blood plasma of adult GBH foraging in the TR compared to those foraging in upstream reference areas and in older birds as compared to their younger cohorts. Concentrations of PCDD/DFs and dioxin-like polychlorinated biphenyls (PCBs) in eggs and nestling tissues of GBH collected from rookeries within the TR floodplain were generally similar among rookeries. Mean concentrations of PCDD/DFs in eggs of GBH ranged from 45 to 67 ng/kg,wet wt for the rookeries studied, with a maximum concentration of 210 ng/kg, wet wt observed. Adipose consistently had the greatest concentration of PCDD/DFs of all tissues collected from nestlings of GBH, ranging from 98 to 430 ng/kg, wet wt. Potential for adverse population-level effects from site-specific contaminant exposures were evaluated by comparison to selected toxicity reference values (TRVs). Minimal risk of adverse population-level effects was predicted when exposures measured in tissues of GBH collected from rookeries within the TR were compared to appropriate TRVs. This prediction is consistent with site-specific measures of population condition, which included clutch size and number of nestlings per successful nest.

Introduction

The Tittabawassee and Saginaw rivers downstream of Midland, MI, USA contain elevated concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs). This contamination is the result of the historical treatment and storage of wastes associated with the manufacture of chlorinated compounds at The Dow Chemical Company, prior to the adoption of modern waste management practices (USEPA 1986). Sediments and associated floodplain soils collected from downstream study areas (SA) were found to contain total concentrations of 2,3,7,8-substituted PCDD/DFs (ΣPCDD/DFs) ranging from $1.0x10^2$ to $5.4x10^4$ ng/kg dry wt, respectively. Mean ΣPCDD/PCDF concentrations in sediments and floodplain soils in the reference area (RA) upstream of Midland were 10- to 20-fold less (Hilscherova *et al.* 2003). The persistence of these compounds in the environment, combined with their toxicity and potential to bioaccumulate, has led to concern over the potential exposures of wildlife species residing in the Tittabawassee and Saginaw River floodplains.

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans often occur in the environment as complex mixtures, commonly with structurally related polychlorinated biphenyls (PCBs). Due to their lipophilic characteristics and their resistance toward metabolism (Mandal 2005) these compounds have the potential to be accumulated through the food web. Threshold toxicological responses of primary concern are mediated through the aryl hydrocarbon receptor (AhR) and include enzyme induction, teratogenicity, immunotoxicity, and adverse effects on reproduction, development, and endocrine functions (Hoffman *et al.* 1987; van den Berg *et al.* 1994a). In particular, AhR-mediated compounds have been shown to decrease hatching success and fledging success in

aquatic avian species (Custer *et al.* 2005; Giesy *et al.* 1994; Gilbertson 1983; Kubiak *et al.* 1989).

The sensitivities of a number of species to AhR-mediated effects have been determined in laboratory studies or inferred from observations of populations exposed in the wild. Sensitivities have been shown to vary among species (Brunström 1988). For example, the domestic chicken (*Gallus gallus*), which is considered the most sensitive avian species, is over 1000-times more sensitive to embryo-lethal effects than is the mallard (*Anas platyrhynchos*) (Head *et al.* 2008). Recent research has suggested that the ligand binding domain (LBD) of the AhR in avian species is highly conserved and can be classified by only a few structural configurations. The specific configuration of the LBD directly affects the binding affinity of ligand (dioxin-like compounds), thereby influencing the organisms response and sensitivity to toxic effects (Head *et al.* 2008; Karchner *et al.* 2006). This confirms the importance of species-specific exposure and response data and its applicability between taxa.

Selecting an appropriate study species is a key element of effective ecological risk assessments (ERAs), especially when site-specific field studies are to be employed (USEPA 1994). Piscivorous birds are frequently selected as receptors for use in evaluating aquatic systems as they can be sensitive to the effects of contaminants and have the potential to accumulate persistent, lipophilic contaminants through trophic-transfer. The great blue heron (*Ardea herodias*; GBH) possesses several characteristics that make it a suitable species to use as a receptor in ERAs concerning bioaccumulative contaminants in aquatic environments. As a long-lived, territorial, apex predator in the aquatic food web, the GBH has great potential to accumulate local contaminants over a

long period of time, especially in areas of its range where foraging is available over the winter months, allowing them to be year-round residents (Butler 1997; Custer *et al.* 1991). Breeding colonies of GBH are more conspicuous to researchers than single-nesting species and the condition of the population can more easily be assessed. The territorial nature of GBH leads to active defense of distinct, identifiable foraging areas proximal to the breeding colony (Marion 1989). Therefore, exposure of GBH to persistent and bioaccumulative compounds potentially has better defined spatial boundaries than do more opportunistically feeding piscivorous birds. GBH have a broad distribution across geographic regions and habitat types, residing in freshwater, estuarine, and marine habitats throughout North America, which makes them a potential receptor for sites of aquatic-based contamination at many different localities (Butler 1992). Previous studies have detected local organochlorine contaminants in the tissues of GBH, which demonstrates their utility in the assessment of site-specific contamination (Custer *et al.* 1997; Elliott *et al.* 2001; Harris *et al.* 2003; Straub *et al.* 2007; Thomas and Anthony 1999).

The primary objective of the present study was to assess the risk that PCDD/DFs in the Tittabawassee River (TR) basin pose to GBH breeding on-site. To characterize PCDD/DF exposure, contaminant concentrations were directly measured in GBH tissues in terms of ΣPCDD/DF, ΣPCB, 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). Concentrations of PCDD/DFs and the patterns of their relative congener concentrations in GBH tissues were also evaluated for spatial trends. Additionally, tissue concentrations were compared

to toxicity reference values (TRVs) to estimate the risk present. Parameters associated with individual and population condition were then assessed and used to confirm risk estimates. Assessments of GBH tissue-based exposure and population condition presented here each represent a separate line of evidence, facilitating a multiple lines of evidence approach to determine the risk present to GBH along the TR (Fairbrother 2003). Integrating the data resulting from these multiple assessments can reduce uncertainty inherent in the risk assessment process and provide sound data for use in decision making regarding the future of the site (Leonards *et al.* 2008).

Methods

Site description

The assessment was conducted in the vicinity of the city of Midland, located in the east-central lower peninsula of Michigan, USA (Figure 4.1). The TR is a tributary of the Saginaw River (SR), which empties into Saginaw Bay and Lake Huron. The TR runs through The Dow Chemical Company (DOW) property, which is located on the southern edge of Midland and is the accepted source of the PCDD/DF contamination (USEPA 1986). The area henceforth referred to as the study area (SA) includes approximately 37 km of the TR and associated floodplain wetlands from DOW to the confluence of the TR and SR and 35 km of the SR until it enters Saginaw Bay. The reference area (RA) is composed of the TR upstream of Midland, together with the Pine and Chippewa Rivers, both of which are tributaries of the TR upstream of Midland. Blood plasma was collected from adult GBH at established trapping stations throughout the reference and study areas.

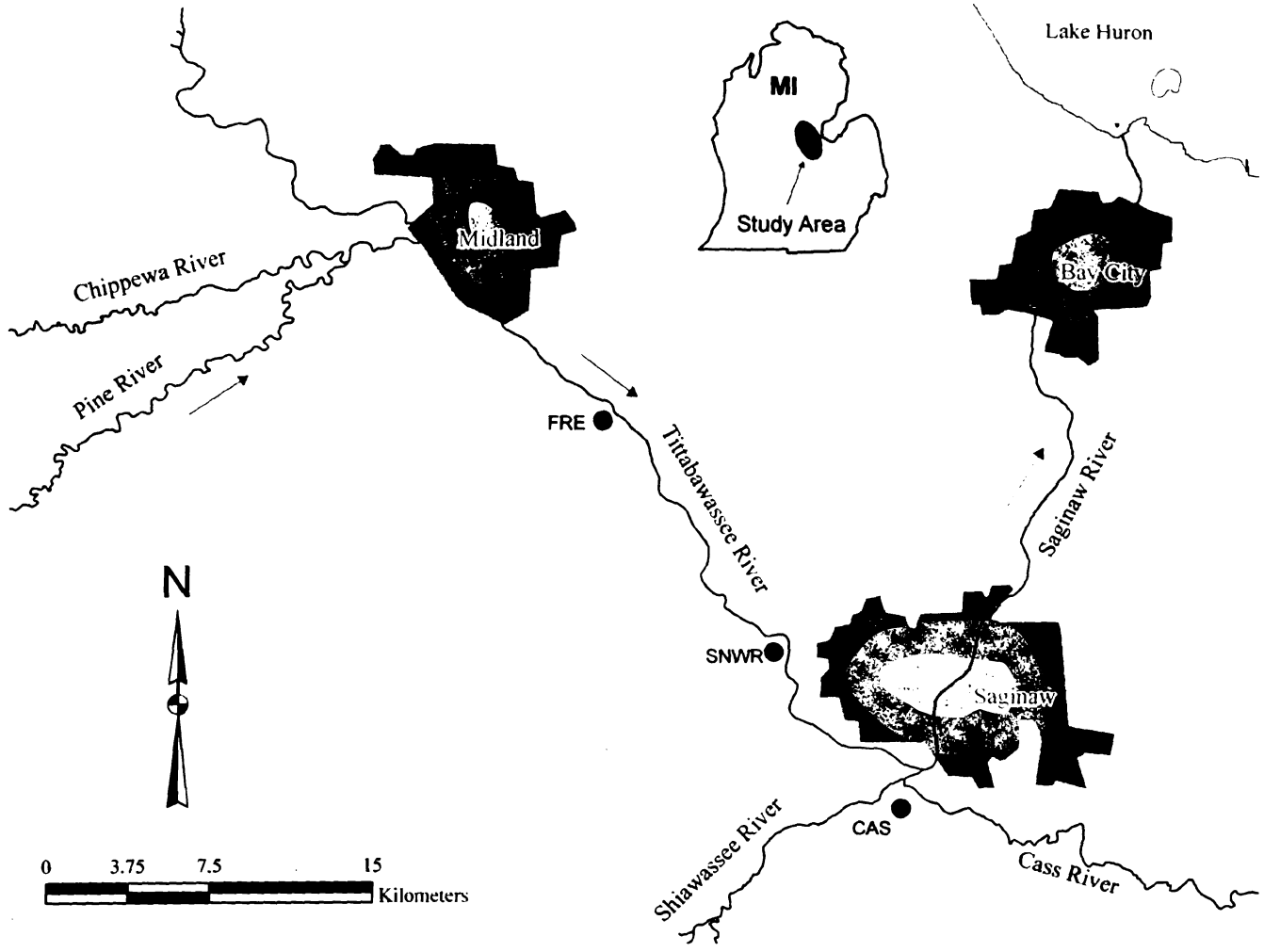


Figure 4.1. Location of great blue heron rookeries within the Tittabawassee River floodplain study area, Midland, MI, USA.

Over the course of the present study 12 adult GBH trapping stations were established, with three in the RA and nine in the SA. Eggs and tissues of GBH nestlings were collected from rookeries located in the SA. Within the SA there were three active GBH rookeries located in the floodplain; the Freeland (FRE), Shiawassee (SNWR), and Cass River (CAS) rookeries. They were located 11, 27, and 36 km downstream of Midland, MI, respectively (Figure 4.1). Established in 2001, FRE was discovered mid-season 2006 and contained 44 nests in 2006 and 46 nests in 2007 (Personal communication, landowner). On the Shiawassee National Wildlife Refuge, SNWR was established in 1999 and at one time contained as many as 161 nests. Nest occupancy of GBH drastically decreased after predatory avian species, including bald eagles (*Haliaeetus leucocephalus*), great horned owls (*Bubo virginianus*), and red-tailed hawks (*Buteo jamaicensis*) established nests in the rookery. It has since been abandoned by GBH. The CAS was located near the confluence of the Cass and Shiawassee rivers in the Shiawassee National Wildlife Refuge and contained approximately 35 nests throughout the present study. No rookeries were discovered in the RA.

Capture and handling of adult GBH

A total of 51 blood samples were collected from adult GBH trapped while foraging along the Tittabawassee, Pine, and Chippewa rivers during 2005-2007. Adult GBH were captured using modified foot hold traps set around feeding stations in predetermined GBH foraging areas. Trapping along this river system was limited to summer months, when the river's water levels were lower and more stable. Individual GBH were fitted with USFWS bands on the tarsus and colored leg bands on the tibia (Simpson and Kelsall

1978). Individuals were aged using plumage characteristics, and were classified as either hatch year (HY) or after hatch year (AHY). In-depth descriptions of adult GBH capture and handling are described in Seston *et al.* (2009).

Nest monitoring and fresh egg sampling

Great blue heron rookeries were visited several times during the 2006 and 2007 nesting season to monitor reproductive success. In mid-Michigan, the GBH nesting season begins in March and runs through mid-July. Parameters of reproductive success monitored include clutch size and the number of nestlings per successful nest. The number of nestlings per successful nest was counted during nestling plasma sampling, when chicks were estimated to be 5 weeks of age. An abbreviated description of the methods is presented here, as they have been described in detail in Seston *et al.* (2009).

Eggs were collected from 24 GBH nests, eight in each of the studied rookeries. Nests were located in Eastern cottonwood (*Populus deltoides*), silver maple (*Acer saccharinum*), or white ash (*Fraxinus americana*) trees at heights ranging from 15 to 25 m. Fresh egg sampling often involved incubation disturbance within the rookery so it was only done when temperatures were greater than 15°C. Viable and nonviable eggs were collected from accessible nests in each rookery. A maximum of one viable egg was collected at random from each accessed nest that contained ≥ two eggs. In addition to viable eggs, all eggs that failed to hatch were collected for analysis of developmental stage and contaminant content. Eggs were weighed, measured, and carefully transported to the laboratory in a crush-proof, water-proof container and kept at 4°C until processing.

Capture and handling of nestling GBH

Blood plasma of nestling GBH was collected from 23 nests (n= 12, 4, and 7 nests at FRE, SNWR, and CAS, respectively). When possible blood plasma of nestlings was collected from the same nests from which eggs were collected. Blood was collected from nestlings when they were approximately 4 to 5 wk old based on methods previously described in Henny and Meeker (1987). At this age, nestlings were still limited to the nest yet had sufficient mass to provide an adequate volume of plasma for residue analyses (McAloney 1973). Individuals were fitted with USFWS bands on the tarsus and colored leg bands on the tibia (Seston *et al.* 2009).

Salvaged nestlings were collected opportunistically following weather events or siblicide. Tissues of salvaged GBH nestlings, including adipose, liver, and muscle, were collected and analyzed from a total of 11 individuals, collected from six nests. Sample size of tissues precluded inter-rookery comparisons. Nestlings were examined for gross external or internal abnormalities including liver, kidney, spleen, intestine, and gonad histology. Nestling stomach contents were analyzed to the lowest taxonomic identification possible to determine the site-specific dietary composition.

Blood plasma sampling

Blood from nestling and adult GBH was drawn from the brachialis vein with needles affixed to sterile syringes, which had been pre-rinsed with sodium heparin solution. The volume of the blood sample procured was determined by calculating 10% of 7% of the bird's body mass (Hoysak and Weatherhead 1991; Sturkie 1986). The volume of blood collected per individual ranged from 5 mL up to 10 mL. The blood sample was then

transferred to a heparinized Vacutainer™ (Becton Dickinson, Franklin Lakes, NJ) for transport back to the field laboratory. Whole blood samples were centrifuged and the plasma (supernatant) was decanted. Both plasma and packed blood cells were stored at -20°C until analysis.

Sample processing and analytical techniques

Concentrations of seventeen 2,3,7,8-substituted PCDD/DF congeners were measured in all samples while concentrations of PCBs and dichloro-diphenyl-trichloroethane (DDT) and related metabolites (DDXs) were determined in a subset of samples. Collected eggs were opened around the girth with a scalpel blade. Contents were then homogenized in a chemically cleaned Omni-mixer, lyophilized, and stored in clean jars until analysis (I-CHEM brand, Rockwood, TN). Concentrations of PCDD/DF in eggs were reported on a fresh weight basis adjusted to account for any desiccation during incubation and storage. Adjusted fresh weight was calculated based on egg volume (Hoyt 1979). The mass of egg contents was determined by subtracting the eggshell mass at the time of processing from the adjusted fresh weight. Tissues collected from salvaged nestlings were homogenized using a chemically cleaned Omni-mixer.

Residues were quantified in accordance with U.S. Environmental Protection Agency (U.S. EPA) Method 8290/1668A with minor modifications (USEPA 1998). Analytical methods have been detailed elsewhere (Fredricks *et al.* 2010b; Seston *et al.* 2010b). Briefly, biotic matrices 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). Losses of congeners during extraction were corrected based on recoveries of ^{13}C-labeled 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. Soxhlet extractions and instrumental analyses were conducted at AsureQuality Ltd, Lower Hutt, New Zealand.

Statistical analyses

Total concentrations of the seventeen 2,3,7,8-substituted PCDD/DF congeners (ΣPCDD/DFs) are reported as the sum of all congeners (ng/kg wet weight (wet wt)). Individual congeners that were less than the limit of quantification were assigned a value of half the sample method detection limit on a per sample basis (ND=0.5DL). Total concentrations of 12 non- and mono-*ortho*-substituted PCB congeners are reported as the sum of these congeners (ng/kg wet wt) (ΣPCBs) for a subset of samples. Concentrations

of $TEQ_{WHO\text{-}Avian}$ (ng/kg wet wt) 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). The term DF-$TEQs_{WHO\text{-}Avian}$ refers to summation of the TEQs of individual PCDD and PCDF congeners while the term PCB-$TEQs_{WHO\text{-}Avian}$ refers to the TEQ from PCBs. Total TEQs refers to the summation of both DF-$TEQs_{WHO\text{-}Avian}$ and PCB-$TEQs_{WHO\text{-}Avian}$. In addition, concentrations of dichloro-diphenyl-trichloroethane (2',4' and 4',4' isomers) and dichloro-diphenyl-dichloroethylene (4',4') were measured in the same subset of samples as PCBs and are reported as the sum of the *o,p* and *p,p* isomers (ΣDDXs; ug/kg wet wt).

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) before statistical analyses. PROC GLM was used to make comparisons for three or more locations. When significant differences among locations were indicated, the Tukey-Kramer test was used to make comparisons between individual locations. PROC TTEST was used to make comparisons between two groups. Differences were considered to be statistically significant at $p < 0.05$.

The experimental unit for residue concentrations in eggs and all nestling tissues was considered to be individual GBH nests. An arithmetic mean was calculated for nests from which multiple samples of the same tissue were collected. The only exception to this was for examining correlations of residue concentrations among various nestling tissues, where the individual nestling was considered the experimental unit. Tissue

correlations were run using Spearman's rank correlation test. Concentrations of ΣPCDD/DF and $TEQ_{WHO\text{-}Avian}$ in GBH nestling plasma and eggs collected from the same nest were compared using PROC REG. Regression analysis was used to develop an equation from which concentrations of ΣPCDD/DF and $TEQ_{WHO\text{-}Avian}$ in eggs could be calculated from concentrations of ΣPCDD/DF and $TEQ_{WHO\text{-}Avian}$ measured in nestling plasma. To better understand the potential distributions of concentrations of DF-$TEQ_{WHO\text{-}Avian}$ and total $TEQ_{WHO\text{-}Avian}$ in eggs and blood plasma of GBH nestlings, a probabilistic modeling approach was used to portray the distributions. Probabilistic models were developed as cumulative frequency distributions based on concentrations of DF-$TEQ_{WHO\text{-}Avian}$ and total $TEQ_{WHO\text{-}Avian}$ in eggs and blood plasma of nestlings. The mean and standard deviation of transformed concentrations values of each sample type were used to generate a sample of 10,000 random concentration values based on a lognormal distribution.

To investigate patterns of relative concentrations of individual congeners in GBH blood plasma, principle component analysis (PCA) was performed with PROC FACTOR using relative orderings of PCDD/DF congener concentrations normalized to the ΣPCDD/DF concentration. Only congeners that were present in ≥60% of the samples were included as factors [TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD), 1,2,3,7,8-pentachlorodibenzofuran (1,2,3,7,8-PeCDF), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF), and octachlorodibenzo-*p*-dioxin (OCDD)]. Samples for which concentrations were less than the limit of quantification had the greatest influence on concentrations of ΣPCDD/DF in

GBH nestling plasma, with a mean difference between using ND=0 and ND=0.5DL for ΣPCDD/DF quantification of 18%.

Selection of toxicity reference values

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 the present study, tissue-specific, egg, plasma, and adipose exposure-based toxicity reference values (TRVs) based on the same or similar compounds were identified from literature and compared to measured site-specific GBH tissue concentrations. Resulting HQs are presented as a range bounded by the LOAEC-associated HQs at the low end and the NOAEC-associated HQs at the high end. It should be noted that the NOAEC and LOAEC associated HQs are a function of the experimental design (dosing regime) and the actual threshold concentration at which effects would be expected to occur lies somewhere within the described range. It has recently been suggested that TRV selection should involve the combination of multiple suitable studies into a dose-response curve to determine the most accurate value (Allard *et al.* 2010), however an inadequate number of suitable studies precluded the use of that approach here.

Toxicity reference values derived from both laboratory- and field-based studies were utilized to assess GBH eggs. The U.S. EPA previously developed egg-based TRVs by taking the geometric mean of the effect concentrations in three double-crested cormorant (*Phalacrocorax auritus*) egg-injection studies (Powell *et al.* 1997a; Powell *et al.* 1997b; Powell *et al.* 1998; USEPA 2003). In each study, dioxin-like compounds were

injected into cormorant eggs that were artificially incubated until hatch. Based on a measurement endpoint of embryo mortality, the resulting $NOAEC_{EGG\text{-}LAB}$ and $LOAEC_{EGG\text{-}LAB}$ was 3670 and 11090 ng total $TEQ_{WHO\text{-}Avian}$ /kg wet wt, respectively. In addition to the aforementioned laboratory-based TRVs, there have been several field studies of GBH from which threshold concentrations of dioxin-like compounds in eggs can be deduced. A GBH rookery with a relatively large mean concentration of 220 ng total $TEQ_{WHO\text{-}Avian}$ /kg wet wt (NOAEC) in eggs was not associated with the number of successful nests and number of fledglings per nest (Elliott *et al.* 2001). Great blue heron eggs with a mean concentration of 360 ng total $TEQ_{WHO\text{-}Avian}$ /kg wet wt (LOAEC) which were collected and artificially incubated produced hatchlings with observed health deficiencies, including decreased body weight, yolk-free body weight, stomach weight, intestine weight, tibia length, and tibia dry, wet, and ash weights (Sanderson *et al.* 1994). Thus a field study based $NOAEC_{EGG\text{-}FIELD}$ and $LOAEC_{EGG\text{-}FIELD}$ of 220 ng total $TEQ_{WHO\text{-}Avian}$ /kg wet wt and 360 ng total $TEQ_{WHO\text{-}Avian}$ /kg wet wt, respectively, was selected.

Studies reporting concentrations of dioxin-like compounds in avian nestling tissues and associated effects are limited, especially for species that would be appropriate comparison to GBH. After applying the 1998 WHO-Avian TEFs to the PCDD/DF and DL-PCB data reported relative to the blood plasma of bald eagle nestlings in British Columbia, a NOAEC of 2.9 ng total $TEQ_{WHO\text{-}Avian}$ /kg was estimated, based on productivity of eagle pairs located in areas of lesser and greater contamination over a 5 yr period (Elliott and Norstrom 1998). This value was selected as the $NOAEC_{NS\text{-}PLASMA}$

for assessing GBH nestling blood plasma. Beyond egg and blood plasma data, one additional study was available for comparison of other tissue concentrations. A group of grey heron nestlings in the UK that were found to have bone abnormalities had adipose concentrations of 640 ng total $TEQ_{WHO\text{-}Avian}$ /kg wet wt ($LOAEC_{NS\text{-}ADIPOSE}$) compared to the group with no observed deformities that had a mean adipose concentration of 300 ng total $TEQ_{WHO\text{-}Avian}$ /kg wet wt ($NOAEC_{NS\text{-}ADIPOSE}$) (Thompson *et al.* 2006). It should be noted that these TEQs were primarily from dioxin-like PCBs.

Results

Adult plasma

Concentrations of ΣPCDD/DF and $DF\text{-}TEQ_{WHO\text{-}Avian}$ in GBH blood plasma were compared spatially and by age category (Table 4.1). In general, concentrations of ΣPCDD/DF were greater in blood plasma of GBH captured in the SA than that of those captured in the RA and in older birds (after hatch year; AHY) as compared to their younger cohorts (hatch year; HY). The greatest mean difference between SA and RA mean ΣPCDD/DF concentrations was for AHY-GBH ($p<0.0001$) while the spatial difference in HY-GBH was less pronounced ($p=0.0700$). As mentioned above, the age of the trapped individual was also associated with plasma concentrations. Concentrations of ΣPCDD/DF were consistently greater in older AHY-GBH than their HY-GBH cohorts, irrespective of study location. This age class trend was statistically significant in the SA ($p=0.0003$). Comparisons based on $DF\text{-}TEQ_{WHO\text{-}Avian}$ were consistent with those of ΣPCDD/DFs (Table 4.1).

Table 4.1. Total concentrations of 2,3,7,8-substituted furan and dioxin (ΣPCDD/DF) and $TEQ_{WHO\text{-}Avian}$ [a] in blood plasma of great blue herons collected during 2005-2007 as a result of trapping along the Chippewa and Tittabawassee River floodplains [b], Midland, MI, USA. Values [c] (pg/mL wet wt) are given as the geometric mean with sample size in parentheses (*n*) over the 95% confidence interval and range (min-max).

	Age of Great Blue Heron	
	HY GBH Plasma	AHY GBH Plasma
ΣPCDD/DF		
Reference	3.5 (8) Aa [d, e]	4.1 (7) Aa
	2.5–5.0	3.1–5.5
	(1.8–5.9)	(1.3–6.6)
Target	9.9 (16) Aa	18 (20) Bb
	7.8–13	15–23
	(5.2–19)	(6.3–41)
$DF\text{-}TEQ_{WHO\text{-}Avian}$ [a]		
Reference	1.2 (8) Aa	1.4 (7) Aa
	0.74–1.9	0.93–2.2
	(0.47–2.1)	(0.74–2.5)
Target	4.6 (16) Aa	9.6 (20) Bb
	3.4–6.2	7.7–12
	(2.0–10)	(3.8–20)

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

[b] Trapping was done in the river channel of the Pine, Chippewa, and Tittabawassee Rivers as previously described in Seston *et al.* 2009.

[c] Values have been rounded and represent only two significant figures

[d] Means identified with the same uppercase letter are not significantly different between locations at the p=0.05 level using Tukey-Kramer means separation test

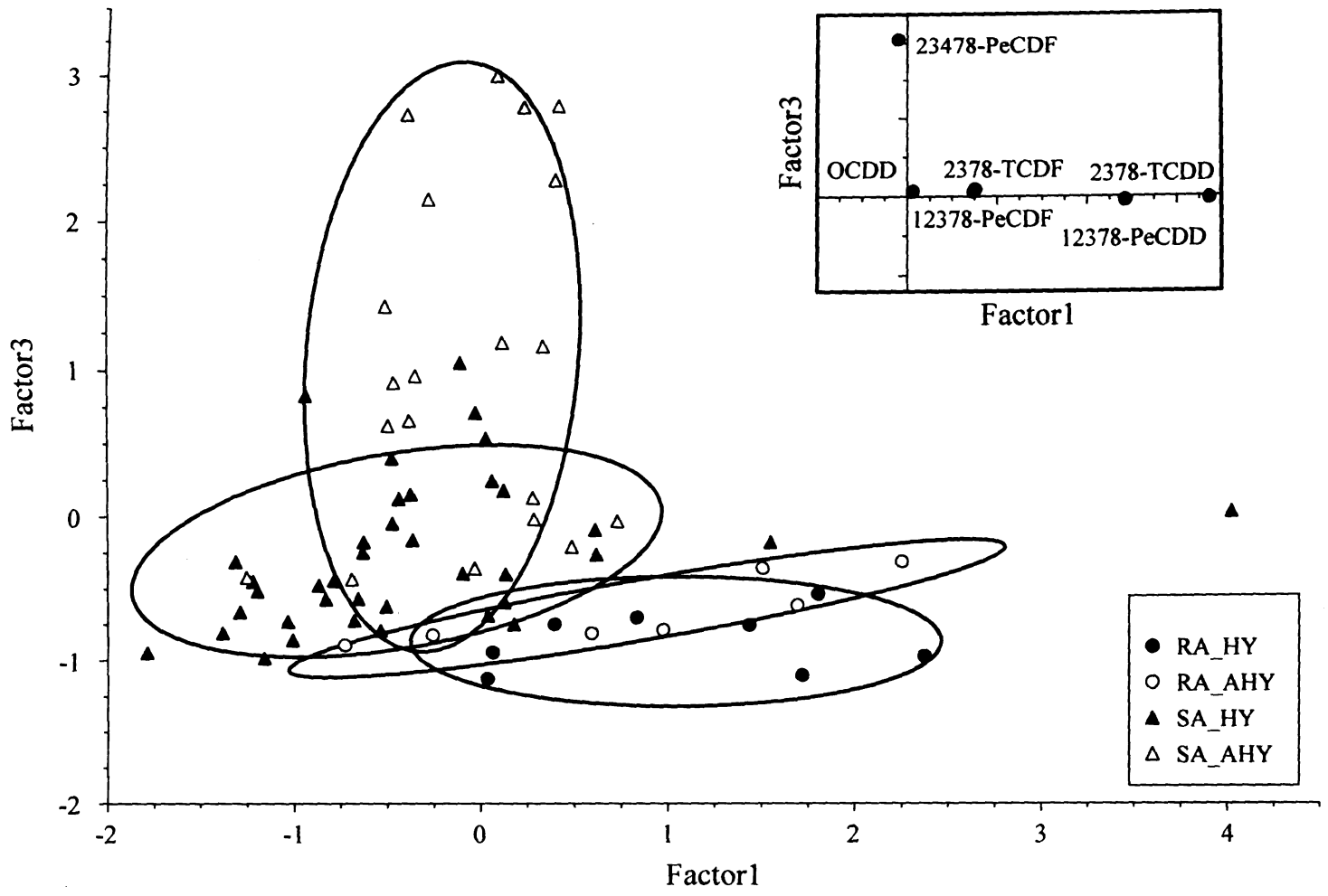
[e] Means identified with the same lowercase letter are not significantly different between age classes at the p=0.05 level using Tukey-Kramer means separation test

Patterns of relative concentrations of individual congeners to ΣPCDD/DF in GBH blood plasma were also associated with the different sampling areas and age classes. The PCA model used to characterize patterns of relative concentrations of congeners in GBH blood plasma explained 75% of the total variance, with PC1 explaining 62% and PC3 explaining 13%. Great blue heron plasma samples were separated by sampling area along PC1, which had a loading score of 0.95 for TCDD, with both HY- and AHY-GBH within the RA having the greatest eigenvectors for PC1. The pattern of congeners in blood plasma from the SA were separated by age along PC3, which had a loading score of 0.996 for 2,3,4,7,8-PeCDF, with AHY-GBH having the greatest eigenvectors for PC3 (Figure 4.2). 2,3,4,7,8-PeCDF contributes a greater percentage to ΣPCDD/DF in blood plasma of AHY-GBH in the SA (31%) than in HY-GBH (12%) (Figure 4.3).

Nest-associated tissues

In general, tissues collected from nests within SA rookeries were similar in analyte concentrations and relative contributions of individual PCDD/DF and dioxin-like PCB congeners. However, a few tissue- and analyte-specific subtle differences and trends were noted. Eggs and nestling plasma, adipose, liver and muscle had similar concentrations and relative congener contributions among the rookeries in the TR SA. Mean concentrations of all residues measured in GBH eggs were not significantly different among rookeries (Table 4.2). A single egg from FRE with an anomalously great concentration was examined as an outlier, however removal of this egg from analysis did not affect the outcome of rookery comparisons (data not presented).

Figure 4.2. Principle component analysis of PCDD/DF concentration congener profiles in blood plasma of great blue heron collected during 2005-2007 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Symbols are labeled by area (RA=Reference Area; SA=Study Area) and age class (HY=hatch year; AHY=after-hatch year). Ellipses indicate 95% confidence intervals of each group. Individual PCDD/DF congener loading scores for each principle component is depicted in the inset. TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin.


23478-PeCDF
OCDD
2378-TCDF
2378-TCDD
12378-PeCDF
12378-PeCDD
Factor3
Factor1
RA_HY
RA_AHY
SA_HY
SA_AHY
Factor3
Factor1
3
2
1
0
-1
-2
-2
-1
0
1
2
3
4

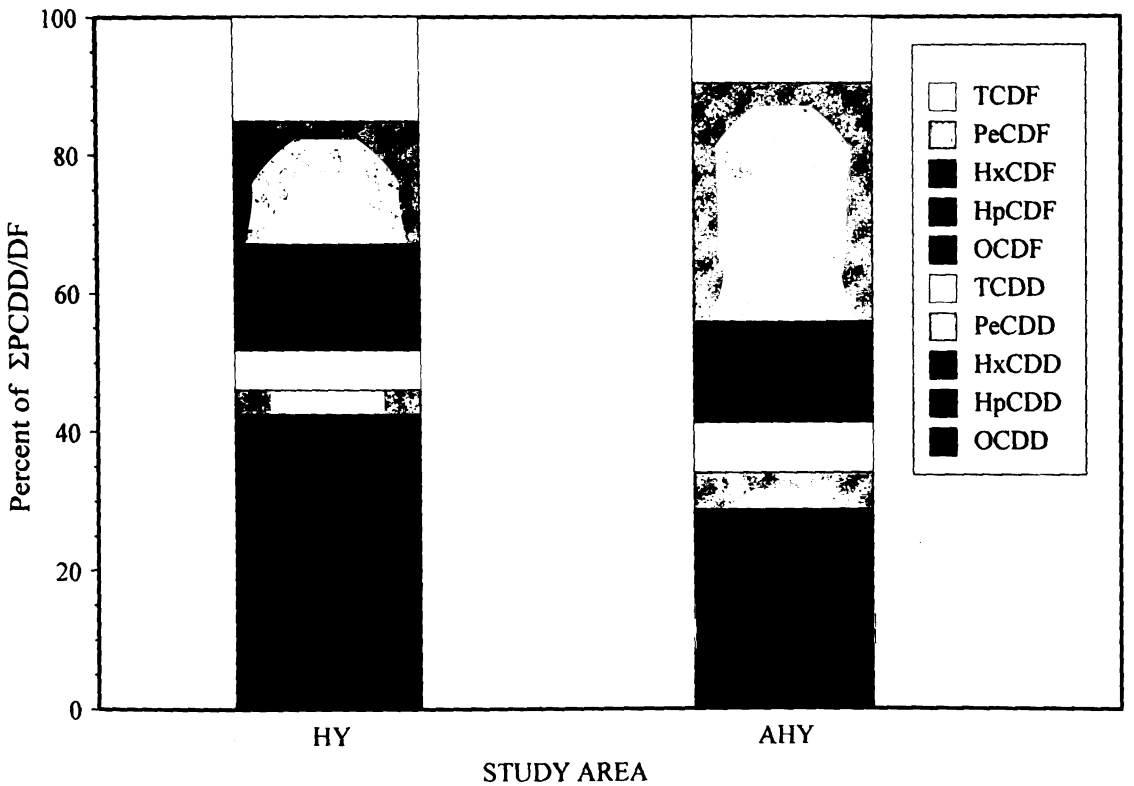


Figure 4.3. Pattern of percent mean ΣPCDD/DF congeners in blood plasma collected from hatch year (HY) and after hatch year (AHY) great blue herons trapped within the Tittabawassee River study area, Midland, MI, USA.

