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A MULTIPLE LINES OF EVIDENCE ECOLOGICAL RISK ASSESSMENT OF GREAT HORNED OWL EXPOSURE TO POLYCHLORINATED DIBENZFURANS AND POLYCHLORINATED DIBENZO-*P*-DIOXINS IN THE TITTABAWASSEE RIVER FLOODPLAIN IN MIDLAND, MI, USA

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By

Sarah Jean Coefield

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ABSTRACT

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The soils and sediments downstream of Midland, Michigan, USA, have elevated polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxin (PCDD) concentrations. To determine if the PCDD/DF concentrations have the potential to adversely affect terrestrial birds, a multiple lines of evidence, site-specific risk assessment for the great horned owl (*Bubo virginianus*; GHO) was conducted. Site-specific GHO dietary exposure, tissue concentrations and population health measures were collected for 115 km of river corridor from 2005-2008. Fifty-five active nests were monitored in 21 breeding territories. The GHO daily dietary exposure estimate was greater in the study area (SA) (3.3 ng $TEQ_{WHO\text{-}Avian}$/kg bw/d) than in the reference area (RA) (0.07 ng/kg bw/d), but was less than the toxicity reference value. Likewise, the geometric mean $TEQ_{WHO\text{-}Avian}$ concentration in GHO plasma was greater in the SA than in the RA for both adult (RA: 3.1 pg $TEQ_{WHO\text{-}Avian}$/mL, SA: 9.4 pg $TEQ_{WHO\text{-}Avian}$/mL) and nestling (RA: 0.82 pg $TEQ_{WHO\text{-}Avian}$/mL, SA: 2.1 pg $TEQ_{WHO\text{-}Avian}$/mL) GHOs, but less than concentrations expected to cause adverse effects. GHO population health and productivity were both greater in the study area than in the reference area. The three lines of evidence suggest GHOs in the Tittabawassee River floodplain are not adversely impacted by PCDD/DFs in the soils and sediments downstream of Midland, MI.

For my family.

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LIST OF ABBREVIATIONS

ADD_{pot}	Potential Average Daily Dose
AhR	Aryl Hydrocarbon Receptor
bw	Body Weight
dl	Detection Limit
dw	Dry Weight
GHO	Great Horned Owl
gm	Geometric Mean
HRGC	High Resolution Gas Chromatography
HRMS	High Resolution Mass Spectrometry
HQ	Hazard Quotient
LOAEL	Lowest Observed Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
PCBs	Polychlorinated Biphenyls
PCDDs	Polychlorinated Dibenzo-*p*-Dioxins
PCDFs	Polychlorinated Dibenzofurans
RA	Reference Area
SA	Study Area
TEFs	Toxic Equivalency Factors
TEQs	TCDD equivalents
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TRV	Toxicity Reference Value
UCL	Upper Confidence Limit
UF	Uncertainty Factor
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Services
WHO	World Health Organization
ww	Wet Weight

INTRODUCTION

Overview

The Tittabawassee River floodplain soils and sediments downstream of Midland, Michigan, USA have elevated concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDD). The PCDD and PCDF (PCDD/DF) concentrations in the floodplain downstream of Midland are 10- to 20-fold greater than those found upstream and are some of the greatest concentrations on record. The sum PCDD/DF (ΣPCDD/DF) concentrations range from $1.0x10^2$ to $5.4x10^4$ ng/kg, dry weight (dw) [1]. Contaminated rivers from other industrialized areas in the eastern United States have ΣPCDD/DF concentrations that range from $1.6x10^2$ to $2.4x10^4$ ng/kg dw with a maximum recorded concentration of ΣPCDD/DFs of $8.2x10^5$ ng/kg dw [2-4].

The PCDD/DFs in the Tittabawassee River floodplain most likely originated at the Dow Chemical Co. in Midland and accumulated over the course of several decades prior to the adoption of modern waste treatment and storage. The Dow Chemical Co., which is currently located on approximately 770 ha in and around Midland, Michigan, has been manufacturing chlorinated compounds for more than 100 years. The company began producing bleach in the late 1800s. After 1914, the chemical company stopped manufacturing bleach, but continued to produce other chlorinated compounds, including chlorinated phenols, chlorobenzenes, liquid chlorine, herbicides and plastics. In order to generate chlorine for its products, the Dow Chemical Co. relied in part on electrolytic processes that used carbon electrodes to generate chlorine from brine. This electrolysis, which was used until the 1980s, was most likely the source of the PCDD/DFs in the Tittabawassee River floodplain. The electrical current may have driven the formation of

chlorinated furans from aromatic forms of carbon in the coal tar used to make the graphite electrodes, along with oxygen and chloride in the brine water.

Prior to 1920, Dow discharged its waste directly into the Tittabawassee River. Over the ensuing decades, the company's waste management strategy evolved to include tertiary treatment with a final effluent filter and holding ponds combined with a revetment groundwater interception system that prevents groundwater contaminants from migrating to the river. Up until the mid-1970s, however, the retaining ponds were susceptible to flooding events, and it is possible dioxins and furans continued to be released into the Tittabawassee River into the 1970s and '80s [5].

Once in the environment, PCDD/DFs have the potential to adversely affect wildlife. Dioxins and furans are persistent, lipophilic compounds that can accumulate in animal tissues and biomagnify up the food chain from contaminated sediments and soils [6-8]. Birds exposed to PCDD/DFs have exhibited behavioral changes, decreased reproductive success, embryotoxicity, edema and developmental deformities [7,9-11].

As predatory birds occupying the top trophic level of the Tittabawassee River floodplain terrestrial ecosystem, great horned owls (*Bubo virginianus*; GHO) have the potential to be exposed to significant dietary concentrations of PCDD/DFs in the floodplain. Because birds are known to be sensitive to PCDD/DFs, the owls may also be more likely to experience adverse effects from this elevated exposure than other animals in the floodplain. Based on these combined characteristics (exposure and sensitivity), the great horned owl was chosen as a biomonitor for the environmental health of the Tittabawassee River floodplain terrestrial ecosystem

The GHO is a large raptor that inhabits a substantial portion of the western hemisphere. GHOs average 1.5 kg in weight and range from 46-63 cm in length [12]. Females are larger than males. The owls are rarely found below 4000 m in South America, but can be found in nearly every climate of North America, including deserts, taiga, deciduous forests, swamps and prairies. In general, GHOs show a preference for fragmented landscapes and their home range will often include fields or cropland. As a reflection of their diverse habitat, the GHO diet can include an array of prey items, ranging from insects and mice to herons and reptiles [12]. GHOs swallow smaller prey whole and will tear apart larger prey items. Several hours after a meal, the owls will egest pellets packed with the undigested fur and bones of their most recent prey.

Great horned owls may mate for at least five years, and possibly for life, though data has not been collected over a pair's lifespan. The owls are highly territorial, and will remain in a territory year-round, although they may migrate if prey populations decline [13]. In the temperate forest, GHOs initiate breeding activity mid-winter, with eggs typically laid by the first weeks of February [14,15]. In warmer climates, GHO breeding activity occurs earlier in the year, while in colder climates eggs may not be laid until May. The incubation period lasts 28-32 d. The owls do not build their own nest, but will take over nests left by red-tailed hawks, crows, eagles, squirrels and great blue herons. They may also nest in artificial nest structures, on ledges, in tree cavities or on the ground [12]. Nestling GHOs are altricial at hatch. They leave the nest before they can fly, typically between 5-7 wks and fledge at 7-9 wks. When out of the nest prior to fledging, the nestlings walk along the ground and climb up trees and branches. Nestlings at this

stage are called "branchers". Adults continue to care for fledglings through the summer and the young birds will often stay in their natal territories until late fall.

Great horned owls have several characteristics desirable in a biomonitor. These include a diet that integrates exposure from multiple trophic levels; a high exposure potential resulting from that diet as well as their year-round occupancy and longevity (up to and exceeding 28 years in the wild) [16]; ease of handling and resilience to human activity; propensity for vocally defending territories which allows for ease of detection and monitoring; they are large enough to obtain blood sample volume that satisfies detection limit requirements for many environmental contaminants; population densities that afford the collection of a meaningful sample size; the GHO ecology and background has been thoroughly studied and understood so deviations in behavior and success can be readily identified; owls are known to be sensitive to various environmental contaminants, including organochlorines [17]; samples from the field study can be related to results from lab studies; GHOs are widespread across North America so results can be related to studies in different regions; and finally, great horned owls are valued by the general public – the GHO's charisma garners popular support for research with the bird and helps secure access to private properties.

Study Objectives

This thesis represents the culmination of a five-year, multifaceted study geared toward answering a single question: Are the great horned owls in the Tittabawassee River floodplain downstream of Midland, MI, at risk for adverse effects from exposure to PCDD/DFs in the floodplain soils and sediments?

To answer that question, this risk assessment employed multiple lines of evidence, including dietary exposure, tissue concentrations and population health and productivity. The convergence or divergence of the three lines of evidence was used to help determine the potential for adverse effects of PCDD/DFs on the great horned owls inhabiting the Tittabawassee River floodplain [18].

Chapter 1

Great Horned Owl (*Bubo Virginianus)* Dietary Exposure to PCDD/DFs in the Tittabawassee River Floodplain in Midland, Michigan, USA

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ABSTRACT

The soils and sediments downstream of Midland, Michigan, USA have elevated polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxin (PCDD) concentrations. As long-lived, resident top predators, great horned owls (*Bubo virginianus*; GHO) have the potential to be exposed to high levels of bioaccumulative compounds such as PCDD/DFs. For this study, site-specific GHO dietary exposure measures were collected for 115 km of river corridor from 2005-2006. The site-specific GHO biomass-based diet was dominated by E. cottontail rabbits and muskrats. Incidental soil ingestion and E. cottontail rabbits were the primary contributors to GHO exposure to PCDD/DFs. The congeners contributing 99% of the GHO diet in the study area (SA) were 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF (2,3,4,7,8-PeCDF = 65%, 2,3,7,8-TCDF = 34%). In the reference area (RA), 2,3,7,8-TCDF (37%), 2,3,7,8-TCDD (22%), 2,3,4,7,8-PeCDF (19%), and 1,2,3,7,8-PeCDD (19%) contributed most of the TEQs observed in the diet. The great horned owl daily dietary exposure estimate was greater in the SA (3.3 ng $TEQ_{WHO\text{-}Avian}$/kg bw/d) than the RA (0.07 ng/kg bw/d). Hazard quotients for the upper 95% confidence limit (UCL) around the geometric mean and no observable adverse effect levels (NOAELs) were <1 for both the RA and SA.

Keywords: Great horned owls, diet, dioxin, furan, risk assessment, raptor, TEQs

INTRODUCTION

Soils and sediments of the mid-Michigan Tittabawassee River floodplain have some of the greatest polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxin (PCDD) concentrations on record [1]. The PCDD/DFs were released prior to the adoption of modern waste treatment and storage over a period spanning several decades of manufacturing chlorinated products at the Dow Chemical Company in Midland, MI, USA [5]. Due to the persistence and lipophilicity of some PCDD/DF congeners, they can accumulate in animal tissues and be biomagnified up the food chain.

The critical toxic effects of PCDD/DF are mediated by the aryl hydrocarbon receptor (AhR), which is a key step for the up-regulation of gene expression and enzyme production that can lead to a variety of toxic effects [6]. Sensitivity of birds to PCDD/DF has been investigated in both laboratory and field studies. Effects can include impaired reproductive success, embryotoxicity, developmental deformities, growth retardation, subcutaneous and pericardial edema, liver damage, immunotoxicity, induction of p450 enzymes and behavioral changes (e.g. decreased nest attentiveness) [7,9-11,19-23].

To determine if the dioxins and furans in the soils and sediment pose a threat to avian wildlife in the Tittabawassee River floodplain, a multiple lines of evidence ecological risk assessment was conducted with the great horned owl (*Bubo virginianus*) as a species representative of terrestrial-based raptors. This study examined dietary exposure, concentrations of PCDD/DF in great horned owl (GHO) plasma, and GHO population health and productivity. The convergence or divergence of the three lines of evidence was used to help determine the potential for adverse effects of PCDD/DF [18].