Table 4.2. Total concentrations of 2,3,7,8-substituted furan and dioxin (ΣPCDD/DF), $TEQ_{WHO\text{-}Avian}$ [a], and co-contaminants in great blue heron eggs collected during 2006-2007 from the Tittabawassee and Saginaw River floodplains, Midland, MI, USA. Values[b] (ng/kg wet wt) are given as the geometric mean (*n*) over the 95% confidence intervals and range in parentheses. DDX values are reported in μg/kg wet wt.

	Rookery		
	FRE	SNWR	CAS
GBH Egg			
DF-$TEQ_{WHO\text{-}Avian}$ [a]	44 (8) A[c]	28 (8) A	27 (8) A
	25–78	17–46	21–35
	(17–150)	(17–99)	(19–44)
PCB-$TEQ_{WHO\text{-}Avian}$ [a]	150 (8) A	110 (2)[d]	130 (8) A
	73–330	–	84–190
	(23–360)	(100–110)	(62–250)
Total $TEQ_{WHO\text{-}Avian}$ [a]	210 (8) A	130 (2)	150 (8) A
	110–390	–	110–220
	(46–430)	(120–140)	(83–290)
ΣPCDD/DF	67 (8) A	50 (8) A	45 (8) A
	39–110	34–73	36–55
	(27–210)	(31–130)	(31–65)
ΣPCB	3.3×10^5 (8) A	1.1×10^5 (2)	2.3×10^5 (8) A
	1.5×10^5–7.5×10^5	–	1.5×10^5–3.2×10^5
	(6.1×10^4–9.0×10^5)	(9.9×10^4–1.2×10^5)	(1.7×10^5–4.9×10^5)
ΣDDX	520 (8) A	320 (2)	680 (8) A
	270–1000	–	420–1100
	(180–1600)	(180-560)	(320–1500)

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

[b] Values have been 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 Tukey-Kramer means separation test

[d] Range reported for sites with 2 samples and were not included in the between location comparisons.

Mean concentrations of most analytes quantified in GBH nestling plasma were not significantly different among rookeries (Table 4.3). However, when ΣPCDD/DFs were normalized using $TEFs_{WHO\text{-}Avian}$, the mean $DF\text{-}TEQ_{WHO\text{-}Avian}$ concentration in nestling plasma from FRE was significantly greater than that from CAS. The significant rookery differences of $DF\text{-}TEQ_{WHO\text{-}Avian}$ concentrations in nestling plasma, and the lack thereof for ΣPCDD/DF concentrations is largely due to a shift in the relative contributions of individual congeners and not a change in ΣPCDD/DFs. 2,3,4,7,8-PeCDF made up a greater proportion of the ΣPCDD/DFs in the upstream FRE nestling plasma while the relative contribution of the less potent OCDD increased in the furthest downstream CAS nestling plasma.

Concentrations of ΣPCDD/DF, $DF\text{-}TEQ_{WHO\text{-}Avian}$, ΣPCB, and $PCB\text{-}\ TEQ_{WHO\text{-}Avian}$ in adipose, liver, and muscle of nestlings were consistently greatest at SNWR and least at CAS (Table 4.3). Even though the relatively small number of samples collected precluded determining statistical significance, the ranges of residue concentrations by rookery did not overlap for a number of tissues. In contrast to nestling plasma, the relative contribution of individual congeners to ΣPCDD/DF was similar among rookeries and tissue types. In GBH eggs, a mean of 40% of the ΣPCDD/DFs was contributed by furan congeners, with 2,3,4,7,8-PeCDF (23%) contributing the greatest proportion (Figure 4.4). 2,3,4,7,8-PeCDF also contributed a significant proportion of the ΣPCDD/DFs in adipose (20%), liver (19%), and muscle (15%) tissues and blood plasma (11%) of nestling GBH. One notable difference was the greater relative proportion of OCDD in GBH nestling blood plasma compared to other nestling tissues.

Table 4.3. Total concentrations of 2,3,7,8-substituted furan and dioxin (ΣPCDD/DF) and $TEQ_{WHO\text{-}Avian}$ in great blue heron nestling tissues[a] collected during 2006-2007 from the Tittabawassee and Saginaw River floodplains, Midland, MI, USA. Values[b] are given as the geometric mean with sample size given in parentheses (*n*) over range (min-max). PCDD/DF and PCB values are reported as ng/kg, wet wt in tissues and pg/mL, wet wt in plasma. DDX values are reported in μg/kg wet wt in tissues and ng/mL in plasma.

	Rookery		
	FRE	SNWR	CAS
GBH NS Adipose			
$DF\text{-}TEQ_{WHO\text{-}Avian}$[d]	110 (1)	140 (3) (65–320)	74 (2) (63–86)
$PCB\text{-}TEQ_{WHO\text{-}Avian}$[d]	560 (1)	800 (3) (380–1400)	250 (2) (210–290)
ΣPCDD/DF	170 (1)	200 (3) (98–430)	120 (2) (100–150)
ΣPCB	9.4×10^5 (1)	1.1×10^6 (3) (4.7×10^5–1.7×10^6)	4.5×10^5 (2) (3.5×10^5–5.7×10^5)
ΣDDX	2000 (1)	1800 (3) (1500–2000)	1700 (2) (1500–1900)
GBH NS Liver			
$DF\text{-}TEQ_{WHO\text{-}Avian}$[d]	3.6 (1)	4.9 (3) (2.0–7.4)	2.6 (2) (2.5–2.8)
$PCB\text{-}TEQ_{WHO\text{-}Avian}$[d]	13 (1)	31 (3) (20–53)	6.5 (2) (5.9–7.1)
ΣPCDD/DF	6.9 (1)	8.5 (3) (4.4–12)	7.2 (2) (5.1–10)
ΣPCB	1.6×10^4 (1)	2.3×10^4 (3) (9.5×10^3–5.8×10^4)	1.3×10^4 (2) (1.3×10^4–1.4×10^4)
ΣDDX	33 (1)	35 (3) (27–55)	40 (2) (31–53)
GBH NS Muscle			
$DF\text{-}TEQ_{WHO\text{-}Avian}$[d]	3.6 (1)	6.8 (3) (2.7–14)	2.1 (2) (1.6–2.7)
$PCB\text{-}TEQ_{WHO\text{-}Avian}$[d]	13 (1)	30 (3) (11–82)	5.9 (2) (4.7–7.3)
ΣPCDD/DF	6.7 (1)	14 (3) (9.3–22)	4.1 (2) (3.3–5.1)

Table 4.3 (cont'd)

ΣPCB	2.0×10^4 (1)	3.9×10^4 (3)	1.3×10^4 (2)
		(1.4×10^4–1.3×10^5)	(1.0×10^4–1.7×10^4)
ΣDDX	51 (1)	87 (3)	46 (2)
		(51–220)	(45–48)
GBH NS Plasma			
DF-$TEQ_{WHO\text{-}Avian}$[d]	1.5 (12) A	1.1 (4) AB	0.65 (7) B
	(0.69–3.5)	(0.70–2.0)	(0.37-1.7)
PCB-$TEQ_{WHO\text{-}Avian}$[d]	2.1 (4)	3.6 (4)	N/A[f]
	(0.90–3.2)	(2.8–6.2)	
ΣPCDD/DF	4.9 (12) A	2.5 (4) A	3.8 (7) A
	(1.8–13)	(1.7–3.6)	(1.9–7.2)
ΣPCB	3.3×10^3 (4)	4.2×10^3 (4)	N/A
	(9.6×10^2–9.1×10^3)	(3.1×10^3–5.4×10^3)	
ΣDDX	6.3 (4)	8.0 (4)	N/A
	(1.8–19)	(5.9–11)	

[a] Nest is considered the experimental unit. A mean value was used for nests from which greater than one sample of the same tissue was collected.

[b] Values have been 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 Tukey-Kramer means separation test

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

[e] Range reported for sites with 2 samples and were not included in the between location comparisons

[f] N/A = no samples collected from this location

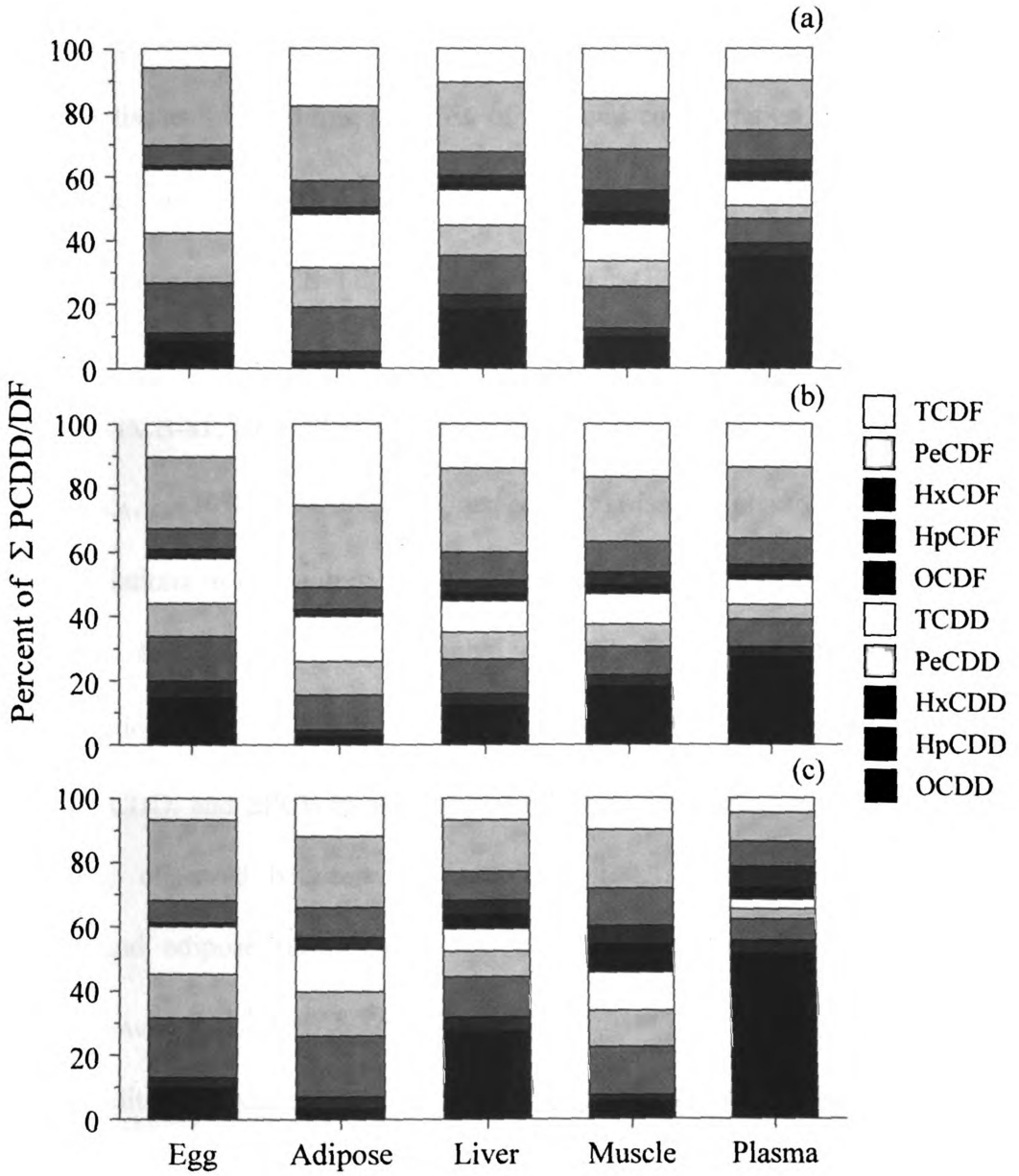


Figure 4.4. Patterns of percent mean ΣPCDD/DF congeners in great blue heron tissues collected from the FRE (a), SNWR (b), and CAS (c) rookeries, located within the Tittabawassee and Saginaw river floodplains, MI, USA.

Similarities were also present in the mean relative contribution of DF-$TEQ_{WHO\text{-}Avian}$ and PCB-$TEQ_{WHO\text{-}Avian}$ to the total concentration of $TEQ_{WHO\text{-}Avian}$ in GBH tissues. In eggs and tissues of nestlings, 71–86% of the total concentration of $TEQ_{WHO\text{-}Avian}$ was attributable to PCB-$TEQ_{WHO\text{-}Avian}$ (Supplemental Data). PCB-126 contributed the greatest proportion to PCB-$TEQ_{WHO\text{-}Avian}$ (43%) in GBH eggs, with PCB-77 (26%) and -81 (24%) also contributing a significant proportion. In tissues of GBH nestlings, PCB-77, PCB-81, and PCB-126 contributed nearly equal proportions of the PCB-$TEQ_{WHO\text{-}Avian}$ (30%, 36%, and 28%, respectively) (data not presented).

Correlations in concentrations of several residues in tissues of GBH nestlings were observed. In individual nestlings, there was a significant positive correlation between concentrations of ΣPCDD/DF, DF-$TEQ_{WHO\text{-}Avian}$, 2,3,4,7,8-PeCDF, 2,3,7,8-TCDF, 2,3,7,8-TCDD, and ΣPCB in adipose and liver (n=11) (Table 4.4). Positive correlations were also observed between concentrations of 2,3,7,8-TCDF and 2,3,7,8-TCDD in plasma and adipose tissue (n=4) and between concentrations of ΣPCDD/DF, DF-$TEQ_{WHO\text{-}Avian}$, and 2,3,4,7,8-PeCDF plasma and liver tissue (n=4) (Table 4.4).

In addition to correlations between concentrations among tissues from the same individual, a relationship was observed between concentrations in egg and nestling blood plasma collected from the same nest, during the same nesting attempt. Concentrations of ΣPCDD/DF (r^2=0.4933 p=0.0160) and DF-$TEQ_{WHO\text{-}Avian}$ (r^2=0.4699 p=0.0199) in egg and nestling blood plasma were correlated (Figure 4.5) (Eqns. 1 and 2). Predicted mean

Table 4-4. Summary statistics for correlations of great blue heron nestling tissue contaminant concentrations. Spearman rank

Table 4.4. Summary statistics for correlations of great blue heron nestling tissue contaminant concentrations. Spearman rank correlations of wet weight contaminant concentrations; including $TEQ_{WHO\text{-}Avian}$ [a] (DF-TEQ), ΣPCDD/DF, 23478-pentachlorodibenzofuran (23478-PeCDF), 2378-tetrachlorodibenzofuran (2378-TCDF), 2378-tetrachlorodibenzo-*p*-dioxin (2378-TCDD), and ΣPCB.

Tissues		Residue					
		DF-TEQ	ΣPCDD/DF	23478-PeCDF	2378-TCDF	2378-TCDD	ΣPCB
y=adipose	R	0.85455	0.91818	0.92727	0.93636	0.81549	0.84545
x=liver	p-value	0.0008	<.0001	<.0001	<.0001	0.0022	0.0010
(N=11)							
y=adipose	R	0.80000	0.80000	0.80000	1.00000	1.00000	0.50000 [c]
x=plasma	p-value	0.2000	0.2000	0.2000	<.0001	<.0001	0.6667
(N=4)							
y=liver	R	1.00000	1.00000	1.00000	0.80000	0.80000	1.00000 [c]
x=plasma	p-value	<.0001	<.0001	<.0001	0.2000	0.2000	<.0001
(N=4)							

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

[b] Comparisons done between tissues collected from the same individual.

[c] For ΣPCB correlations involving plasma, N=3

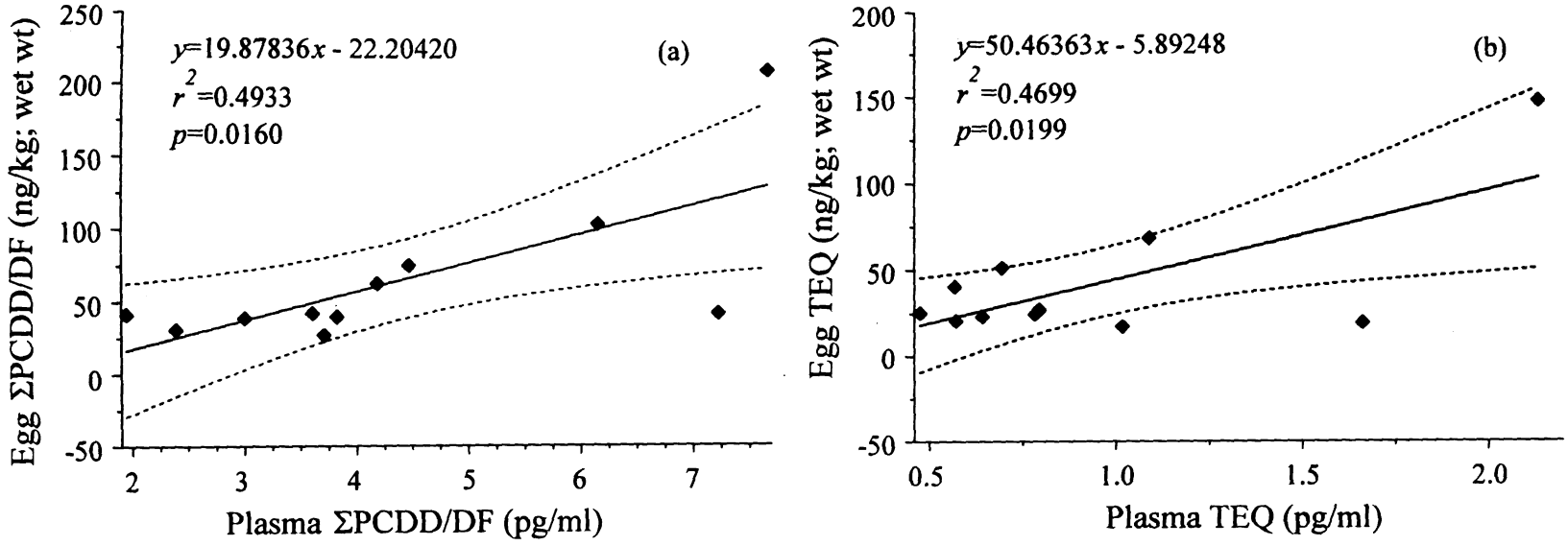


Figure 4.5. Plasma to egg relationship for ΣPCDD/DF (a) and DF-$TEQ_{WHO\text{-}Avian}$ (b) for great blue herons from the Tittabawassee and Saginaw river floodplains (N=11). Line of best fit with 95% confidence intervals.

concentrations of ΣPCDD/DF and DF-$TEQ_{WHO\text{-}Avian}$ and ranges, along with corresponding measured egg concentrations from each rookery, are listed in Table 4.5.

ΣPCDD/DF: Egg (ng/kg, wet wt) = 19.87836[plasma(pg/mL,wet wt)] – 22.20420 **(1)**

DF-$TEQ_{WHO\text{-}Avian}$: Egg (ng/kg, wet wt) = 50.46363[plasma(pg/mL,wet wt)] –5.89248 **(2)**

Risk assessment

Predicted probabilistic distributions of expected cumulative percent frequencies based on concentrations of DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ in eggs and blood plasma of nestling GBH were compared to selected TRVs. Predicted distributions of concentrations of both DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ in GBH eggs were not greater than the $NOAEC_{EGG\text{-}LAB}$ or $LOAEC_{EGG\text{-}LAB}$ (Figure 4.6). Based on concentrations of DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ in GBH eggs, less than 1.0% and 34% of the cumulative frequency was greater than the $NOAEC_{EGG\text{-}FIELD}$, respectively, and less than 1.0% and 10% of the cumulative frequency was greater than the $LOAEC_{EGG\text{-}FIELD}$, respectively. Based on the predicted distributions of concentrations of DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ in blood plasma of nestling GBH, 4% and 70% of the cumulative frequency was greater than the $NOAEC_{NS\text{-}PLASMA}$.

Table 4.5. Measured and predicted and measured concentrations of ΣPCDD/DFs and DF- $TEQ_{WHO\text{-}Avian}$ [a] in great blue heron eggs collected from rookeries within the Tittabawassee and Saginaw river floodplains during 2006-2007. Predicted concentrations were calculated using plasma to egg relationship developed from eggs and nestling plasma collected from the same nest[b,c]. Egg concentrations[d] are reported in ng/kg, wet wt.

	Rookery					
	Freeland		Shiawassee		Cass River	
	Predicted *n*=7	Measured *n*=8	Predicted *n*=4	Measured *n*=8	Predicted *n*=1	Measured *n*=8
ΣPCDD/DF						
Arithmetic mean ± SD	100±81	80±58	30±19	56±32	N/C[e]	46±12
Geomean (95% CI)	64 (22–180)	67 (39–110)	25 (8.1–80)	50 (34–73)	N/C	45 (36–55)
Range	13–230	27–210	11–49	31–130	120	31–65
$DF\text{-}TEQ_{WHO\text{-}Avian}$ [a]						
Arithmetic mean ± SD	110±52	55±41	55±31	34±28	N/C	28±9.3
Geomean (95% CI)	93 (52–170)	44 (25–78)	48 (20–120)	28 (17–46)	N/C	27 (21–35)
Range	39–170	17–150	30–94	17–98	13	19–44

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

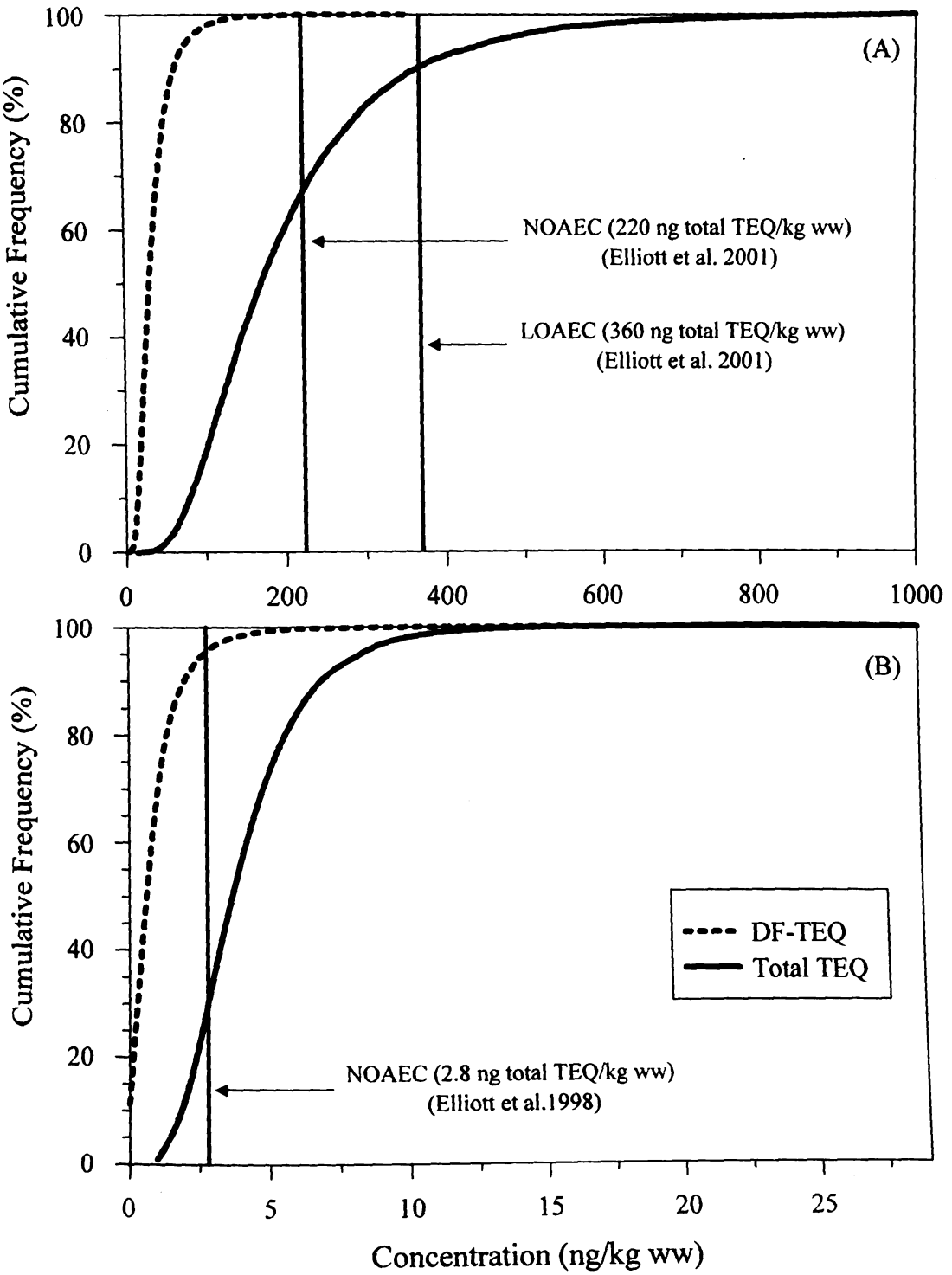
[b] Predicted ΣPCDD/DF concentrations were calculated using the equation: egg (ng/kg, wet wt) = 19.87836[plasma(pg/mL,wet wt)] – 22.20420 (r^2=0.4933 p=0.0160).

[c] Predicted DF- $TEQ_{WHO\text{-}Avian}$ concentrations were calculated using the equation: egg (ng/kg, wet wt) = 50.46363[plasma(pg/mL,wet wt)] – 5.89248 (r^2=0.4699 p=0.0199).

[d] Values have been rounded and represent only two significant figures.

[e] Arithmetic and geometric means not calculated for samples with n=1.

Figure 4.6. Modeled probabilistic distribution of expected cumulative percent frequencies for great blue heron egg $TEQ_{WHO\text{-}Avian}$ concentrations ng/kg wet wt in site-specific eggs collected from the river floodplains near Midland, Michigan in 2005-2007. Concentrations of DF- $TEQ_{WHO\text{-}Avian}$ and total $TEQ_{WHO\text{-}Avian}$ indicated by dashed and solid lines, respectively. NOAEC and LOAEC indicated by vertical bars.


(A)
Cumulative Frequency (%)
100
80
60
40
20
0
NOAEC (220 ng total TEQ/kg ww)
(Elliott et al. 2001)
LOAEC (360 ng total TEQ/kg ww)
(Elliott et al. 2001)
0
200
400
600
800
1000
(B)
Cumulative Frequency (%)
100
80
60
40
20
0
DF-TEQ
Total TEQ
NOAEC (2.8 ng total TEQ/kg ww)
(Elliott et al.1998)
0
5
10
15
20
25
Concentration (ng/kg ww)

Hazard quotients were calculated by comparing measured tissue concentrations to the selected TRVs. Upper 95% confidence level (UCL; of the geometric mean) concentrations of DF-$TEQs_{WHO\text{-}Avian}$ in GBH eggs (n=24) collected from within the SA were not greater than either of the egg-based NOAEC or LOAEC TRVs, resulting in HQs less than 1.0. Conversely, 95% UCL concentrations of total $TEQs_{WHO\text{-}Avian}$ in GBH eggs (n=18) did marginally exceed the $NOAEC_{EGG\text{-}FIELD}$ TRV, resulting in a HQ of approximately 1.0 (Figure 4.7).

Similar trends in HQs were observed for GBH nestling blood plasma as for GBH eggs. The 95% UCL DF-$TEQs_{WHO\text{-}Avian}$ concentrations in GBH nestling blood plasma did not exceed the $NOAEC_{NS\text{-}PLASMA}$ for any rookery, thus resulting in less than 1.0. When based on total $TEQs_{WHO\text{-}Avian}$ and the $NOAEC_{NS\text{-}PLASMA}$, HQs calculated from 95% UCL concentrations were greater than 1.0 for blood plasma of nestlings from FRE and SNWR. Concentrations of total $TEQs_{WHO\text{-}Avian}$ in blood plasma of nestlings were not available from CAS for comparison (Figure 4.7).

Comparisons of the geometric mean and 95% UCL concentrations of DF-$TEQs_{WHO\text{-}Avian}$ in adipose of nestling GBH to both the $NOAEC_{NS\text{-}ADIPOSE}$ and $LOAEC_{NS\text{-}ADIPOSE}$ resulted in HQs less than 1.0 (Figure 4.7). Total $TEQs_{WHO\text{-}Avian}$ geometric mean and 95% UCL concentrations exceeded both the $NOAEC_{NS\text{-}ADIPOSE}$ and $LOAEC_{NS\text{-}ADIPOSE}$, resulting in HQs greater than 1.0. Although concentrations of total $TEQs_{WHO\text{-}Avian}$ did

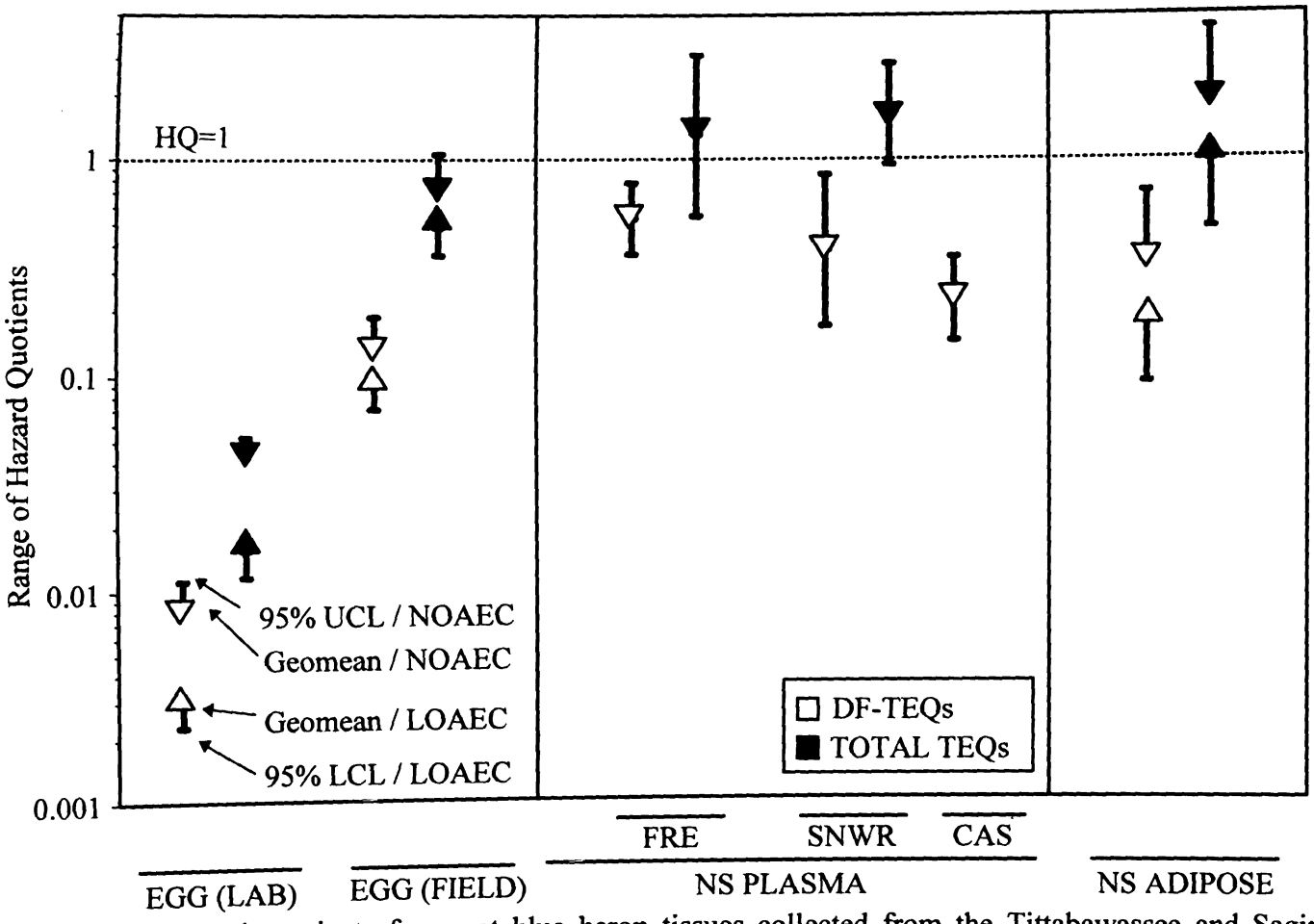


Figure 4.7. Range of hazard quotients for great blue heron tissues collected from the Tittabawassee and Saginaw River floodplains. Upright triangles represent values based on lowest observed adverse effect levels (LOAECs) and inverted triangles represent values based on no observed adverse effect levels (NOAECs). 95% confidence limits also presented.

exceed the associated TRVs, none of the bone abnormalities observed in the study upon which the TRV was based were observed in any GBH nestlings in the present study.