As top terrestrial predators in the Tittabawassee River floodplain, great horned owls are a useful indicator species. Owls are opportunistic predators, whose prey can include both terrestrial (rabbits, shrews, mice) and aquatic (muskrats, waterfowl, crayfish) pathways of exposure. Due to its dietary composition, year-round residency, longevity (up to 28 years in the wild) [16], and relatively great ingestion rate [24], the GHO could be exposed to significant concentrations of PCDD and PCDFs in its diet. While there is little information on the effects of PCDD/DF on GHOs, owls have been shown to be sensitive to the effects of other environmental contaminants, including organochlorines, organophosphates and metals, and thus have been suggested as useful environmental sentinels [17,25-28].

Herein are the results of a site-specific dietary exposure assessment for resident GHOs exposed to PCDD/DFs associated with the Tittabawassee River and its floodplain. Toxicity reference values (TRVs) based on laboratory-based studies in which birds were exposed to PCDD/DF and other AhR-active compounds were developed. These avian TRVs were then compared to a site-specific dietary exposure for GHOs inhabiting the floodplain, expressed as potential average daily dose (ADD_{pot}), to determine the risk of adverse effects from feeding within the floodplain.

MATERIALS AND METHODS

Site Description

The Tittabawassee River study area (SA) includes sediments and floodplain soils associated with 72 km of the Tittabawassee and Saginaw rivers in Michigan (Figure 1.1). The upstream and downstream boundaries of the SA are Midland, MI and Saginaw Bay, respectively. The floodplain is characterized by intermittent agricultural (37%) and wooded (41%) areas. The floodplains along the Tittabawassee River are periodically inundated, usually during high flows in the spring and following major storm events. Releases from the Sanford dam upstream of Midland can cause daily changes in water levels and flow rates in the Tittabawassee River. The reference areas (RAs) lie directly upstream of the SA in the Tittabawassee river watershed and include 16 km along the Pine River, 12 km along the Chippewa River and 15 km of the Tittabawassee River. These areas represent background levels of PCDD/DFs in the region [1].

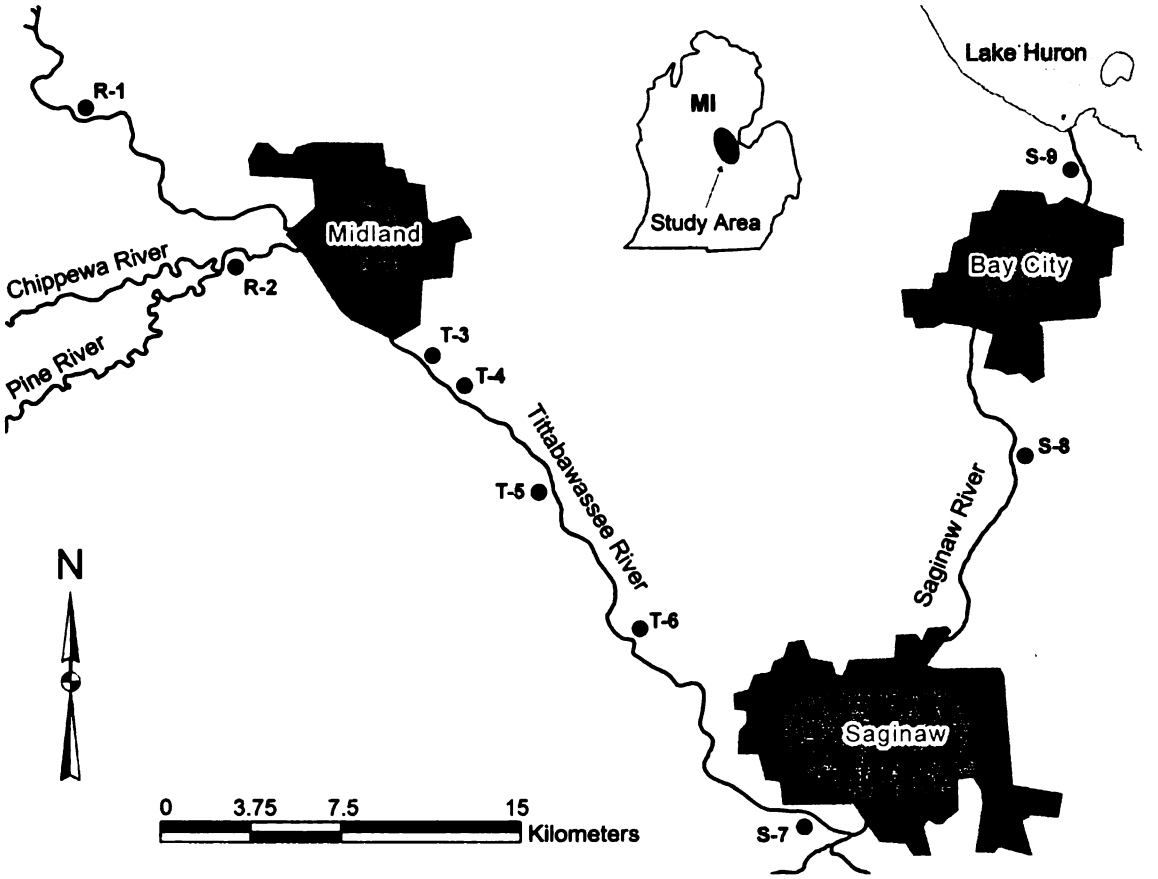

Figure 1.1. Study locations within the Chippewa, Tittabawassee, and Saginaw River floodplains, Michigan, USA. Reference areas (R-1 and R-2); Tittabawassee River study areas (T-3 to T-6); and Saginaw River study areas (S-7 to S-9).

Nests

Great horned owls will readily occupy artificial nesting structures [27,29]. By taking advantage of this behavior researchers can encourage owls to nest in accessible locations and trees that can be safely climbed. For this study, 75 cone-shaped nests were constructed by weaving dogwood and willow through chicken wire following previously described methods [27]. Two to three nests were deployed in each of 9 RA and 13 SA GHO territories that were identified through call-response surveys and incidental observations (Chapter 2). Additional nests were installed in areas with good habitat but unconfirmed GHO occupation. In total, 45 nests were placed in the RAs over ~35 km of river, while 37 nests were placed in the SA spanning ~41 km of river.

Nest trees were chosen based on several qualifying factors, including distance from the river, ease of GHO access, and the presence of nearby trees for roosting. Only deciduous trees were used, and included swamp willows (*Salix nigra*), hackberries (*Celtis occidentalis*), sugar maples (*Acer saccharum)*, black walnut (*Juglans nigra*), white ash (*Fraxinus americana*), cottonwood (*Populus deltoids*), red maple (*Acer rubrum*), bur oak (*Quercus macrocarpa*), red oak (*Quercus rubra*), white oak (*Quercus alba*), aspen (*Populus grandidentata*) and sycamore (*Platanus occidentalis*). Nests were installed 9-21 m from the ground, with an average nest height of 15 m. Adjustable hose clamps were used to hold the nest in the tree, preferably in at the junction of three limbs. The hose clamps' circumferences were adjusted to match the girths of limbs and trunks during a yearly maintenance visit. After installation was complete, the hose clamps were spray painted with matte-brown paint to better match the surrounding limbs. A matte-brown

aluminum sheeting was installed around the trunk of the tree below the nest as a predator guard.

Prey remains: collection and identification

Great horned owls swallow whole prey and later egest pellets of packed fur, feathers, and bones. Larger prey items are generally torn apart and consumed in manageable portions [30]. The pellets can be used to identify the owls' prey species. In 2005 and 2006, pellets and prey remains (hereafter referred to jointly as prey remains) were collected from GHO nests and the ground beneath nests and roosting trees when nestlings were approximately six weeks old. Larger prey items with meat still attached were identified, noted, and returned to the nest for further consumption. The samples were gathered in plastic bags labeled with a unique nest identification number, date, location and collection point (e.g. ground, nest, roost tree). Upon arrival at the field laboratory, the prey remains were transferred into labeled paper bags and treated with naphthalene to kill invertebrates. The treated prey remains were then stored in a secure dry area for moisture elimination. Once the prey remains dried, they were sterilized by autoclave and analyzed for composition.

Prey remains from the nest were combined with items collected on the ground directly below the nest for analysis. This was done to account for the fact that larger animals may be present in more than one pellet, and analysis of individual pellets could overestimate the presence of larger prey items [30]. Roost tree samples were analyzed separately. Prey remains were examined by carefully separating bones from fur and feathers using forceps and scalpels. The bones were identified to the lowest taxonomical

ranking possible. The Michigan State University museum's collection was used as a reference.

Diet reconstruction

The GHO diet can be reconstructed by examining unconsumed prey and skeletal remains in pellets [30-33]. The site-specific diet was constructed on a frequency basis by determining the minimum number of individuals in each taxonomic category necessary to account for the bones present in the prey remains. This frequency-based diet was then used to construct the GHO diet on a biomass basis. The percent biomass was determined by multiplying the number of individuals in a taxonomic group by the mean adult mass for that group [33], using appropriate mammal and bird guide books [34,35] and a reference for Michigan crayfish [36]. Since the distribution of sizes of individuals in the diet was unknown, the average size of adults was applied as a conservative exposure, such that the maximum most likely exposure would be predicted.

Dietary Item Sampling

Great horned owl dietary items identified in the prey remains were sampled from the reference and study areas. Samples collected include small mammals (shrews (*Blarina brevicauda* and *Sorex cinereus*), meadow voles (*Microtus pennsylvanicus*), flying squirrels (*Glaucomys sabrinus*), white-footed mice (*Peromyscus leucopus*), and deer mice (*Peromyscus maniculatus)*), cottontail rabbits (*Sylvilagus floridanus*), turkeys (*Meleagris gallopavo*), muskrats (*Ondatra zibethicus*), passerine nestlings (*Sialia sialis,*

Troglodytes aedon, and *Tachycineta bicolor*), and crayfish. The sampling efforts have been previously described by Zwiernik et al. [37] and Fredricks et al. 2009 [38]. Dietary items were sampled from two locations in the RA (R-1, R-2) and seven locations in the SA (T3-T6 and S7-S9) (Figure 1.1).

Identification and Quantification of PCDD/DF congeners

Concentrations of seventeen 2,3,7,8-substituted PCDD/DF congeners were measured in prey items and soil sampled from the reference and study area floodplains. Small mammals were depurated prior to whole-body homogenization and analysis for PCDD/DF concentrations. Crayfish and rabbits were analyzed for whole-body PCDD/DF concentrations. Muskrats were analyzed for whole body PCDD/DF concentrations minus the pelage. Passerine nestlings were analyzed for whole body PCDD/DF concentrations minus beaks, legs and feathers. Turkey muscle and skin PCDD/DF concentrations were used to substitute for waterfowl, which were not sampled from the floodplains.