Population condition

Measures of population condition were similar among all studied rookeries (Table 4.6). Mean clutch sizes for GBH from FRE, SNWR, and CAS were not significantly different. Additionally, the mean number of nestlings per successful nest was not significantly different among rookeries. The number of nestlings per nest was not significantly correlated with concentrations of total $TEQs_{WHO\text{-}Avian}$ in eggs or blood plasma, adipose, liver, or muscle of GBH nestlings.

Discussion

Tissue-based exposure

Spatial trends in the concentrations of ΣPCDD/DFs and the relative contribution of PCDDs and PCDFs were consistent between adult GBH blood plasma and prey items (Seston *et al.* 2010b). Concentrations of highly lipophilic compounds in blood plasma are largely influenced by the absorption of contaminants in recently ingested food items from the gastrointestinal tract. Differences in concentrations of residues in the blood plasma of both HY- and AHY-GBH trapped in the RA as compared to those trapped in the SA are in agreement with the conclusion that GBH were consistently foraging in the area of the study site in which they were trapped. The greater mean concentrations of ΣPCDD/DFs in blood plasma from GBH trapped in the SA than that in blood plasma

Table 4.6. Reproductive parameters for great blue heron rookeries located in the Tittabawassee and Shiawassee river floodplains from 2006-2007. Values are given as the arithmetic mean ± 1 SD over the sample size given in parentheses (*n*).

	Rookery		
	FRE	SNWR	CAS
Clutch size [a]	3.8±1.0 A [b]	4.0±1.6 A	3.9±1.4 A
	(22)	(9)	(23)
Nestlings / successful nest [d]	2.7±0.86 A	2.5±1.1 A	3.4±1.2 A
	(21)	(13)	(8)

[a] Number of eggs per nest

[b] Means identified with the same letter are not significantly (p=0.05) among rookeries (across) using the Tukey-Kramer means separation test.

[c] Nestlings / successful nest calculated as the number of chicks present at 4-5 weeks of age.

from GBH trapped in the RA is spatially consistent with predicted average daily doses (ADD_{pot}) based on site-specific prey item concentrations. The ADD_{pot} in the RA and SA, based on an assumption of 100% site use, were 0.97 and 44 to 52 ng ΣPCDD/DF/kg bw/d, respectively (Seston *et al.* 2010b). The relative contributions of PCDDs and PCDFs to ΣPCDD/DFs in GBH blood plasma and prey items also exhibited consistent spatial trends. Great blue heron blood plasma from the SA had a greater relative contribution of PCDFs to ΣPCDD/DFs compared to those from the RA. This trend was also seen in fish, mammals, and other birds in the RA and SA (Coefield *et al.* 2010b; Coefield *et al.* 2010a; Fredricks *et al.* 2010a; Fredricks *et al.* 2010b; Seston *et al.* 2010b; Seston *et al.* 2010a; Zwiernik *et al.* 2008b).

Concentrations of ΣPCDD/DFs in GBH blood plasma were associated with the age of individual. The association between concentrations of ΣPCDD/DFs in GBH blood plasma and age, in which nestling < HY-GBH < AHY-GBH is similar to the trend between ΣPCDD/DF concentrations in blood plasma of great horned owl nestlings and adults along the TR (Coefield *et al.* 2010b). This phenomenon is consistent with developmental dilution in the younger birds (Kunisue *et al.* 2006) and duration of exposure. As a long-lived species that can defend its foraging territories over multiple years, AHY-GBH are potentially exposed longer than nestling or HY-GBH.

Patterns of relative concentrations of individual congeners in blood plasma from the more highly exposed GBH trapped in the SA were also associated with age class. In the SA, both HY- and AHY-GBH blood plasma congener profiles were dominated by furans but there was a shift in predominant congeners between age classes. 2,3,4,7,8-PeCDF was the predominant congener in blood plasma from AHY-GBH whereas 2,3,7,8-TCDF

and 2,3,4,7,8-PeCDF were nearly equal in HY-GBH. The shift in predominance between these two congeners by age class has previously been observed in common cormorants and albatross (Kubota *et al.* 2004; Kunisue *et al.* 2006). Field studies have reported negligible bioaccumulation of TCDF from prey items in Forster's terns and herring gulls (Braune and Norstrom 1989; Kubiak *et al.* 1989). This may be due to preferential metabolism of 2,3,7,8-TCDF, as a number of studies have reported in various avian species exposed to mixtures of AhR-active compounds (Elliott *et al.* 1996; Kubota *et al.* 2005; Senthil Kumar *et al.* 2002). Data on avian toxicokinetics from controlled laboratory studies are limited, but mammalian studies have shown the rate of metabolism of 2,3,7,8-TCDF to be elevated with increased concentrations of dioxin-like compounds and the subsequent induction of cytochrome P450 1A1 and/or 1A2 while 2,3,4,7,8-PeCDF is preferentially sequestered in the liver (van den Berg *et al.* 1994b; Zwiernik *et al.* 2008a). Due to these differences in persistence, it is unsurprising the older GBH in the SA would have a relatively greater proportion of PCDD/DF in their body due to the greater bioaccumulation of 2,3,4,7,8-PeCDF compared to younger GBH.

Although spatial trends were observed in the relative congener contributions in blood plasma of adult GBH, the congener patterns among other tissues were similar among rookeries. As a migratory species, there is potential for GBH to accumulate contaminants while on their wintering grounds and then later deposit these compounds into eggs (Yates *et al.* 2010). The small variability among congener patterns in the various tissues within and among the three SA rookeries is consistent with adult GBH from all the rookeries foraging in areas with a common source. The similarity of congener profiles in tissues of GBH and those in site-specific prey items strongly suggests that the majority of the

resident GBH PCDD/DF exposure is site-specific and not from wintering grounds. Moreover, nestlings are limited to resources collected proximal to the nest by parent birds. Thus the similarity of congener profiles in both eggs and nestling tissues from rookeries within the TR floodplain again suggests the contaminants in each are site-specific and not acquired from wintering grounds.

Albeit of site-specific origin, the concentrations of ΣPCDD/DFs found in GBH tissues were less than expected based on those measured in a second site-specific avian piscivore. The belted kingfisher (*Ceryle alcyon*; BKF) was assessed within the TR floodplain using similar exposure studies (Seston *et al.* 2010a). Mean concentrations of ΣPCDD/DFs were greater in both eggs and nestling tissues of belted kingfisher compared to those of GBH. Despite the difference in concentrations, the relative contributions of individual PCDD/DF congeners were similar between the two species, indicating they are both being exposed to the same PCDD/DF mixture. The observed difference in tissue concentrations is likely a result of variation in metabolic rate or the proportion of diet collected from the study area due to differences in foraging range size. During the breeding season, GBH may travel a mean distance of 3.2 to 6.4 km from their rookery to foraging grounds (Dowd and Flake 1985; Marion 1989; Thompson 1978) whereas BKF forage 0.92 to 2.9 km proximal to their nest burrow (Davis 1982; Mazeika *et al.* 2006).

Risk characterization

The risk of site-specific adverse effects can be assessed by the use of probabilistic modeling of site-specific concentrations and comparison of the frequency distributions to

appropriate TRVs. The most conservative estimate of risk for GBH eggs was the comparison of concentrations of total TEQ to the $NOAEC_{EGG\text{-}FIELD}$ and $LOAEC_{EGG\text{-}FIELD}$. Compared to the $NOAEC_{EGG\text{-}FIELD}$ and $LOAEC_{EGG\text{-}FIELD}$, 34% and 10% of the predicted frequency distribution of concentrations of total $TEQs_{WHO\text{-}Avian}$ in GBH eggs exceeded these values, respectively. Importantly, the actual effect threshold for individuals is likely between the established no-and lowest-effect TRVs. When compared to the $NOAEC_{EGG\text{-}LAB}$ or $LOAEC_{EGG\text{-}LAB}$, 0% of the frequency distribution for concentrations of $DF\text{-}TEQs_{WHO\text{-}Avian}$ or total $TEQs_{WHO\text{-}Avian}$ in GBH eggs exceeded either value. Based on conservatively selected egg-based TRVs (see *Uncertainty assessment*), the potential for effects on individual GBH is minimal. Based on the predicted distributions of concentrations of $DF\text{-}TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ in blood plasma of nestling GBH, 4% and 70% of the cumulative frequency was greater than the $NOAEC_{NS\text{-}PLASMA}$. However, there is no $LOAEC_{NS\text{-}PLASMA}$ for comparison, so it is not clear where the effect threshold occurs.

The degree of concern associated with the potential presence of site-specific adverse effects can also be assessed by use of the traditional point estimate HQ approach. Despite the conservative nature of the selected TRVs, egg-based HQs were less than or equal to 1.0 when based on NOAECs and the 95% UCL concentrations of $DF\text{-}TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$, which is consistent with a conclusion that adverse effects would not be expected. For SA rookeries, HQs for blood plasma of nestling GBH were less than 1.0 based on the 95% UCL concentrations of $DF\text{-}TEQs_{WHO\text{-}Avian}$ and marginally

greater than 1.0 when based on 95% UCL concentrations of total $TEQs_{WHO\text{-}Avian}$, again indicating a minimal expectation for risk of adverse effects.

Hazard quotients calculated from 95% UCL concentrations of total $TEQs_{WHO\text{-}Avian}$ in GBH nestling adipose tissue did exceed 1.0 based on both the $NOAEC_{NS\text{-}ADIPOSE}$ and $LOAEC_{NS\text{-}ADIPOSE}$, but did not exceed 10. This would suggest there is the potential for individual GBH nestlings to exhibit the bone abnormalities observed in the grey heron nestlings (Thompson *et al.* 2006). This difference in apparent sensitivity may be due to site-specific differences in the environmental mixture of PCDD/DFs and dioxin-like PCBs present in each study area. Additionally, the potential role of additional unknown stressors, such as co-contaminants which may be associated with the bone abnormality observed in the grey heron nestlings, must also be taken into consideration. None of the salvaged nestlings or other nestlings monitored along the TR exhibited this deformity.

In addition to dioxin-like compounds, the potential for adverse effects of site-specific ΣDDX concentrations was assessed. GBH appear to be relatively insensitive to the effects of DDE contamination, as an egg with a concentration of 78 mg/kg successfully pipped (Vermeer and Reynolds 1970). Other studies have found mean DDE concentrations in eggs ranging from 0.086 to 16 mg/kg and reported no adverse impacts on breeding success (Blus *et al.* 1980; Harris *et al.* 2003; Laporte 1982; Thomas and Anthony 1999). These observations suggest that egg ΣDDX concentrations reported in the present study would not cause adverse effects on breeding success of GBH along the TR floodplain.

The prediction of minimal potential for adverse effects from the aforementioned tissue-based assessments is consistent with site-specific measures of population

condition. The clutch size of GBH is reported to range from 2 to 6 eggs per nest (Butler 1992), with rookery averages ranging from 2.8 to 4.4 eggs per nest (Elliott *et al.* 1989; Straub *et al.* 2007; Thomas and Anthony 1999). Clutch sizes observed in nests within the TR floodplain are within these reported ranges. Literature values for the number of nestlings per successful nest range from rookery averages of 3.09 to 3.22 nestlings per nest (Straub *et al.* 2007), which is similar to values observed along the TR.

Uncertainty assessment

The greatest limiting factor in the assessment of risk to avian species exposed to dioxin-compounds is the lack of studies reporting effect threshold concentrations, especially for wild species. Thus, TRV selection often introduces significant uncertainty to the risk assessment, especially in cases where comprehensive site-specific data sets describe exposure with great certainty. HQs greater than 1.0 are indicative of exposures that exceed the threshold for adverse effects and that there is the potential for effects to occur. However, such assessments are conservative when extrapolating from individuals to populations, as they are based on assumptions of maximal exposure and do not account for compensatory mechanisms associated with resource availability (Fairbrother 2001). Therefore, HQ values greater than 1.0 do not necessarily translate into population-level or ecologically relevant adverse effects (Blankenship *et al.* 2008).

Each TRV selected to assess the risk to GBH based on egg concentrations present along the TR floodplain has associated uncertainties. The values based on the double-crested cormorant egg injection studies were considered to be appropriate for use in the present study because they were derived from a related avian piscivore which has a

similar sensitivity to dioxin-like compounds as GBH (Sanderson and Bellward 1995). Furthermore, both the double-crested cormorant and GBH have similar AhR ligand binding domain constructs, which suggests the two species will respond similarly to dioxin-like compounds (Head *et al.* 2008; Karchner *et al.* 2006). Although the species appear to be similar in sensitivity to dioxin-like compounds, the route of exposure in the aforementioned double-crested cormorant studies and the GBH being assessed along the TR is different. Residues in the GBH eggs are maternally deposited compared to being artificially introduced as in the study conducted with the double-crested cormorant eggs. This difference in route of exposure adds some uncertainty when using this TRV in the risk assessment (Heinz *et al.* 2009; Hoffman *et al.* 1996). Selected TRVs based on field studies of GBH reduce the uncertainty associated with interspecies comparisons and differences routes of exposure, but add uncertainty associated with confounding environmental factors such as weather events, cyclical population trends of both predators and prey, ecological relevance of observed endpoints, and the potential for unknown co-contaminants. In general, the uncertainties associated with field studies will tend to add conservative bias to the selected TRV if the same co-contaminant issues are not present at the assessment site.

Studies conducted on various raptor species have measured concentrations of dioxin-like compounds in blood plasma, but none have reported a concentration at which reproduction was impaired. A NOAEC of 0.8 ng PCB-$TEQs_{WHO\text{-}Avian}$ /kg nestling plasma was reported based on reproductive parameters for great horned owl (GHO) nestlings exposed to PCBs in Michigan (Strause *et al.* 2007a). Within the same study site as the GBH being assessed in the present study, GHO nestlings and adults had 95% UCL

plasma concentrations of 2.6 ng DF-TEQ/kg and 14 ng DF-TEQ/kg, respectively (Coefield *et al.* 2010b). Since no reproductive impairments were observed, these values are also considered to be plasma-based NOAECs. The bald eagle $NOAEC_{NS\text{-}PLASMA}$ of 2.9 ng total $TEQs_{WHO\text{-}Avian}$ /kg selected for assessing GBH nestlings in the present study was the greatest concentration among those selected as potentially appropriate for use in the present assessment, thus theoretically being closest to the effect concentration.

There is additional uncertainty associated with the application of $TEFs_{WHO\text{-}Avian}$ to concentrations of PCDD/DFs. Recent studies have found that TCDD, TCDF, and 2,3,4,7,8-PeCDF may not be equipotent in birds, as was concluded by the most recent assessment by the WHO (Cohen-Barnhouse *et al.* 2010; Hervé *et al.* 2010b; Hervé *et al.* 2010a; van den Berg *et al.* 1998). Based on the results of egg injection studies in Japanese quail (Cohen-Barnhouse *et al.* 2010), a species that falls into the same broad category of sensitivity to dioxin-like compounds as GBH, TCDF and 2,3,4,7,8-PeCDF were found to be 2- and 6-fold more potent, respectively, than TCDD at causing embryolethality. By applying these relative potencies to the concentrations observed in eggs of GBH collected in the present study, concentrations expressed as DF-TEQs increased approximately 3-fold. Despite the increase in DF-TEQs seen by using these revised relative potencies for TCDF and 2,3,4,7,8-PeCDF, minimal potential for adverse effects would be expected based on comparisons to selected TRVs.

Plasma to egg relationship

In addition to assessing the risk that site-specific concentrations of PCDD/DFs pose to resident GBH along the TR, a relationship between concentrations of these compounds

in egg and nestling plasma was developed. Concentrations in eggs are often regarded as the most important, as embryonic development and hatching success are considered some of the most sensitive endpoints for PCDD/DFs, PCBs, and many other environmental contaminants. The nest is more susceptible to parental abandonment as a result of disturbance while it contains eggs than later in the nesting season. For GBH, incubating adults from nearby nests or potentially the entire rookery will flush from the nest during egg collection, leaving the eggs susceptible to drops in temperature and predation. The development of a relationship between concentrations of PCDD/DFs in nestling plasma and eggs would allow researchers to limit time in the rookery to after hatch out when rookery disturbance may have a lesser impact on nest abandonment and/or success. Additionally, nestling plasma sampling is non-destructive, which is beneficial when working with endangered or threatened species. Not collecting an egg also eliminates the need to account for sampling in hatch success calculations. Predicting concentrations of PCDD/DFs in eggs from those in nestling plasma is an effective, non-destructive method. Use of this method does presuppose that foraging habits of adults remain similar throughout the nesting cycle, so that adults during egg formation are exposed similarly to nestlings. This type of relationship has also previously been developed for great horned owls and bald eagles (Strause *et al.* 2007b).

Conclusions

Great blue heron nesting within the TR floodplain are exposed to greater concentrations of PCDD/DFs compared to those from associated reference areas. Comparison of observed concentrations of PCDD/DFs and dioxin-like PCBs in tissues of

GBH nesting within the TR floodplain to threshold effect concentrations suggest there is minimal to no risk of adverse effects present. Minimal to no risk of adverse effects was also predicted from a dietary exposure assessment of GBH in this same system (Seston *et al.* 2010b). Conclusions from each of these lines of evidence are in agreement with site-specific measures of GBH population condition.

Acknowledgements

The authors would like to thank all staff and students of the Michigan State University – Wildlife Toxicology Laboratory field crew; namely M. Nadeau, W. Folland, D. Hamman, and S. Plautz for their tree-climbing abilities, along with E. Koppel, J. Moore, L. Williams, and C. Bartrem. We gratefully acknowledge M. Fales for his design and fabrication of specialized equipment which was pivotal to the success of this research. Additionally, we would like to recognize P. Bradley, M. Kramer, and N. Ikeda for their assistance in the laboratory. The following people were key to the success of the project, J. Dastyck and S. Kahl of the United States Fish and Wildlife Service – Shiawassee National Wildlife Refuge for their assistance and access to the refuge; Saginaw County Parks and Recreation Commission for access to Imerman Park; Tittabawassee Township Park Rangers for access to Tittabawassee Township Park and Freeland Festival Park; and Michael Bishop of Alma College for guidance as our Master Bander. We would also like to acknowledge the greater than 50 cooperating landowners throughout the study area who have granted us access to their property, making our research possible. Thanks also to S. Roark for reviewing drafts of this manuscript.

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 this study that involved the use of animals were conducted using the most humane means possible. To achieve that objective, all aspects of the study were performed following standard operation procedures (GBH adult handling 03/05-036-00; GBH nest monitoring 05/07-066-00; Field studies in support of TR ERA 03/04-042-00; Protocol for fish sampling 03/04-043-00) 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 SC1254 for GBH/SC permit for fish (Zwiernik)/SC permit for amphibians (Zwiernik); USFWS Migratory Bird Scientific Collection Permit MB1000062-0; and subpermitted under US Department of the Interior Federal Banding Permit 22926) are on file at MSU-WTL.

Supplemental Information

Table 4.7. Concentrations of 2378-TCDD equivalents (TEQs[a]) in eggs and nestling tissues of great blue herons collected during 2006–2007 from rookeries within the Tittabawassee and Saginaw River floodplains, Midland, MI, USA. Values (ng/kg wet wt) are given as geometric mean (sample size) over the 95% confidence interval.

	Eggs	Plasma	Adipose	Liver	Muscle
PCDD-$TEQ_{WHO\text{-}Avian}$[b]					
	16 (24)	0.33 (23)	43 (6)	1.3 (6)	1.5 (6)
	12–20	0.25–0.43	29–63	0.83–2.1	0.74–3.2
PCDF-$TEQ_{WHO\text{-}Avian}$[c]					
	16 (24)	0.72 (23)	66 (6)	2.4 (6)	2.4 (6)
	13–21	0.52–0.99	31–140	1.2–4.6	0.98–6.1
non-*ortho* PCB-$TEQ_{WHO\text{-}Avian}$[d]					
	130 (18)	2.6 (8)	480 (6)	16 (6)	14 (6)
	90–180	1.7–4.0	220–1000	6.6–40	4.7–43
mono-ortho PCB-$TEQ_{WHO\text{-}Avian}$[e]					
	9.8 (18)	0.14 (8)	29 (6)	0.71 (6)	0.93 (6)
	6.9–14	0.083–0.24	15–57	0.38–1.3	0.35–2.5
Total $TEQ_{WHO\text{-}Avian}$					
	170 (18)	4.2 (8)	620 (6)	21 (6)	19 (6)
	130–230	2.9–6.1	300–1300	9.6–46	6.8–55

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

[b] PCDD-$TEQ_{WHO\text{-}Avian}$= summation of the TEQs of individual PCDD congeners

[c] PCDF-$TEQ_{WHO\text{-}Avian}$= summation of the TEQs of individual PCDF congeners

[d] non-*ortho* PCB-$TEQ_{WHO\text{-}Avian}$= summation of the TEQs of individual non-*ortho* substituted PCB congeners

[e] mono-*ortho* PCB-$TEQ_{WHO\text{-}Avian}$= summation of the TEQs of individual mono-*ortho* substituted PCB congeners

Table 4.8. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in blood plasma of great blue herons collected during 2005-2007 as a result of trapping within the Chippewa and Tittabawassee River floodplains, Midland, MI, USA. Values[a] (ng/kg wet wt) are given as the arithmetic mean[b] ± 1 SD over the range.

	Reference Areas		Study Areas	
	HY	AHY	HY	AHY
Chemical[c]	*n*=8	*n*=7	*n*=16	*n*=20
2378-TCDF	0.27±0.14	0.39±0.17	2.7±1.9	1.6±1.3
	0.095–0.48	0.21–0.61	0.76–7.3	0.12–5.9
	2ND	3ND		
23478-PeCDF	0.27±0.15	0.34±0.18	1.6±1.3	6.4±4.1
	0.13–0.48	0.15–0.56	0.40–4.8	0.75–13
	2ND			
12378-PeCDF	0.094		0.96±0.79	0.71±0.53
		0.10–0.14	0.16–2.9	0.12–1.7
	7ND	5ND		4ND
234678-HxCDF			0.38	0.27±0.20
				0.084–0.53
	8ND	7ND	15ND	14ND
123789-HxCDF	8ND	7ND	16ND	20ND
123678-HxCDF		0.16	0.26±0.13	0.42±0.17
			0.10–0.44	0.20–0.91
	8ND	6ND	10ND	5ND
123478-HxCDF		0.29	0.67±0.46	1.1±0.56
			0.12–1.5	0.21–2.4
	8ND	6ND	4ND	
1234789-HpCDF				0.15
	8ND	7ND	16ND	19ND
1234678-HpCDF			0.31±0.19	0.56±0.41
			0.089–0.64	0.11–1.0
	8ND	7ND	7ND	14ND
12346789-OCDF			0.60	1.0±0.43
				0.46–1.5
	8ND	7ND	15ND	16ND
2378-TCDD	0.50±0.29	0.62±0.32	0.52±0.31	1.4±0.58
	0.22–1.0	0.23–1.1	0.19–1.3	0.35–2.1
		1ND	1ND	

Table 4.8 (cont'd)

12378-PeCDD	0.36±0.14	0.37±0.17	0.38±0.22	1.2±0.71
	0.18–0.53	0.14–0.52	0.13–0.76	0.25–3.2
	1ND	3ND	3ND	1ND
123789-HxCDD				0.49
	8ND	7ND	0.12–0.15	
123678-HxCDD			14ND	19ND
	0.25–0.59	0.35±0.11	0.43±0.23	1.1±0.64
	6ND	0.20–0.44	0.18–0.75	0.30–2.6
123478-HxCDD		3ND	7ND	3ND
				0.39±0.21
	8ND	7ND	0.18–0.33	0.16–0.64
1234678-HpCDD	0.50±0.085	0.27	14ND	15ND
	0.45–0.60		0.53±0.25	0.67±0.42
	5ND	6ND	0.25–0.94	0.12–1.5
12346789-OCDD	1.2±0.47	1.1±0.80	5ND	9ND
	0.76–1.8	0.46–2.5	2.3±1.6	5.1±6.2
	4ND	1ND	0.62–7.0	0.32–26
			1ND	1ND

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 4.9. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in eggs of great blue herons collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (ng/kg wet wt) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	FRE *n*=8	SNWR *n*=8	CAS *n*=8
2378-TCDF	3.2±3.5	5.2±2.8	3.1±1.8
	0.30–10	0.44–9.2	1.1–5.8
23478-PeCDF	20±15	14±16	11±5.0
	4.2–48	3.7–51	5.6–20
12378-PeCDF	0.49±0.47	1.0±0.62	0.42±0.37
	0.07–1.2	0.47–1.9	0.18–1.3
	2ND	2ND	
234678-HxCDF	0.87±0.71	0.83±0.57	0.55±0.27
	0.30–2.1	0.39–1.8	0.28–0.98
		3ND	
123789-HxCDF	8ND	8ND	8ND
123678-HxCDF	1.9±1.8	1.3±0.87	1.5±0.82
	0.43–4.4	0.62–3.3	0.75–3.1
123478-HxCDF	3.6±6.5	1.7±1.6	1.1±0.58
	0.31–20	0.55–5.1	0.53–2.0
		1ND	
1234789-HpCDF	0.22±0.20		
	0.10–0.46		
	5ND	8ND	8ND
1234678-HpCDF	0.46±0.36	0.71±0.41	0.31±0.13
	0.20–1.3	0.27–1.1	0.12–0.53
		5ND	
12346789-OCDF	0.17±0.098		
	0.085–0.34	0.085–0.45	0.071–0.12
	3ND	6ND	6ND
2378-TCDD	18±19	8.7±7.8	7.0±2.7
	5.9–65	3.2–27	4.3–8.2
12378-PeCDD	12±7.6	5.6±3.3	6.2±1.6
	4.2–28	3.1–13	4.2–8.2
123789-HxCDD	0.65±0.29	0.78±0.35	0.58±0.21
	0.27–1.1	0.41–1.3	0.30–0.93
		4ND	
123678-HxCDD	9.4±6.9	5.6±2.9	6.5±1.9

Table 4.9 (cont'd)

	2.7–23	2.5–10	3.9–9.1
123478-HxCDD	1.9±1.0	1.1±0.51	1.3±0.80
	0.69–3.9	0.50–1.8	0.55–2.9
1234678-HpCDD	2.1±1.8	2.4±1.4	1.4±0.82
	0.88–6.7	1.3–5.3	0.67–3.3
		1ND	
12346789-OCDD	5.1±3.7	6.8±5.1	4.5±2.1
	0.90–12	2.1–17	1.3–6.9

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 4.10. Concentrations of selected co-contaminants in eggs of great blue herons collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (µg/kg wet wt) are given as the arithmetic mean ± 1 SD over the range.

Chemical[b]	FRE *n*=8	SNWR *n*=2	CAS *n*=8
PCB 77	0.59±0.45 0.055–1.4	0.78–0.96	0.64±0.43 0.093–1.6
PCB 81	0.51±0.36 0.030–1.1	0.30–0.33	0.30±0.16 0.086–0.54
PCB 126	1.0±0.65 0.15–1.9	0.26–0.30	0.69±0.34 0.40–1.3
PCB 169	0.10±0.067 0.026–0.22	0.023–0.029	0.074±0.048 0.038–0.17
PCB 105	86±60 11–180	23–27	49±14 32–67
PCB 114	9.4±6.3 1.0–19	1.9–2.7	5.6±2.4 2.9–11
PCB 118	300±230 37–610	56–74	150±80 87–340
PCB 123	6.8±4.4 0.78–14	1.8–2.1	4.7±2.2 2.8–9.3
PCB 156	34±22 5.7–69	6.8–7.6	22±7.9 14–37
PCB 157	7.6±5.1 1.1–15	1.4–1.9	5.0±1.9 3.0–8.5
PCB 167	15±10 3.3–31	3.3–4.3	10±2.1 7.5–14
PCB 189	4.0±2.6 0.65–7.6	0.79–0.81	2.7±1.1 1.4–5.1
2,4'-DDT[b]	0.77±1.6 0.050–4.1	0.090–0.30	0.11±0.071 0.016–0.23
2',4'-DDE[c]	2ND 670±480		500±200
4, 4'-DDT	180–1600 9.6±19	180–560	320–820 0.66±0.68
	0.23–55	0.77–1.4	0.15–2.0

Table 4.10 (cont'd)

[a] Values have been rounded and represent only two significant figures

[b] DDT = dichloro-diphenyl-trichloroethane

[c] DDE = dichloro-diphenyl-dichloroethylene

Table 4.11. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in blood plasma of great blue herons nestlings collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (ng/kg wet wt) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	FRE *n*=12	SNWR *n*=4	CAS *n*=7
2378-TCDF	0.67±0.64	1.1±1.5	0.26±0.19
	0.080–2.2	0.18–3.4	0.11–0.74
	2ND		1ND
23478-PeCDF	0.57±0.45	0.84±1.1	0.27±0.22
	4.2–48	0.21–2.5	0.11–0.74
12378-PeCDF	0.36±0.30	0.36±0.45	0.15±0.12
	0.025–0.95	0.072–1.0	0.073–0.33
	3ND		3ND
234678-HxCDF	0.044±0.0091	0.032	
	0.037–0.054		
	9ND	3ND	7ND
123789-HxCDF	12ND	4ND	7ND
123678-HxCDF	0.097±0.030	0.14±0.12	0.079
	0.064–0.13	0.074–0.32	
	7ND		6ND
123478-HxCDF	0.21±0.14	0.18±0.21	0.14±0.085
	0.057–0.53	0.053–0.42	0.055–0.22
	2ND	1ND	4ND
1234789-HpCDF	12ND	4ND	7ND
1234678-HpCDF	0.12±0.070	0048±0.019	0.11±0.032
	0.033–0.25	0.034–0.069	0.062–0.13
	2ND	1ND	2ND
12346789-OCDF	0.18±0.10		0.091
	0.11–0.26		
	10ND	4ND	6ND
2378-TCDD	0.40±0.32	0.42±0.46	0.20±0.12
	0.11–1.2	0.16–1.1	0.12–0.34
	2ND		4ND
12378-PeCDD	0.26±0.15	0.37±0.44	
	0.16–0.59	0.11–0.88	0.079–0.31

Table 4.11 (cont'd)

	5ND	1ND	5ND
123789-HxCDD	12ND	4ND	7ND
123678-HxCDD	0.17±0.10	0.30±0.21	
	0.068–0.31	0.11–0.77	0.060–0.26
	6ND		5ND
123478-HxCDD	0.085	0.048	
	11ND	3ND	7ND
1234678-HpCDD	0.20±0.095	0.15±0.11	0.17±0.036
	0.069–0.32	0.081–0.27	0.13–0.21
	2ND	1ND	3ND
12346789-OCDD	2.3±2.0	0.91±0.44	2.5±2.0
	0.30–7.0	0.39–1.3	0.61–6.1

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 4.12. Concentrations of selected co-contaminants in blood plasma of great blue herons nestlings collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values (ng/kg wet wt) are given as the arithmetic mean ± 1 SD over the range.

Chemical	FRE *n*=4	SNWR *n*=4	CAS *n*=0
PCB 77	13±9.0 3.2–24	25±14 16–45	
PCB 81	7.0±3.1 2.6–9.7	15±6.9 11–25	
PCB 126	8.4±7.7 1.7–19	8.7±2.5 6.2–12	
PCB 169	1.2±0.13 0.99–1.3	1.6±0.39 1.2–2.1	
PCB 105	940±740 230–2000	890±240 600–1200	
PCB 114	69±54 16–150	71±19 50–94	
PCB 118	2700±2100 580–5700	2600±590 2000–3300	
PCB 123	61±44 15–120	67±16 56–91	
PCB 156	300±230 58–610	280±63 220–360	
PCB 157	66±52 13–140	63±12 52–79	
PCB 167	140±100 28–280	150±27 120–180	
PCB 189	36±25 9.2–69	37±6.3 32–44	
2,4'-DDT [a]	0.0094±0.0045 0.0038–0.014	0.028±0.028 0.012–0.071	
2',4'-DDE [b]	8.4±7.2 1.8–19	8.1±2.1 5.6–11	
4, 4'-DDT	0.087±0.062 0.032–0.17	0.081±0.083 0.020–0.20	

[a] DDT = dichloro-diphenyl-trichloroethane

[b] DDE = dichloro-diphenyl-dichloroethylene

Table 4.13. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in adipose of great blue heron nestlings collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (ng/kg wet wt) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	FRE *n*=1	SNWR *n*=3	CAS *n*=2
2378-TCDF	30	20±19 0.57–37	13–15
23478-PeCDF	33	21±23 1.0–46	17–32
12378-PeCDF	6.7	2.2±1.7 0.26–3.3	3.4–4.3
234678-HxCDF	2.3	1.2±1.1 0.029–2.3	1.8–3.4
123789-HxCDF	1ND	3ND	2ND
123678-HxCDF	6.7	2.9±2.6 0.058–5.2	3.1–7.2
123478-HxCDF	4.7	1.8±1.6 0.16–3.3	3.0–5.0
1234789-HpCDF	1ND	3ND	2ND
1234678-HpCDF	2.8	1.4±1.2 0.056–2.2	2.4–5.1
12346789-OCDF	0.57	1.1 2ND	2ND
2378-TCDD	28	16±16 0.22–32	15–17
12378-PeCDD	21	12±12 0.11–24	14–21
123789-HxCDD	2.2	1.1–2.1 1ND	2.3–3.0
123678-HxCDD	18	9.2±8.8 0.078–18	13–24
123478-HxCDD	3.2	1.7–2.7	2.5–3.3

Table 4.13 (cont'd)

		1ND	
1234678-HpCDD	5.3	1.9±1.6	
		0.074–2.9	4.2–5.4
12346789-OCDD	3.3	2.6±2.0	
		0.55–4.6	2.6–4.7

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 4.14. Concentrations of selected co-contaminants in adipose of great blue heron nestlings collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (µg/kg wet wt) are given as the arithmetic mean ± 1 SD over the range.