PCDD/DFs were quantified in accordance with EPA Method 8290 with minor modifications (U.S. Environmental Protection Agency (USEPA) 1998). Briefly, samples were homogenized with anhydrous sodium sulfate and Soxhlet extracted in hexane:dichloromethane (1:1) for 18 hr. Before extraction, known amounts of ^{13}C-labeled internal standards were added to the sample as internal standards. The extraction solvent was exchanged to hexane and the extract was concentrated to 10 mL. Ten percent of this extract was removed for lipid content determination. Extracts were initially purified by treatment with concentrated sulfuric acid. The extract was then

passed through a silica gel column containing silica gel and sulfuric acid silica gel and eluted with hexane. The extract received additional column chromatography by elution through acidic alumina which resulted in two fractions: The first fraction eluted contained most PCBs and pesticide compounds, while the second fraction contained dioxins and furans. The second fraction eluted from the alumina column was then passed through a carbon column packed with 1 g of activated carbon-impregnated silica gel. The first fraction, eluted with various solvent mixtures, was combined with the fraction one eluate from the acidic alumina column and retained for possible co-contaminant analyses. The second fraction, eluted with toluene, contained the 2,3,7,8-substituted PCDD/DFs. Components were analyzed using HRGC-HRMS, a Hewlett-Packard 6890 GC (Agilent Technologies, Wilmington, DE) connected to a MicroMass® high resolution mass spectrometer (Waters Corporation, Milford, MA). PCDF and PCDD congeners were separated on a DB-5 capillary column (Agilent Technologies, Wilmington, DE) coated at 0.25 μm (60 m x 0.25 mm i.d.). The mass spectrometer was operated at an EI energy of 60 eV and an ion current of 600 μA. Congeners were identified and quantified by use of single ion monitoring (SIM) at the two most intensive ions at the molecular ion cluster. Concentrations of certain PCDD/DF congeners, particularly TCDD and 2,3,7,8-tetrachlorodibenzofuran (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 matrix spikes, blanks, and duplicates. Concentrations of $TEQs_{WHO\text{-}Avian}$ (ng/kg ww) were calculated for PCDD/DFs by summing the product of the concentration of each congener, multiplied by its avian TEF [39].

Potential average daily dose (ADDpot)

The potential average daily dose (ADD_{pot}) for GHOs was calculated using the equation for wildlife dose from the Wildlife Exposure Factors Handbook [40] and the site-specific, biomass-based GHO diet (Equation 1).

$$ADD_{pot} = \Sigma(C_k \times FR_k \times NIR_k) \quad (1)$$

Where:

C_k = concentration of $TEQ_{WHO\text{-}Avian}$ (ww) in the kth prey item category of the GHO diet.

FR_k = Fraction of GHO diet (based on mass) represented by the kth prey item

NIR_k = Normalized GHO ingestion rate of the kth prey item (prey (g, ww)/ bw (g, ww)/d).

Two approaches using Equation 1 were used to estimate the ADD_{pot} from the measured tissue residue concentrations in prey items: (1) The geometric mean concentration and 95% upper confidence limit of the geometric mean (95 UCL_{gm}) of items in the kth prey item category were input into Equation 1, with results of each representing the central tendency and upper end of the ADD_{pot}, respectively. Hereafter, this method is referred to as the 'traditional' approach.

(2) The ADD_{pot} was also estimated using a resampling approach. This approach avoids assumptions about the distributions of data in the dietary item categories, and avoids the uncertainty resulting from the use of summary statistics such as the geometric mean and 95 UCL_{gm} as inputs to Equation 1. Using R (version 2.10.0, R Foundation for

Statistical Computing, Vienna, Austria, 2009, www.R-project.org) the ADD_{pot} was repeatedly calculated (Equation 1), each time drawing a single randomly sampled dietary item (Ck) from each dietary category (k). The results of 50,000 iterations were used to establish the distribution of the ADD_{pot}. The median and 95th centile of that distribution were used to represent the central tendency and upper end of the ADD_{pot}. The resampling was performed on the site-specific, measured dietary item concentrations rather than on a continuous distribution inferred from the measured data or a range of exposure parameters, and therefore remains free from assumptions about the distribution of the data. A similar method was used in an assessment of mink exposure to PCDD/DFs in the Tittabawassee River floodplain [37].

A site-use factor of 1 was applied based on the conservative assumption that GHOs obtain 100% of their diet from within the 100-year floodplain. The normalized ingestion rate (0.072g/g bw/day) was derived from the ingestion rates reported by Craighead and Craighead [15] for the GHO.

To the extent possible, concentrations in site-collected prey items were used to create the ADD_{pot} were matched precisely with the prey items in the site-specific GHO diet. However, not all prey items identified from the GHO prey remains were sampled from the floodplain. As necessary, concentration data were assigned based on similarity in feeding habits and routes of exposure. The substitutions were chosen to be conservative and the potential for bias that could have been introduced by these substitutions is discussed.

Incidental soil ingestion was included in the estimates of ADD_{pot}. The top 15 cm of soil was collected from the floodplain in areas adjacent to the small mammal trapping grids in the study and reference areas. Soil was included in dietary calculations as 1.4% of the GHO diet, which is less than the soil ingestion rate suggested for the red fox

(2.8%) [40], but more than the 0% suggested for red tail hawks [41]. PCDD/DFs in soil were assumed to be 80% bioavailable [42].

Statistical Analyses

All statistical analyses were performed using Systat 11 software (Systat Software, Inc. 2004, Richmond, CA, www.systat.com). Normality was tested using the Shapiro-Wilks test. Log-normally distributed data were log-transformed before statistical analysis. A Student's *t*-test was used to evaluate the significance of differences between reference and study area prey item PCDD/DF concentrations expressed as sum avian TEQs.

Hazard Assessment

Hazard quotients (HQs) were used to determine potential for adverse effects from GHO dietary exposure to PCDD/DFs. The HQs were calculated by dividing ADD_{pot} dietary TEQs by no-observed-adverse-effect-level (NOAEL) toxicity reference values (TRVs) from two avian feeding studies (Table 1.1), (Equation 2).

$$HQ = \frac{ADD_{pot}\ (ng\ TEQ_{WHO\text{-}Avian}/kg\ bw/day)}{Dietary\ TRV} \qquad (2)$$

Because there is no single definitive toxicity reference value for GHO exposure to PCDD/DFs, TRVs for GHO dietary exposure were derived from a combination of chronic laboratory studies. The studies were chosen because they measured ecologically relevant endpoints such as reproductive success and hatch success. A chronic feeding study has been conducted in which over the course of two years, screech owls (*Otus asio*)

were administered 3 mg/kg, ww Aroclor 1248 in their diet [43]. Aroclor 1248 is a PCB mixture with a dioxin toxicity equivalency factor of $2.57x10^{-4}$ kg/kg in birds [44]. The study examined number of eggs laid, young hatched and young fledged. No adverse effects were observed in that study, which suggests a NOAEL of 3 mg/kg, ww Aroclor 1248. By applying this factor to the NOAEL, we attained a NOAEL of 105 ng/kg/day TEQs. Using the allometric relationships, body weight and food consumption given by Sample et al (1996) [45] we estimated a TRV expressed as a daily dose of 14.5 ng/kg bw/d.

When rink-necked pheasants (*Phasianus colchinus*) were injected weekly intraperitoneally with 2,3,7,8-TCDD for 10 wk [10] egg production and hatchability was less at 1µg/kg/week (140 ng/kg/d) [45]). No effects were observed at 0.1 µg/kg/week (14 ng/kg/d). Thus, NOAEL and LOAEL of 14 ng $TEQ_{WHO\text{-}Avian}$/kg bw/d and 140 ng $TEQ_{WHO\text{-}Avian}$/kg bw/d were chosen as TRVs in the present study.

Table 1.1. Dietary-based toxicity reference values (TRVs) for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) for great horned owl (*Bubo virginianus*) potential average daily dose (ADDpot)

	TRV	Study Type	Species	Reference
Dietary dose (ng/kg bw/day)				
NOAEL	14.5	feeding	*Otus asio*	McLane 1980
NOAEL	14	i.p. injection	*Phasianus colchicus*	Nosek 1992/ Sample 1996

RESULTS

Diet reconstruction

GHO pellets and prey remains were collected from 13 nests over the 2005 and 2006 breeding seasons. The prey remains were pooled for composition analysis and a total of 465 individual prey items were identified. Numerically, meadow voles and other herbivorous small mammals (e.g. white-footed and deer mice) dominated the GHO diet (36% and 27%, respectively). On a biomass basis, however, E. cottontail rabbits and muskrats represented the bulk of GHO dietary intake (57% and 21%, respectively). The GHO biomass-based diet also included meadow voles (6.5%), waterfowl (5.2%), passerine birds (5.5%), herbivorous small mammals (2.03%), star-nosed moles (0.09%), short-tail shrews (0.04%) and crayfish (0.04%) (Figure 1.2).

Residues in prey items

Eight of the 11 prey items identified in the site-specific GHO diet were sampled from the study and reference area floodplains and concentrations of PCDD/DF measured. The dietary items for which concentrations of PCDD/DFs were available composed 93% of the biomass of the GHO diet (Table 1.2). For the remaining three items (7.5 % of the diet by biomass) substitutions of measured prey item concentrations were made using surrogate prey items having comparable or greater body burdens by weight. Masked and short-tailed shrew have similar diets to moles and had the greatest PCDD/DF concentrations of all prey items collected on site, and thus the concentrations for these two small mammals were used as a surrogates for star-nosed mole. For fox squirrel and waterfowl, data from separate Tittabawassee River floodplain studies, where wild game

species were collected and edible portions analyzed for PCDD/DFs [46,47]. In those studies there was not a significant difference in PCDD/DF concentration in cottontail rabbit muscle and fox squirrel muscle collected from the same locations, although this similarity results primarily because concentrations of the majority of congeners were less than the method detection limits. Therefore, the whole body rabbit concentrations from the present study, which at TR-6 were approximately 70-fold greater than rabbit muscle from the same area, were used as a surrogate for the squirrel portion of the diet. From the same wild game study [46,47] wood duck muscle had 6-fold greater PCDD/DF concentrations than turkey muscle, and approximately 30-fold greater PCDD/DF concentrations than muscle from Canada geese collected in the vicinity of S-7 (Figure 1.1). However, the duck and goose tissue were not available from locations upstream of S-7 on the Tittabawassee River, therefore concentrations in turkey muscle with skin from T-3 (n=11), T-6 (n=12), and T-8 (n=8), were selected as a surrogates for waterfowl. The geometric mean concentrations of measured dietary items were greater in the study area than the reference area (student's *t*-test, $p < 0.05$).

The geometric mean of the sampled prey item PCDD/DF concentrations when normalized to TCDD ($TEQ_{WHO\text{-}Avian}$) ranged from 8 ng/kg for the meadow vole to ng/kg for combined masked and short-tailed shrews (surrogates for the star-nosed mole) in the study area. Geometric mean $TEQ_{WHO\text{-}Avian}$ concentrations in the reference area ranged from 0.21 ng/kg for turkey, which was used as a surrogate for waterfowl, to 7.4 ng/kg for passerine nestlings.

Geometric mean $TEQ_{WHO\text{-}Avian}$ of the sampled prey items dl- PCB concentrations in the SA ranged from 2.8 ng/kg for small mammals to 11 for passerine nestlings (Table 1.3). Geometric mean $TEQ_{WHO\text{-}Avian}$ of the sampled prey items dl-PCB concentrations in the SA ranged from 2.8 ng/kg for small mammals to 10.6 for passerine nestlings, and in the RA ranged from 3.2 ng/kg for small mammals to 12.3 for passerine

nestlings (Table 1.3). In general, the lower end of the PCB concentrations reflects the limit of detection for PCB congeners

Potential average daily dose (ADDpot)

Using the traditional approach to estimating ADDpot, the geometric mean GHO ADDpot of $TEQ_{WHO\text{-}Avian}$ from PCDD/DF was 47-fold greater in the study area than the reference area, and the upper end estimate based on the 95 UCL_{gm} was approximately 90- fold greater in the study area than the reference area (Table 1.4) Using the resampling method, the median and 95th centile of the GHO ADDpot of $TEQ_{WHO\text{-}Avian}$ from PCDD/DF were 76- and 200-fold greater in the study area than the reference area, respectively (Table 1.4). Cottontail rabbits and incidental soil ingestion contributed the majority of the GHO ADDpot (Figure 1.3).

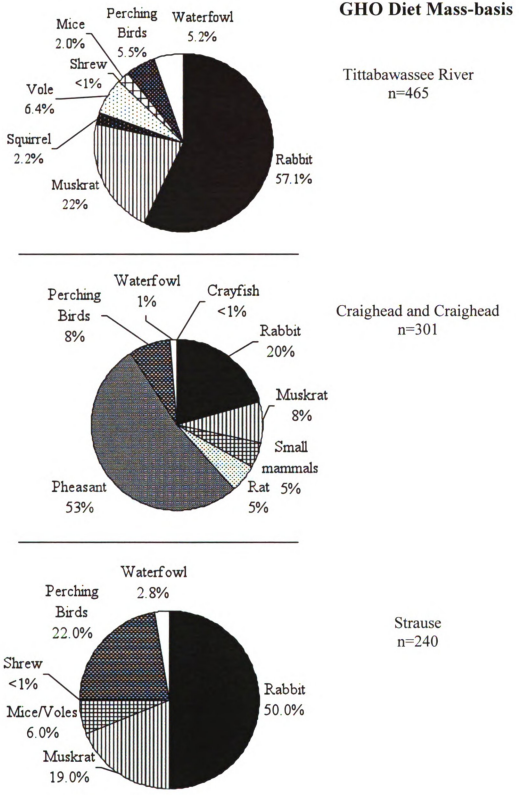

Figure 1.2. Great horned owl (*Bubo virginianus*) mass-based site-specific and literature-based dietary compositions. Literature-based diets are from Strause 2008 and Craighead 1956.

Table 1.2. Geometric mean and 95% UCL 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent (TEQWHO-Avian) concentrations in great horned owl (*Bubo virginianus*) prey items collected from the reference and study areas in the Tittabawassee River floodplain (MI, USA).

	Reference			Study		
	n	Geomean	95% UCL	*n*	Geomean	95% UCL
Dietary component concentrations ng TEQWHO-Avian/kg wet wt						
Rabbit	5	0.66	0.76	11	26	94
Muskrat	9	0.19	0.22	13	27	36
Squirrel[a]		0.66	0.76		26	94
Meadow Vole	2	0.26	0.34	23	7.5	13
Short-tailed Shrew	8	2.30	4.1	10	100	240
Star-nosed Mole[b]	14	5.70	11	43	610	1030
Other herbivorous small mammals[c]	40	1.40	1.7	163	83	102
Passerine Birds[d]	7	7.90	10	8	205	244
Waterfowl[e]	12	0.21	0.27	31	20	33
Crayfish	5	0.91	2	23	66	96
Soil	13	8.80	13	33	2800	4800

[a] E. Cottontail data used for squirrel

[b] Short-tail and masked shrew data used for mole

[c] Small mammals include white-footed mice, deer mice, flying squirrels, chipmunks, and meadow jumping mice

[d] Nestling passerines

[e] Turkey data used for waterfowl

Table 1.3 Geometric mean and 95% UCL 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent ($TEQ_{WHO\text{-}Avian}$) concentrations from PCBs in great horned owl (*Bubo virginianus*) prey items collected from the reference and study areas in the Tittabawassee River.

	Reference (RA)			Study (SA)		
PCB	*n*	Geomean	95% UCL	*n*	Geomean	95% UCL
Dietary component concentrations ng PCB $TEQ_{WHO\text{-}Avian}$/kg wet wt						
Muskrat	2	2.9	3.1	8	3.5	12.4
Meadow Vole	0	NA	NA	2	4.6	5.1
Short-tailed Shrew	0	NA	NA	1	4	4
Other herbivorous small mammals[a]	1	3.2	3.2	8	2.8	5.5
Passerine birds[b]	16	12.3	36.5	36	10.6	63.4
Turkey	0	NA	NA	19	3.9	4.5
Crayfish	0	NA	NA	3	4.2	5.3

[a] Small mammals include white-footed mice, deer mice, flying squirrels, chipmunks, and meadow jumping mice

[b] Nestling passerines

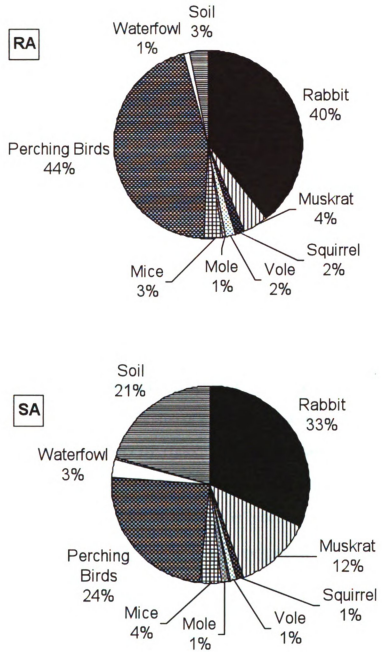

Figure 1.3. Prey item contributions to GHO geometric mean ADD_{pot} in the reference (RA) and study (SA) areas in the Tittabawassee River floodplain in Midland, MI, USA.

Congeners

Furan congeners contributed the majority of the PCDD/DF in the ADD_{pot} in the study area, but only about half of the total PCDD/DF TEQ in the reference area (Figure 1.4). With the addition of soil ingestion, 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF contributed 99% of the PCDD/DF TEQs in the SA GHO diet (2,3,4,7,8-PeCDF = 65%, 2,3,7,8-TCDF = 34%). In the reference area the primary congeners were 2,3,7,8-TCDF (37%), 2,3,7,8-TCDD (22%), 2,3,4,7,8-PeCDF (19%), and 1,2,3,7,8-PeCDD (19%) (Figure 1.4).

Because the data set for dl-PCBs was limited and because many PCB congeners were at or below detection limits, it was not possible to precisely quantify the relative contribution of dl-PCB congeners in the GHO diet. However, the available data suggest that in the RA, where PCDD/DF and PCB represent background concentrations, dl-PCBs may contribute a substantially greater portion than PCDD/DF to the $TEQ_{WHO\text{-}Avian}$. In contrast, in the SA dl-PCB concentrations appear at background, or only slightly above background, and the relative contribution of dl-PCB reflects is in general inversely related to the magnitude of PCDD/DF concentrations. For example, in "other small mammals" and passerines, $TEQ_{WHO\text{-}Avian}$ from PCBs contributes about 3% to 5% of that of $TEQ_{WHO\text{-}Avian}$ from PCDD/DF, whereas in meadow voles $TEQ_{WHO\text{-}Avian}$ from PCBs is about 60% of that of $TEQ_{WHO\text{-}Avian}$ from PCDD/DF. The proportion of the TEQ in the diet of GHO that was contributed by dioxin-like PCB congeners was approximately 3% in the study area.

Table 1.4. Potential average daily dose (ADDpot) (ng TEQWHO-Avian/kg bw/day) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents from PCDD/DF for great horned owls (*Bubo virginianus*) in the Tittabawassee River floodplain from the site-specific and two literature-based diets.

	Traditional Approach ADDpot Geometric mean (95% UCLgm) ng TEQWHO-Avian/kg bw/day		Resampling Approach ADDpot 50th centile (95th centile) ng TEQWHO-Avian/kg bw/day	
	Reference Area	Study Area	Reference Area	Study Area
Site-specific	0.07 (0.09)	3.3 (7.1)	0.07 (0.23)	5.0 (47)
1940s Michigan [15]	0.09 (0.13)	5.8 (9.6)	0.06 (0.23)	7.2 (22)
Kalamazoo [26]	0.16 (0.20)	5.7 (9.5)	0.14 (0.81)	7.6 (45)

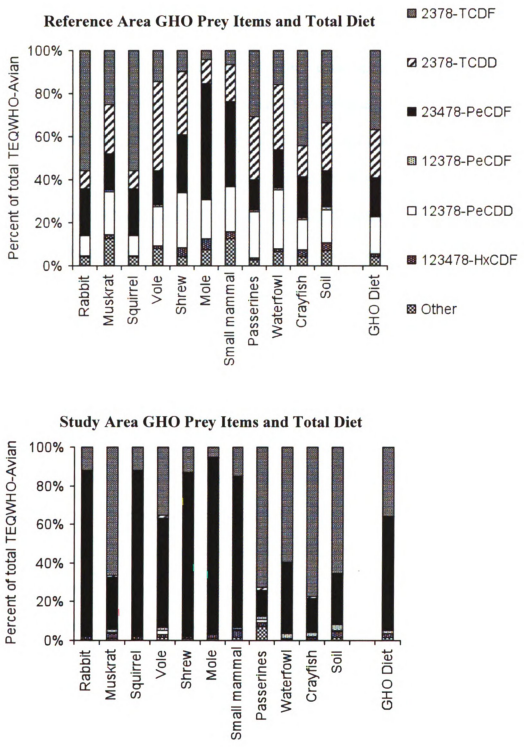

Figure 1.4. Prey item contributions to TEQWHO-Avian in the great horned owl (*Bubo virginianus*) diet in the Tittabawassee River reference and study areas.

Hazard Assessment

Hazard quotients were calculated based on the 95% UCL (traditional approach) for the site-specific diet. The HQs were less than 1.0 for the site-specific diet in both the reference and study areas, regardless of which TRV was used for the HQ calculation in Equation 2. The dietary exposure *HQ* in the reference area was 0.005 (Nosek NOAEL and 95% UCL_{gm} *ADDpot*). The dietary exposure HQ in the study area was 0.51 (Nosek NOAEL and 95% UCL_{gm} ADD_{pot}). In addition, the HQs were less than 1.0 based on the geometric mean and 95% UCL_{gm} concentrations for dietary item categories, but using dietary proportions from the literature-based diets instead of site-specific proportions (Figure 1.5).

HQs were also calculated using the median and 95th centile (resampling method) of the GHO ADDpot. HQs based on median and 95th centile ADDpot in the reference areas were 0.005 and 0.016, respectively. In the study area, HQs for the median and 95th centile ADDpot were 0.36 and 3.4, respectively, based on the NOAEL [11]. Similarly, using the resampling approach to estimating GHO ADDpot with the dietary item concentrations from the present study but using literature-based diets [15,26], the HQs based on median estimates of ADDpot were less than 1.0, whereas HQs based on 95th centile estimates of the ADDpot were 1.3 and 3.2 (Fig. 1.5). As in the traditional method, all estimates of ADDpot were at least 3-fold less than the LOAEL TRV.