Chemical	FRE *n*=1	SNWR *n*=3	CAS *n*=2
PCB 77	3.3	5.4±3.2	
		2.7–9.0	2.2–2.5
PCB 81	1.7	3.0±1.9	
		1.2–5.0	0.18–0.33
PCB 126	2.0	2.9±1.6	
		1.1–3.9	0.69–1.2
PCB 169	0.15	0.18±0.094	
		0.081–0.27	0.086–0.11
PCB 105	190	250±140	
		90–360	73–110
PCB 114	14	19±10	
		7.2–26	7.0–11
PCB 118	610	810±440	
		300–1100	200–350
PCB 123	13	18±9.0	
		7.2–24	5.4–8.3
PCB 156	63	79±41	
		32–110	32–45
PCB 157	13	18±8.7	
		8.1–25	6.9–11
PCB 167	25	32±15	
		17–47	19–30
PCB 189	7.4	8.8±4.3	
		4.2–13	3.8–5.4
2,4'-DDT[b]	0.19	0.77±0.45	
		0.26–1.1	0.038–0.054
2',4'-DDE[c]	2000	1800±240	
		1500–2000	1500–1900
4, 4'-DDT	9.4	9.9±10	
		3.4–22	5.5–6.6

[a] Values have been rounded and represent only two significant figures

Table 4.14 (cont'd)

[b] DDT = dichloro-diphenyl-trichloroethane

[c] DDE = dichloro-diphenyl-dichloroethylene

Table 4.15. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in liver of great blue heron nestlings collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (ng/kg wet wt) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	FRE *n*=1	SNWR *n*=3	CAS *n*=2
2378-TCDF	0.74	47±80 0.52–140	0.42–0.49
23478-PeCDF	1.3	36±58 0.66–100	0.83–1.3
12378-PeCDF	0.20	0.33–31	
		1ND	2ND
234678-HxCDF		0.16–3.5	
	1ND	1ND	2ND
123789-HxCDF	1ND	3ND	2ND
123678-HxCDF	0.18	4.1±6.8 0.14–12	
123478-HxCDF	0.24	4.9±8.1 0.13–14	2ND
1234789-HpCDF	1ND	3ND	2ND
1234678-HpCDF		0.16–5.4	
	1ND	1ND	2ND
12346789-OCDF	1ND	3ND	2ND
2378-TCDD	0.77	13±22 0.43–39	0.48–0.48
12378-PeCDD	0.65	11±17 0.34–31	0.43–0.60
123789-HxCDD		3.5	
	1ND	2ND	2ND
123678-HxCDD	0.66	9.0±15 0.30–26	0.46–0.51
123478-HxCDD		0.21–4.3	
	1ND	1ND	2ND
1234678-HpCDD	0.30	2.6±3.8 0.24–7.0	0.29–0.38
12346789-OCDD	1.3	2.7±3.4 0.70–6.6	0.74–4.1

[a] Values have been rounded and represent only two significant figures

Table 4.15 (cont'd)

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 4.16. Concentrations of selected co-contaminants in liver of great blue heron nestlings collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values (µg/kg wet wt) are given as the arithmetic mean ± 1 SD over the range.

Chemical	FRE *n*=1	SNWR *n*=3	CAS *n*=2
PCB 77	0.11	0.22±0.085 0.14–0.31	0.057–0.076
PCB 81	0.091	0.140±0.053 0.097–0.20	0.0015–0.0058
PCB 126	0.040	0.071±0.070 0.024–0.15	0.022–0.024
PCB 169	0.0038	0.0050±0.0047 0.0013–0.010	0.0023–0.0026
PCB 105	3.6	6.5±4.7 2.1–11	2.8–2.9
PCB 114	0.26	0.49±0.38 0.15–0.90	0.23–0.25
PCB 118	9.3	19±17 5.5–37	7.5–8.6
PCB 123	0.23	0.41±0.31 0.16–0.76	0.13–0.14
PCB 156	1.1	1.9±1.5 0.67–3.5	0.99–1.2
PCB 157	0.24	0.39±0.31 0.16–0.75	0.21–0.26
PCB 167	0.49	0.88±0.78 0.35–1.8	0.55–0.64
PCB 189	0.12	0.19±0.14 0.076–0.35	0.11–0.21
2,4'-DDT [a]	0.0049	0.0077±0.0059 0.0031–0.014	0.027 1ND
2',4'-DDE [b]	33	37±16 27–55	31–53
4, 4'-DDT	0.011	0.013±0.0086 0.0062–0.023	0.022 1ND

Note: Values have been rounded and represent only two significant figures

[a] DDT = dichloro-diphenyl-trichloroethane

[b] DDE = dichloro-diphenyl-dichloroethylene

Table 4.17. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in muscle of great blue heron nestlings collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[a] (ng/kg wet wt) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	FRE *n*=1	SNWR *n*=3	CAS *n*=2
2378-TCDF	1.1	2.1±1.1 0.95–3.0	0.37–0.42
23478-PeCDF	0.83	2.9±2.1 0.68–4.8	0.42–0.92
12378-PeCDF	0.41	0.33–0.50	
234678-HxCDF	1ND	1ND 3ND	2ND 2ND
123789-HxCDF	1ND	3ND	2ND
123678-HxCDF	0.22	0.50±0.38 0.22–0.93	
123478-HxCDF	1ND	0.40–0.44 1ND	2ND
1234789-HpCDF	1ND	3ND	2ND
1234678-HpCDF	1ND	0.31 2ND	2ND
12346789-OCDF	1ND	0.62 2ND	2ND
2378-TCDD	0.77	1.5±1.4 0.57–3.1	0.33–0.70
12378-PeCDD	0.54	1.1±0.96 0.41–2.2	0.39–0.56
123789-HxCDD	1ND	3ND	2ND
123678-HxCDD	0.39	0.92±0.64 0.38–1.6	0.24–0.61
123478-HxCDD	1ND	3ND	2ND
1234678-HpCDD		0.48±0.19	

Table 4.17 (cont'd)

	1ND	0.27–0.62	2ND
12346789-OCDD	0.66	2.2±1.1	
		1.5–3.4	2ND

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 4.18. Concentrations of selected co-contaminants in muscle of great blue heron nestlings collected during 2006-2007 from rookeries within the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values (µg/kg wet wt) are given as the arithmetic mean ± 1 SD over the range.

Chemical	FRE *n*=1	SNWR *n*=3	CAS *n*=2
PCB 77	0.089	0.25±0.16 0.083–0.40	0.048–0.063
PCB 81	0.037	0.12±0.11 0.028–0.24	0.0034–0.0079
PCB 126	0.043	0.14±0.16 0.034–0.33	0.016–0.027
PCB 169	0.0051	0.014±0.010 0.0067–0.025	0.0018–0.0020
PCB 105	3.8	13±13 2.7–28	2.0–3.3
PCB 114	0.35	1.1±1.2 0.23–2.4	0.15–0.31
PCB 118	13	35±37 8.9–77	6.5–11
PCB 123	0.30	0.93±0.98 0.21–2.0	0.065–0.21
PCB 156	1.6	4.6±5.2 1.1–11	0.75–1.3
PCB 157	0.30	1.0±1.1 0.25–2.3	0.16–0.31
PCB 167	0.79	2.3±2.6 0.63–5.3	0.43–0.86
PCB 189	0.18	0.57±0.64 0.14–1.3	0.088–0.18
2,4'-DDT [a]	0.019	0.043±0.010 0.031–0.050	0.0077–0.025
2',4'-DDE [b]	51	110±95 51–220	45–48
4, 4'-DDT	0.061	0.16±0.11 0.068–0.27	0.084–0.090

Note: Values have been rounded and represent only two significant figures

[a] DDT = dichloro-diphenyl-trichloroethane

[b] DDE = dichloro-diphenyl-dichloroethylene

References

Allard, P., Fairbrother, A., Hope, B. K., Hull, R. N., Johnson, M. S., Kapustka, L., Mann, G., McDonald, B., and Sample, B. E. (2010). Recommendations for the development and application of wildlife toxicity reference values. *Integrated Environmental Assessment and Management* **6**(1), 28-37.

Blankenship, A. L., Kay, D. P., Zwiernik, M. J., Holem, R. R., Newsted, J. L., Hecker, M., and Giesy, J. P. (2008). Toxicity reference values for mink exposed to 2,3,7,8-tetrachlodibenzo-*p*-dioxin (TCDD) equivalents (TEQs). *Ecotoxicology and Environmental Safety* **69**(3), 325-349.

Blus, L. J., Henny, C. J., and Kaiser, T. E. (1980). Pollution ecology of breeding great blue herons in the Columbia Basin, Oregon and Washington. *The Murrelet* **61**(2), 63-71.

Braune, B. M., and Norstrom, R. J. (1989). Dynamics of organochlorine compounds in herring gulls: III. Tissue distribution and bioaccumulation in Lake Ontario gulls. *Environmental Toxicology and Chemistry* **8**(10), 957-968.

Brunström, B. (1988). Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. *Poultry Science* **67**(1), 52-57.

Butler, R. W. (1992). Great blue heron. A. Poole, P. Stettenheim, & F. Gill (Eds.), *The Birds of North America*, No. 25. Philadelphia: The Academy of Natural Sciences; Washington, DC: The American Ornithologists's Union.

Butler, R. W. (1997). The Great Blue Heron: A Natural History and Ecology of a Seashore Sentinel, UBC Press, Vancouver, BC.

Coefield, S. J., Giesy, J. P., Fredricks, T. B., Seston, R. M., Nadeau, M. W., Tazelaar, D. L., Moore, J. N., Kay, D. P., Roark, S. A., and Zwiernik, M. J. (2010a). Great horned owl (*Bubo virginianus*) dietary exposure to PCDD/DFs in the Tittabawassee River floodplain in Midland, Michigan, USA. *Environmental Toxicology and Chemistry* (in press).

Coefield, S. J., Zwiernik, M. J., Fredricks, T. B., Seston, R. M., Nadeau, M. W., Tazelaar, D. L., Kay, D. P., Newsted, J. L., and Giesy, J. P. (2010b). Ecological risk assessment of great horned owls (*Bubo virginianus*) exposed to PCDDs/PCDFs in the Tittabawassee River floodplain in Midland, Michigan, USA. *Environmental Toxicology and Chemistry* (in press).

Cohen-Barnhouse, A. M., Zwiernik, M. J., Link, J. E., Fitzgerald, S. D., Kennedy, S. W., Giesy, J. P., Wiseman, S. B., Yang, Y., Jones, P. D., Wan, Y., Collins, B., Newsted, J. L., Kay, D. P., and Bursian, S. J. (2010). Sensitivity of Japanese quail (*Coturnix japonica*), common pheasant (*Phasianus colchicus*) and white leghorn

chicken (*Gallus gallus domesticus*) embryos to *in ovo* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF). *Toxicological Sciences* (in press).

Custer, C. M., Custer, T. W., Rosiu, C. J., Melancon, M. J., Bickham, J. W., and Matson, C. W. (2005). Exposure and effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in tree swallows (*Tachycineta bicolor*) nesting along the Woonasquatucket River, Rhode Island, USA. *Environmental Toxicology and Chemistry* **24**(1), 93-109.

Custer, T. W., Hines, R. K., Melancon, M. J., Hoffman, D. J., Wickliffe, J. K., Bickham, J. W., Martin, J. W., and Henshel, D. S. (1997). Contaminant concentrations and biomarker response in great blue heron eggs from 10 colonies on the upper Mississippi River, USA. *Environmental Toxicology and Chemistry* **16**(2), 260-271.

Custer, T. W., Rattner, B. A., Ohlendorf, H. F., and Melancon, M. J. (1991). Herons and egrets as proposed indicators of estuarine contamination in the United States. *Acta XX Congressus Internationalis Ornithologici* **20**, 2474-2479.

Davis, W. J. (1982). Territory size in *Megacerlye alcyon* along a stream habitat. *The Auk* **99**, 353-362.

Dowd, E. M., and Flake, L. D. (1985). Foraging habitats and movements of nesting great blue herons in a prairie river ecosystem, South Dakota. *Journal of Field Ornithology* **56**(4), 379-387.

Elliott, J. E., Butler, R. W., Norstrom, R. J., and Whitehead, P. E. (1989). Environmental contaminants and reproductive success of great blue herons *Ardea herodias* in British Columbia, 1986-87. *Environmental Pollution* **59**, 91-114.

Elliott, J. E., Harris, M. L., Wilson, L. K., Whitehead, P. E., and Norstrom, R. J. (2001). Monitoring temporal and spatial trends in polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in eggs of great blue heron (*Ardea herodias*) on the coast of British Columbia, Canada, 1983-1998. *Ambio* **30**(7), 416-428.

Elliott, J. E., and Norstrom, R. J. (1998). Chlorinated hydrocarbon contaminants and productivity of bald eagle populations on the Pacific coast of Canada. *Environmental Toxicology and Chemistry* **17**(6), 1142-1153.

Elliott, J. E., Wilson, L. K., Langelier, K. W., and Norstrom, R. J. (1996). Bald eagle morality and chlorinated hydrocarbon contaminants in livers from British Columbia, Canada, 1989-1994. *Environmental Pollution* **94**(1), 9-18.

Fairbrother, A. (2003). Lines of evidence in wildlife risk assessments. *Human and Ecological Risk Assessment* **9**, 1475-1491.

Fairbrother, A. (2001). Putting the impacts of environmental contamination into perspective. In *Ecotoxicology of Wild Mammals* (R.F.Shore and B.A.Rattner, Eds.), pp. 671-689. John Wiley & Sons Ltd., West Sussex, England.

Fredricks, T. B., Giesy, J. P., Coefield, S. J., Seston, R. M., Haswell, M. M., Tazelaar, D. L., Bradley, P. W., Moore, J. N., Roark, S. A., and Zwiernik, M. J. (2010a). Dietary exposure of three passerine species to PCDD/DFs from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. *Environmental Monitoring and Assessment*; available online DOI: 110.1007/s10661-010-1319-5.

Fredricks, T. B., Zwiernik, M. J., Seston, R. M., Coefield, S. J., Plautz, S. C., Tazelaar, D. L., Shotwell, M. S., Bradley, P. W., Kay, D. P., and Giesy, J. P. (2010b). Passerine exposure to primarily PCDFs and PCDDs in the river floodplains near Midland, Michigan, USA. *Archives of Environmental Contamination and Toxicology* **58**, 1048-1064.

Giesy, J. P., Ludwig, J. P., and Tillitt, D. E. (1994). Dioxins, Dibenzofurans, PCBs and Colonial Fish-Eating Water Birds. In *Dioxin and Health* (A.Schecter, Ed.), pp. 249-307. Plenum Press, New York, NY.

Gilbertson, M. (1983). Etiology of chick edema disease in herring gulls in the lower Great Lakes. *Chemosphere* **12**(3), 357-370.

Harris, M. L., Elliott, J. E., Butler, R. W., and Wilson, L. K. (2003). Reproductive success and chlorinated hydrocarbon contamination of resident great blue herons (*Ardea herodias*) from coastal British Columbia, Canada, 1977 to 2000. *Environmental Pollution* **121**, 207-227.

Head, J. A., Hahn, M. E., and Kennedy, S. W. (2008). Key amino acids in the aryl hydrocarbon receptor predict dioxin sensitivity in avian species. *Environmental Science & Technology* **42**(19), 7535-7541.

Heinz, G. H., Hoffman, D. J., Klimstra, J. D., Stebbins, K. R., Kondrad, S. L., and Erwin, C. A. (2009). Species differences in the sensitivity of avian embryos to methylmercury. *Archives of Environmental Contamination and Toxicology* **56**, 129-138.

Henny, C. J., and Meeker, D. L. (1987). An evaluation of blood plasma for monitoring DDE in birds of prey. *Environmental Pollution (Series A)* **25**, 291-304.

Hervé, J. C., Crump, D., Jones, S. P., Mundy, L. J., Giesy, J. P., Zwiernik, M. J., Bursian, S. J., Jones, P. D., Wiseman, S. B., Wan, Y., and Kennedy, S. W. (2010a).

Cytochrome P4501A Induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and two chlorinated dibenzofurans in primary hepatocyte cultures of three avian species. *Toxicol. Sci.* **113**(2), 380-391.

Hervé, J. C., Crump, D., Mclaren, K., Giesy, J. P., Zwiernik, M. J., Bursian, S. J., and Kennedy, S. W. (2010b). 2,3,4,7,8-Pentachlorodibenzofuran is a more potent cytochrome P4501A inducer than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in herring gull hepatocyte cultures. *Environmental Toxicology and Chemistry*; available online DOI: 10.1002/etc.255.

Hilscherova, K., Kannan, K., Nakata, H., Hanari, N., Yamashita, N., Bradley, P. W., McCabe, J. M., Taylor, A. B., and Giesy, J. P. (2003). Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. *Environmental Science and Technology* **37**(3), 468-474.

Hoffman, D. J., Rattner, B. A., Sileo, L., Docherty, D. E., and Kubiak, T. J. (1987). Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. *Environmental Research* **42**, 176-184.

Hoffman, D. J., Rice, C. P., and Kubiak, T. J. (1996). PCBs and dioxins in birds. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations* (W.N.Beyer, G.H.Heinz, and A.W.Redmon-Norwood, Eds.), pp. 165-207. Lewis Publishers, Boca Raton, FL, USA.

Hoysak, D. J., and Weatherhead, P. J. (1991). Sampling blood from birds - a technique and an assessment of its effect. *The Condor* **93**, 746-752.

Hoyt, D. F. (1979). Practical methods of estimating volume and fresh weight of bird eggs. *The Auk* **96**, 73-77.

Karchner, S. I., Franks, D. G., Kennedy, S. W., and Hahn, M. E. (2006). The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor. *PNAS* **103**(16), 6252-6257.

Kubiak, T. J., Harris, H. J., Smith, L. M., Schwartz, T. R., Stalling, D. L., Trick, J. A., Sileo, L., Docherty, D., and Erdman, T. C. (1989). Microcontaminants and reproductive impairment of the Forster's tern in Green Bay, Lake Michigan-1983. *Archives of Environmental Contamination and Toxicology* **18**, 706-727.

Kubota, A., Iwata, H., Tanabe, S., and Tobata, S. (2005). Hepatic CYP1A induction by dioxin-like compounds, and congener-specific metabolism and sequestration in wild common cormorants from Lake Biwa, Japan. *Environmental Science and Technology* **39**(10), 3611-3619.

Kubota, A., Iwata, H., Tanabe, S., Yoneda, K., and Tobata, S. (2004). Levels and toxicokinetic behaviors of PCDD, PCDF, and coplanar PCB congeners in common cormorants from Lake Biwa, Japan. *Environmental Science & Technology* **38**(14), 3853-3859.

Kunisue, T., Nakanishi, S., Oka, N., Sato, F., Tsurumi, M., and Tanabe, S. (2006). Dioxins and related compounds in albatrosses from the Torishima Island, Japan: accumulation features by growth stage and toxicological implications. *Environmental Science and Technology* **40**, 6919-6927.

Laporte, P. (1982). Organochlorine residues and eggshell measurements of great blue heron eggs from Quebec. *Colonial Waterbirds* **5**, 95-103.

Leonards, P. E. G., van Hattum, B., and Leslie, H. (2008). Assessing the risks of persistent organic pollutants to top predators: A review of approaches. *Integrated Environmental Assessment and Management* **4**(4), 386-398.

Mandal, P. K. (2005). Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology: Part B* **175**, 221-230.

Marion, L. (1989). Territorial feeding and colonial breeding are not mutually exclusive: the case of the grey heron (*Ardea cinerea*). *The Journal of Animal Ecology* **58**(2), 693-710.

Mazeika, S., Sullivan, P., Watzin, M. C., and Hession, W. C. (2006). Differences in the reproductive ecology of belted kingfishers (*Ceryle alcyon*) across streams with varying geomorphology and habitat quality. *Waterbirds* **29**(3), 258-270.

McAloney, K. (1973). The breeding biology of the great blue heron on Tobacco Island, Nova Scotia. *Canadian Field-Naturalist* **87**, 137-140.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Stromborg, K. L., Kubiak, T. J., Giesy, J. P., and Bursian, S. J. (1997a). Organochlorine contaminants in double-crested cormorants from Green Bay, Wisconsin: II. Effects of an extract derived from cormorant eggs on the chicken embryo. *Archives of Environmental Contamination and Toxicology* **32**, 316-322.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Kelly, M. E., Stromborg, K. L., Melancon, M. J., Fitzgerald, S. D., and Bursian, S. J. (1998). Effects of 3,3',4,4',5-pentachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin injected into the yolks of double-crested cormorant (*Phalacrocorax auritus*) eggs prior to incubation. *Environmental Toxicology and Chemistry* **17**(10), 2035-2040.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Powell, J. F., Restum, J. C., Stromborg, K. L., Giesy, J. P., and Bursian, S. J. (1997b). Effects of 3,3',4,4',5-

pentachlorobiphenyl (PCB 126), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), or an extract derived from field-collected cormorant eggs injected into double-crested cormorant (*Phalacrocorax auritus*) eggs. *Environmental Toxicology and Chemistry* **16**(7), 1450-1455.

Sanderson, J. T., Elliott, J. E., Norstrom, R. J., Whitehead, P. E., Hart, L. E., Cheng, K. M., and Bellward, G. D. (1994). Monitoring biological effects of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in great blue heron chicks (*Ardea herodias*) in British Columbia. *Journal of Toxicology and Environmental Health* **41**, 435-450.

Sanderson, J. T., and Bellward, G. D. (1995). Hepatic microsomal ethoxyresorufin-*O*-deethylase-inducing potency in ovo and cytosolic Ah receptor binding affinity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: comparison of four avian species. *Toxicology and Applied Pharmacology* **132**, 131-145.

Senthil Kumar, K., Kannan, K., Giesy, J. P., and Masunaga, S. (2002). Distribution and elimination of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, and p,p'-DDE in tissues of bald eagles from the upper peninsula of Michigan. *Environmental Science & Technology* **36**(13), 2789-2796.

Seston, R. M., Giesy, J. P., Fredricks, T. B., Tazelaar, D. L., Coefield, S. J., Bradley, P. W., Roark, S. A., Newsted, J. L., Kay, D. P., and Zwiernik, M. J. (2010a). Multiple lines of evidence risk assessment of belted kingfisher exposed to PCDFs and PCDDs in Tittabawassee River floodplain, Midland, MI, USA. *Environmental Toxicology and Chemistry* (in review).

Seston, R. M., Zwiernik, M., Fredricks, T., Coefield, S., Tazelaar, D., Hamman, D., Paulson, J., and Giesy, J. (2009). Utilizing the great blue heron (*Ardea herodias*) in ecological risk assessments of bioaccumulative contaminants. *Environmental Monitoring and Assessment* **157**(1), 199-210.

Seston, R. M., Zwiernik, M. J., Fredricks, T. B., Tazelaar, D. L., Coefield, S. J., Bradley, P. W., Newsted, J. L., Roark, S. A., Kay, D. P., and Giesy, J. P. (2010b). Dietary exposure of great blue heron (*Ardea herodias*) to PCDD/DFs in the Tittabawassee River floodplain, Michigan, USA. *Ecotoxicology and Environmental Safety* (in review).

Simpson, K., and Kelsall, J. P. (1978). Capture and banding of adult great blue herons at Pender Harbour, British Columbia. *Proceedings Colonial Waterbird Group* **3**, 71-78.

Straub, C. L., Maul, J. D., Halbrook, R. S., Spears, B., and Lydy, M. J. (2007). Trophic transfer of polychlorinated biphenyls in great blue heron (*Ardea herodias*) at Crab Orchard National Wildlife Refuge, Illinois, United States. *Archives of Environmental Contamination and Toxicology* **52**(4), 572-579.

Strause, K. D., Zwiernik, M. J., Im, S. H., Bradley, P. W., Moseley, P. P., Kay, D. P., Park, C. S., Jones, P. D., Blankenship, A. L., Newsted, J. L., and Giesy, J. P. (2007a). Risk assessment of great horned owls (*Bubo virginianus*) exposed to polychlorinated biphenyls and DDT along the Kalamazoo River, Michigan, USA. *Environmental Toxicology and Chemistry* **26**(7), 1386-1398.

Strause, K. D., Zwiernik, M. J., Im, S. H., Newsted, J. L., Kay, D. P., Bradley, P. W., Blankenship, A. L., Williams, L. L., and Giesy, J. P. (2007b). Plasma to egg conversion factor for evaluating polychlorinated biphenyl and DDT exposures in great horned owls and bald eagles. *Environmental Toxicology and Chemistry* **26**(7), 1399-1409.

Sturkie, P. D. (1986). Body Fluids: Blood. In *Avian Physiology* (P.D.Sturkie, Ed.), pp. 102-129. Springer-Verlag, New York, NY, USA.

Thomas, C. M., and Anthony, R. G. (1999). Environmental contaminants in the great blue herons (*Ardea herodias*) from the Lower Columbia and Willamette Rivers, Oregon and Washington, USA. *Environmental Toxicology and Chemistry* **18**(12), 2804-2816.

Thompson, D. H. (1978). Feeding areas of great blue herons and great egrets nesting within the floodplain of the upper Mississippi River. *Proceedings of the Colonial Waterbird Group* **2**, 202-213.

Thompson, H. M., Fernandes, A., Rose, M., White, S., and Blackburn, A. (2006). Possible chemical causes of skeletal deformities in grey heron nestlings (*Ardea cinerea*) in North Nottinghamshire, UK. *Chemosphere* **65**, 400-409.

US Environmental Protection Agency (USEPA). Michigan Dioxin Studies. Dow Chemical Wastewater Characterization Study. Tittabawassee River Sediments and Native Fish. EPA-905/4-88-003. 1986. U.S. Environmental Protection Agency, Region V, Westlake, OH.

US Environmental Protection Agency (USEPA). Field Studies for Ecological Risk Assessment. 540-F-94-014. 1994. Washington, D.C., US Environmental Protection Agency.

US Environmental Protection Agency (USEPA). Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Revision 1. Method 8290A. SW-846. 1998. Washington, DC, US Environmental Protection Agency.

US Environmental Protection Agency (USEPA). Analyses of laboratory and field studies of reproductive toxicity in birds exposed to dioxin-like compounds for use in ecological risk assessment. EPA/600/R-03/114F. 2003.

van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., and Zacharewski, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* **106**(12), 775-792.

van den Berg, M., Craane, B. L. H. J., Sinnige, T., van Mourik, S., Dirksen, S., Boudewijn, T., van der Gaag, M., Lutke-Schipholt, I. J., Spenkelink, B., and Brouwer, A. (1994a). Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (*Phalacrocorax carbo*) after in ovo exposure. *Environmental Toxicology and Chemistry* **13**(5), 803-816.

van den Berg, M., De Jongh, J., Poiger, H., and Olson, J. R. (1994b). The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Critical Reviews in Toxicology* **24**(1), 1-74.

Vermeer, K., and Reynolds, L. M. (1970). Organochlorine residues in aquatic birds in the Canadian Prairie Provinces. *Canadian Field-Naturalist* **84**, 117-129.

Yates, M. A., Fuller, M. R., Henny, C. J., Seegar, W. S., and Garcia, J. (2010). Wintering area DDE source of migratory white-faced ibis revealed by satellite telemetry and prey sampling. *Ecotoxicology* **19**, 153-162.

Zwiernik, M. J., Bursian, S. J., Aylward, L. L., Kay, D. P., Moore, J., Rowlands, C., Woodburn, K., Shotwell, M., Khim, J. S., Giesy, J. P., and Budinsky, R. A. (2008a). Toxicokinetics of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF in mink (*Mustela vison*) at ecologically relevant exposures. *Toxicological Sciences* **105**(1), 33-43.

Zwiernik, M. J., Kay, D. P., Moore, J. N., Beckett, K. J., Khim, J. S., Newsted, J. L., Roark, S. A., and Giesy, J. P. (2008b). Exposure and effects assessment of resident mink (*Mustela vison*) exposed to polychlorinated dibenzofurans and other dioxin-like compounds in the Tittabawassee River basin, Midland, Michigan, USA. *Environmental Toxicology and Chemistry* **27**(10), 2076-2087.

CHAPTER 5

Multiple lines of evidence risk assessment of belted kingfisher exposed to PCDFs and PCDDs in the Tittabawassee River floodplain, Midland, MI, USA

Rita M. Seston[1], John P. Giesy[1,2,3], Timothy B. Fredricks[1], Dustin L. Tazelaar[4], Sarah J. Coefield[1], Patrick W. Bradley[4], Shaun A. Roark[5], John L. Newsted[5], Denise P. Kay[5], and Matthew J. Zwiernik[4]

[1]Zoology Department, Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA

[2]Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, S7J 5B3, Canada

[3]Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, SAR, China

[4]Department of Animal Science, Michigan State University, East Lansing, MI 48824, USA

[5]ENTRIX, Inc., Okemos, MI, USA

Abstract

Concentrations of dioxin-like compounds, primarily polychlorinated dibenzofurans (PCDFs), in soils and sediments of the Tittabawassee River (TR) and associated floodplains downstream of Midland, Michigan (USA) are greater than upstream sites. As a result of these elevated concentrations, a site-specific risk assessment of belted kingfisher (BKF) breeding in the assessment area was conducted. To reduce the uncertainty associated with predicting exposure from abiotic matrices, concentrations of residues were quantified in site-specific prey items and also in eggs and nestlings BKF. Simultaneously, site-specific assessments of reproductive effort and success of BKF were conducted. Dietary exposure, expressed as the potential average daily dose, based on site-specific concentrations of PCDFs, polychlorinated dibenzo-*p*-dioxins (PCDDs), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in prey items was consistently greater along the TR than in associated reference areas (RAs) and the further downstream sites in the Saginaw River (SR). Concentrations of PCDD/DFs in eggs and nestlings of BKF were associated with sampling area, being greater in both eggs and nestlings of BKF nesting along the TR compared to those of BKF from upstream reference areas. Geometric mean concentrations of PCDD/DFs were 130 and 200 ng/kg, wet wt in eggs and nestlings of BKF, respectively, collected from nests along the TR. Potential for adverse population-level effects associated with site-specific diet and egg contaminant exposures were evaluated by comparison to dietary and egg-based toxicity reference values (TRVs). Minimal risk of adverse population-level effects was predicted based on either measured dietary- or tissue-based exposures. This conclusion was

consistent with site-specific measures of population condition, which included clutch size, hatching success, and fledging success.

Introduction

Concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) in the Tittabawassee River (TR) and associated floodplains downstream of Midland, MI, USA are greater than at upstream locations and regional background concentrations. The presence of PCDD/DFs is the result of historical chemical production and associated waste management practices (USEPA 1986). Sediments and floodplain soils downstream of Midland, MI contain total concentrations of the seventeen 2,3,7,8-substituted PCDD/DF congeners (ΣPCDD/DFs) ranging from $1.0x10^2$ to $5.4x10^4$ ng/kg dry wt, respectively. In contrast, concentrations of ΣPCDD/DFs in sediments and soils from upstream reference areas were 10- to 20-fold less (Hilscherova *et al.* 2003). The elevated concentrations of PCDD/DFs within the TR floodplain led to concerns about their potential effects on resident wildlife species in the TR and Saginaw River (SR) floodplains.

Both PCDD/DFs and dioxin-like polychlorinated biphenyls (PCBs) are persistent in the environment and due to their lipophilic nature tend to biomagnify (Mandal 2005). Additionally, these compounds are known to cause an array of negative effects in mammalian and avian species. The PCDD/DF and PCB congeners with the greatest toxic potency act via a common mechanism, the aryl hydrocarbon receptor (AhR) and effects include enzyme induction, immunotoxicity, and adverse effects on reproduction, development, and endocrine functions (Hoffman *et al.* 1987; van den Berg *et al.* 1994a).

In particular, exposure to compounds that bind to the AhR can result in lesser hatching and fledging success of bird species (Custer *et al.* 2005; Giesy *et al.* 1994; Gilbertson 1983; Kubiak *et al.* 1989; Ludwig *et al.* 1993).

The sensitivities of a number of species to AhR-mediated effects have been determined in laboratory studies or inferred from observations of populations exposed in the wild. Sensitivities have been shown to vary among species (Brunström 1988). For example, the domestic chicken (*Gallus gallus*), which is considered the most sensitive avian species, is more than 1000-times more sensitive to embryo-lethal effects than is the mallard (*Anas platyrhyncos*) (Head *et al.* 2008). One theory that has been proposed to account for these differences in avian sensitivity is that toxicity can be attributed to variations in the affinity of dioxin-like compounds to the ligand-binding domain (LBD) of the AhR (Head *et al.* 2008). Specifically, differences in three different amino acid sequences in the LBD of the AhR among avian species leads to differential binding of ligands, which subsequently results in differential sensitivities (Karchner *et al.* 2006). These differences can be used to classify birds into groups with different sensitivities to AhR-mediated effects.