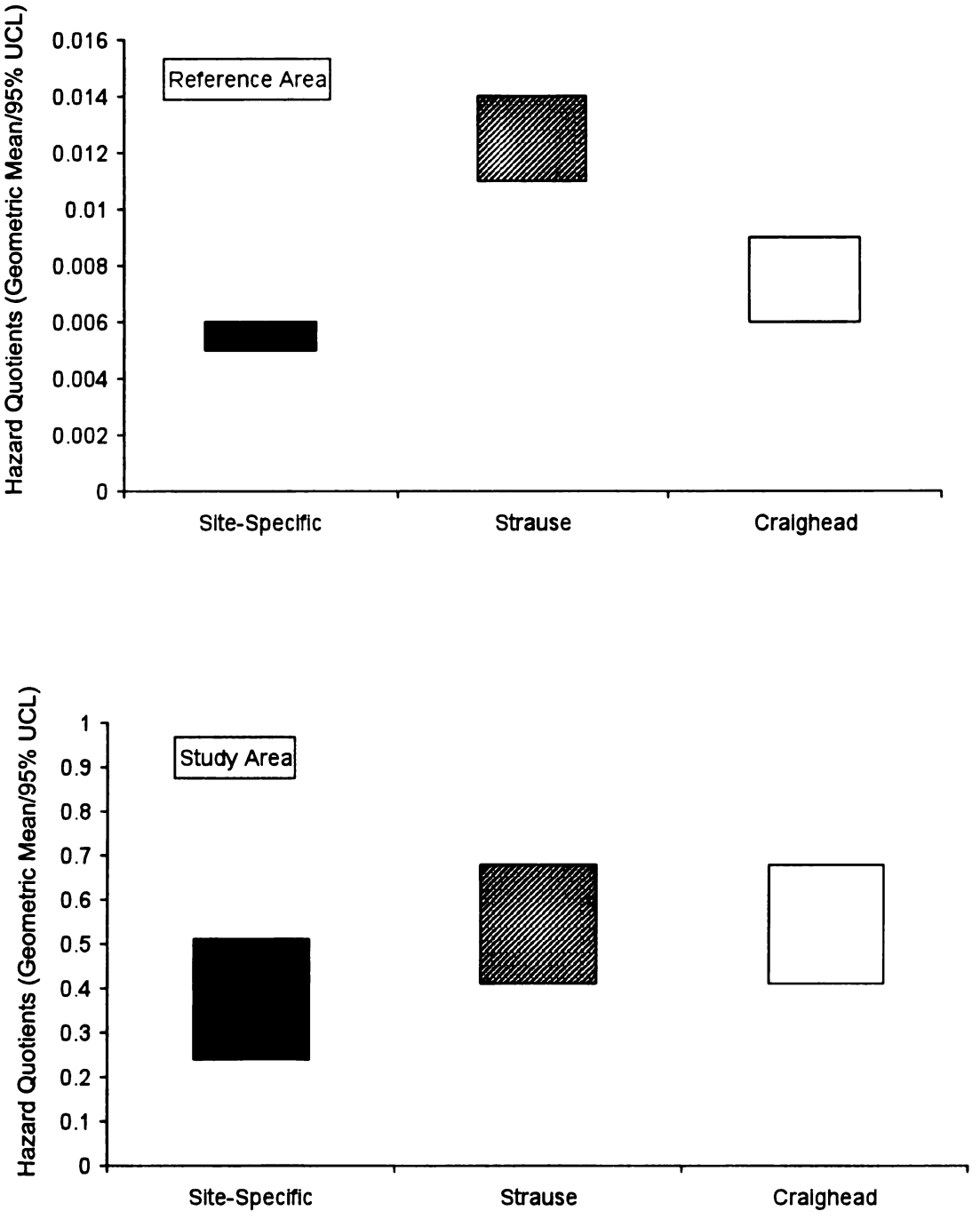

Figure 1.5. Hazard quotients for great horned owl (*Bubo virginianus*) dietary exposure to polychlorinated dibenzofurans and polychlorinated dibenzo-*p*-dioxins in the Tittabawassee River floodplain in Midland, MI, based on a site-specific and two literature-based diets [15,26].

DISCUSSION

Site-specific diet

The great horned owl diet varies in both composition and integration of trophic levels. The GHO diet has been well-characterized [15,33,48-56], and while it consistently comprises small mammals, birds, and invertebrates, the diet has been noted for its variability among habitats. A marked difference in dietary composition between agricultural and rangeland regions was observed in a correlation between habitat and dietary composition in Idaho [50]. Furthermore, the GHO diet typically integrates multiple trophic levels. Changes in species composition for the same prey class, such as shifts from herbivorous small mammals to omnivorous or carnivorous species, can change the trophic level exposure characteristics. This is important when assessing bioaccumulative contaminants. As a result, characterizing a site-specific diet is generally preferable for assessing GHO exposures in wildlife toxicology studies [27].

The diet of GHOs in the Tittabawassee River floodplain differs from other reported Michigan GHO diets. Pheasants dominate the Craighead [15] diet (52.5% of diet by biomass) (Figure 1.2). The Craigheads studied GHOs in Michigan in the 1950s, a time when pheasants and rats were much more prevalent. The pheasant population dropped precipitously in the 1960s, possibly because of severe winters, pesticides, fungicides, and habitat loss [57]. If the Craighead and Craighead diet was used rather than a site-specific diet, the predicted GHO dietary exposure would have been approximately 1.8-fold greater (Table 1.3). This is because the avian tissues that dominated the 1940s diet generally have greater concentrations of PCDD/DF than the

rabbits that predominate in the site-specific Tittabawassee River[26] GHO diet. However, it is notable that based on the resampling method, the 95th centile ADD_{pot} estimate in the Craighead and Craighead diet is less than half that of the site-specific diet. This results because the lesser contribution of rabbit in the Craighead and Craighead diet reduces the skewing effect of the two rabbit samples from T-6 on the ADD_{pot} distribution.

Compared to the site-specific diet, the Strause et al [26] diet shows a preponderance of passerines (Figure 1.2). Because passerine nestlings in the SA had greater $TEQ_{WHO\text{-}Avian}$ concentrations than most other GHO prey items collected from the floodplain (Table 1.2), an ADD_{pot} based on the Strause diet [26], like the Craighead diet, would overestimate GHO exposure to PCDD/DFs (Table 1.4), although the upper end estimates from the resampling approach were similar between the Strause and site-specific diets.

The site-specific Tittabawassee River GHO diet is numerically dominated by meadow voles and other small mammals. On a biomass basis, however, these only contribute 6.5% and 2% of the GHO diet, and only 1% and 4% of the geometric mean GHO dietary exposure to PCDD/DFs. Rabbits contribute the highest proportion of geometric mean GHO dietary exposure (33%) (Figure 1.3).

The prey items with the least tissue concentrations of PCDD/DFs (rabbits, muskrats and meadow voles) make up 85% of the GHO biomass-based diet. As a result, even though GHOs consume shrews, passerine birds and soil, which have greater PCDD/DF concentrations, GHO dietary exposure is low, particularly when compared to the TRVs from the studies by Nosek and McLane. Rabbits, muskrats and meadow voles eat plants, which do not readily take up dioxin-like chemicals from soil. When plants do

take up the chemicals, they are not readily translocated to the leaves [58,59]. The resulting low concentrations in plants lead directly to low concentrations in the GHO's herbivorous prey.

Despite the greater estimated dietary exposure to PCDD/DF that resulted from using dietary proportions derived from the literature, the central tendency of all ADD_{pot} estimates was less than the NOAEL TRV (Figures 1.5 and 1.6). The upper end estimates of ADD_{pot} using the site-specific and literature-based diets with the resampling approach exceeded the NOAEL TRV, but none exceeded the LOAEL TRV (Figure 1.6). This further underscores the conclusion that GHO dietary exposure to PCDD/DFs in the Tittabawassee River SA is unlikely to cause adverse effects.

Uncertainty

Incidental soil ingestion accounted for 21% and 28% of the GHO ADD_{pot} to $TEQ_{WHO\text{-}Avian}$ but there are uncertainties surrounding raptor soil ingestion and the bioavailability of PCDD/DFs in floodplain soil. It has been suggested that incidental soil ingestion by terrestrial predators is approximately 2.8% of its diet (dry wt), based on red fox stomach content analysis [60]. Due to differences in hunting modes, the fox value may overestimate raptor incidental soil ingestions. Great horned owls spend little time on the ground, and will tear apart large prey items in a tree. Based on raptor feeding habits, 0% soil ingestion for red tail hawks and barn owls has been recommended [41]. This is likely an underestimate, however, because the small mammals the owls prey on live in constant contact with dirt, and owls catch their mammalian prey on the ground. To compromise, we chose a soil ingestion rate of 1.4%.

Soil PCDD/DF concentrations in the Tittabawassee River floodplain are some of the greatest concentrations ever observed [1]. Because the soil PCDD/DF concentrations are so great, ignoring incidental soil ingestion would underestimate owls' exposure. However, choosing an unrealistic soil ingestion rate would result in an unreasonable exposure estimate. It is possible that even 1.4% is an overestimate of GHO soil ingestion, but it provides a conservative, and therefore more protective, measure of GHO PCDD/DF exposure. To quantify the effect of uncertainty in soil ingestion rate, ADD_{pot} was estimated with soil ingestion set to zero and to 2.8% of daily food ingestion. The median and 95th centile ADD_{pot} with soil at zero were 3.6 and 46 ng $TEQ_{WHO\text{-}Avian}$/kg bw/d, respectively, whereas with soil at 2.8% the median and 95th centile ADD_{pot} were and 6.4 and 48 ng $TEQ_{WHO\text{-}Avian}$/kg bw/d, respectively. Therefore, if zero and 2.8% are considered reasonable bounds on the soil ingestion, then the median estimate is ± 30% to 40% of the median value in Table 1.4. Uncertainty due to soil ingestion has a negligible effect on the upper end of the estimate.

Along with soil ingestion rate, there is uncertainty surrounding the bioavailability of PCDD/DFs from soil. Bioavailability can differ between soil type and species [61]. A bioavailable fraction estimate of 0.8 was selected. In feeding study with White Leghorn chickens, it was found that congeners with fewer chlorines were more bioavailable from sandy loam [42]. For example, tetra-substituted congeners were from 70-80% bioavailable. Tetrachlorinated congeners dominate the TEQs in the Tittabawassee River floodplain soils (67% 2,3,7,8-TCDF and 1% 2,3,7,8-TCDD) and the soil in the Tittabawassee River has been classified as sandy loam [62]. Because conditions in the Tittabawassee River floodplain align with those included in the study, we chose the

conservative 80% bioavailability estimate. This estimate should be protective and is considerably higher than the bioavailability observed for PCDD/DFs in Tittabawassee River soil samples fed to rats and pigs [61].

Because concentrations of PCDD/DF were not available for some prey items, surrogate values were used to fill in GHO exposure. These substitutions are unlikely to result in an underestimation of GHO exposure to PCDD/DFs. The nestling passerine TEQ concentrations were greater than concentrations in adult robins sampled from the study area; star-nosed moles were assigned shrew concentrations, and the shrews had the greatest PCDD/DF concentrations of any animal tissue collected from the TR floodplain. While squirrel muscle data were available from a wild game study, the whole-body rabbit TEQ concentrations was substituted because they were 70-fold greater than the concentrations in squirrel muscle and because they included fatty tissues and livers that are likely to be more representative of the tissues to which GHOs would be exposed. Waterfowl data were not available in most TR locations, so we assigned turkey muscle with skin to waterfowl. Although wood duck at TR-7 (the only location where data were available) had greater TEQ concentrations than turkey at TR-7, the geometric mean concentration in turkey at all sites was greater than wood duck. Because the turkey samples were for muscle with skin, it is likely that these underestimate the concentration in whole body waterfowl. Whole body waterfowl concentrations were not available. To consider the potential effect on the ADD_{pot}, the ADD_{pot} was calculated with waterfowl data (1) increased 6.3-fold to approximate a diet of wood duck, and (2) decreased 4.5 fold to approximate a diet of Canada goose. These modifications resulted in an increase of 12% and a decrease of 2%, respectively, the estimate of ADD_{pot}.

Another significant uncertainty in interpretation of these results is that of spatial resolution. The GHO ADD_{pot} presented here assume equal likelihood of feeding throughout the SA, yet home range or territory of any one GHO is less than the total area of the SA. In addition, samples of most prey items were taken at a few discreet locations throughout the floodplain. It is therefore important to recognize that a single point estimate (or distribution) of the ADD_{pot} for the SA characterizes a hypothetical GHO that feeds at random on samples collected from each dietary item sampling location. Because the dietary item sampling locations were selected to represent depositional areas where exposures would be the greatest, it is likely that the geometric mean and median ADD_{pot} are overestimates of the true 'average' ADD_{pot}.

Because the assessment was based on concentrations of $TEQs_{WHO\text{-}Avian}$ contributed by PCDD and PCDF in the diet of GHO and did not consider other residues such as PCBs and polychlorinated napthalenes (PCNs) that could contribute to the TEQ the HQ could be underestimates. While concentrations of PCBs were not measured in all samples, concentrations of the dl-PCBs were measured in a few representative samples 556 (Table 1.3). PCB concentrations were at or near detection limits in many samples, and RA and SA PCB concentrations were similar. A rough estimate of the GHO SA ADD_{pot} obtained using only the dietary categories available in Table 1.3, suggests including dl-PCBs in the ADD_{pot} calculation would increase the estimate by about 8% in the SA. Based on this and the fact that in this area the contribution of PCNs is less than that of PCBs [63] and the relative potencies of PCNs [64] indicates that there was little bias in the estimates due to the presence of PCBs or PCNs.

Resampling and traditional methodologies

While both ADD_{pot} analysis methods, traditional and resampling implementations of the equation from the wildlife exposure factors handbook, gave similar results (Table 1.4), the resampling method presents a more complete picture of GHO exposure. This is because the resampling method does not require potentially faulty assumptions about the distribution of the dietary item data; rather, the method uses the measured data and exposure factor assumptions to create the distribution of potentially dietary concentrations or does. In contrast, the traditional method uses the equation to combine summary statistics (mean, geometric mean, median and confidence limits) for each dietary category, but the combined summary statistics are not necessarily an accurate estimate of the summary statistic for the resultant distribution.

The median GHO SA dietary exposure estimate obtained from resampling and the geometric mean ADD_{pot} determined with the traditional method are generally similar (Table 1.3). However, in the traditional method, using the 95% upper confidence limit on the geometric mean of the concentration in each dietary item category yields a different and considerably lesser upper end estimate than does the 95^{th} centile in the resampling approach. The resampling approach identifies two "outlying" or extreme clumps in the distribution of GHO exposure estimates (Figure 1.6). These clumps are the result of two individual rabbit samples from the study area. Because rabbits compose 57% of the GHO biomass-based diet, any randomly sampled iteration of the diet that includes one of these two samples is distinctly greater than iterations that do not. Concentrations of TEQ in these cottontail rabbits, both sampled at T-6 (Figure 1.1), were 534 ng $TEQ_{WHO\text{-}Avian}$/kg and 1074 ng $TEQ_{WHO\text{-}Avian}$/kg. Without these two rabbits, the geometric mean

$TEQ_{WHOAvian}$ concentration for rabbits in the study area would have been 12 ng/kg, ww, and the SA GHO ADD_{pot}, based on the traditional method, would have been 2.7 ng/kg bw/d. The median of the resampling approach would be nearly unchanged, while the upper end estimate would be greatly reduced.

The two extreme values for rabbits were included in the dietary exposure calculations because they represent the concentration variations GHOs in the Tittabawassee River may be exposed to in their daily predation in the floodplain. The resampling approach that builds the distribution ADD_{pot} better illustrates the range of possible exposure. Furthermore, the variation evident in the distribution of the GHO ADD_{pot} gets a step closer to addressing the uncertainty discussed above regarding spatial variation in exposure within the Tittabawassee River floodplain. This is variation that may be more easily overlooked using the traditional method of estimating the ADD_{pot}.

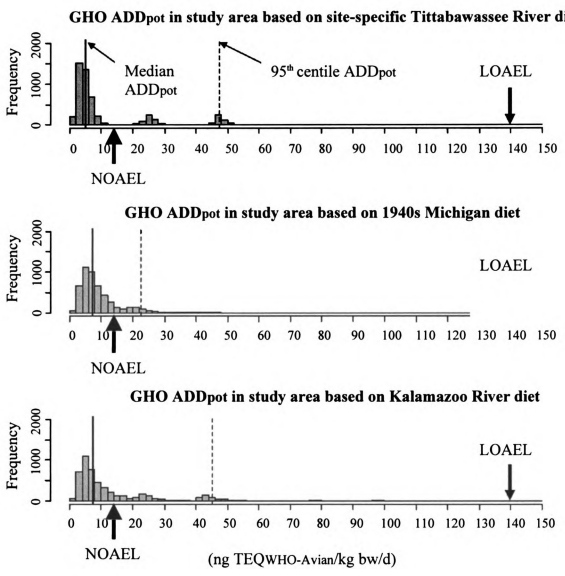

Figure 1.6. Distribution of great horned owl (*Bubo virginianus*) daily dietary exposure estimates in the Tittabawassee River study area based on resampling dietary items 10,000 times. The 50th centile from the resampling is shown (median) along with the 95th centile ADDpot.

The greatest uncertainty in the risk characterization presented here is most likely in the denominator of Equation 2. Although there is reassurance in the convergence of the screech owl Aroclor feeding study and the pheasant dioxin intraperitoneal injection study to a single NOAEL of 14, there remains considerable uncertainty in comparison of these no-effect concentrations to the predominately furan exposure GHO on the Tittabawassee River. It is likely that the TRV from the Nosek study is conservative when applied to the GHO, and that even with HQs slightly greater than 1.0, adverse effects on reproductive effects would be unlikely. Since the TRV values used in this study were for pheasants and screech owls it is assumed that the relative sensitivity of the GHO is similar to these species. Recent information on the cause of variation in sensitivities among birds is due to differences in the ligand-binding region of the AhR [65]. The fact that the pheasant and GHO have the same sequence in the ligand binding region of the AhR, suggests that they should have similar sensitivities to AhR agonists.

Congeners

The GHO diet congener profiles were determined by summing the weighted congener profiles of the GHO's prey items (the average congener contribution for each prey item was multiplied by that prey item's contribution to the GHO's biomass-based diet). The resulting SA and RA GHO diet congener profiles were representative of the prey species the owls consume. The great 2,3,4,7,8-PeCDF contribution to many of the prey species TEQ values carries over to the GHO diet congener profile. This is consistent with the congener profiles from adult and nestling GHO plasma, thereby increasing confidence in the dietary exposure assessment (Figure 1.7).

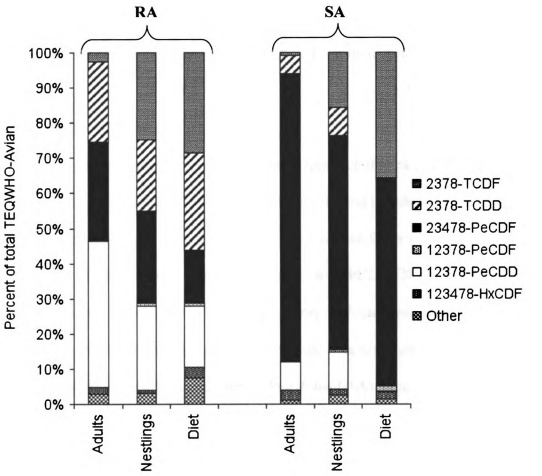

Figure 1.7. Congener contributions to $TEQ_{WHO\text{-}Avian}$ concentrations in great horned owl (*Bubo virginianus*) nestling and adult plasma and diets in the Tittabawassee River floodplain in Midland, MI.

Hazard Assessment

GHO dietary exposure estimates in the present study indicate that adverse effects are unlikely. All median and geometric mean exposure estimates were at least 2-fold less than the NOAEL TRV. Although upper end estimates in some cases exceeded the NOAEL TRV by 3-fold, the greatest upper end exposure estimates were about 3-fold less than the LOAEL TRV [10]. Even allowing for a 10% increase due to potential dl-PCB

contribution and a 10% increase that might result from surrogate concentrations in some prey item categories, this suggests GHOs in the Tittabawassee River floodplain are at little or no risk for effects from consuming PCDD/DFs in their diet.

Multiple lines of evidence

This study was part of a multiple lines of evidence approach that examined GHO dietary exposure to PCDD/DF, tissue PCDD/DF concentrations, and population health. The conclusions from this study, that GHOs in the Tittabawassee River floodplain are not at risk for adverse effects resulting from dietary exposure to PCDD/DFs, converge with the results from both the tissue-based exposure assessment, which found that GHO plasma PCDD/DF concentrations are less than those expected to produce adverse effects, and the results from population health studies, which found that GHO reproduction, survival and abundance are not negatively impacted (Chapter 2).

Chapter 2

Ecological Risk Assessment of Great Horned Owls (*Bubo* virginianus) Exposed to PCDDs/PCDFs in the Tittabawassee River Floodplain in Midland, Michigan, USA

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ABSTRACT

The soils and sediments downstream of Midland, Michigan, USA have elevated polychlorinated dibenzofuran (PCDF) and polychlorinated dibenzo-*p*-dioxin (PCDD) concentrations. To determine if the PCDD/DF concentrations have the potential to adversely affect terrestrial birds, a site-specific, multiple lines of evidence risk assessment for the great horned owl (*Bubo virginianus*; GHO) was conducted. As long-lived resident top predators, great horned owls have the potential to be exposed to high levels of bioaccumulative compounds such as PCDD/DFs. From 2005-2008, blood plasma samples were collected from adult and nestlings GHOs. Addled eggs were also collected. Population health measures, including abundance and reproductive success, were collected for 115 km of river corridor. Fifty-five active nests were monitored in 21 breeding territories from 2005-2008. The geometric mean $TEQ_{WHO\text{-}Avian}$ concentration in GHO plasma was greater in the study area (SA) than in the reference area (RA) for both adult (RA: 3.1 ng $TEQ_{WHO\text{-}Avian}$/kg, SA: 9.4 ng $TEQ_{WHO\text{-}Avian}$/kg) and nestling (RA: 0.82 ng $TEQ_{WHO\text{-}Avian}$/kg, SA: 2.1 ng $TEQ_{WHO\text{-}Avian}$/kg) GHOs, but lesser than concentrations expected to cause adverse effects. Concentrations of $TEQ_{WHO\text{-}Avian}$ in addled eggs were also greater in the SA than the RA (50 ng/kg and 7.3 ng/kg, respectively), but less than concentrations expected to cause adverse effects. The GHO population health and productivity were both greater in the study area than in the reference area and were similar to other GHO populations, which suggests that the GHO population in the Tittabawassee River floodplain is in good health.

Key words: Great horned owl, raptors, dioxin, furan, TEQs, ERA, plasma, population health, reproductive success

INTRODUCTION

Decades of chlorine compound manufacture at the Dow Chemical Co. in Midland, MI, USA, led to the inadvertent contamination of the Tittabawassee River floodplain in mid-Michigan, USA. The soils and sediments downstream of Midland have some of the greatest concentrations of polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxin (PCDDs) ever recorded [1]. The chemicals were released prior to the adoption of modern waste treatment and storage [5].

The Tittabawassee River regularly overflows its banks, depositing contaminated sediment within the terrestrial habitat of the 100-year floodplain. A five-year, multiple lines of evidence risk assessment was conducted to the exposure to and effects of PCDDs/DFs on great horned owls (*Bubo virginianus;* GHO). Great horned owls have many characteristics desirable in an avian biomonitor [66]. As long-lived (up to 28 years in the wild [16]), non-migratory, territorial tertiary predators, owls have the potential to be exposed to significant contaminant concentrations. In addition, owls are relatively easy to work with. Because they do not build their own nests and will readily occupy man-made nests, researchers can install nests in trees that can be safely climbed. Nestling GHOs are easy to handle and can be returned to their nests without fear of abandonment, and adult territoriality can be used to both locate territories [27,67] and capture adults [68] for banding and collection of samples. The GHO has been previously monitored to study PCBs [25,26] and dieldrin [69] in the environment and the owls have been suggested as useful environmental sentinels [27].

The GHO was selected as a terrestrial receptor species because dioxin-like chemicals are known to adversely avian wildlife by causing developmental deformities, reduced productivity and decreased nest attentiveness [7,9]. In addition, laboratory studies have confirmed avian exposure to PCDD/DFs can result in embryotoxicity, growth retardation, subcutaneous and pericardial edema, liver damage, immunotoxicity, and induction of p450 enzymes [10,11,20,21,23]. While there is little information on the effects of PCDD/DF on GHO, owls have been shown to be sensitive to the effects of other environmental contaminants, including organochlorines, organophosphates and metals [17].

This study was part of a site-specific, multiple lines of evidence risk assessment. Here we report measures associated with GHO population status, including abundance, density and reproductive success, and compare them to expected outcomes predicted by comparing concentrations of PCDD/DF in GHO blood plasma and addled eggs to toxicity reference values (TRV). The third line of evidence, dietary exposure to PCDD/DFs is reported elsewhere (Chapter 1). Examining three lines of evidence provided greater confidence in the assessment of the potential for the PCDD/DFs in the floodplain soils and sediments to adversely affect resident great horned owls [18].