The primary objective of the present study was to characterize the exposure of belted kingfishers (*Ceryle alcyon*; BKF) foraging and nesting within the TR floodplain to 2,3,7,8-substituted PCDD/DFs congeners and predict the associated risk of effects. Species-specific characteristics of the BKF give it a great potential for exposure and make it well suited for study using a multiple-lines-of-evidence approach to ecological risk assessment. The lines of evidence used here include (1) predicted exposure to contaminants through the diet, (2) measured concentrations in egg and nestlings of BKF,

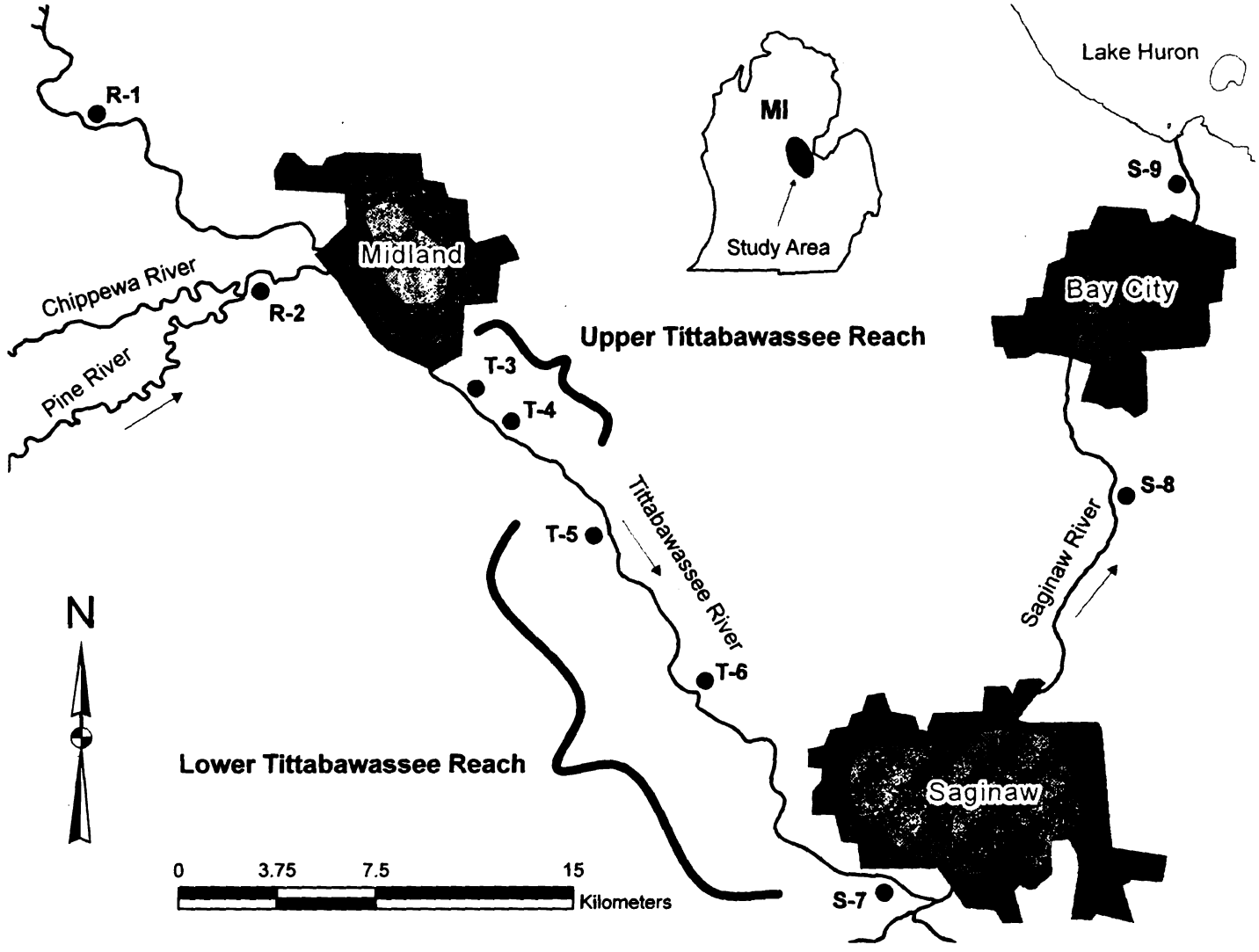
and (3) site-specific measures of BKF population condition, including clutch size, hatching success, and fledging success. Concentrations of ΣPCDD/DF were measured in dietary items and BKF tissues collected from reference and study areas. These concentrations were also expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents ($TEQ_{WHO\text{-}Avian}$) based on World Health Organization 2,3,7,8-TCDD equivalency factors for birds ($TEF_{WHO\text{-}Avian}$) (van den Berg *et al.* 1998). Spatial trends in concentrations and relative congener contributions in dietary items and BKF tissues were also evaluated. Measured exposures were compared to appropriate toxicity reference values (TRVs) to estimate the risk present. Integrating the data resulting from these multiple assessments reduces the uncertainty inherent in the risk assessment process (Fairbrother 2003; Leonards *et al.* 2008; USEPA 1998) and provides better information for use in risk management decisions.

Methods

Site description

The assessment was conducted in the vicinity of the city of Midland, located in the east-central lower peninsula of Michigan (USA) (Figure 1). The TR is a tributary of the Saginaw River (SR), which empties into Saginaw Bay and Lake Huron. The TR runs through The Dow Chemical Company (DOW), which is the accepted source of the PCDD/DF contamination (USEPA 1986). The area henceforth referred to as the study area (SA) includes approximately 37 km of the TR (sites T-3 to T-6) stretching downstream from DOW to the convergence of the TR and SR and 35 km of the SR (sites S-7 to S-9) until it enters Saginaw Bay. Sampling sites selected in the SA were chosen to

Figure 5.1. Assessment area along the Chippewa, Tittabawassee, and Saginaw river floodplains, Michigan, USA. Sampling locations for dietary components of belted kingfisher were located in the Reference area (R-1 and R-2); Upper Tittabawassee River (T-3 and T-4); Lower Tittabawassee River (T-5, T-6, and S-7); and Saginaw River (S-8 and S-9). Tissues of belted kingfisher were collected in the reference area (RA) and along the Tittabawassee River (study area; SA).


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

characterize maximal exposure potential designated as "worst case scenario" locations based on a previous study that measured concentrations of PCDD/DFs in soils and sediments (Hilscherova *et al.* 2003). The willingness of landowners to provide access was also a factor in site selection. The reference area (RA) includes the TR upstream of Midland, together with the Pine and the Chippewa River, both of which are tributaries of the TR upstream of Midland. Reference area sampling locations were on the upstream TR (R-1) and on the Pine River, just upstream of its confluence with the Chippewa River (R-2). Sampling areas were assessed both individually and in spatial groups based on characteristics of the river and associated floodplain. Spatial groupings included reference (RA) R-1 and R-2, upper Tittabawassee River (UTR) T-3 and T-4, lower Tittabawassee River (LTR) T-5 to S-7, and Saginaw River (SR) S-8 and S-9. Components of the diet, including soil, sediment, and prey items were collected at each sampling area whereas BKF tissues were collected from nests and were designated to either RA or SA.

Receptor Species Selection

Selection of a receptor species is a key element in effective risk assessment, particularly when site-specific field studies are to be employed. The belted kingfisher was selected as a receptor species because it possesses characteristics desirable to investigate exposure and potential effects of PCDD/DFs via an aquatic exposure pathway. As top aquatic food web predators with high food consumption rates, BKF have a relatively great potential for exposure (Hamas 1994; Vessel 1978). Additionally, BKF excavate subterranean burrows in which they nest, thus adult and nestling BKF have

intimate contact with river bank soils. Because BKF are territorial of distinct foraging ranges proximal to the nest burrow, the spatial boundaries of the area from which nestling BKF are exposed can be better defined than other species (Davis 1980). Their widespread distribution has led to BKF being included in other ecological assessments (Baron *et al.* 1997; Evers and Lane 2000; Moore *et al.* 1999).

Diet-based exposure assessment

Collection of prey items. Prey items of BKF, including forage fish, crayfish, and amphibians were collected and concentrations of PCDD/DFs (n=188) and PCBs (n=20) determined. Prey items were collected from nine sampling locations (Figure 5.1). The sampling scheme maximized information on dietary exposure including geographically associated contaminant variability and trends. Detailed sample collection methods have previously been described (Seston *et al.* 2009).

Dietary exposure calculations. Exposure of BKF to PCDD/DFs via the diet was estimated by use of information provided in the U.S. Environmental Protection Agency (USEPA) Wildlife Exposure Factors Handbook (WEFH) (USEPA 1993). Major factors influencing dietary exposure included body mass (BW), daily food intake rate [FIR; g wet weight (wet wt) food/g body weight (bw)/d], dietary concentrations (C), and proportion of foraging time spent on-site (AUF). A mean body mass of 147 g and a body-weight normalized FIR of 0.50 kg food(wet wt) $\cdot$kg $bw^{-1} \cdot d^{-1}$ for BKF is given in the USEPA WEFH. The potential average daily dose (ADD_{pot}; ng/kg bw/d) was calculated

using equation 4-3 of the WEFH (USEPA 1993). Incidental sediment ingestion was also included in the ADD_{pot} using equation 4-23 (USEPA 1993).

The relative proportion of each type of prey to the BKF diet was determined through collection and identification of prey remains from active nest chambers located in the assessment area. Collected remains were sorted to prey item type by distinct elements, such as fish otoliths, pharyngeal arches, and dentary bones, crayfish chelipeds, and amphibian femurs or pelvic girdles. Reach-specific concentrations of PCDD/DFs in prey items were multiplied by their relative contribution to the dietary composition. Exposure was estimated using the geometric mean and associated 95% confidence interval of concentrations of residues in prey items from each reach (RA, UTR, LTR, and SR). PCBs were not measured in all frogs or crayfish, thus the data set is incomplete for calculating total $TEQ_{WHO\text{-}Avian}$ exposure associated with PCDD/DFs and PCBs. Where both PCDD/DF and PCB data were available, concentrations of total $TEQs_{WHO\text{-}Avian}$ in frogs and crayfish were less than proximally collected fish. To estimate a conservative exposure of BKF to total $TEQs_{WHO\text{-}Avian}$ a dietary composition of 100% fish was utilized.

Tissue-based exposure assessment

Nest excavation and monitoring. Active BKF nest burrows were located via canoe surveys of the rivers in the assessment area during 2005-2007 from mid-April to mid-May and again in late June to search for any pairs that may have re-nested. Burrows were considered active when there was evidence of fresh digging around and beneath the burrow entrance, the presence of tracks left by the feet of BKF along the base of the

entrance, and/or defensive behavior of BKFs in the area. Further confirmation of burrow activity was gained by use of an infrared video camera that was inserted into the burrow opening to visualize the nest chamber.

Once a burrow was deemed active, the location of the nest chamber was determined by using a folding wooden ruler to approximate the length and angle of the burrow tunnel. A hole was dug behind the approximate location of the nest chamber, and then the leading edge of the excavation pit was slowly moved forward until a small opening was made in the rear of the nest chamber (Mazeika *et al.* 2006). After the nest chamber was located, a wooden panel with an access door and video-port was installed to allow access for nest monitoring and sample collection. The entire excavation was then covered with a sheet of 2.0 cm exterior grade plywood and a tarpaulin to prevent water and predators entrance to the excavation or nesting chamber. It was optimal to perform the excavation once the clutch was complete and incubation had begun to lessen the risk of nest abandonment by adults (Davis 1982).

Nest abandonments attributed to influences other than contaminants were not included in comparisons of nesting success between study locations. The excavation process employed in the present study could have been disruptive to normal behavior of nesting BKF. Nest abandonment was considered to be due to excavation if either of the following two conditions were met (1) the nest contained an incomplete clutch at the time of excavation and egg laying never resumed, or (2) the nest contained eggs at the time of excavation and they were never warm after excavation. These conditions also required an indication that the nest was active at the time of excavation, such as presence of defensive adults and/or warm eggs. During early May 2006, the assessment area

received approximately 7.5 cm of rain within 24 h. Water clarity remained reduced for several days afterward, which likely reduced foraging success of BKF. Previous observations have shown that BKF will abandon their foraging areas when turbidity increases as a result of heavy rains (Davis 1980; Salyer and Lagler 1949). Nests of BKF that were active prior to the rain and then abandoned up to three days following the rain event were considered to have done so in response to the decreased foraging success proximal to the nest during incubation.

Nests were monitored from late-April to mid-July. Nests with complete clutches were checked every other day to determine hatch date, nestling status, and fledge date. All nestlings were banded with U.S. Fish and Wildlife Service (USFWS) bands. Nest success, clutch size, hatching success, and fledging success were all used to assess the reproductive effort and success of BKFs. Clutch size was not adjusted for egg sampling, as collection was done during incubation when the clutch was already complete. However, hatching and fledging success were calculated based on an adjusted clutch size since the fertility and hatchability of the collected egg was unknown at collection. Adjusted clutch size was defined as the clutch size excluding any eggs that were collected. Hatching success (number of eggs that hatch per adjusted clutch size) and fledging success (number of nestlings that fledge per number of eggs that hatched) were adjusted to account for egg collection. Mortality of nestling BKF is low and generally occurs early in the nestling period (Hamas 1975). Since nestlings were collected past midway into their development, it was assumed that any nestlings collected would have successfully fledged provided the remaining portion of the nesting attempt was successful. Thus, fledging success was not adjusted for sampled nestlings. Nestlings

reaching 25 d of age were considered successfully fledged. Reproductive parameters for all clutches were included in comparisons up to the point that they were preyed upon or abandoned due to human interference or the aforementioned rain event.

Various nest activities, including incubation, hatching, and feeding of nestlings, were recorded using the camera at the rear of the nest chamber, and during routine handling, nestling BKF were monitored for gross external abnormalities. Additionally, adult BKF were trapped and banded with USFWS bands to allow determination of nest site fidelity and survival.

Collection of BKF tissues. Both eggs and nestlings of BKF were collected from each available nest chamber located within the assessment area for quantification of PCDD/DFs. One egg was selected at random from each clutch. Mass, length, and three width measurements were recorded for each egg. Addled or abandoned eggs were also collected for possible quantification of PCDD/DF congeners. Addled eggs were defined as those which failed to hatch 2 d post hatch of other eggs in the clutch. Abandoned eggs were defined as those which were cold on three consecutive visits to the nest. Individual eggs were wrapped in chemically-cleaned foil and placed inside a glass jar (I-CHEM brand, Rockwood, TN) for storage and transport to the laboratory. One nestling from each nest was randomly selected for collection at age 15 d and euthanized via cervical dislocation. Individual collected nestlings were stored in similar jars for storage and transport.

During the 2005-2007 breeding seasons, a total of 37 nest chambers were excavated. Eggs were collected from 6 and 19 unique clutches in the RA and SA, respectively. A

total of 5 and 12 nestlings were collected from unique broods in the RA and SA, respectively. Of the collected nestlings, 9 (3 RA and 6 SA) were collected from nests from which an egg was also analyzed.

Sample processing and analytical techniques

Concentrations of seventeen 2,3,7,8-substituted PCDD/DF congeners were measured in all samples while concentrations of PCBs and dichloro-diphenyl-trichloroethane (DDT) and related metabolites (DDXs) were determined in a subset of samples. Collected eggs were opened around the girth with a scalpel blade. Contents were then homogenized in a chemically cleaned Omni-mixer, lyophilized, and stored in chemically cleaned jars until analysis (I-CHEM brand, Rockwood, TN). Concentrations of PCDD/DF in eggs were reported on a fresh weight basis adjusted to account for any desiccation during incubation and storage. Adjusted fresh weight was calculated based on egg volume (Hoyt 1979). The mass of egg contents was determined by subtracting the eggshell mass at the time of processing from the adjusted fresh weight. Nestlings were homogenized in a chemically-cleaned Omni-mixer, without stomach contents, feathers, legs below the tibiotarsus, or the beak.

Residues were quantified in accordance with USEPA Method 8290/1668A with minor modifications (USEPA 1998). Analytical methods have been detailed elsewhere (Fredricks *et al.* 2010b; Seston *et al.* 2010b). Briefly, biotic matrices 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). Losses of congeners during extraction were corrected based on recoveries of ^{13}C-labeled as outlined in USEPA 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. Soxhlet extractions and instrumental analyses were conducted at AsureQuality Ltd, Lower Hutt, New Zealand.

Statistical analyses

Total concentrations of the seventeen 2,3,7,8-substituted PCDD/DF congeners (ΣPCDD/DFs) are reported as the sum of all congeners (ng/kg wet weight (wet wt)). Individual congeners for which concentrations were less than the limit of quantification were assigned a value of half the sample method detection limit on a per sample basis. Total concentrations of the twelve dioxin-like non- and mono-*ortho*-substituted PCB congeners are reported as the sum of these congeners (ng/kg wet wt) (ΣPCBs) for a

subset of samples. Concentrations of $TEQ_{WHO\text{-}Avian}$ (ng/kg wet wt) were calculated for both PCDD/DFs and dioxin-like 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). Total TEQs throughout this manuscript refers to the summation of TEQs from ΣPCDD/DFs (DF-$TEQs_{WHO\text{-}Avian}$) and ΣPCBs (PCB-$TEQs_{WHO\text{-}Avian}$). 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 wet wt) 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. Values that were not normally distributed were transformed using the natural log (ln) before statistical analyses. PROC TTEST was used to make comparisons between the RA and SA. PROC GLM was used to make comparisons for three or more locations. When significant differences among locations were indicated, the Tukey-Kramer test was used to make comparisons between individual locations. The association between concentrations of ΣPCDD/DF or DF-$TEQs_{WHO\text{-}Avian}$ and hatching success was evaluated with Spearman's correlation coefficients for nesting attempts in which both data were collected. Statistical significance was inferred at $p<0.05$.

To better understand the potential distributions of concentrations of DF-$TEQ_{WHO\text{-}Avian}$ and total $TEQ_{WHO\text{-}Avian}$ in eggs of BKF, a probabilistic modeling approach was

used to portray the distributions. Probabilistic models were developed as cumulative frequency distributions based on concentrations of DF-$TEQ_{WHO\text{-}Avian}$ and total $TEQ_{WHO\text{-}Avian}$ in eggs. The mean and standard deviation of transformed concentrations of each sample type were used to generate 10,000 iterations of random concentration values based on a lognormal distribution.

Selection of toxicity reference values

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 the present study, matrix-specific toxicity reference values (TRVs) based on the same or similar compounds were identified from literature and compared to measured site-specific exposures of BKF. Resulting HQs are presented as a range bounded by the LOAEC-associated HQs at the low end and the NOAEC-associated HQs at the high end. It should be noted that the NOAEC and LOAEC associated HQs are a function of the experimental design (dosing regimen) and the actual threshold concentration at which effects would be expected to occur somewhere within the described range. It has recently been suggested that TRV selection should involve the combination of multiple suitable studies into a dose-response curve to determine the most accurate value (Allard *et al.* 2010). However an inadequate number of suitable studies precluded the use of that approach in the present study.

Laboratory studies of effects from dietary exposure to PCDD/DFs are limited for avian species. The TRV selected for use in this assessment was derived from a study that dosed adult hen ring-necked pheasants (*Phasianus colchicus*) with TCDD through

intraperitoneal (IP) injection (Nosek *et al.* 1992). 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 $LOAEC_{DIET}$ for daily exposure of 140 ng TCDD/kg/d. Adverse effects were not present at the next smaller dose, which was determined to be the $NOAEC_{DIET}$ for dietary exposure (14 ng TCDD/kg/d).

The number of laboratory studies that report the effects of PCDD/DFs from egg-based exposures is limited. The USEPA previously developed egg-based TRVs by taking the geometric mean of the effect concentrations in three double-crested cormorant (*Phalacrocorax auritus*) egg-injection studies (Powell *et al.* 1997a; Powell *et al.* 1997b; Powell *et al.* 1998; USEPA 2003). Based on a measurement endpoint of embryo mortality, the resulting $NOAEC_{EGG}$ and $LOAEC_{EGG}$ was 3670 and 11090 ng total $TEQ_{WHO\text{-}Avian}$ /kg wet wt, respectively. No studies reporting effects of dioxin-like compounds in nestlings were available for comparison.

Results

PCDD/DFs and PCBs in BKF prey

Concentrations of the contaminants of concern varied among sampling reach and prey type, ranging from 1.8 ng ΣPCDD/DFs/kg and 0.29 ng $DF\text{-}TEQ_{WHO\text{-}Avian}$/kg in the RA to 3300 ng ΣPCDD/DFs/kg and 1900 ng $DF\text{-}TEQ_{WHO\text{-}Avian}$/kg in the SA (Table 5.1). Consistent spatial trends in concentrations of ΣPCDD/DFs and $DF\text{-}TEQ_{WHO\text{-}Avian}$ were

Table 5.1. $TEQ_{WHO\text{-}Avian}$[a] in prey items of belted kingfisher collected during 2004-2006 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[b] (ng/kg ww) are given as the geometric mean $TEQ_{WHO\text{-}Avian}$ and sample size in parentheses (n) over the 95% confidence interval and range (min-max).

	Reach			
	Reference Area	Upper Tittabawassee	Lower Tittabawassee	Saginaw River
Frog				
ΣPCDD/DF	5.5 (29) A	49 (51) B	100 (55) C	6.7 (12) A
	4.3-6.9	33-73	78-140	4.7-9.4
	(1.8-21)	(4.4-920)	(17-3300)	(3.3-26)
ΣPCB	N/A	N/A	1300 (4)	N/A
			880-2000	
			(940-1700)	
$DF\text{-}TEQ_{WHO\text{-}Avian}$	1.0 (29) A	20 (51) B	56 (55) C	2.9 (12) A
	0.81–1.3	13–31	42–76	1.9–4.5
	(0.29–3.4)	(1.1–460)	(9.1–1900)	(1.5–14)
$PCB\text{-}TEQ_{WHO\text{-}Avian}$	N/A[c]	N/A	1.5 (4)	N/A
			0.86–2.5	
			(1.1–2.0)	
Crayfish				
ΣPCDD/DF	5.0 (5) A	140 (7) B	360 (8) C	50 (8) D
	1.8–13	83–240	200–650	34–72
	(2.3–12)	(86–340)	(140–1300)	(28–110)
ΣPCB	N/A	–[e] (2)	– (1)	N/A
		–	–	
		(2200-2200)	(2100)	
$DF\text{-}TEQ_{WHO\text{-}Avian}$	0.91 (5) A	55 (7) B	160 (8) C	27 (8) B
	0.29–2.8	24–130	110–250	18–41
	(0.34–2.9)	(12–190)	(75–420)	(13–61)
$PCB\text{-}TEQ_{WHO\text{-}Avian}$	N/A	– (2)	– (1)	N/A
		–	–	
		(3.3-4.3)	(5.3)	
Forage Fish				
ΣPCDD/DF	– (2)	– (2)	260 (5) A	59 (4) B
	–	–	95–730	26–130

Table 5.1 (cont'd)

	(4.1–5.1)	(200–220)	(83–610)	(37–210)
ΣPCB	– (2)	– (2)	18000 (5) A	25000 (4) A
	–	–	4600–72000	10000–62000
	(880–1000)	(9300–15000)	(7100–110000)	(15000–47000)
DF-$TEQ_{WHO-Avian}$	– (2)	– (2)	180 (5) A	33 (4) B
	–	–	66–480	20–56
	(0.90–0.91)	(130–170)	(74–440)	(25–53)
PCB-$TEQ_{WHO-Avian}$	– (2)	– (2)	31 (5) A	55 (4) A
	–	–	10–92	12–250
	(1.3–1.8)	(30–39)	(13–100)	(24–150)

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

[b] Values have been rounded and represent only two significant figures

[c] N/A = no samples collected from this location

[d] Means identified with the same uppercase letter are not significantly different among reach at the p=0.05 level using Tukey-Kramer means separation test.

[e] Geometric mean and confidence intervals not calculated for sites with fewer than 3 samples. These sites were not included in reach comparisons.

observed for each type of prey. Concentrations were least in the RA, greater in UTR, greatest in LTR. Concentrations in prey items from the SR were intermediate to those from the RA and UTR. Mean concentrations of ΣPCDD/DFs were 9-, 18-, and 1- fold greater in frogs, 28-, 72-, and 10-fold greater in crayfish, and 47-, 58-, and 13-fold greater in forage fish collected from the UTR, LTR, and SR than those from the RA, respectively. Mean concentrations of DF-$TEQ_{WHO\text{-}Avian}$ were 20-, 56-, and 3-fold greater in frogs, 60-, 180-, and 30-fold greater in crayfish, and 170-, 200-, and 36-fold greater in forage fish collected from the UTR, LTR, and SR when compared to the RA, respectively. Concentrations of ΣPCBs in prey items followed a different spatial trend than that of ΣPCDD/DFs, being greatest in the SR. A more detailed description of the spatial trends in concentrations of PCDD/DFs and PCBs in the prey items, along with discussion of relative congener concentrations, is available is Seston *et al.* (2010b).

Spatial trends were also seen in the relative contribution of DF-$TEQ_{WHO\text{-}Avian}$ and PCB-$TEQ_{WHO\text{-}Avian}$ to total $TEQ_{WHO\text{-}Avian}$ in prey items. A majority of the total $TEQ_{WHO\text{-}Avian}$ in fish from the RA and SR were attributed to PCB-$TEQs_{WHO\text{-}Avian}$ (63% and 61%, respectively), in contrast to fish collected from the Tittabawassee River that had a majority of their total $TEQ_{WHO\text{-}Avian}$ attributed to DF- $TEQ_{WHO\text{-}Avian}$ (81% and 85% for the UTR and LTR, respectively). PCB-$TEQs_{WHO\text{-}Avian}$ were dominated by PCB-126, PCB-77, and PCB-81 in all reaches.

Dietary exposure

Prey remains collected from BKF nest chambers revealed the site-specific diet to be 90.2% fish, 5.4% crayfish, and 4.4% frogs. Using this site-specific diet, the ADD_{pot} was consistently greatest within reaches of the Tittabawassee River when compared to either the RA or SR, regardless of whether it was based on ΣPCDD/DF, DF-$TEQ_{WHO\text{-}Avian}$, or total $TEQ_{WHO\text{-}Avian}$ (Table 5.2). ΣPCDD/DF ADD_{pot} to BKF was 44- to 54-fold greater along the Tittabawassee River and 12-fold greater along the SR when compared to the RA. When normalized to DF-$TEQ_{WHO\text{-}Avian}$, fold differences in ADD_{pot} increased, being 150- to 190-fold greater along the Tittabawassee River and 35-fold greater along the SR when compared to the RA. Site-specific prey items contained contaminant burdens that were quite similar (wet wt), with forage fish composites being slightly greater than crayfish and frogs. Based on a diet of 100% fish, the ADD_{pot} of DF-$TEQ_{WHO\text{-}Avian}$ ranged from 0% to 6.6% greater, resulting in a slightly more conservative estimate. The ADD_{pot} expressed as total $TEQ_{WHO\text{-}Avian}$ based on a 100% fish diet was 76- to 92-fold greater along the Tittabawassee River and 38-fold greater along the SR when compared to the RA.

PCDD/DFs and PCBs in BKF tissues

Concentrations of measured residues were consistently greater in tissues of BKF collected within the SA compared to those of the RA. However, the differences varied in their statistical significance (Table 5.3). Mean concentrations of ΣPCDD/DFs and DF-$TEQ_{WHO\text{-}Avian}$ were significantly greater in eggs of BKF collected from the SA compared to those from the RA (4- and 6-fold greater, respectively; $p<0.0001$). Although mean

Table 5.2. Predicted daily dietary dose of ΣPCDD/DFs and $TEQs_{WHO\text{-}Avian}$ [a] (ng/kg body weight/d [b, c]) for adult belted kingfisher breeding during 2004-2006 within the Chippewa, Tittabawassee, and Saginaw river floodplains, Midland, Michigan, USA, based on the geometric mean (95% confidence interval) of site-specific dietary items.

Study Area	ΣPCDD/DFs	DF-$TEQ_{WHO\text{-}Avian}$ [a]	Total $TEQ_{WHO\text{-}Avian}$ [a, d]
Reference [e]			
	2.6 (2.1–3.5)	0.46 (0.44–0.53)	1.2 (1.1–1.4)
Upper Tittabawassee River [f, g, h]			
	114 (100–130)	70 (60–83)	91 (80–110)
Lower Tittabawassee River [i]			
	140 (54–370)	88 (34–230)	110 (39–290)
Saginaw River			
	31 (13–110)	16 (9.5–32)	46 (15–140)

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

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

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

[d] Total $TEQ_{WHO\text{-}Avian}$ based on a diet of 100% fish due to lack of PCB data in frogs and crayfish

[e] Two fish composite samples collected from Reference reach. Range represents daily dietary dose based on minimum-maximum of fish concentrations.

[f] Off-site diet assumed to be equal to reference area diet.

[g] Upper Tittabawassee River reach includes sites T-3 and T-4

[h] Two fish composite samples collected from Upper Tittabawassee River reach. Range represents daily dietary dose based on minimum-maximum of fish concentrations.

[i] Lower Tittabawassee includes sites T-5, T-6, and S-7

Table 5.3. Total concentrations of 2,3,7,8-substituted furan and dioxin (ΣPCDD/DF) and $TEQ_{WHO\text{-}Avian}$ [a] belted kingfisher eggs and nestlings collected during 2005-2007 from the Chippewa, Tittabawassee and Saginaw River floodplains, Midland, MI, USA. Values[b] (ng/kg ww) are given as the geometric mean (*n*) over the 95% confidence intervals and range in parentheses. ΣPCB and ΣDDX values are reported in μg/kg ww.

	BKF Eggs		BKF Nestlings	
	RA	SA	RA	SA
DF-TEQs	15 (6) A 6.3–35 (6.5–53)	84 (19) B 62–110 (36–260)	5.0 (5) a 2.9–8.6 (2.6–8.5)	95 (12) b 73–120 (49–180)
PCB-TEQs	29 (3) 12–74 (19–39)	64 (11) 41–99 (23–270)	6.0 (4) a 3.8–9.6 (4.1–8.5)	35 (9) b 14–87 (9.4–590)
Total TEQs	47 (3) A 10–210 (26–87)	170 (11) B 120–240 (79–520)	9.7 (4) a 8.0–12 (8.6–11)	160 (9) b 100–250 (110–660)
ΣPCDD/DF	30 (6) A 14–62 (13–73)	130 (19) B 100–170 (63–370)	9.6 (5) a 5.0–18 (4.7–20)	200 (12) b 120–340 (82–1300)
ΣPCB	120 (3) 20–660 (52–190)	130 (11) 81–220 (42–650)	11 (4) a 7.3–15 (8.3–14)	67 (9) b 35–130 (23–430)
ΣDDX	440 (3) 43–4600 (210–1300)	540 (11) 310–940 (120–1500)	140 (4) 43–430 (82–390)	210 (9) 160–280 (120–350)

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

[b] Values have been rounded and represent only two significant figures.

[c] Means identified with a unique capitalized letter are significantly different between locations (across) at the p=0.05 level, for BKF eggs.

[d] Means identified with a unique lowercase letter are significantly different between locations (across) at the p=0.05 level, for BKF nestlings.

concentrations of PCB-$TEQ_{WHO\text{-}Avian}$ were 2-fold greater in eggs of BKF collected from the SA than those from the RA, the difference was not significant (p=0.0767). Mean concentrations of ΣPCB and ΣDDX in eggs of BKF were similar between sampling locations (p=0.7686 and p=0.7372, respectively).

The trend in nestling contaminant concentrations was similar to that observed for eggs of BKF. Mean concentrations of ΣPCDD/DFs and DF-$TEQ_{WHO\text{-}Avian}$ were significantly greater in nestlings of BKF collected from the SA compared to those fromthe RA (21- and 19-fold greater, respectively; p<0.0001). In contrast to eggs, mean concentrations of ΣPCB and PCB-$TEQ_{WHO\text{-}Avian}$ were significantly greater in nestlings of BKF collected from the SA compared to those from the RA (6- and 6-fold, respectively; p=0.0014 and p=0.0167, respectively). Mean concentrations of ΣDDX in nestlings of BKF were similar between sampling locations (p=0.1492).

Spatial trends in relative contribution of PCDDs, PCDFs, and individual congeners to ΣPCDD/DFs and DF- $TEQ_{WHO\text{-}Avian}$ in eggs of BKF were observed. Eggs of BKF collected from the SA had a greater percent contribution of furans to both ΣPCDD/DFs and DF- $TEQ_{WHO\text{-}Avian}$ (66% and 73%, respectively) compared to those collected from the RA (27% and 29%, respectively). Predominant congeners of ΣPCDD/DFs in eggs of BKF from the RA included TCDD (20%) and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) (17%), in contrast to 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (39%) which was the predominant congener in eggs collected from the SA (Figure 5.2). When normalized to DF-$TEQ_{WHO\text{-}Avian}$, the predominant congeners remained the same in each

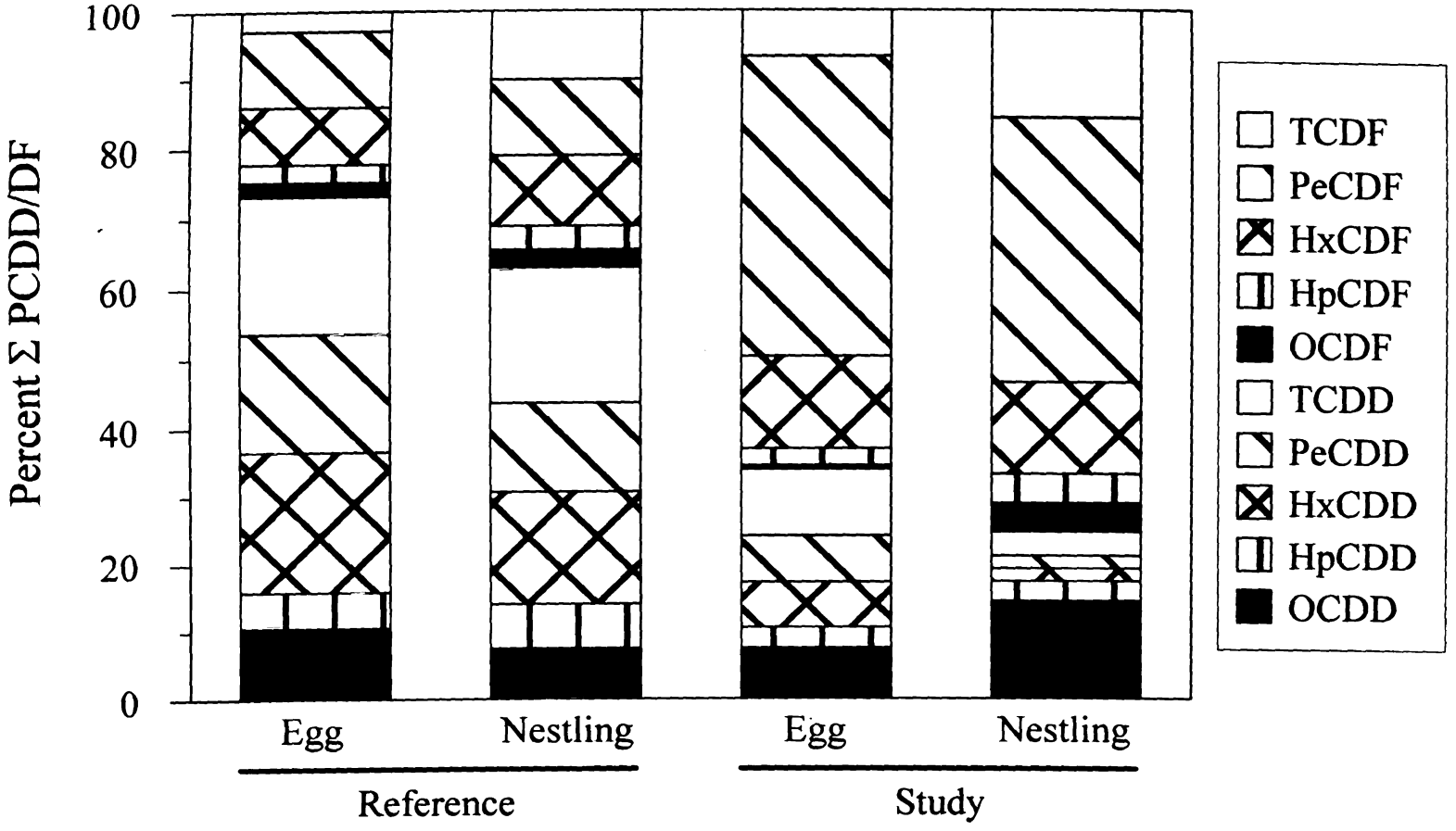


Figure 5.2. Percent mean contribution of individual 2,3,7,8-substituted congeners to ΣPCDD/DF in eggs and nestlings of belted kingfisher collected from reference (RA) and study (SA) areas.

area, with TCDD (36%) and 1,2,3,7,8-PeCDD (34%) in eggs of BKF collected from the RA and 2,3,4,7,8-PeCDF (59%) in eggs collected from the SA.

Similar spatial trends in relative contribution of PCDDs, PCDFs, and individual congeners to ΣPCDD/DFs and DF- $TEQ_{WHO\text{-}Avian}$ were also observed in nestlings of BKF. Nestlings of BKF collected from the SA had a greater percent contribution of furans to both ΣPCDD/DFs and DF- $TEQ_{WHO\text{-}Avian}$ (75% and 90%, respectively) compared to those collected from the RA (37% and 38%, respectively). Predominant congeners of ΣPCDD/DFs in nestlings of BKF from the RA included TCDD (19%) and 1,2,3,7,8-PeCDD (13%), in contrast to 2,3,4,7,8-PeCDF (30%) which was the predominant congener in nestlings collected from the SA (Figure 5.2). When normalized to DF- $TEQ_{WHO\text{-}Avian}$, the predominant congeners remained the same in each area, with TCDD (36%) and 1,2,3,7,8-PeCDD (25%) in nestlings of BKF collected from the RA and 2,3,4,7,8-PeCDF (57%) in nestlings collected from the SA.

There was also a spatial trend in the relative contribution of DF-$TEQ_{WHO\text{-}Avian}$ and PCB-$TEQ_{WHO\text{-}Avian}$ to total $TEQ_{WHO\text{-}Avian}$ in the tissues of BKF. In the RA, PCBs accounted for the greatest proportion of total $TEQ_{WHO\text{-}Avian}$ in both eggs and nestlings of BKF (64% and 62%, respectively). Conversely, PCDD/DFs accounted for a majority of the total $TEQ_{WHO\text{-}Avian}$ in both eggs and nestlings of BKF collected from the SA (59% and 72%, respectively). However, PCB-$TEQs_{WHO\text{-}Avian}$ were dominated by PCB-126, PCB-77, and PCB-81 in each sampling location.

Risk assessment

Estimates of dietary exposure based on the geometric means of measured concentrations of DF-TEQs$_{WHO\text{-}Avian}$ and total TEQs$_{WHO\text{-}Avian}$ from Tittabawassee River reaches were greater than the diet-based NOAEC TRV, which resulted in HQs that were greater than 1.0 (Figure 5.3). Along the Tittabawassee River, maximum calculated HQs, based on the most conservative parameters of 100% site use and 95% upper confidence limit (95% UCL) of measured DF-TEQs$_{WHO\text{-}Avian}$ and total TEQs$_{WHO\text{-}Avian}$, ranged between 5.0 and 20. Dietary exposure-based estimates based on the 95% UCL of measured concentrations of DF-TEQs$_{WHO\text{-}Avian}$ and total TEQs$_{WHO\text{-}Avian}$ compared to the diet-based LOAEC TRV resulted in HQs less than 1.0, regardless of reach.

Hazard quotients based on the 95% UCL of the geometric mean concentrations of DF-TEQs$_{WHO\text{-}Avian}$ (n=19) and total TEQs$_{WHO\text{-}Avian}$ (n=18) in BKF eggs collected from the RA or SA were not greater than 1.0 when compared to either the NOAEC$_{EGG}$ or LOAEC$_{EGG}$ TRV (Figure 5.4). Based on predicted probabilistic distributions of concentrations of either DF-TEQs$_{WHO\text{-}Avian}$ or total TEQs$_{WHO\text{-}Avian}$, 100% of the expected cumulative percent frequencies were below both the NOAEC$_{EGG}$ and LOAEC$_{EGG}$ TRVs (Figure 5.5).

Population condition

During the 2005-2007 breeding seasons, a total of 37 nest chambers were excavated and monitored for reproductive effort and success. Of the studied nests, 27 contained eggs upon excavation while the remaining 10 were found with nestlings. The number of

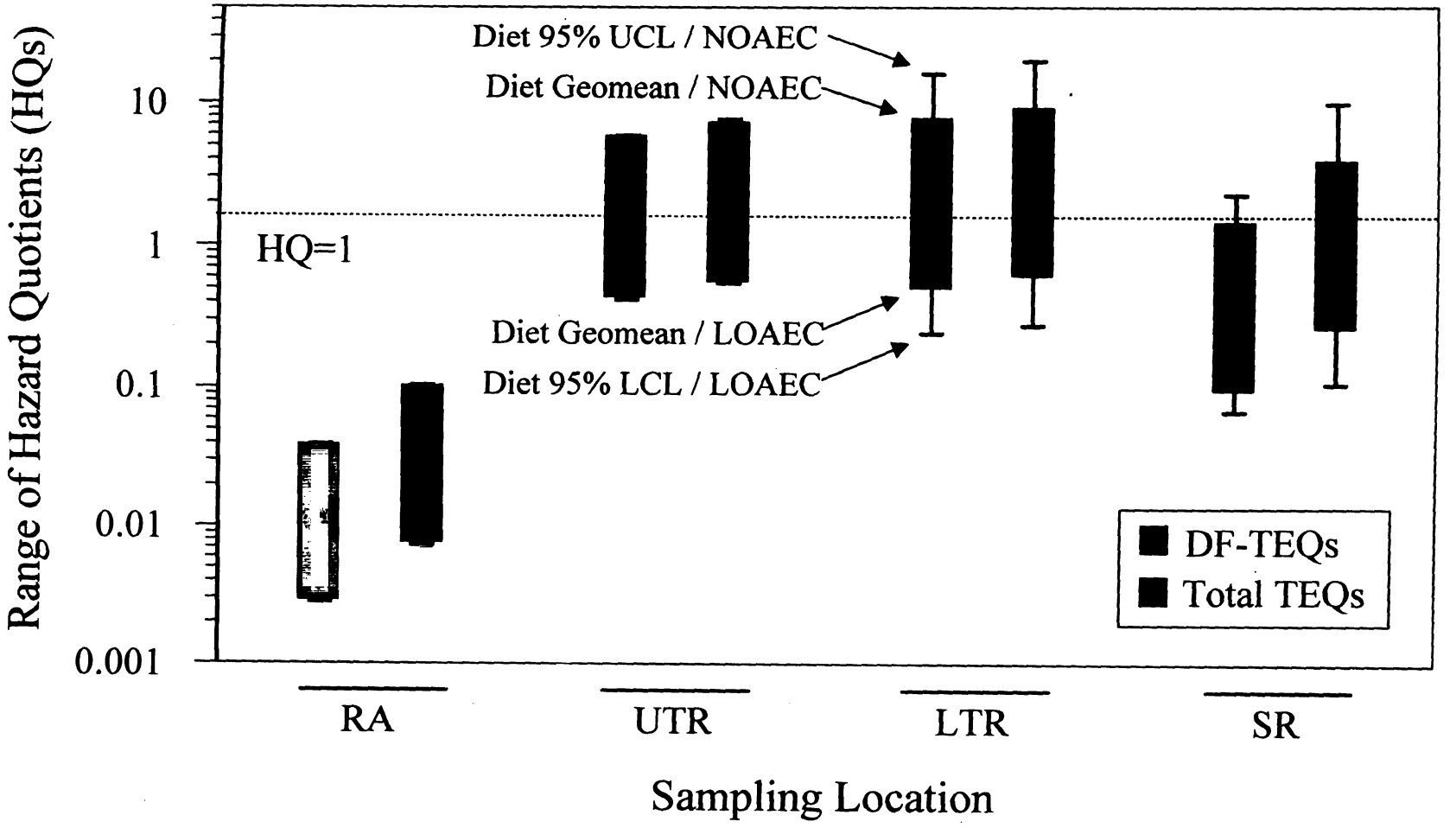


Figure 5.3. Range of hazard quotients for dietary-based exposure of belted kingfisher to DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ along the Chippewa, Tittabawassee, and Saginaw river floodplains.

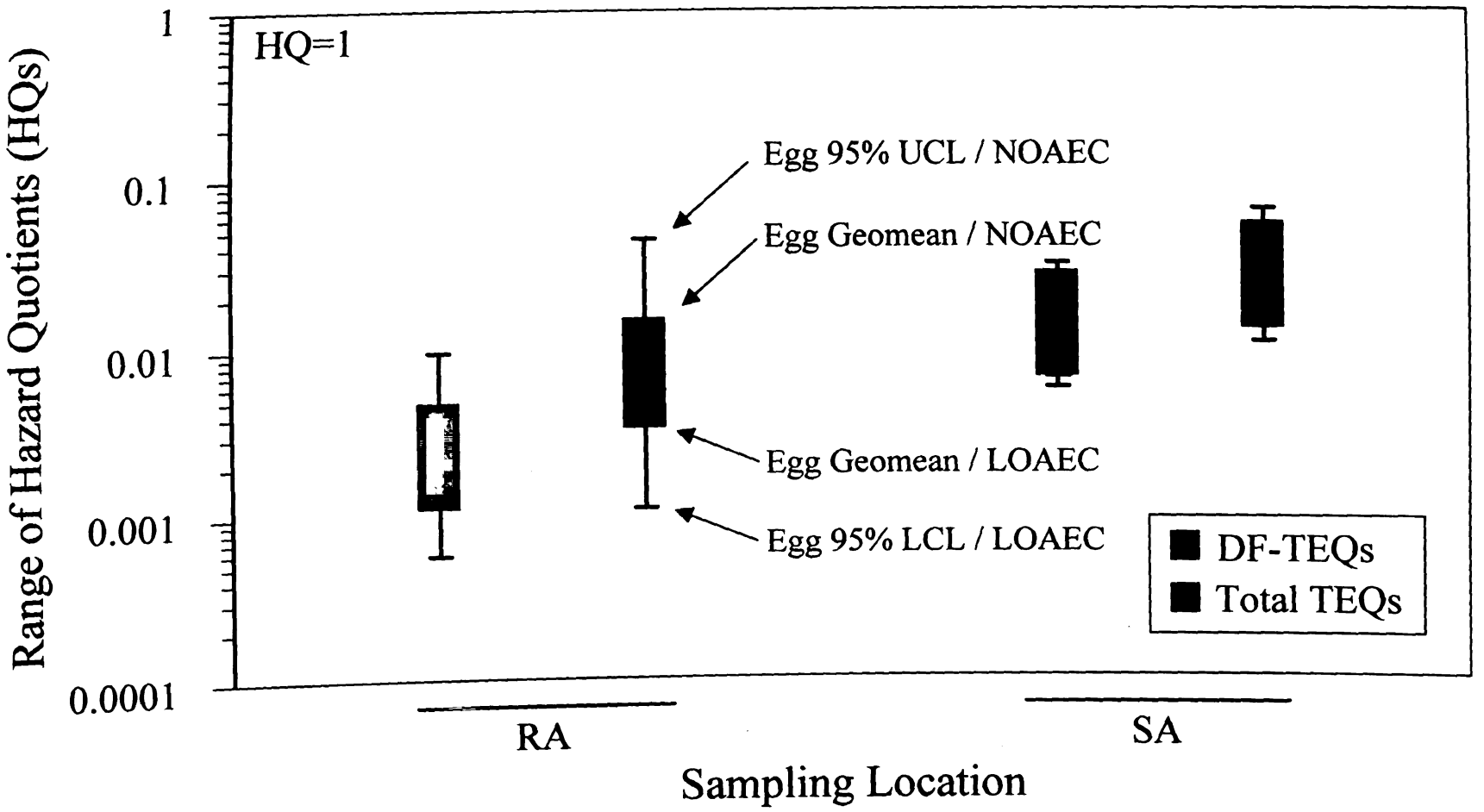


Figure 5.4. Range of hazard quotients for DF-TEQs$_{WHO\text{-}Avian}$ and total TEQs$_{WHO\text{-}Avian}$ in eggs of belted kingfisher from within the Chippewa and Tittabawassee river floodplains.

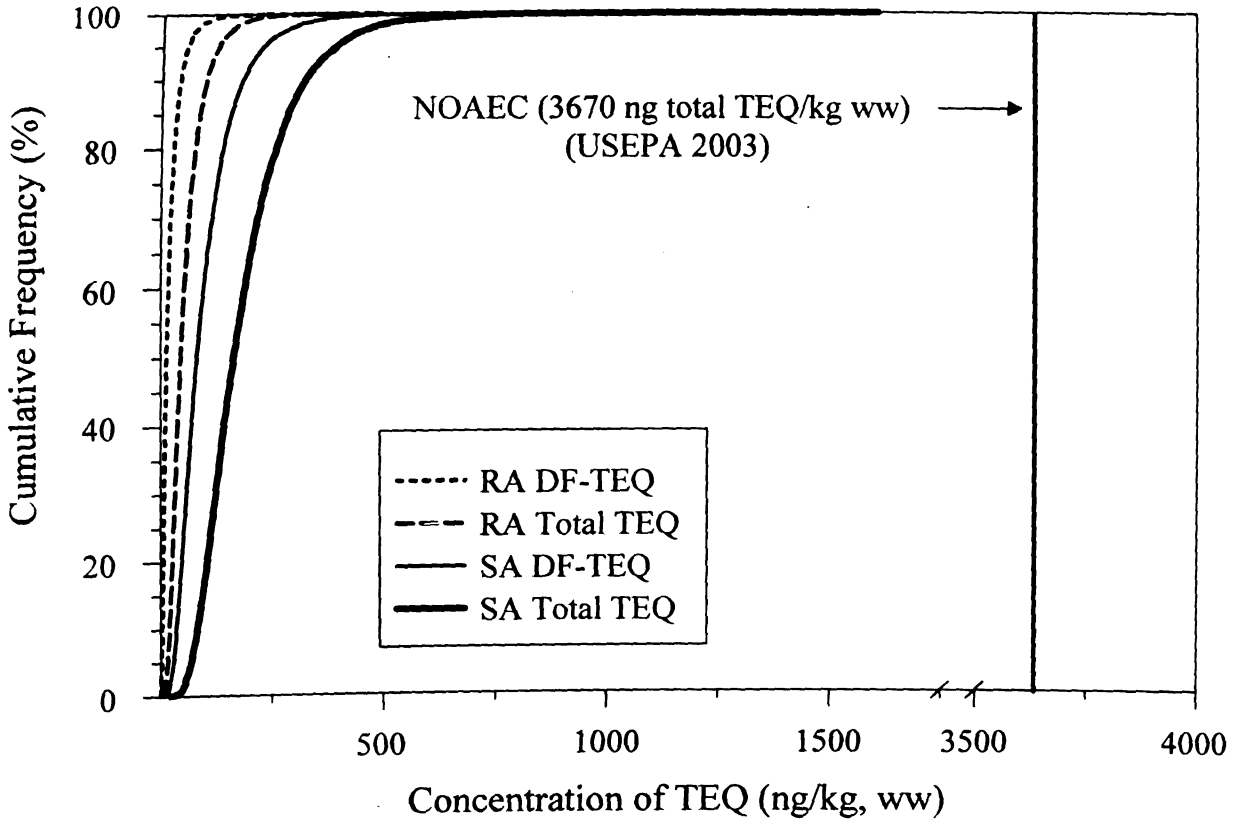


Figure 5.5. Modeled probabilistic distribution of expected cumulative percent frequencies for concentrations of $TEQ_{WHO\text{-}Avian}$ in eggs of belted kingfisher collected from the river floodplains near Midland, MI in 2005-2007. Sampling locations included a reference area (RA) and study area (SA). Vertical bar represents NOAEC (USEPA 2003). LOAEC (11090 ng total $TEQ_{WHO\text{-}Avian}$/kg ww) not included on plot. Note break in x-axis.

eggs per clutch ranged from six to seven for nests in both the RA (n=6) and SA (n=16). The most frequently occurring number of eggs per clutch was six in nests in the RA and seven in nests in the SA. A statistically significant difference in mean (±SD) clutch size of BKF was noted between the RA (6.3±0.52) and SA (6.9±0.34; p=0.0477).

Hatching success was not correlated with concentrations of ΣPCDD/DFs in eggs of BKF for clutches with both data points measured. Eggs from the RA had lesser concentrations of ΣPCDD/DFs but similar hatching success compared to the downstream SA, which resulted in a slightly positive correlation coefficient (R=0.29, p=0.3576). Similarly, no significant correlation existed between concentrations of DF-$TEQs_{WHO\text{-}Avian}$ and hatching success (R=0.24, p=0.4592; Figure 5.6). A limited number of clutches (n=3) had both hatching success and concentration of total $TEQs_{WHO\text{-}Avian}$, precluding a correlation assessment.

Hatching success was similar between the RA and SA, regardless of whether hatching success calculations included extrinsic influences or were done on a per egg or per nest basis (Table 5.4). When only examining eggs that were incubated until hatch, hatching success was 100% (16/16) and 95% (40/42) for eggs from the RA and SA, respectively. When clutches which were partially incubated and abandoned were included, hatching success was reduced to 73% (16/22) and 73% (40/55) for eggs from the RA and SA, respectively. If all eggs were included, hatching success was further reduced yet remained similar between areas, at 38% (16/42) and 31% (40/128) for eggs from the RA and SA, respectively. The percentage of nests that successfully hatched at least one egg was also similar between study areas, at 75% (3/4) and 78% (7/9) for the RA and SA,

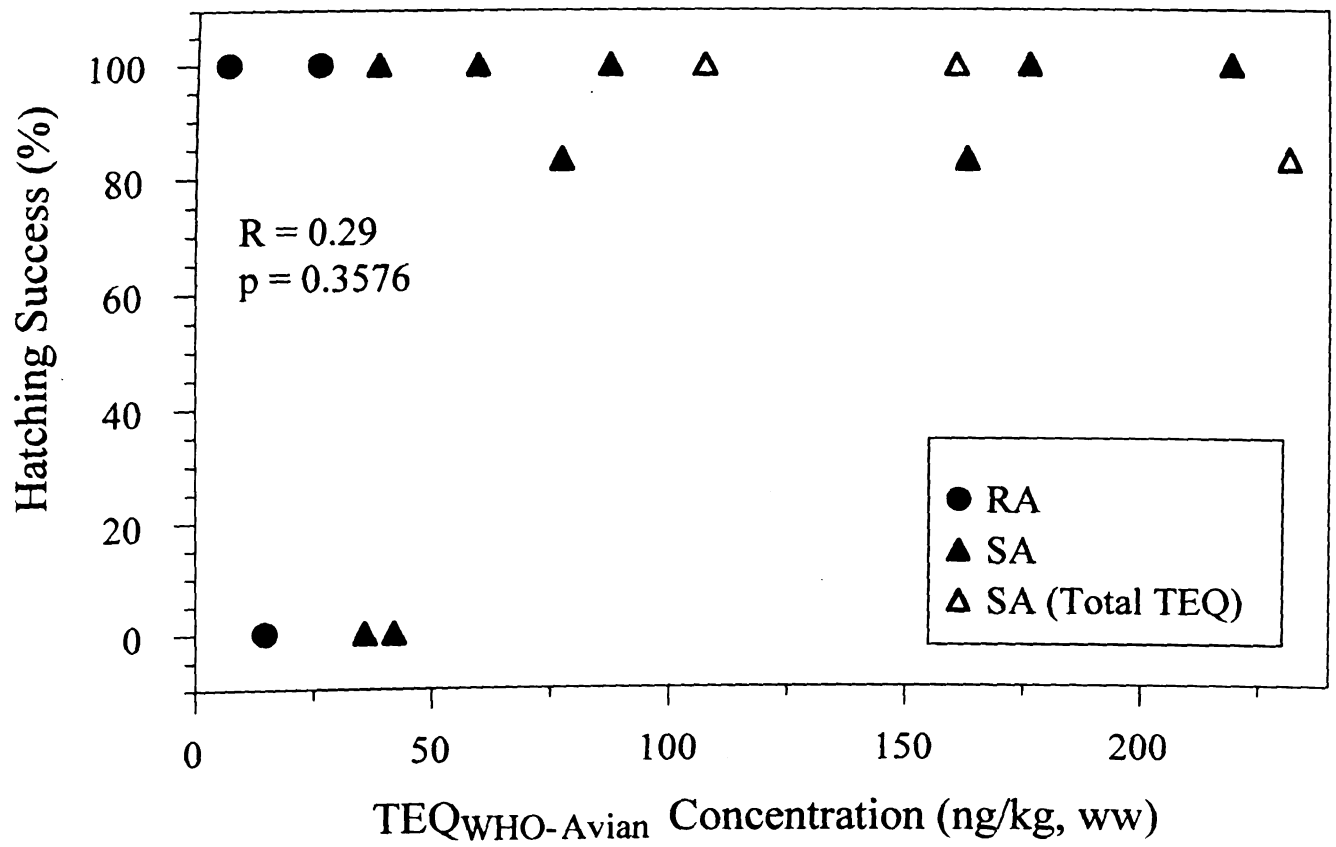


Figure 5.6. Correlation plot of percent hatching success and DF-TEQs$_{WHO\text{-}Avian}$ in eggs of belted kingfisher for nesting attempts with data collected for both variables from the river floodplains near Midland, Michigan during 2005–2007. R- and *p*-values and sample size indicated; RA=reference area and SA=study area. Nesting attempts with data for both hatching success and concentrations of total TEQs$_{WHO\text{-}Avian}$ also plotted but not included in correlation analysis.

Table 5.4. Parameters of reproductive effort and success of belted kingfishers breeding along the Chippewa and Tittabawassee river floodplains during 2005-2007.

Reproductive Parameter	RA	SA
Incubation Period		
No. of nests	7	20
No. successful[a]	3	7
No. failed[b]	4	13
No. abandoned (unknown)	1	2
No. abandoned (disturbance)[c]	2	5
No. abandoned (2006 rain)	1	6
No. of eggs	42	128
No. incubated	22	55
No. successful	16	40
No. failed	0	2
No. abandoned (unknown)	6	13
Excluded from hatching success calculations		
No. abandoned (disturbance)	11	27
No. abandoned (2006 rain)	7	39
No. missing	6	7
No. of eggs hatched		
No. of eggs hatched/eggs incubated	16/22 (73%)	40/55 (73%)
No. of eggs hatched/egg fully incubated	16/16 (100%)	40/42 (95%)
Nestling Period		
No. of nests	5	15
No. successful[a]	3	12
No. where all nestlings disappeared	1	2
No. abandoned	1	1
No. of nestlings	28	83
No. abandoned/missing/depredated	12	29
No. dead in nest	0	1
No. reaching 25 d age	15	52
No. of nestlings fledged		
No. of nestlings fledged (excluding abandoned/missing/depredated)	15/15 (100%)	52/53 (98%)
No. of nestling fledged/all nestlings	15/28 (54%)	52/83 (63%)

[a] successful = at least one egg hatched or at least one nestling fledged

[b] failed = no eggs hatched

[c] See *Methods* for definition of disturbance and 2006 rain event

respectively, excluding those nests which were abandoned as result of human disturbance or the 2006 rain event.

Measures of fledging success were also similar between the RA and SA (Table 5.4). Mortality of nestlings that was likely unrelated to exposure to contaminants, such as those that were abandoned, depredated, or were otherwise missing from the nest, was excluded from the calculation of fledging success. By excluding these nestlings, fledging success was 100% (15/15) and 98% (52/53) for nestlings from the RA and SA, respectively. When abandoned, depredated, and missing nestlings were included, fledging success was reduced yet remained similar between the study location, at 54% (15/28) and 63% (52/83) for nestlings from the RA and SA, respectively.

A number of band recoveries were observed during the present study. One adult BKF breeding in the RA and fourteen adults breeding in the SA were banded. Band return monitoring occurred for one and two breeding seasons in the RA and SA, respectively. A breeding pair banded during the 2006 breeding season was recaptured during the 2007 breeding season. The pair was nesting in a new burrow located within 500m of the previous year's burrow. Another adult female banded during the 2006 breeding season was recaptured in 2008 at a new burrow with a new mate. All band recoveries were made in the SA of adults initially banded in the SA.

Discussion

BKF as receptor species

Belted kingfishers possess desirable characteristics for use in assessing ecological effects of contaminants in the environment. As inhabitants of varied riverine and

lacustrine habitats with a widespread distribution across North America, BKF are a species likely to be present in areas of aquatic-based contamination. They are also a highly charismatic species that is easily recognized by the public. Additional attributes that make BKF ideal for assessing bioaccumulative compounds include their high trophic status, defined foraging territories, and great food consumption rate. However, BKF can be a difficult species to study in the field. In contrast to box-nesting passerine species that are often used as receptor species, BKF are a subterranean nesting species and have not been shown to nest in artificial structures. As such, time and effort must be expended in locating and accessing nests for monitoring and sample collection, which is difficult without causing undue disturbance to the nest. Furthermore, BKF often appear to be nest-site limited (Cornwell 1963; Davis 1982), which may make it difficult to obtain the necessary sample size in a short-term study. In spite of these potential disadvantages, BKF should still be considered an optimal piscivorous avian species to study because their defined foraging range allows for greater spatial resolution of their dietary exposure compared to other species commonly used, such as the great blue heron (*Ardea herodias*; GBH) or black-crown night heron (*Nycticorax nycticorax*).

Dietary exposure

The observed trends in concentrations and relative contribution of individual congeners of PCDD/DFs in prey items of BKF were consistent with those observed for dietary items and tissues of other receptor species studied within the SA (Coefield *et al.* 2010; Fredricks *et al.* 2010a; Fredricks *et al.* 2010b; Seston *et al.* 2010b; Seston *et al.* 2010a; Zwiernik *et al.* 2008b). The pattern observed is likely the result of the

downstream movement of this historical contamination from the upstream source. When expressed as dietary exposure, the relative proportion of DF-$TEQ_{WHO\text{-}Avian}$ to ΣPCDD/DF for ADD_{pot} of BKF was 18% in the RA, while in the SA it ranged from 61% to 63% and 52% for reaches along the TR and SR, respectively. The greater percentages observed along the TR and SR were due to prey items from those reaches having greater relative TCDD potency of ΣPCDD/DF, from primarily TCDF and secondarily from 2,3,4,7,8-PeCDF.

Greater concentrations of PCBs in forage fish from the SR compared to those from the TR are most likely due to historical PCB contamination in areas downstream of the TR, including the Saginaw River and Saginaw Bay (Kannan *et al.* 2008) as well as some lesser sources upstream of the RA. Anadromous fishes from Saginaw Bay can move up into the TR, but a dam located in Midland, MI may impede the movement of these fish into the RA. As such, the total $TEQ_{WHO\text{-}Avian}$ ADD_{pot} was only 13-20% greater than the DF-$TEQ_{WHO\text{-}Avian}$ ADD_{pot} along the reaches of the TR, but was 62% and 65% greater in the RA and SR, respectively.

Tissue exposure

Consistency in the relative contribution of individual congeners to PCDD/DF observed between prey items of BKF and both eggs and nestlings of BKF is likely resultant of site-specific dietary exposure. TCDF was the predominant congener present in prey items sampled from the SA, with 2,3,4,7,8-PeCDF also having a significant presence. In eggs and nestlings of BKF, the overall congener profile was similar to that observed in prey items, although there was a change in the predominant congener from

TCDF to 2,3,4,7,8-PeCDF. This pattern was also observed in other species foraging in the SA, including GBH, great horned owls, and several passerine species (Coefield *et al.* 2010; Fredricks *et al.* 2010a; Fredricks *et al.* 2010b; Seston *et al.* 2010b; Seston *et al.* 2010a; Zwiernik *et al.* 2008b). Bioaccumulation of TCDF from prey items in Forster's terns and herring gulls has been reported to be negligible (Braune and Norstrom 1989; Kubiak *et al.* 1989). This may be due to preferential metabolism of TCDF as reported in various avian species that have been exposed to mixtures of AhR-active compounds (Elliott *et al.* 1996; Kubota *et al.* 2005; Senthil Kumar *et al.* 2002). While data on avian toxicokinetics from controlled laboratory studies are limited, mammalian studies have shown the rate of metabolism of TCDF to be elevated with increased concentrations of dioxin-like compounds and the subsequent induction of cytochrome P450 1A1 and/or 1A2 while 2,3,4,7,8-PeCDF is preferentially sequestered in the liver (van den Berg *et al.* 1994b; Zwiernik *et al.* 2008a). This difference in toxicokinetics is a likely explanation for the observed difference in the relative contribution of the two furan congeners from diet to tissue observed for BKF in the present study.

The relative contribution of individual PCDD/DF congeners was consistent between eggs and nestlings of BKF, which supports the conclusion that tissue exposure was the result of site-specific dietary exposure. Nestlings concentrations are generally considered more representative of local contamination as they are confined to the nest and rely on food brought to them by adults (Neigh *et al.* 2006; Olsson *et al.* 2000). During breeding and nestling rearing, adult BKF have a foraging range that is typically limited to 0.92 to 2.9 km proximal to the nest (Davis 1982; Mazeika *et al.* 2006). Although the BKF is generally a seasonally migratory species in this area of its range (Hamas 1994), because

PCDD/DF congener patterns of contamination in sediments and prey are similar to that observed in tissues of BKF, it can be concluded that the contamination measured in eggs and nestlings of BKF are site-specific.

Risk characterization

Based on the selected TRVs and calculated exposures for BKF foraging along the TR, there is the potential for adverse effects on hatching success, but the likelihood is small given the conservative nature of the assessment. For instance, based on the 95% UCL of measured concentrations of DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ compared to the $NOAEC_{DIET}$ TRV, maximum calculated HQs along the Tittabawassee River ranged between 1.0 and 20 and were less than 1.0 when based on $LOAEC_{DIET}$. Since the HQs derived from comparisons of the $NOAEC_{DIET}$ and $LOAEC_{DIET}$ to the range of BKF dietary exposure bracket an HQ of 1.0, it is possible that none of the dietary exposures predicted here exceed the actual effect threshold concentration, given that the actual effects threshold lies somewhere between these values. However, it is important to note that forage fish, which were the primary component of the BKF diet, were composite samples of all individual fish captured at each location per sampling period. While this provided an accurate estimate of the central tendency of concentration estimates in forage fish, it limited the characterization of the variability of concentrations among all forage fish at each sampling location. Thus, the upper end estimates of dietary exposure presented here, and the risk associated with that exposure, may be an underestimate.

Despite the conservative nature of the selected TRVs, egg-based HQs were less than 1.0 when based on NOAECs and the 95% UCL concentrations of DF-$TEQs_{WHO\text{-}Avian}$ and

total $TEQs_{WHO\text{-}Avian}$, which is consistent with the other lines of evidence that concluded that adverse effects would not be expected. The few other studies which have monitored contaminants in tissues of BKF have generally reported concentrations of total PCBs and methylmercury (Baron *et al.* 1997; Evers and Lane 2000; Fecteau 2008; Heinz *et al.* 1984; Hudson River Natural Resource Trustees 2004). A study of BKF along the PCB-contaminated Hudson River reported concentrations of total $TEQs_{WHO\text{-}Avian}$ in eggs of BKF (Custer *et al.* 2010) that ranged from 96 to 5200 ng/kg with a geometric mean of 620 ng/kg. In comparison, eggs of BKF collected along the Hoosic, Mohawk, and Connecticut Rivers, considered to be the reference area in that study, contained concentrations of total $TEQs_{WHO\text{-}Avian}$ ranging from 50 to 870 ng/kg with a geometric mean of 120 ng/kg. PCBs accounted for 98% and 92% of the total $TEQs_{WHO\text{-}Avian}$ concentration in eggs of BKF collected along the Hudson River and associated reference areas, respectively. No association was found between these concentrations of total TEQs and reproductive impairment. When the predicted frequency distribution for concentrations of DF-$TEQs_{WHO\text{-}Avian}$ or total $TEQs_{WHO\text{-}Avian}$ in BKF eggs from the RA and SA of the present study were compared to the Hudson River geometric mean of 620 ng/kg, 0% and <1% of eggs of BKF exceeded either value, respectively (Figure 5). This comparison to a field-derived NOAEC also suggests that adverse effects on reproductive success would not be expected along the TR. There is some uncertainty in comparing these two studies given that TEQs along the Hudson River were primarily from PCBs whereas those along the TR were primarily from PCDFs and secondarily from PCDDs.

In addition to dioxin-like compounds, the potential for adverse effects of site-specific ΣDDX concentrations was assessed. Previous studies have found mean DDE

concentrations in eggs of great blue heron ranging from 0.086 to 16 mg/kg had no adverse impacts on breeding success (Blus *et al.* 1980; Harris *et al.* 2003; Laporte 1982; Thomas and Anthony 1999). However, concentrations of DDE greater than 8 mg/kg were associated with decreases in clutch size and productivity in black-crowned night herons (Henny *et al.* 1984). For reproductive effects in bald eagles, a threshold concentration of 6 mg DDE/kg has been suggested (Elliott and Harris 2001). Eggs of BKF collected from the TR assessment area were well below these thresholds, with mean concentrations of ΣDDX <1.0 mg/kg. A lack of adverse reproductive effects was observed with similar concentrations of ΣDDX in BKF collected from the Hudson River assessment area (Custer *et al.* 2010). These observations suggest that egg ΣDDX concentrations reported in the present study would not cause adverse effects on reproductive success of BKF along the TR floodplain.