MATERIALS AND METHODS

Site Description

The Tittabawassee River study area (SA) includes sediments and floodplain soils associated with 72 km of the Tittabawassee and Saginaw rivers in Michigan (Figure 2.1). The upstream and downstream boundaries of the SA are Midland, MI, and Saginaw Bay, respectively. The floodplain is characterized by intermittent agricultural (37%) and wooded (41%) areas. The floodplains along the Tittabawassee River are periodically inundated, usually during high flows in the spring and following major storm events. Releases from the Sanford Lake hydro-electric dam upstream of Midland cause daily changes in water levels and flow rates in the Tittabawassee River.

The reference areas (RAs) lie directly upstream of the SA in the Tittabawassee river watershed and include 16 km along the Pine River, 12 km along the Chippewa River and 15 km of the Tittabawassee River. These areas represent background levels of PCDD/DFs in the region [1].

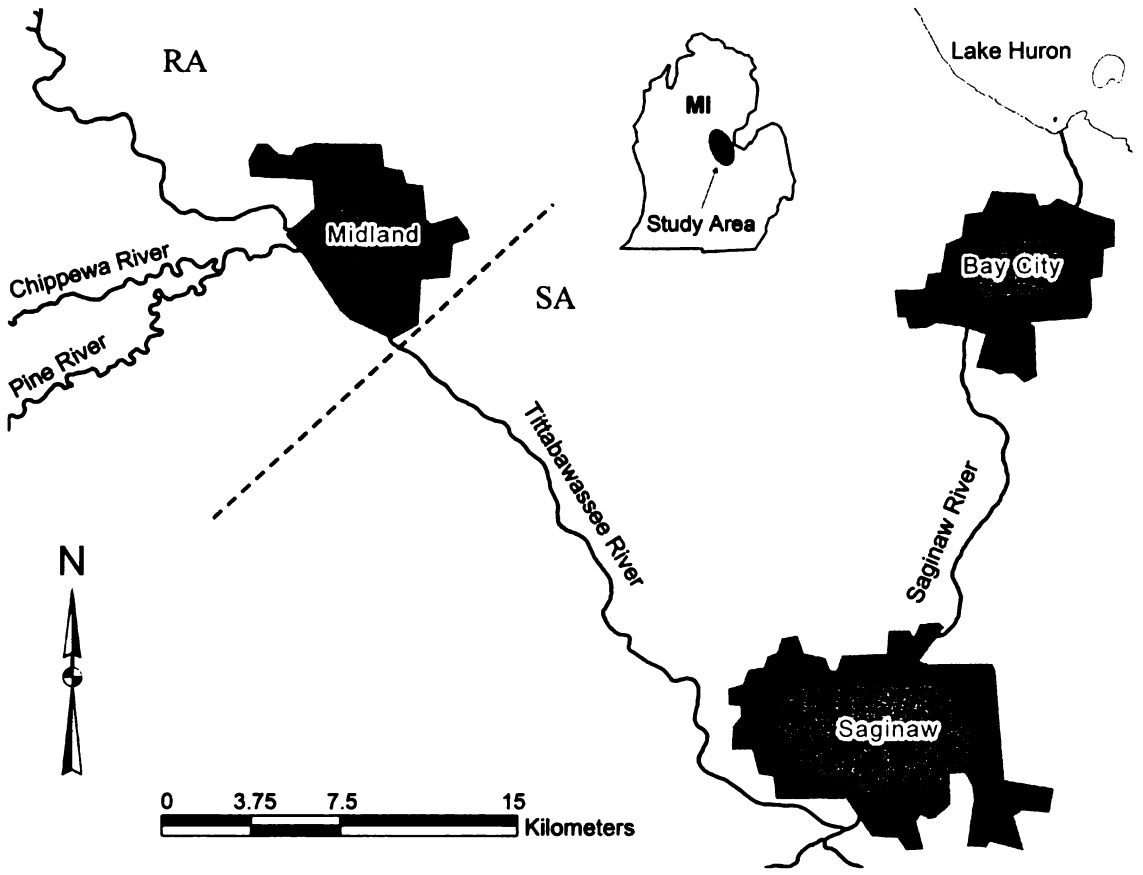

Figure 2.1. Study locations within the Chippewa, Tittabawassee, and Saginaw River floodplains, Michigan, USA. Reference areas include the Pine and Chippewa rivers and the Tittabawassee River from the Sanford Dam to the convergence with the Chippewa River. The study area includes the Tittabawassee River from Midland, MI to the convergence with the Cass River, and the Saginaw River to the Saginaw Bay.

Nests

Great horned owls will readily occupy artificial nesting structures [27,29]. By taking advantage of this behavior, researchers can encourage owls to nest in accessible locations and trees that can be safely climbed. For this study, 75 cone-shaped nests were constructed by weaving dogwood and willow through chicken wire following previously described methods [27]. The nests were deployed in GHO territories that were identified through call-response surveys and incidental observations. Two to three nests were installed per breeding territory, with additional nests installed in areas with good habitat but unconfirmed GHO occupation.

Nest trees were chosen based on several qualifying factors, including distance from the river, ease of GHO access, and the presence of nearby trees for roosting. Only deciduous trees were used, and included black willows (*Salix nigra*), hackberries (*Celtis occidentalis*), sugar maples (*Acer saccharum)*, black walnut (*Juglans nigra*), white ash (*Fraxinus americana*), cottonwood (*Populus deltoids*), red maple (*Acer rubrum*), bur oak (*Quercus macrocarpa*), red oak (*Quercus rubra*), white oak (*Quercus alba*), aspen (*Populus grandidentata*) and sycamore (*Platanus occidentalis*). Nests were installed 9-21 m from the ground, with an average nest height of 15 m. Adjustable hose clamps were used to hold the nest in the tree, preferably at the junction of three limbs. The hose clamps' circumferences were adjusted to match the girths of limbs and trunks during a yearly maintenance visit. After installation was complete, the hose clamps were spray-painted with matte-brown paint to better match the surrounding limbs. A matte-brown

aluminum sheeting was installed around the trunk of the tree below the nest as a predator guard.

Collection of blood plasma from nestlings and adults and addled eggs

Great horned owl nests were accessed when the nestlings were 5-7 wks old and weighed at least 0.75 kg. At this age the young birds have sufficient mass to safely take a blood sample large enough for analysis. They are also young enough to remain in the nest or are easy to retrieve if they have branched. Nestlings were lowered to the ground in individual cloth bags and weighed with a spring scale (Model 42500, Pesola AG, Switzerland). While the nestlings were in-hand, measurements of culmen, bill depth, eighth primary, foot pad, and hallux were recorded. Nestlings were identified by attaching a USFWS #9 band. Nestling physical condition and health were noted and the nestling hydrated with 5-10 ml Gatorade® before being returned to the nest.

Blood was collected from adults and nestlings using the same procedure. Blood was drawn from the brachial vein using a 22.5 gauge needle (Becton Dickinson, Franklin Lakes, NJ 07417) affixed to a sterile syringe pre-rinsed with sodium heparin solution. The blood sample volume procured was determined by calculating 10% of 7% of the bird's body mass [70,71]. The sample was transferred to a labeled and heparinized green-capped Vacutainer™ (Becton Dickinson) for transport back to the field laboratory. Whole blood samples were centrifuged at 1200 rpm for 10 min within 12 h of collection and the plasma (supernatant) was decanted into solvent-rinsed scintillation vials. Both plasma and packed cells were stored at -20°C until analysis.

Addled eggs were collected concurrent with the nestling blood draw and during visits to failed nests. Eggs were transported to the laboratory in a crush-proof, water-

proof container and kept at 4°C until processing. Length, width, whole-egg weight, and whole-egg water volume were measured. Egg contents were homogenized in solvent-rinsed blenders and frozen until time of analysis. Residue concentrations were corrected for moisture loss and reported as fresh wet weight [72].

Trapping of Adults

Adult GHOs were trapped from June to October, 2005-2007. The adult GHO trapping scheme utilized GHO territoriality by luring the owls into mist nets at night with a decoy and territorial hoots. Mist nets (Avinet Inc, 210 denier/4-ply, 127 mm mesh, 2.6m x 9m) were deployed in a triangle formation around a plastic decoy great horned owl positioned on a 1.5 m post. A CD player directly beneath the decoy broadcast territorial GHO hoots once per minute. The mist nets were set up in shaded areas to decrease the visibility of the nets and poles. Sheep bells were positioned on the mist nets to alert researchers when an owl flew into the net. Trapping efforts were initiated at sunset and continued until an owl was successfully caught or the sun rose

Researchers in blinds approximately 0.5-1 km from the trapping location were alerted by voice-activated two-way radios that broadcast the ringing bells when an owl flew into the net. Owls were removed from nets within minutes of capture. The adult work-up paralleled the nestling procedures. All work was completed using head lamps for illumination, and once the blood sample was collected, the USFWS #9 band attached, and measurements were completed, the headlamps were extinguished to give the owl a few minutes to readjust to the darkness before its release.

GHO observations

Nests were monitored for incubation onset from the last week of January through the third week of February. Great horned owl nest occupation (incubation onset) was confirmed with both aerial and land-based observations. Ground-based observations were conducted from a distance of 100 m with the aid of a spotting scope. That distance was maintained to avoid spooking the owl and exposing the eggs to low temperatures and predators. Aerial observations were conducted in 2006 and 2007 using binoculars from a Bell JetRanger® helicopter. Nests with confirmed activity were monitored on a weekly to biweekly basis. This monitoring was used to determine hatch-out date and also note nest failures. If a nest was abandoned for two visits in a row, the tree was climbed and any unhatched eggs were collected and processed. Nestling ages, as determined by nest observations, were used to schedule nestling blood draws. Incidental observations of GHO activity, territory occupation, and fledge success were made during adult trapping attempts, vocalization recording and surveys. These data were collected and included in productivity measurements.

Relative abundance and productivity

Occupancy was determined with both call-response surveys and breeding season observations. Call-response surveys were conducted to determine relative GHO abundance along the Chippewa, Pine, and Tittabawassee river corridors during the period of June-October during 2004-2007. The surveys were conducted under dry, windless conditions, by use of previously described methods [27] with minor alterations. The surveys were conducted from a canoe in the evening and early morning, initiating at

sunset and ending at sunrise. Surveyors stopped at predetermined points spaced 500 m apart. The survey began with a 2 min listening period, followed by a 5 min calling period, and a final 3 min listening period. During the calling period, a CD of a territorial GHO hoot was broadcast from the canoe once every minute. Great horned owl responses to the call were recorded and classified as juveniles, territorial pairs and non-territory-holding owls.

Relative abundance was classified as GHO territories/river km. To avoid overestimating territory density, all responses in a single territory were counted as one response, even if owls were hooting for more than one transect and more than one owl responded. Juvenile and adult responses at a single territory were grouped together in order to measure relative territory density rather than actual number of GHOs present. Fledgling responses were later used to calculate reproductive success.

Productivity was expressed as the number of nestlings fledged/breeding attempt [73]. Nests at which GHOs demonstrated breeding activity (female sitting low in the nest) were considered active and were identified in late January and early February. Fledging was confirmed through call-response surveys and visual observations during nest visits.