Despite elevated exposure of BKF to PCDD/DFs in the SA compared to the RA, there were no discernable differences in parameters of reproductive success between the two locations. The average clutch size observed here is similar to that reported in other studies, which is reported to vary between five to eight eggs, with six or seven eggs occurring most often (Bent 1940; Hamas 1975). A similar percentage of nests that successfully hatched at least one egg has been reported in a previous study (Custer *et al.* 2010). When influences of human disturbance and depredation were excluded, rates of hatching and fledging success observed here were comparable to those reported in previous studies (Custer *et al.* 2010; Davis 1980; Hamas 1975). Both the RA and SA had a robust population of American mink (*Mustela vison*) (Zwiernik *et al.* 2008b), which was the primary predator of nestlings of BKF in the assessment area.

Uncertainty assessment

For the study described herein, exposure and effects assessments were conducted based on both dietary and tissue exposure, facilitating a multiple lines of evidence approach. Discrepancies in the outcomes of the two methodologies were examined in terms of the uncertainties and associated conservative bias in order to weight each line appropriately. In the assessment of dietary exposure, uncertainty is often introduced by making assumptions in regards to dietary composition and the proportion of the diet that is collected from the area of concern. For tissue-based assessments, uncertainty is often associated with site-use characteristics as well as spatial, temporal, and individual variations in exposure. Many of the uncertainties associated with both exposure assessments were greatly reduced in the present study through the collection of extensive site-specific data. Uncertainties may also be present in the effects assessment as a result of comparing across species and exposure scenarios due to limited toxicological data available for comparison. For the two exposure and effects assessments of BKF presented here the least uncertainty is associated with the tissue-based approach, which is derived from a more robust and directly linked data set.

Uncertainties in the quantification of the dietary exposure of BKF were minimized through the identification of a site-specific dietary composition and the subsequent collection of the appropriate prey items. However, unlike the sampling of BKF tissues, prey items were collected at fewer time points, thus it is possible that there were temporal variations in the contaminant burdens of prey items that were not captured. The techniques used to determine dietary composition did integrate over the duration of

incubation of eggs through nestling rearing but did not directly identify prey items consumed during egg laying. To investigate the potential influence of dietary composition, the predicted dietary dose was calculated with the determined site-specific diet (90.2% fish, 5.4% crayfish, 4.4% frog), a literature-based diet (58% fish, 41% crayfish; (Salyer and Lagler 1949)) and an equally opportunistic diet (33% of each fish, crayfish, and frog). Use of the site-specific diet consistently resulted in the greatest predicted dietary dose, thus making this the most conservative estimate. Additional conservative bias was introduced by the assumption of 100% site use as the BKF was very likely forced to forage off-site in non-flowing water during times of extreme water turbidity associated with heavy rains (Davis 1980; Hamas 1975). Therefore, while dietary exposure is thoroughly characterized with an extensive amount of site-specific data, there remains a considerable potential to overestimate exposure due to the aforementioned factors.

Laboratory studies of effects from dietary exposure to PCDD/DFs are limited for avian species. The TRV selected for use in this assessment was derived from a study in which adult hen ring-necked pheasants (*Phasianus colchicus*) were dosed with TCDD through intraperitoneal (IP) injection (Nosek *et al.* 1992). There are a few factors that make the use of this TRV conservative in determining the potential hazard of these compounds to BKF. First, in Nosek et al. (1992) the hens were exposed to TCDD via IP injection rather than a true dietary-based exposure, potentially resulting in greater exposure efficiency than that of a true dietary exposure. Dietary exposure also allows greater potential for metabolism and excretion prior to reaching target tissues. Additionally, the ring-necked pheasant is not a piscivorous species, but a galliformes,

which are generally considered to have greater sensitivity to dioxin-like compounds (Brunström 1988; Brunström and Reutergardh 1986; Powell *et al.* 1996; Powell *et al.* 1997a). Variability in sensitivity among species has been attributed to differences in the amino acid sequence of the ligand binding domain of the AhR (Head *et al.* 2008; Karchner *et al.* 2006) with ring-necked pheasants having the construct which classifies them as moderately sensitive whereas the BKF construct classifies them as insensitive. Furthermore, dosing in the Nosek study was done with only a single congener (TCDD), whereas the BKF being assessed are exposed to a complex mixture of dioxin-like compounds. Although previously assumed to be equitable in toxicity when normalized using $TEFs_{WHO\text{-}Avian}$, findings from recent studies investigating the relative potencies of individual PCDD/DF congeners suggest that this may not hold true in every exposure scenario (Hervé *et al.* 2010b; Hervé *et al.* 2010a).

Tissue-based exposure assessments integrate exposure characteristics including site use, dietary composition, and contaminant metabolic potentials that may vary over time and space. Directly measuring residue levels in tissues requires the collection of fewer data and eliminates any uncertainties associated with modeling the exposure (Leonards *et al.* 2008). To illustrate this, a screening level ERA of fish-eating birds exposed to PCDD/DFs along the TR predicted there to be much greater concentrations in eggs than what were directly measured in site-specific samples (Galbraith Environmental Sciences LLC. 2003). In the screening-level assessment, concentrations of $DF\text{-}TEQs_{WHO\text{-}Avian}$ in eggs of fish-eating birds were predicted through the application of biomagnification factors (BMFs) to concentrations measured in fish collected from the TR. Concentrations in eggs were predicted to be 1031 ng $DF\text{-}TEQs_{WHO\text{-}Avian}$/kg, wet wt, based on the mean

concentration of DF-TEQs$_{WHO\text{-}Avian}$ in collected fish. Eggs of BKF collected from the SA had a geometric mean concentration of 84 ng DF-TEQs$_{WHO\text{-}Avian}$/kg, wet wt, and a maximum of 260 ng DF-TEQs$_{WHO\text{-}Avian}$/kg, wet wt. Factors which may have resulted in the overestimation of exposure in the screening level assessment include; collection of unrepresentative prey items, inaccurate dietary composition, assumptions regarding foraging range and site use, and inaccurate BMFs. The direct quantification of residues in both eggs and nestlings of BKF collected from within the TR floodplain eliminated these uncertainties from the assessment, allowing for a more accurate characterization of tissue-based exposure.

The TRVs selected to assess the risk to BKF based on egg concentrations present along the TR floodplain also have associated uncertainties. The values based on the double-crested cormorant egg injection studies were considered to be appropriate for use in the present study because they were derived from another avian piscivore. Furthermore, both the double-crested cormorant and BKF have similar AhR ligand binding domain constructs, which suggests the two species will respond similarly to dioxin-like compounds (Head *et al.* 2008; Karchner *et al.* 2006). Although the species appear to be similar in sensitivity to dioxin-like compounds, the route of exposure in the aforementioned double-crested cormorant studies and the BKF being assessed along the TR is different. Residues in the BKF eggs are maternally deposited rather than being artificially introduced as in the study conducted with the double-crested cormorant eggs. This difference in route of exposure adds uncertainty when using this TRV in the risk assessment (Heinz *et al.* 2009; Hoffman *et al.* 1996).

Hazard quotients are generally used in screening for the potential for risk and not for direct risk characterization. That HQs based on the predicted dietary exposure exceeded 1.0 in some instances is not incompatible with HQs less than 1.0 for the tissue-based assessment or for the inability of the study to identify adverse effects on individual and population condition, despite the extensive dataset. HQs greater than 1.0 indicate exposures that exceed an effect concentration given the present dataset and conservative biases associated with uncertainties. Additionally, the HQ approach may be conservative when extrapolating from individuals to populations, as they are usually based on laboratory exposures and do not account for compensatory mechanisms associated with wild populations (Fairbrother 2001). Conversely, the limited number of endpoints generally measured in laboratory studies may not capture an effect that could have implications at the population level. Thus, there is uncertainty inherent in the HQ approach, meaning that HQ values greater than 1.0 do not necessarily translate into population-level or ecologically relevant adverse effects (Blankenship *et al.* 2008).

The hazard assessment based on tissue exposure resulted in HQs less than 1.0. This held true even when the assessment was made more conservative by including an interspecies uncertainty factor of three. The robust dataset for contaminant concentrations in eggs of BKF also allowed for the use of a probabilistic assessment, which gives a more complete picture of the exposure distribution and associated risk. The findings of the probabilistic assessment were consistent with the prediction of minimal risk and with field measured parameters associated with individual and population condition.

The accuracy and certainty of the assessment hazard in the HQ approach and the assessment of risk in the probabilistic approach are each dependent on the quality applicability of the dose response curves used to establish effect concentrations. The greater certainty in both of exposure and effects assessment based on contaminant concentrations in eggs of BKF within the TR floodplain gives greater weight to the conclusions drawn from that line of evidence.

Conclusions

The BKF proved to be a useful receptor species in the assessment of PCDD/DFs along the TR. Given its relatively small, distinct foraging range, the exposure of BKF has a greater spatial resolution than most other fish-eating birds. Belted kingfisher foraging along the TR were predicted to be more greatly exposed to PCDD/DFs than those in associated RAs. This spatial trend was consistent with what was noted in concentrations of PCDD/DFs in both eggs and nestlings of BKF. Overall, the weighted exposure and effects assessment suggests that BKF along the TR are exposed to significant concentrations of PCDD/DFs, however, presently there is minimal potential for adverse effects. This conclusion was consistent with direct field measures of individual and population condition. The greatest remaining uncertainty for the assessment of risk to BKF along the TR include temporal and species variability in forage fish contaminant burdens and uncertainties associated with threshold effects concentrations for both dietary exposure and internal doses (tissue concentrations).

Acknowledgements

The authors would like to thank all staff and students of the Michigan State University – Wildlife Toxicology Laboratory field crew; namely M. Nadeau, D. Hamman, W. Folland, E. Koppel, and L. Williams. We gratefully acknowledge M. Fales for his design and fabrication of specialized equipment which was pivotal to the success of this research. Additionally, we would like to recognize M. Kramer and N. Ikeda for their assistance in the laboratory. The following people were key to the success of the project, J. Dastyck and S. Kahl of the USFWS – Shiawassee National Wildlife Refuge; Saginaw County Parks and Recreation Commission for access to Imerman Park; Tittabawassee Township Park Rangers for access to Tittabawassee Township Park and Freeland Festival Park; Tom Lenon for access to Chippewa Nature Center; and Michael Bishop of Alma College for guidance as our Master Bander. We also acknowledge the greater than 50 cooperating landowners throughout the study area who made our research possible through granting us access to their property. Funding was provided through an unrestricted grant from The Dow Chemical Company, Midland, Michigan to J. Giesy and M. Zwiernik of Michigan State University.

Animal Use

All aspects of the present study that involved the use of animals were conducted using the most humane means possible. To achieve that objective, all aspects of the present study were performed following standard operation procedures (Protocol for belted kingfisher monitoring and tissue collection 05/07-071-00; Field studies in support of TR ERA 03/04-042-00; Protocol for fish sampling 03/04-043-00) 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 SC1254 for BKF/SC permit for fish (Zwiernik)/SC permit for amphibians (Zwiernik); USFWS Migratory Bird Scientific Collection Permit MB1000062-0; and subpermitted under US Department of the Interior Federal Banding Permit 22926) are on file at MSU-WTL.

Supplemental Information

Table 5.5. Concentrations of 2378-TCDD equivalents (TEQs[a]) in eggs and nestlings of belted kingfisher collected during 2005–2007 from within the Chippewa and Tittabawassee River floodplains, Midland, MI, USA. Values[b] (ng/kg ww) are given as the geometric mean (sample size) over the 95% confidence interval.

	BKF Eggs		BKF Nestlings	
	RA	SA	RA	SA
PCDD-$TEQ_{WHO\text{-}Avian}$[c]	10 (6) 3.9–28	19 (19) 15–26	3.1 (5) 1.6–6.0	8.5 (12) 0.74–3.2
PCDF-$TEQ_{WHO\text{-}Avian}$[d]	4.1 (6) 2.4–7.0	59 (19) 40–87	1.8 (5) 1.2–2.9	86 (12) 65–110
non-*ortho* PCB-$TEQ_{WHO\text{-}Avian}$[e]	26 (3) 11–63	58 (11) 37–89	5.6 (4) 3.5–9.0	32 (9) 12–81
mono-ortho PCB-$TEQ_{WHO\text{-}Avian}$[f]	2.5 (3) 6.9–14	5.5 (11) 3.2–9.3	0.40 (4) 0.26–0.63	2.6 (9) 1.3–5.1
Total $TEQ_{WHO\text{-}Avian}$	47 (3) 10–210	170 (11) 120–240	9.7 (4) 8.0–12	170 (9) 110–250

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

[b] Values have been rounded and represent only two significant figures

[b] PCDD-$TEQ_{WHO\text{-}Avian}$= summation of the TEQs of individual PCDD congeners

[c] PCDF-$TEQ_{WHO\text{-}Avian}$= summation of the TEQs of individual PCDF congeners

[e] non-*ortho* PCB-$TEQ_{WHO\text{-}Avian}$= summation of the TEQs of individual non-*ortho* substituted PCB congeners

[f] mono-*ortho* PCB-$TEQ_{WHO\text{-}Avian}$= summation of the TEQs of individual mono-*ortho* substituted PCB congeners

Table 5.6. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in eggs and nestlings of belted kingfisher collected during 2005-2007 within the Chippewa and Tittabawassee River floodplains, Midland, MI, USA. Values[a] (ng/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

	BKF Eggs		BKF Nestlings	
	RA	SA	RA	SA
Chemical[c]	*n*=6	*n*=19	*n*=5	*n*=12
2378-TCDF	0.82±0.46	7.4±3.8	0.91±0.42	26±10
	0.22–1.3	1.6–14	0.52–1.6	12–49
23478-PeCDF	3.4±2.1	67±57	0.87±0.41	63±38
	1.3–7.2	6.7–180	0.61–1.6	12–140
12378-PeCDF	0.30±0.14	5.1±2.9	0.27±0.026	15±7.9
	0.21–0.66	0.88–10	0.24–0.29	6.3–34
	3ND	1ND	2ND	
234678-HxCDF	0.46±0.17	1.5±1.1		2.3±2.2
	0.21–0.66	0.47–4.6	0.15–0.16	0.69–7.0
	1ND		3ND	
123789-HxCDF				0.54–0.99
	6ND	19ND	5ND	10ND
123678-HxCDF	1.0±0.48	4.1±3.1		5.6±5.6
	0.49–1.6	1.1–12	0.25–2.7	1.0–18
	1ND		3ND	
123478-HxCDF	1.4±0.95	16±15	0.23±0.096	26±27
	0.62–2.7	2.1–57	0.17–0.34	3.0–77
	1ND		2ND	
1234789-HpCDF		0.52±0.18		3.7±3.7
		0.28–0.77		0.40–9.9
	6ND	11ND	5ND	7ND
1234678-HpCDF	0.31±0.077	2.8±1.3	0.16	18±31
	0.22–0.39	1.2–5.8		1.2–99
	2ND	1ND	4ND	
12346789-OCDF		0.84±0.60	0.16	25±44
		0.36–2.5		0.34–130
	6ND	5ND	4ND	2ND
2378-TCDD	8.5±9.7	13±9.1	2.0±0.97	5.6±1.6
	1.8–27	3.8–46	0.76–3.2	2.5–8.2

Table 5.6 (cont'd)

12378-PeCDD	6.6±5.7 1.7–17	10±7.3 2.5–29	1.4±0.85 0.69–2.9	3.1±0.94 1.3–4.6
123789-HxCDD	0.78±0.29 0.43–1.1 2ND	1.1±0.68 0.21–2.8 5ND	5ND	0.86±0.80 0.23–1.9 8ND
123678-HxCDD	5.5±3.8 1.1–11	7.0±5.3 2.1–21	1.5±1.3 0.33–3.6	3.3±3.6 1.4–14
123478-HxCDD	1.6±0.50 0.70–1.9 1ND	1.9±1.4 0.60–5.2 3ND	0.53±0.49 0.23–1.1 2ND	0.72±0.45 0.34–1.6 5ND
1234678-HpCDD	1.6±0.98 0.77–3.5	3.7±1.4 2.0–6.2	0.60±0.43 0.32–1.2 1ND	15±29 0.96–97
12346789-OCDD	3.4±2.6 1.4–8.3	9.0±5.2 1.8–20	0.67±0.088 0.52–0.75	85±190 1.1–650

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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 5.7. Concentrations of selected co-contaminants in eggs and nestlings of belted kingfisher collected during 2005-2007 within the Chippewa and Tittabawassee River floodplains, Midland, MI, USA. Values[a] (μg/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

	BKF Eggs		BKF Nestlings	
Chemical[c]	RA *n*=3	SA *n*=11	RA *n*=4	SA *n*=9
PCB 77	0.099±0.035 0.074–0.14	0.34±0.25 0.11–0.96	0.043±0.016 0.028–0.061	0.49±0.98 0.039–3.1
PCB 81	0.047±0.014 0.036–0.063	0.11±0.073 0.025–0.23	0.010±0.0077 0.0037–0.021	0.44±1.1 0.011–3.5
PCB 126	0.18±0.066 0.10–0.23	0.45±0.54 0.085–2.0	0.026±0.0067 0.020–0.034	0.17±0.18 0.053–0.64
PCB 169	0.025±0.0.006 4 0.0018–0.030	0.041±0.035 0.014–0.13	0.0030–0.0040	0.011±0.0038 0.0063–0.015
PCB 105	16±11 4.6–27	1ND 43±49 7.6–180	2ND 2.0±0.65 1.3–2.7	5ND 26±43 5.0–140
PCB 114	1.2±0.66 0.52–1.8	4.0±4.5 0.79–17	0.16±0.045 0.10–0.20	2.0±3.1 0.44–10
PCB 118	56±45 19–110	100±91 26–350	6.6±1.4 5.2–8.2	58±66 14–230
PCB 123	1.2±0.0.75 0.53–2.0	2.7±3.2 0.32–11	0.14±0.072 0.067–0.23	1.6±2.6 0.23–8.5
PCB 156	6.7±4.2 2.4–11	14±13 3.6–47	1.0±0.28 0.82–1.4	5.7±5.2 1.7–19
PCB 157	1.7±1.2 0.54–2.9	3.3±3.1 0.76–11	0.21±0.071 0.17–0.32	1.2±1.2 0.36–4.3
PCB 167	4.0±3.2	6.8±6.3	0.49±0.058	2.7±1.9

Table 5.7 (cont'd)

	1.2–7.5	2.0–23	0.43–0.57	0.89–7.4
PCB 189	0.69±0.51	1.4±1.2	0.098±0.020	0.60±0.49
	0.24–1.3	0.45–4.5	0.081–0.13	0.19–1.8
2,4'-DDT [c]	0.78±0.99	0.36±0.18		0.075±0.045
	0.035–1.9	0.084–0.62	0.012–0.031	0.016–0.15
			2ND	1ND
2',4'-DDE [d]	600±580	690±500	170±150	220±78
	200–1300	120–1500	82–390	120–350
4, 4'-DDT	6.4±1.7	20±16	0.26±0.32	1.3±1.2
	5.0–8.3	4.0–55	0.0055–0.69	0.028–3.1

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[c] DDT = dichloro-diphenyl-trichloroethane

[d] DDE = dichloro-diphenyl-dichloroethylene

Table 5.8. Concentrations of seventeen 2,3,7,8-substituted furan and dioxin congeners in soils collected during 2006 from nest chambers of belted kingfishers within the Chippewa and Tittabawassee River floodplains, Midland, MI, USA. Values[a] (ng/kg ww) are given as the arithmetic mean[b] ± 1 SD over the range.

Chemical[c]	Reference Area *n*=2	Study Area *n*=12
2378-TCDF		3200±4300
	7.5–8.1	12–15000
23478-PeCDF		1000±1500
	0.64–0.73	1.7–5100
12378-PeCDF		1300±1900
	1.1–1.3	2.4–6800
234678-HxCDF	0.22	110±130
		0.21–430
	1ND	
123789-HxCDF		22±27
		1.2–90
	2ND	2ND
123678-HxCDF	0.19	180±240
		0.29–850
	1ND	
123478-HxCDF		990±1300
	0.22–0.47	1.1–4500
1234789-HpCDF	0.10	82±96
		0.13–320
	1ND	
1234678-HpCDF		800±1200
	0.20–0.94	2.4–4300
12346789-OCDF		1500±2400
	0.35–1.1	3.6–8600
2378-TCDD	0.13	7.4±11
		0.26–40
	1ND	
12378-PeCDD	0.21	9.6±12
		0.22–36
	1ND	2ND

Table 5.8 (cont'd)

123789-HxCDD	0.25	15±20
		0.27–55
	1ND	2ND
123678-HxCDD	0.30	28±43
		0.34–130
	1ND	
123478-HxCDD	0.12	7.5±9.2
		0.13–23
	1ND	4ND
1234678-HpCDD		520±810
	0.73–3.0	4.3–2300
12346789-OCDD		6300±10000
	5.0–19	32–29000
SUM PCDD/DFs		16000±16000
	17–35	62–50000

[a] Values have been rounded and represent only two significant figures

[b] Concentrations below limit of detection treated as zero in the calculation of arithmetic means

[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

References

Allard, P., Fairbrother, A., Hope, B. K., Hull, R. N., Johnson, M. S., Kapustka, L., Mann, G., McDonald, B., and Sample, B. E. (2010). Recommendations for the development and application of wildlife toxicity reference values. *Integrated Environmental Assessment and Management* **6**(1), 28-37.

Baron, L. A., Ashwood, T. L., Sample, B. E., and Welsh, C. (1997). Monitoring bioaccumulation of contaminants in the belted kingfisher (*Ceryle alcyon*). *Environmental Monitoring and Assessment* **47**, 153-165.

Bent, A. C. (1940). Life histories of North American cuckoos, goatsuckers, hummingbirds, and their allies. (No. 176). U.S. National Museum Bulletin.

Blankenship, A. L., Kay, D. P., Zwiernik, M. J., Holem, R. R., Newsted, J. L., Hecker, M., and Giesy, J. P. (2008). Toxicity reference values for mink exposed to 2,3,7,8-tetrachlodibenzo-*p*-dioxin (TCDD) equivalents (TEQs). *Ecotoxicology and Environmental Safety* **69**(3), 325-349.

Blus, L. J., Henny, C. J., and Kaiser, T. E. (1980). Pollution ecology of breeding great blue herons in the Columbia Basin, Oregon and Washington. *The Murrelet* **61**(2), 63-71.

Braune, B. M., and Norstrom, R. J. (1989). Dynamics of organochlorine compounds in herring gulls: III. Tissue distribution and bioaccumulation in Lake Ontario gulls. *Environmental Toxicology and Chemistry* **8**(10), 957-968.

Brunström, B. (1988). Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. *Poultry Science* **67**(1), 52-57.

Brunström, B., and Reutergardh, L. (1986). Differences in sensitivity of some avian species to the embryotoxicity of a PCB, 3,3',4,4'-tetrachlorobiphenyl, injected into the eggs. *Environmental Pollution Series A-Ecological and Biological* **42**, 37-45.

Coefield, S. J., Zwiernik, M. J., Fredricks, T. B., Seston, R. M., Nadeau, M. W., Tazelaar, D. L., Kay, D. P., Newsted, J. L., and Giesy, J. P. (2010). Ecological risk assessment of great horned owls (*Bubo virginianus*) exposed to PCDDs/PCDFs in the Tittabawassee River floodplain in Midland, Michigan, USA. *Environmental Toxicology and Chemistry* (in press).

Cornwell, G. W. (1963). Observations on the breeding biology and behavior of a nesting population of belted kingfisher. *The Condor* **65**(5), 426-431.

Custer, C. M., Custer, T. W., Rosiu, C. J., Melancon, M. J., Bickham, J. W., and Matson, C. W. (2005). Exposure and effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in tree

swallows (*Tachycineta bicolor*) nesting along the Woonasquatucket River, Rhode Island, USA. *Environmental Toxicology and Chemistry* **24**(1), 93-109.

Custer, T. W., Custer, C. M., and Gray, B. R. (2010). Polychlorinated biphenyls, dioxins, furans, and organochlorine pesticides in belted kingfisher eggs from the upper Hudson River basin, New York, USA. *Environmental Toxicology and Chemistry* **29**(1), 99-110.

Davis, W. J. (1980). The belted kingfisher, *Megaceryle alcyon*: its ecology and territoriality. Cincinnati, OH, University of Cincinnati.

Davis, W. J. (1982). Territory size in *Megacerlye alcyon* along a stream habitat. *The Auk* **99**, 353-362.

Elliott, J. E., and Harris, M. L. (2001). An ecotoxicological asessment of chlorinated hydrocarbon effects on bald eagle poplulations. *Reviews in Toxicology* **4**, 1-60.

Elliott, J. E., Wilson, L. K., Langelier, K. W., and Norstrom, R. J. (1996). Bald eagle morality and chlorinated hydrocarbon contaminants in livers from British Columbia, Canada, 1989-1994. *Environmental Pollution* **94**(1), 9-18.

Evers, D. C., and Lane, O. (2000). Assessing methylmercury availability in Maine's aquatic systems with the belted kingfisher, 1998-99. BRI2000-01.

Fairbrother, A. (2003). Lines of evidence in wildlife risk assessments. *Human and Ecological Risk Assessment* **9**, 1475-1491.

Fairbrother, A. (2001). Putting the impacts of environmental contamination into perspective. In *Ecotoxicology of Wild Mammals* (R.F.Shore and B.A.Rattner, Eds.), pp. 671-689. John Wiley & Sons Ltd., West Sussex, England.

Fecteau, C. S. (2008). The Effects of Mercury on the Productivity of Belted Kingfishers (*Ceryle alcyon*) on Three Rivers in the Eastern United States. University of Southern Maine.

Fredricks, T. B., Giesy, J. P., Coefield, S. J., Seston, R. M., Haswell, M. M., Tazelaar, D. L., Bradley, P. W., Moore, J. N., Roark, S. A., and Zwiernik, M. J. (2010a). Dietary exposure of three passerine species to PCDD/DFs from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, Michigan, USA. *Environmental Monitoring and Assessment*; available online DOI: 110.1007/s10661-010-1319-5.

Fredricks, T. B., Zwiernik, M. J., Seston, R. M., Coefield, S. J., Plautz, S. C., Tazelaar, D. L., Shotwell, M. S., Bradley, P. W., Kay, D. P., and Giesy, J. P. (2010b). Passerine exposure to primarily PCDFs and PCDDs in the river floodplains near

Midland, Michigan, USA. *Archives of Environmental Contamination and Toxicology* **58**, 1048-1064.

Galbraith Environmental Sciences LLC. Tittabawassee River Aquatic Ecological Risk Assessment. 2003. Newfane, Vermont.

Giesy, J. P., Ludwig, J. P., and Tillitt, D. E. (1994). Dioxins, Dibenzofurans, PCBs and Colonial Fish-Eating Water Birds. In *Dioxin and Health* (A.Schecter, Ed.), pp. 249-307. Plenum Press, New York, NY.

Gilbertson, M. (1983). Etiology of chick edema disease in herring gulls in the lower Great Lakes. *Chemosphere* **12**(3), 357-370.

Hamas, M. J. (1975). Ecological and physiological adaptations for breeding in the belted kingfisher (*Megaceryle alcyon*). University of Minnesota, Minneapolis.

Hamas, M. J. (1994). Belted kingfisher (*Cerlye alcyon*). A. Poole, P. Stettenheim, & F. Gill (Eds.), *The Birds of North America*, No. 25. Philadelphia: The Academy of Natural Sciences; Washington, DC: The American Ornithologists's Union.

Harris, M. L., Elliott, J. E., Butler, R. W., and Wilson, L. K. (2003). Reproductive success and chlorinated hydrocarbon contamination of resident great blue herons (*Ardea herodias*) from coastal British Columbia, Canada, 1977 to 2000. *Environmental Pollution* **121**, 207-227.

Head, J. A., Hahn, M. E., and Kennedy, S. W. (2008). Key amino acids in the aryl hydrocarbon receptor predict dioxin sensitivity in avian species. *Environmental Science & Technology* **42**(19), 7535-7541.

Heinz, G. H., Swineford, D. M., and Katsma, D. E. (1984). High PCB residues in birds from the Sheboygan River, Wisconsin. *Environmental Monitoring and Assessment* **4**, 155-161.

Heinz, G. H., Hoffman, D. J., Klimstra, J. D., Stebbins, K. R., Kondrad, S. L., and Erwin, C. A. (2009). Species differences in the sensitivity of avian embryos to methylmercury. *Archives of Environmental Contamination and Toxicology* **56**, 129-138.

Henny, C. J., Blus, L. J., Krynitsky, A. J., and Bunck, C. M. (1984). Current impact of DDE on black-crowned night-herons in the intermountain west. *Journal of Wildlife Management* **48**(1), 1-13.

Hervé, J. C., Crump, D., Jones, S. P., Mundy, L. J., Giesy, J. P., Zwiernik, M. J., Bursian, S. J., Jones, P. D., Wiseman, S. B., Wan, Y., and Kennedy, S. W. (2010a). Cytochrome P4501A Induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and two

chlorinated dibenzofurans in primary hepatocyte cultures of three avian species. *Toxicol. Sci.* **113**(2), 380-391.

Hervé, J. C., Crump, D., Mclaren, K., Giesy, J. P., Zwiernik, M. J., Bursian, S. J., and Kennedy, S. W. (2010b). 2,3,4,7,8-Pentachlorodibenzofuran is a more potent cytochrome P4501A inducer than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in herring gull hepatocyte cultures. *Environmental Toxicology and Chemistry*; available online DOI: 10.1002/etc.255.

Hilscherova, K., Kannan, K., Nakata, H., Hanari, N., Yamashita, N., Bradley, P. W., McCabe, J. M., Taylor, A. B., and Giesy, J. P. (2003). Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. *Environmental Science and Technology* **37**(3), 468-474.

Hoffman, D. J., Rattner, B. A., Sileo, L., Docherty, D. E., and Kubiak, T. J. (1987). Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. *Environmental Research* **42**, 176-184.

Hoffman, D. J., Rice, C. P., and Kubiak, T. J. (1996). PCBs and dioxins in birds. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations* (W.N.Beyer, G.H.Heinz, and A.W.Redmon-Norwood, Eds.), pp. 165-207. Lewis Publishers, Boca Raton, FL, USA.

Hoyt, D. F. (1979). Practical methods of estimating volume and fresh weight of bird eggs. *The Auk* **96**, 73-77.

Hudson River Natural Resource Trustees. (2004). Data report for the collection of eggs from spotted sandpipers, American woodcock, belted kingfisher, American robin, red-winged blackbird, and eastern phoebe associated with the Hudson River from Hudson Falls to Schodack Island, New York. Hudson River natural resource damage assessment. 9-17-2004. Silver Spring, MD, USA, U.S Department of Commerce, National Oceanic and Atmospheric Administration.

Kannan, K., Yun, S., Ostaszewski, A., McCabe, J., MacKenzie-Taylor, D., and Taylor, A. (2008). Dioxin-like toxicity in the Saginaw River watershed: Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in sediments and floodplain soils from the Saginaw and Shiawassee Rivers and Saginaw Bay, Michigan, USA. *Archives of Environmental Contamination and Toxicology* **54**(1), 9-19.

Karchner, S. I., Franks, D. G., Kennedy, S. W., and Hahn, M. E. (2006). The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor. *PNAS* **103**(16), 6252-6257.

Kubiak, T. J., Harris, H. J., Smith, L. M., Schwartz, T. R., Stalling, D. L., Trick, J. A., Sileo, L., Docherty, D., and Erdman, T. C. (1989). Microcontaminants and reproductive impairment of the Forster's tern in Green Bay, Lake Michigan-1983. *Archives of Environmental Contamination and Toxicology* **18**, 706-727.

Kubota, A., Iwata, H., Tanabe, S., and Tobata, S. (2005). Hepatic CYP1A induction by dioxin-like compounds, and congener-specific metabolism and sequestration in wild common cormorants from Lake Biwa, Japan. *Environmental Science and Technology* **39**(10), 3611-3619.

Laporte, P. (1982). Organochlorine residues and eggshell measurements of great blue heron eggs from Quebec. *Colonial Waterbirds* **5**, 95-103.

Leonards, P. E. G., van Hattum, B., and Leslie, H. (2008). Assessing the risks of persistent organic pollutants to top predators: A review of approaches. *Integrated Environmental Assessment and Management* **4**(4), 386-398.

Ludwig, J. P., Auman, H. J., Kurita, H., Ludwig, M. E., Campbell, L. M., Giesy, J. P., Tillitt, D. E., Jones, P., Yamashita, N., Tanabe, S., and Tatsukawa, R. (1993). Caspian tern reproduction in the Saginaw Bay ecosystem following a 100-Year flood event. *Journal of Great Lakes Research* **19**(1), 96-108.

Mandal, P. K. (2005). Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. *Journal of Comparative Physiology: Part B* **175**, 221-230.

Mazeika, S., Sullivan, P., Watzin, M. C., and Hession, W. C. (2006). Differences in the reproductive ecology of belted kingfishers (*Ceryle alcyon*) across streams with varying geomorphology and habitat quality. *Waterbirds* **29**(3), 258-270.

Moore, D. R. J., Sample, B. E., Suter, G. W., Parkhurst, B. R., and Teed, R. S. (1999). A probabilistic risk assessment of the effects of methylmercury and PCBs on mink and kingfishers along East Fork Poplar Creek Oak Ridge, Tennessee, USA. *Environmental Toxicology and Chemistry* **18**(12), 2941-2953.

Neigh, A. M., Zwiernik, M. J., Bradley, P. W., Kay, D. P., Park, C. S., Jones, P. D., Newsted, J. L., Blankenship, A. L., and Giesy, J. P. (2006). Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund Site, MI, USA. *Environmental Toxicology and Chemistry* **25**(2), 428-437.

Nosek, J. A., Craven, S. R., Sullivan, J. P., Hurley, S. S., and Peterson, R. E. (1992). Toxicity and reproductive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in ring-necked pheasant hens. *Journal of Toxicology and Environmental Health* **35**, 187-198.

Olsson, A., Ceder, K., Bergman, Å., and Helander, B. (2000). Nestling blood of the white-tailed sea eagle (*Haliaeetus albicilla*) as an indicator of territorial exposure to organohalogen compounds - an evaluation. *Environmental Science and Technology* **34**, 2733-2740.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Stromborg, K. L., Kubiak, T. J., Giesy, J. P., and Bursian, S. J. (1997a). Organochlorine contaminants in double-crested cormorants from Green Bay, Wisconsin: II. Effects of an extract derived from cormorant eggs on the chicken embryo. *Archives of Environmental Contamination and Toxicology* **32**, 316-322.

Powell, D. C., Aulerich, R. J., Stromborg, K. L., and Bursian, S. J. (1996). Effects of 3,3,',4,4',-tetrachlorobiphenyl, 2,3,3',4,4'-pentachlorobiphenyl, and 3,3',4,4',5-pentachlorobiphenyl on the developing chicken embryo when injected prior to incubation. *Journal of Toxicology and Environmental Health* **49**, 319-338.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Kelly, M. E., Stromborg, K. L., Melancon, M. J., Fitzgerald, S. D., and Bursian, S. J. (1998). Effects of 3,3',4,4',5-pentachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin injected into the yolks of double-crested cormorant (*Phalacrocorax auritus*) eggs prior to incubation. *Environmental Toxicology and Chemistry* **17**(10), 2035-2040.

Powell, D. C., Aulerich, R. J., Meadows, J. C., Tillitt, D. E., Powell, J. F., Restum, J. C., Stromborg, K. L., Giesy, J. P., and Bursian, S. J. (1997b). Effects of 3,3',4,4',5-pentachlorobiphenyl (PCB 126), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), or an extract derived from field-collected cormorant eggs injected into double-crested cormorant (*Phalacrocorax auritus*) eggs. *Environmental Toxicology and Chemistry* **16**(7), 1450-1455.

Salyer, J. C., and Lagler, K. F. (1949). The eastern belted kingfisher, *Megaceryle alcyon alcyon* (Linnaeus), in relation to fish management. *Transactions of the American Fisheries Societies* **76**, 97-117.

Senthil Kumar, K., Kannan, K., Giesy, J. P., and Masunaga, S. (2002). Distribution and elimination of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, and p,p'-DDE in tissues of bald eagles from the upper peninsula of Michigan. *Environmental Science & Technology* **36**(13), 2789-2796.

Seston, R. M., Fredricks, T. B., Tazelaar, D. L., Coefield, S. J., Bradley, P. W., Newsted, J. L., Roark, S. A., Kay, D. P., Giesy, J. P., and Zwiernik, M. J. (2010a). Tissue-based risk assessment of great blue heron (*Ardea herodias*) exposed to PCDD/DFs in the Tittabawassee River floodplain, MI, USA. *Environmental Toxicology and Chemistry* (in press).

Seston, R. M., Zwiernik, M., Fredricks, T., Coefield, S., Tazelaar, D., Hamman, D., Paulson, J., and Giesy, J. (2009). Utilizing the great blue heron (*Ardea herodias*)

in ecological risk assessments of bioaccumulative contaminants. *Environmental Monitoring and Assessment* **157**(1), 199-210.

Seston, R. M., Zwiernik, M. J., Fredricks, T. B., Tazelaar, D. L., Coefield, S. J., Bradley, P. W., Newsted, J. L., Roark, S. A., Kay, D. P., and Giesy, J. P. (2010b). Dietary exposure of great blue heron (*Ardea herodias*) to PCDD/DFs in the Tittabawassee River floodplain, Michigan, USA. *Ecotoxicology and Environmental Safety* (in review).

Thomas, C. M., and Anthony, R. G. (1999). Environmental contaminants in the great blue herons (*Ardea herodias*) from the Lower Columbia and Willamette Rivers, Oregon and Washington, USA. *Environmental Toxicology and Chemistry* **18**(12), 2804-2816.

US Environmental Protection Agency (USEPA). Michigan Dioxin Studies. Dow Chemical Wastewater Characterization Study. Tittabawassee River Sediments and Native Fish. EPA-905/4-88-003. 1986. U.S. Environmental Protection Agency, Region V, Westlake, OH.

US Environmental Protection Agency (USEPA). Wildlife Exposure Factors Handbook. EPA/600/R-93/187. 1993. Washington, DC 20460, U.S. Environmental Protection Agency.

US Environmental Protection Agency (USEPA). Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Revision 1. Method 8290A. SW-846. 1998. Washington, DC, US Environmental Protection Agency.

US Environmental Protection Agency (USEPA). Analyses of laboratory and field studies of reproductive toxicity in birds exposed to dioxin-like compounds for use in ecological risk assessment. EPA/600/R-03/114F. 2003.

US Environmental Protection Agency (USEPA), and Risk Assessment Forum. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F, 1-124. 1998. Washington D.C., US Environmental Protection Agency.

van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., and Zacharewski, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* **106**(12), 775-792.

van den Berg, M., Craane, B. L. H. J., Sinnige, T., van Mourik, S., Dirksen, S., Boudewijn, T., van der Gaag, M., Lutke-Schipholt, I. J., Spenkelink, B., and

Brouwer, A. (1994a). Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (*Phalacrocorax carbo*) after in ovo exposure. *Environmental Toxicology and Chemistry* **13**(5), 803-816.

van den Berg, M., De Jongh, J., Poiger, H., and Olson, J. R. (1994b). The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Critical Reviews in Toxicology* **24**(1), 1-74.

Vessel, R. D. (1978). Energetics of the belted kingfisher (*Megaceryle alcyon*). Corvallis, OR, Oregon State University.

Zwiernik, M. J., Bursian, S. J., Aylward, L. L., Kay, D. P., Moore, J., Rowlands, C., Woodburn, K., Shotwell, M., Khim, J. S., Giesy, J. P., and Budinsky, R. A. (2008a). Toxicokinetics of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF in mink (*Mustela vison*) at ecologically relevant exposures. *Toxicol. Sci.* **105**(1), 33-43.

Zwiernik, M. J., Kay, D. P., Moore, J. N., Beckett, K. J., Khim, J. S., Newsted, J. L., Roark, S. A., and Giesy, J. P. (2008b). Exposure and effects assessment of resident mink (*Mustela vison*) exposed to polychlorinated dibenzofurans and other dioxin-like compounds in the Tittabawassee River basin, Midland, Michigan, USA. *Environmental Toxicology and Chemistry* **27**(10), 2076-2087.

CHAPTER 6

Conclusions

Rita Marie Seston

Comparison of Receptor Species

Selection of receptor species is a critical step of the problem formulation process of ecological risk assessments. The great blue heron (*Ardea herodias*; GBH) and belted kingfisher (*Ceryle alcyon*; BKF) are often selected as receptor species for ecological risk assessments to determine the potential for effects of persistent bioaccumulative contaminants in aquatic environments. Both species have similar diets, however their exposure characteristics including metabolic rates, foraging range, and time on-site may differ. Furthermore, issues of maximal obtainable sample size and level of effort required to reach sample size requirements will be different between the two species depending on site characteristics such as size and available habitat types. Matching receptor characteristics to site characteristics and the risk management goals ultimately determines the utility of any ecological risk assessment. Herein, the exposures of BKF and GBH breeding within a river system contaminated with dioxin-like compounds are compared. To provide risk assessors with information on species characteristics and suitability, a number of exposure characteristics were compared between species to assess spatial trends, spatial resolution, data interpretability, and required level of effort.

As a result of historical chemical production and management of associated wastes, the Tittabawassee (TR) and Saginaw Rivers (SR) downstream of Midland, MI, USA contain elevated concentrations of polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs). The persistence of these compounds in the environment, combined with their toxicity and potential to bioaccumulate, has led to concern over the potential exposures of wildlife species foraging in the Tittabawassee and Saginaw River

floodplains. To understand both exposure and associated effects, field studies were conducted over several years in support of a large scale, site-specific ecological risk assessment (ERA) utilizing multiple lines of evidence. To most accurately assess an ecosystem as complex as the Tittabawassee River floodplain, the site-specific ERA employed many different receptor species representative of various feeding guilds and exposure pathways.

This dissertation details the exposure of GBH and BKF foraging and breeding within the TR floodplain to the seventeen 2,3,7,8-substituted PCDD/DFs (ΣPCDD/DFs). Due to the tendency of PCDD/DFs to bioaccumulate through trophic transfer, species located near the top of food webs have the greatest potential for exposure. Additionally, it has been determined that avian species are more sensitive to the toxic effects of PCDD/DFs than are mammals, fish, amphibians, or reptiles. As maximally-exposed, potentially highly-sensitive species, the GBH and BKF were selected as top-tier avian receptors of an aquatic-based exposure pathway.

Although both the BKF and GBH share an exposure pathway and several characteristics which make both of them desirable species to investigate exposure and potential effects of PCDD/DFs, there are a few key differences. As top aquatic food web predators, both the BKF and GBH have a relatively great potential for exposure as a result of biomagnification. Each species has a widespread distribution throughout North America, making them potential receptors for ecological assessments of aquatic environments in various localities. Many of the differences between the two species are related to their difference in body size. Although the dietary composition is similar between the two species, there may be differences in the size-class of prey consumed. As

the smaller species, BKF have a greater food consumption rate normalized to body weight. Additionally, BKF defend defined territories and foraging areas proximal to its nest burrow (0.92 to 2.9 km (Davis 1982; Mazeika *et al.* 2006)) in contrast to the GBH which defends territories within 3.2 to 6.5 km of the breeding colony (Dowd and Flake 1985; Marion 1989; Peifer 1979; Thompson 1978).

Concentrations of PCDD/DFs and PCBs were quantified in the dietary components of GBH and BKF. The diet of both the GBH and BKF is primarily composed of fish, but also includes crayfish and amphibians. Consistent spatial trends in concentrations of ΣPCDD/DFs and DF-$TEQ_{WHO\text{-}Avian}$ were observed for each type of prey. Concentrations were least in the RA, greater in the UTR, and greatest in the LTR. Concentrations of PCDD/DFs in prey items from the SR were intermediate to those from the RA and UTR (Table 6.1). Mean concentrations of ΣPCDD/DFs were 8-, 18-, and 1-fold greater in frogs, 21-, 49-, and 7-fold greater in crayfish, and 36-, 71-, and 23-fold greater in forage fish collected from the UTR, LTR, and SR than those from the RA, respectively. Mean concentrations of DF-$TEQ_{WHO\text{-}Avian}$ were 17-, 52-, and 3-fold greater in frogs, 43-, 120-, and 20-fold greater in crayfish, and 130-, 240-, and 64-fold greater in forage fish collected from the UTR, LTR, and SR when compared to the RA, respectively. Greater fold differences observed in $TEQ_{WHO\text{-}Avian}$ are a result of prey items from the TR and SR having greater relative TCDD potency of ΣPCDD/DF, primarily from 2,3,7,8-TCDF and secondarily from 2,3,4,7,8-PeCDF. Concentrations of ΣPCDD/DFs in estimated diets of BKF were 29-, 59-, and 16-fold greater and 32-, 65-, and 20-fold greater for GBH compared to the RA for UTR, LTR, and SR, respectively

Table 6.1. $TEQ_{WHO\text{-}Avian}$[a] in prey items of belted kingfisher and great blue heron collected during 2004-2006 from the Chippewa, Tittabawassee, and Saginaw River floodplains, Midland, MI, USA. Values[b] (ng/kg lw) are given as the geometric mean $TEQ_{WHO\text{-}Avian}$ and sample size in parentheses (n) over the 95% confidence interval and range (min-max).

	Reference Area	Upper Tittabawassee	Lower Tittabawassee	Saginaw River
Frog				
TCDD	22	65	52	24
	16–31	53–79	42–63	20–28
	(3.4–82)	(13–800)	(11–310)	(14–37)
TCDF	22	790	2800	110
	15–32	460–1300	2200–3700	98–130
	(4.3–230)	(21–19000)	(310–68000)	(84–150)
4-PeCDF	9.9	270	970	51
	7.3–13	170–430	720–1300	33–79
	(2.3–38)	(15–3800)	(190–23000)	(30–410)
ΣPCDD/DF	430 (29)	3300 (51)	7700 (55)	520 (12)
	330–540	2200–4900	6000–9900	440–620
	(98–1500)	(260–68000)	(1000–160000)	(410–1100)
PCB-126	N/A	N/A	240 (4)	N/A
			130-440	
			(140-310)	
$DF\text{-}TEQ_{WHO\text{-}Avian}$	80 (29)	1300 (51)	4200 (55)	230 (12)
	60–110	840–2100	3200–5500	180–280
	(19–330)	(79–23000)	(600–99000)	(160–600)
Total $TEQ_{WHO\text{-}Avian}$	N/A[c]	N/A	24000 (4)	N/A
			5200–110000	
			(9800–97000)	
Crayfish				
TCDD	11	61	86	30
	2.3–55	45–83	55–130	12–77
	(3.9–75)	(36–93)	(35–180)	(7.3–120)
TCDF	41	2900	8300	1500
	11–160	1200–6800	6400–11000	910–2400
	(15–260)	(490–9400)	(5700–15000)	(680–3300)
4-PeCDF	15	620	2000	220

Table 6.1 (cont'd)

	2.9–81	310–1300	1600–2400	140–340
	(3.9–120)	(170–1500)	(1300–2600)	(110–570)
ΣPCDD/DF	480 (5)	9900 (7)	24000 (8)	3200 (8)
	130–1800	5800–17000	19000–30000	2000–5300
	(180–2500)	(5800–28000)	(17000–35000)	(1600–7700)
PCB-126	N/A	– [d] (2)	– (1)	N/A
		–	–	
		(330–430)	(360)	
DF-$TEQ_{WHO\text{-}Avian}$	88 (5)	3800 (7)	11000 (8)	1800 (8)
	20–390	1700–8400	8600–14000	1100–2900
	(27–590)	(780–11000)	(8000–18000)	(840–4200)
Total $TEQ_{WHO\text{-}Avian}$	N/A	– (2)	– (1)	N/A
		–	–	
		(4900–8200)	(14000)	
Forage Fish				
TCDD	– (2)	– (2)	100	57
	–	–	46–220	20–170
	4.0–5.3	86–89	(37–200)	(33–140)
TCDF	– (2)	– (2)	3800	970
	–	–	1700–8500	450–2100
	9.6–11	1800–2000	(1400–7500)	(690–1932)
4-PeCDF	– (2)	– (2)	620	140
	–	–	180–2100	48–440
	(1.1–1.8)	(280–410)	(160–2000)	(85–390)
ΣPCDD/DF	– (2)	– (2)	6900 (5)	2200 (4)
	–	–	2700–17000	750–6300
	(80–120)	(3300–3600)	(2200–15000)	(1200–5700)
PCB-126	– (2)	– (2)	1300 (5)	2000 (4)
	–	–	320–5200	710–5700
	(60–64)	(460–760)	(500–8100)	(1100–4800)
DF-$TEQ_{WHO\text{-}Avian}$	– (2)	– (2)	4600 (5)	1200 (4)
	–	–	1900–11000	540–2800
	(18–21)	(2400–2500)	(1700–10000)	(840–2600)
Total $TEQ_{WHO\text{-}Avian}$	– (2)	– (2)	5500 (5)	3400 (4)
	–	–	2300–13000	930–12000
	(52–52)	(2900–3100)	(2000–11000)	(1600–10000)

Table 6.1 (cont'd)

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

[b] Values have been rounded and represent only two significant figures

[c] N/A = no samples collected from this location

[d] Geometric mean and confidence intervals not calculated for sites with fewer than 3 samples. These sites were not included in reach comparisons.

(Table 6.2). To facilitate direct comparisons between prey and receptor tissues, all concentrations are reported on a lipid-normalized basis.

A spatial trend similar to that observed for prey items was observed in concentrations of measured residues in eggs and nestlings of BKF but not in those of GBH. Concentrations of ΣPCDD/DFs were 4- and 5-fold greater in BKF eggs collected from the UTR and LTR compared to those collected in the RA, respectively (Table 6.3). This trend was most pronounced for concentrations of 2,3,4,7,8-PeCDF in eggs of BKF collected from the UTR and LTR, which were 10- and 23-fold greater, respectively, than in eggs from the RA. Concentrations of ΣPCDD/DFs were greater in BKF nestlings from downstream study areas compared to the RA, however the spatial trend between the UTR and LTR was not observed due to elevated concentrations of octachlorodibenzo-*p*-dioxin (OCDD) in BKF nestlings collected from the UTR (Table 6.4). Similar to eggs, the concentrations of the site-specific 2,3,4,7,8-PeCDF in BKF nestlings exhibited the same spatial gradient, being 33- and 52-fold greater in the UTR and LTR, respectively, than those collected from the RA. Concentrations of DF-$TEQs_{WHO\text{-}Avian}$ were 5- and 7-fold greater in BKF eggs and 10- and 15-fold greater in BKF nestlings from the UTR and LTR, respectively, compared to those collected in the RA. Concentrations of ΣPCDD/DFs and DF-$TEQs_{WHO\text{-}Avian}$ in both eggs and livers of nestling GBH were similar among all studied rookeries (Table 6.3, 6.4).

Consistent spatial trends in relative contributions of DF-$TEQs_{WHO\text{-}Avian}$ and PCB-$TEQs_{WHO\text{-}Avian}$ to total $TEQs_{WHO\text{-}Avian}$ between prey items and receptor tissues were observed. In fish collected from the RA, PCBs accounted for the greatest proportion of the total $TEQs_{WHO\text{-}Avian}$ (63%), compared to those collected from the Tittabawassee

Table 6.2. Total concentrations of 2,3,7,8-substituted furan and dioxin (ΣPCDD/DF) and $TEQ_{WHO\text{-}Avian}$ [a] in the diet of BKF and GBH collected during 2005-2006 from the Tittabawassee River floodplain, Midland, Michigan, USA, expressed with varying dietary compositions. Values[b] (ng/kg lw) are given as the geometric mean over the 95% confidence intervals.

	33% Fish-33% Crayfish- 33% Frog	70% Fish-20% Crayfish-10% Frog	100% Fish	BKF Diet	GBH Diet
TCDD	76 55–110	88 58–140	97 60–160	94 59–150	96 60–150
TCDF	3200 2000–5200	3300 2000–5700	3100 1700–5500	3100 1800–5500	3100 1700–5500
4-PeCDF	730 450–1200	650 350–1200	520 230–1200	560 270–1200	540 250–1200
ΣPCDD/DF	8900 6100–13000	7600 4700–13000	5700 3000–11000	6200 3500–11000	5900 3200–11000
PCB-126	550 270–1200	820 350–2000	1000 410–2600	960 390–1300	1000 400–
DF-TEQs	4300 2700–6900	4300 2500–7400	3800 2100–7100	3900 2200–7100	3900 2100–7100
Total TEQs	12000 3300–51000	7300 2700–24000	4600 2500–8500	5700 2600–14000	5100 2600–11000

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

[b] Values have been rounded and represent only two significant figures.

[c] BKF diet = 90.2% fish, 5.4% crayfish, and 4.4% frog.

[d] GBH diet = 96% fish, 2% crayfish, and 2% frog.

Table 6.3. Total concentrations of 2,3,7,8-substituted furan and dioxin (ΣPCDD/DF) and $TEQ_{WHO\text{-}Avian}$ [a] in eggs of belted kingfisher and great blue heron collected during 2005-2007 from the Chippewa, Tittabawassee and Saginaw River floodplains, Midland, MI, USA. Values [b] (ng/kg lw) are given as the geometric mean (*n*) over the 95% confidence intervals and range in parentheses.

	BKF Eggs			GBH Eggs
	RA	UTR	LTR	SA
TCDD	72 (6) 22–240 (22–470)	200 (8) 110–370 (64–680)	130 (11) 94–190 (53–270)	180 (24) 130–230 (80–1200)
TCDF	9.6 (6) 4.8–19 (3.7–18)	77 (8) 49–120 (24–130)	100 (11) 63–180 (20–220)	57 (24) 36–88 (5.0–320)
4-PeCDF	41 (6) 19–90 (14–120)	410 (8) 160–1100 (88–2600)	940 (11) 610–1500 (420–2200)	240 (24) 180–320 (82–920)
ΣPCDD/DF	410 (6) 170–990 (140–1300)	1600 (8) 900–2900 (820–5500)	2100 (11) 1500–2900 (1200–3900)	1100 (24) 920–1300 (690–4000)
PCB-126	3700 (3) 1700–7800 (2900–5200)	8900 (4) 2000–40000 (3400–30000)	3200 (7) 2100–5100 (1200–5900)	14000 (18) 9400–20000 (2700–39000)
DF-TEQs	210 (6) 77–550 (67–920)	960 (8) 490–1900 (380–3800)	1400 (11) 980–2000 (750–2800)	670 (24) 530–850 (320–2800)
Total TEQs	750 (3) 130–4400 (360–1500)	3500 (4) 1200–9700 (1600–7800)	2000 (7) 1400–3000 (1100–3900)	3700 (18) 2700–5000 (850–8000)

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

[b] Values have been rounded and represent only two significant figures.

Table 6.4. Total concentrations of 2,3,7,8-substituted furan and dioxin (ΣPCDD/DF) and $TEQ_{WHO\text{-}Avian}$ [a] nestlings of belted kingfisher and great blue heron collected during 2005-2007 from the Chippewa, Tittabawassee and Saginaw River floodplains, Midland, MI, USA. Values[b] (ng/kg lw) are given as the geometric mean (*n*) over the 95% confidence intervals and range in parentheses.

	BKF Whole-Body Nestlings			BKF Liver	GBH Liver
	RA	UTR	LTR	LTR	SA
TCDD	46 (5)	– (2)	110 (9)	88 (3)	18 (6)
	22–94	–	92–140	56–140	11–30
	(26–94)	(110–120)	(59–150)	(71–97)	(11–35)
TCDF	22 (5)	– (2)	510 (9)	320 (3)	21 (6)
	14–33	–	370–690	110–940	10–43
	(13–29)	(480–520)	(270–950)	(240–530)	(11–68)
4-PeCDF	21 (5)	– (2)	1100 (9)	1200 (3)	39 (6)
	11–41	–	560–2100	480–3200	19–80
	(12–46)	(680–880)	(280–3800)	(790–1600)	(19–31)
ΣPCDD/DF	250 (5)	– (2)	3200 (9)	2800 (3)	210 (6)
	130–480	–	2000–5300	1600–4900	130–340
	(140–570)	(4100–9200)	(1500–11000)	(2200–3300)	(120–360)
PCB-126	650 (4)	2700 (1)	2800 (7)	1200 (3)	1000 (6)
	250–1700	–	1400–5500	470–3100	480–2100
	(380–1200)	–	(1500–13000)	(820–1700)	(820–2100)

Table 6.4 (cont'd)

DF-TEQs	130 (5) 71–230 (85–240)	– (2) – (1400–1700)	2000 (9) 1400–3100 (1100–4900)	1800 (3) 790–4100 (1200–2300)	100 (6) 55–180 (56–220)
Total TEQs	240 (4) 140–430 (170–350)	2300 (1) – –	3600 (7) 1900–6600 (2000–13000)	2100 (3) 1000–4300 (1500–2600)	570 (6) 250–1300 (220–1400)

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

[b] Values have been rounded and represent only two significant figures.

River where the greatest proportion of total $TEQs_{WHO\text{-}Avian}$ were attributed to DF-$TEQs_{WHO\text{-}Avian}$ (81% and 85% for the UTR and LTR, respectively). Concentrations of total $TEQs_{WHO\text{-}Avian}$ were dominated by PCB-$TEQs_{WHO\text{-}Avian}$ in fish collected from the Saginaw River (61% PCB- $TEQ_{WHO\text{-}Avian}$). In both eggs and nestlings of BKF from the RA, PCB-$TEQs_{WHO\text{-}Avian}$ accounted for the greatest proportion of total $TEQs_{WHO\text{-}Avian}$ (69% and 61%, respectively). Conversely, DF-$TEQs_{WHO\text{-}Avian}$ accounted for the greatest proportion of total $TEQs_{WHO\text{-}Avian}$ in both eggs and nestlings of BKF collected from the UTR (51% and 73%, respectively) and LTR (64% and 66%, respectively). This observation was in contrast to eggs and livers of nestling GBH collected from along the TR, in which PCB-$TEQs_{WHO\text{-}Avian}$ (79% and 81%, respectively) accounted for the greatest proportion of total $TEQs_{WHO\text{-}Avian}$. PCB-$TEQs_{WHO\text{-}Avian}$ were dominated by congeners PCB-126, PCB-77, and PCB-81 in all reaches.

The observed spatial trends in concentrations of PCDD/DFs and PCBs in prey of BKF and GBH were consistent with those observed for prey and tissues of other receptor species studied within the SA (Coefield *et al.* 2010; Fredricks *et al.* 2010a; Fredricks *et al.* 2010b; Seston *et al.* 2010). The occurrence of greater concentrations of PCDD/DFs further downstream in the TR is likely the result of the downstream movement of this historical contamination from the source near Midland, MI. The observation that concentrations of PCBs were greater in forage fish from the SR compared to those from the TR is consistent with historical PCB contamination in areas downstream of the TR, including the Saginaw River and Saginaw Bay (Kannan *et al.* 2008) as well as some lesser sources upstream of the RA. Anadromous fishes from Saginaw Bay can move up

into the TR, but there is a dam which may impede the movement of these fish into the RA. Furthermore, comparisons between fish and the more spatially confined frogs and crayfish (Hazlett *et al.* 1974; Martof 1953), found that fish generally contained greater concentrations of PCBs than frogs or crayfish collected from within the same reach. This also suggests that fish may be acquiring PCBs from areas other than the TR. Additionally, there may be incidental PCB inputs from the urbanized Midland, MI area.

The observed difference in the relative concentrations of PCBs between BKF and GBH may also be due to differences in contribution from the SR or offsite sources. Belted kingfisher nesting along the TR have a lesser proportion of the total TEQs in their tissues attributable to PCBs than GBH nesting in similar areas. This could be from a difference in size of foraging ranges between the two species. To estimate how closely concentrations in receptor tissues match those in site-specific prey, spatial trends in the ratio of the ubiquitious PCB-126 to the site-specific 2,3,4,7,8-PeCDF in diet and receptor tissues were compared. In prey, listed in descending order, this ratio is greatest in the RA, then the SR, and nearly equal in the UTR and LTR. Both egg and nestlings of BKF exhibited a similar spatial trend of this ratio. In contrast, the ratio in GBH egg and nestling liver collected from rookeries along the TR most closely resembles that of prey and BKF tissues from the RA. This dissimilarity could be a result the two species utilizing different foraging grounds, with BKF exhibiting a greater spatial resolution in their exposure. Additionally, as a larger species, it is possible that GBH target larger fish that are more likely to be moving longer distances and integrating contaminants over a larger spatial scale.

Thus, it can be seen that either BKF or GBH may be better suited as a receptor species based on site characteristics and assessments goals. Sites with areas of widespread contamination may be better represented by GBH, which integrate exposure over a large area. Additionally, many GBH nests may be monitored simultaneously within a single breeding colony to assess any potential impacts on reproductive and population health. By contrast, assessments of sites with a point-source may be better understood by employing BKF, whose exposure has a greater spatial resolution and may be used to evaluate concentration gradients. Each species has qualities that make it a good receptor as long as consideration is given to the different exposure characteristics of each species and which would be most appropriate for a given assessment.

Overall Conclusions

This dissertation details the exposure of GBH and BKF foraging and breeding within the TR floodplain to the seventeen 2,3,7,8-substituted PCDD/DFs and the associated risk of adverse effects. Exposure was determined in both modeled dietary exposure and measured concentrations in receptor specific tissues. Simultaneously, site-specific population health of both BKF and GBH was monitored. Each measure was then combined in the framework of a multiple lines of evidence assessment to minimize uncertainty in conclusions. Over the course of this assessment, BKF and GBH breeding along the TR successfully reproduced despite elevated exposure to PCDD/DFs.

Concentrations of PCDD/DFs in tissues of both BKF and GBH were greater at downstream SAs compared to upstream RAs. For BKF, this spatial trend was seen in eggs and nestlings. In GBH, this comparison was only available for blood plasma of

adults foraging in the river channel, as no rookeries were found in the RA. Elevated exposures in the SA was composed primarily of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and secondarily 2,3,7,8-tetrachlorodibenzofuran (TCDF), which are specific to the historical releases from DOW. Although GBH eggs and nestlings from within the SA also contained these site-specific congeners, their concentrations were lesser than expected based on predicted dietary exposures. Additionally, eggs and nestlings of GBH had greater relative concentrations of PCBs compared to BKF, suggesting they may be foraging partially off-site.

Site-specific dietary- and tissue-based exposures for both the BKF and GBH were compared to toxicity reference values (TRVs) to estimate the potential for adverse effects. Egg-based exposures based on DF-$TEQs_{WHO\text{-}Avian}$ and total $TEQs_{WHO\text{-}Avian}$ for both the BKF and GBH within the SA were at or below the no observed adverse effect concentration (NOAEC). Dietary-based exposures did exceed associated NOAECs for both species but were below the lowest observed adverse effect concentrations (LOAECs). It is important to note that there is more uncertainty associated with the prediction of dietary exposures compared to measured tissue concentrations due to the greater number of assumptions that are necessary. Additionally, the best available TRVs for dietary exposure were based on intraperitoneal injections which likely overestimate exposures compared to actual ingestions. The prediction of minimal risk of adverse effects from the dietary- and tissue-based assessments is in agreement with observations of population health of BKF and GBH along the TR. Therefore, the overall conclusion of the research presented here is that the populations of BKF and GBH breeding along the TR are not at risk despite elevated concentrations of PCDD/DFs in the diet and tissues.

From this research, several areas were identified which should be addressed in future work. Firstly, a better understanding of the foraging habits of GBH would greatly reduce the uncertainty currently associated with their predicted dietary exposure and may explain the presence of greater proportion of PCBs in their tissues. The location and study of a breeding rookery(s) in the RA would also add great value to the current study, allowing direct comparison to regional background concentrations in tissues and reproductive success. Furthermore, although there is great certainty in the site-specific exposure assessments conducted here, further research needs to be done to expand upon the TRVs available to assess risk in ecological studies. There is currently a large gap in toxicological data based on ecologically relevant endpoints, particularly those based on dietary exposure. Lastly, because the research presented here failed to find any significant population-level or individual-based effects as a result of contaminant exposure, any remediation actions suggested or taken along the TR should primarily focus on the habit-based effects of those actions. This is of particular concern for BKF which is often limited by the presence of suitable banks for nest sites. The alteration or removal of that habitat would likely have immediate and long-lasting adverse effects on the breeding population of BKF along the TR.

References

Coefield, S. J., Zwiernik, M. J., Fredricks, T. B., Seston, R. M., Nadeau, M. W., Tazelaar, D. L., Kay, D. P., Newsted, J. L., and Giesy, J. P. (2010). Ecological risk assessment of great horned owls (*Bubo virginianus*) exposed to PCDDs/PCDFs in the Tittabawassee River floodplain in Midland, Michigan, USA. *Environmental Toxicology and Chemistry* (in press).

Davis, W. J. (1982). Territory size in *Megacerlye alcyon* along a stream habitat. *The Auk* **99**, 353-362.

Dowd, E. M., and Flake, L. D. (1985). Foraging habitats and movements of nesting great blue herons in a prairie river ecosystem, South Dakota. *Journal of Field Ornithology* **56**(4), 379-387.

Fredricks, T. B., Giesy, J. P., Coefield, S. J., Seston, R. M., Tazelaar, D. L., Roark, S. A., Kay, D. P., Newsted, J. L., and Zwiernik, M. J. (2010a). Multiple lines of evidence risk assessment of terrestrial passerines exposed to PCDFs and PCDDs in the Tittabawassee River floodplain, Midland, Michigan, USA. *Human and Ecological Risk Assessment* (in press).

Fredricks, T. B., Zwiernik, M. J., Seston, R. M., Coefield, S. J., Tazelaar, D. L., Roark, S. A., Kay, D. P., Newsted, J. L., and Giesy, J. P. (2010b). Multiple lines of evidence risk assessment of tree swallows exposed to PCDFs and PCDDs in the Tittabawassee River floodplain, Midland, Michigan, USA. *Environmental Toxicology and Chemistry* (in review).

Hazlett, B., Rittschof, D., and Rubenstein, D. (1974). Behavioral biology of the crayfish *Orconectes virilis* I. Home range. *American Midland Naturalist* **92**(2), 301-319.

Kannan, K., Yun, S., Ostaszewski, A., McCabe, J., MacKenzie-Taylor, D., and Taylor, A. (2008). Dioxin-like toxicity in the Saginaw River watershed: Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in sediments and floodplain soils from the Saginaw and Shiawassee Rivers and Saginaw Bay, Michigan, USA. *Archives of Environmental Contamination and Toxicology* **54**(1), 9-19.

Marion, L. (1989). Territorial feeding and colonial breeding are not mutually exclusive: the case of the grey heron (*Ardea cinerea*). *The Journal of Animal Ecology* **58**(2), 693-710.

Martof, B. (1953). Home range and movements of the green frog, *Rana clamitans*. *Ecology* **34**(3), 529-543.

Mazeika, S., Sullivan, P., Watzin, M. C., and Hession, W. C. (2006). Differences in the reproductive ecology of belted kingfishers (*Ceryle alcyon*) across streams with varying geomorphology and habitat quality. *Waterbirds* **29**(3), 258-270.

Peifer, R. W. (1979). Great blue herons foraging for small mammals. *The Wilson Bulletin* **91**(4), 630-631.

Seston, R. M., Giesy, J. P., Fredricks, T. B., Tazelaar, D. L., Coefield, S. J., Bradley, P. W., Roark, S. A., Newsted, J. L., Kay, D. P., and Zwiernik, M. J. (2010). Multiple lines of evidence risk assessment of belted kingfisher exposed to PCDFs and PCDDs in Tittabawassee River floodplain, Midland, MI, USA. *Environmental Toxicology and Chemistry* (in review).

Thompson, D. H. (1978). Feeding areas of great blue herons and great egrets nesting within the floodplain of the upper Mississippi River. *Proceedings of the Colonial Waterbird Group* **2**, 202-213.