Quantification of PCDD/DF

Concentrations of PCDD/DFs in addled eggs and plasma of adult and nestling GHOs were quantified in accordance with EPA Method 8290 with minor modifications [74]. Briefly, samples were homogenized with anhydrous sodium sulfate and Soxhlet extracted in hexane:dichloromethane (1:1) for 18 hr. Before extraction, known amounts of ^{13}C -

labeled internal standards were added to the sample as internal standards. The extraction solvent was exchanged to hexane and the extract was concentrated to 10 mL. Ten percent of this extract was removed for lipid content determination. Extracts were initially purified by treatment with concentrated sulfuric acid. The extract was then passed through a silica gel column containing silica gel and sulfuric acid silica gel and eluted with hexane. The extract received additional column chromatography by elution through acidic alumina which resulted in two fractions: The first fraction eluted contained most PCBs and pesticide compounds, while the second fraction contained dioxins and furans. The second fraction eluted from the alumina column was then passed through a carbon column packed with 1 g of activated carbon-impregnated silica gel. The first fraction, eluted with various solvent mixtures, was combined with the fraction one eluate from the acidic alumina column and retained for possible co-contaminant analyses. The second fraction, eluted with toluene, contained the 2,3,7,8-substituted PCDD/DFs. Components were analyzed using HRGC-HRMS, a Hewlett-Packard 6890 GC (Agilent Technologies, Wilmington, DE) connected to a MicroMass® high resolution mass spectrometer (Waters Corporation, Milford, MA). PCDF and PCDD congeners were separated on a DB-5 capillary column (Agilent Technologies, Wilmington, DE) coated at 0.25 μm (60 m x 0.25 mm i.d.). The mass spectrometer was operated at an EI energy of 60 eV and an ion current of 600 μA. Congeners were identified and quantified by use of single ion monitoring (SIM) at the two most intensive ions at the molecular ion cluster. Concentrations of certain PCDD/DF congeners, particularly TCDD and 2,3,7,8-tetrachlorodibenzofuran (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 matrix spikes, blanks, and duplicates. Concentrations of $TEQs_{WHO\text{-}Avian}$ (ng/kg ww) were calculated for PCDD/DFs by summing the product of the concentration of each congener, multiplied by its avian TEF [39].

Statistical Analysis

All statistical analyses were performed using Systat 11 software (Systat Software, Inc. 2004, Richmond, CA, www.systat.com). Normality was tested using the Shapiro-Wilks test, and non-normal data were log-transformed before statistical analysis. A Student's *t*-test was used to evaluate the significance of differences between reference and study area plasma PCDD/DF concentrations expressed as sum avian TEQs. Due to the small sample size (n=4), relative abundance data were analyzed with a nonparametric test (Mann-Whitney U). Statistics were not used for productivity data (n=1).

Hazard Assessment

Hazard quotients (HQ) were used to determine potential for adverse effects from GHO exposure to PCDD/DFs. The HQs were calculated by dividing concentrations of $TEQs_{WHO\text{-}Avian}$ in blood plasma and addled eggs by respective no-observed-adverse-effect-level (NOAEL) toxicity reference values (TRVs) based on both field (Table 2.1) and laboratory studies (Table 2.2). The upper and lower 95% confidence intervals around the geometric mean concentration of $TEQs_{WHO\text{-}Avian}$ in blood plasma of nestlings were divided by plasma-based TRVs. Since a small number of addled eggs and distributions of concentration data precluded calculation of a 95% UCL, HQs were

calculated by dividing the maximum and minimum concentrations of $TEQ_{WHO\text{-}Avian}$ by the TRVs based on concentrations in eggs.

Table 2.1. Toxicity reference values (TRVs) for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in nestling great horned owl (*Bubo virginianus*) plasma

	Tissue-based TRV	Study Type	Species	Reference
Plasma TEQ concentrations (ng/kg wet wt)				
NOAEL	0.8	Field	*Bubo virginianus*	Strause (unpublished)
NOAEL	2.9	Field	*Haliaeetus leucocephalus*	Elliott 1998

Table 2.2. Tissue-based toxicity reference values (TRVs) for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQWHO-Avian) for great horned owl (*Bubo virginianus*) eggs.

	Tissue based TRV	Study Type	Reference
Egg TEQ concentrations (ng/kg wet wt)			
NOAEL	1800	feeding	McLane 1980
NOAEL	100	egg injection	Nosek 1993
NOAEL	230	egg injection	Hoffman 1998

Toxicity reference values

Because there is no single definitive toxicity reference value for GHO exposure to PCDD/DFs, TRVs were derived from a combination of field and chronic laboratory studies. The selection criteria for utilized studies included exposure compound relevance, species relevance, and response measures associated with ecologically relevant endpoints such as reproductive success and hatch success. Few studies included measures of adult exposure to the primary compounds of concern, PCDD/DFs, and none could be found that measured concentrations of these compounds in blood plasma.

Two field based studies were included in the derivation of site specific TRVs (Table 2.1). In these studies, blood plasma concentrations of dioxin-like compounds were directly compared to key reproductive endpoints. In an examination of GHO exposure to polychlorinated biphenyls in Kalamazoo, MI, Strause et al [25] measured avian TEQs in nestling GHO plasma. The field-based no-observed-adverse-effect-level

(NOAEL) from that study was 0.80 ng $TEQ_{WHO\text{-}Avian}$/kg nestling plasma. By applying 1998 WHO TEFs to concentration data reported for bald eagle nestlings in British Columbia, we estimated a second field-based NOAEL of 2.9 ng $TEQ_{WHO\text{-}Avian}$/kg nestling plasma [75]. It should be noted that because neither study identified a lowest-observed-adverse-effect-level, plasma HQs < 1.0 result in a likely absence of hazard while plasma HQs > 1.0 are difficult to interpret because the plasma concentrations at which a hazard exists is unknown.

Three laboratory studies that measured TEQs in bird eggs were chosen as TRVs for TEQ concentrations in GHO addled eggs. In a chronic feeding study, McLane and Hughes [43] administered 3 mg/kg, ww, Aroclor 1248 in the diet of screech owls over the course of two years. No adverse effects were observed in this study at the greatest mean egg concentration, 7.0 mg Aroclor 1248/kg. Aroclor 1248 is a PCB mixture with a dioxin toxicity equivalency factor of 2.57×10^{-4} kg/kg in birds [44]. By applying this factor to the NOAEL, we attained a NOAEL of 1800 ng $TEQ_{WHO\text{-}Avian}$/kg egg (Table 2.2). 2,3,7,8-TCDD injected into the albumin of ring-necked pheasant (*Phasianus colchinus*) eggs resulted in embryo mortality and hepatic ethoxyresorufin *O*-deethylase (EROD) induction [11]. The NOAEL and LOAEL from that study (100 ng 2,3,7,8-TCDD/kg and 1000 ng 2,3,7,8-TCDD/kg , respectively) were chosen as TRVs for addled eggs. After injecting kestrel egg air cells with PCB 126 and PCB 77, Hoffman et al identified NOAELs of 0.23 mg PCB 126/kg and 100 mg PCB 77/kg [22]. By applying WHO avian TEFs [39] and calculating the geometric mean of the two congener trials, a NOAEL of 230 ng $TEQs_{WHO\text{-}Avian}$/kg was identified as a potential TRV. Since the amino acid sequence of the ligand binding domain of the Ah receptor (AhR) is the same

for the GHO, bald eagle, American kestrel and common pheasant, all of these species are classified as moderately sensitive to PCDD/DFs species [65] (Kennedy unpublished data).

RESULTS

TEQ in blood plasma and eggs

Concentrations of $TEQs_{WHO\text{-}Avian}$ in blood plasma were different between adults and juveniles and between the study and reference areas. Concentrations of $TEQ_{WHO\text{-}Avian}$ in blood plasma were significantly greater in adults than in nestling GHOs residing in the same area (t-test, $p<0.05$). Thus, for spatial analysis, adult and nestling plasma data were analyzed separately. Of the blood plasma samples collected from 61 GHO in 20 territories, between 2005 and 2008 15 were from the reference area (5 adults and 10 nestlings) and 46 were from the study area (12 adults and 34 nestlings). The geometric mean $TEQ_{WHO\text{-}Avian}$ concentration in adult blood plasma was significantly greater in the study area than the reference area (Mann-Whitney U, $p<0.05$). Concentrations of $TEQ_{WHO\text{-}Avian}$ in plasma of adults from the reference area ranged from 2.0 to 5.2 ng/kg , with a geometric mean of 3.1 ng $TEQs_{WHO\text{-}Avian}$/kg plasma. Concentrations of $TEQs_{WHO\text{-}Avian}$ in blood plasma of adult GHO from the reference area ranged from 3.3 to 38 ng/kg with a geometric mean of 9.4 ng $TEQs_{WHO\text{-}Avian}$/kg plasma (Table 2.3).

Similarly, the geometric mean concentration of $TEQ_{WHO\text{-}Avian}$ in nestling plasma was significantly greater in the study area (t-test, $p<0.05$). Concentrations of $TEQ_{WHO\text{-}Avian}$ in blood plasma of nestlings from the reference area ranged from 0.25 to 4.0 ng/kg,

with a geometric mean of 0.82 ng TEQWHO-Avian/kg plasma. Concentrations of TEQWHO-Avian in blood plasma of nestlings from the study area ranged from 0.57 to 8.6 ng/kg, with a geometric mean of 2.1 ng TEQWHO-Avian/kg plasma (Table 2.3).

Between 2005 and 2008, addled eggs were collected from five GHO nests (2 RA, 3 SA). The small sample size precluded statistical analysis and calculation of 95% UCL. However, as with the plasma data, the geometric mean TEQWHO-Avian concentration in study area eggs was greater (50 ng TEQWHO-Avian/kg egg) than in the reference area. Concentrations of TEQWHO-Avian in eggs from the study area ranged from 14 to 168 ng TEQWHO-Avian/kg. Concentrations of TEQWHO-Avian in addled, GHO eggs from the reference area ranged from 3.8 to 14 ng/kg, with a geometric mean of 7.3 ng TEQWHO-Avian/kg (Table 2.3).

Table 2.3. Geometric mean, 95% UCL and ranges of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQWHO-Avian) in great horned owl (*Bubo virginianus*) adult and nestling plasma and addled eggs from the reference and study areas along the Tittabawassee River in Midland, Michigan, USA.

	Reference TEQWHO-Avian Concentrations				Study TEQWHO-Avian Concentrations			
Tissue	*n*	Range	Geomean	95% UCL	*n*	Range	Geomean	95% UCL
Adult Plasma (ng/kg)	5	2.0-5.2	3.1	4.6	12	3.3-38	9.4	14
Nestling Plasma (ng/kg)	10	0.25-4.0	0.82	1.5	34	0.57-8.6	2.1	2.6
Addled Egg	2	3.8-14	7.3		3	14-168	50	

PCDD and PCDF Congeners

Patterns of relative concentrations of congeners in eggs and blood plasma of adults and nestlings from the reference and study areas were dissimilar. However, the 5 to 7 congeners that accounted for approximately 90% of the total $TEQs_{WHO\text{-}Avian}$ were consistent among areas, albeit in very different proportions (Figure 2.2). The primary congeners contributing to TEQ concentrations in the nestling plasma from the reference areas were 1,2,3,7,8-PeCDD (21%); 2,3,4,7,8-PeCDF (25%); 2,3,7,8-TCDD (24%);and 2,3,7,8-TCDF (26 Concentrations of $TEQ_{WHO\text{-}Avian}$ in blood plasma of adult GHO in the reference areas were composed primarily of 1,2,3,7,8-PCDD (42%); 2,3,4,7,8-PeCDF (28%); and 2,3,7,8-TCDD (23%). 2,3,7,8-TCDF only contributed 2% to reference adult $TEQs_{WHO\text{-}Avian}$ concentrations. The primary congeners contributing to TEQ in the addled egg collected from the reference area were 2,3,4,7,8-PeCDF (37%); 2,3,7,8-TCDD (29%); 1,2,3,7,8-PeCDD (25%) and 2,3,7,8-TCDF (6%).

The relative contributions of congeners to $TEQs_{WHO\text{-}Avian}$ in blood plasma of both nestling and adult GHO in the study area were dominated by 2,3,4,7,8-with contributions of 64% and 83%, respectively. The other congeners contributing TEQs in blood plasma of nestlings were 1,2,3,7,8-PeCDD (10%); 2,3,7,8-TCDF (13%); and 2,3,7,8-TCDD (8%). The other congeners contributing to TEQ in blood plasma of adult GHO included 1,2,3,7,8-PeCDD (8%); 2,3,7,8-TCDD (5%); and 2,3,7,8-TCDF (1%). The pattern of relative concentrations of congeners in addled eggs from the study area was different than that in blood plasma of adult GHO. The predominant congeners contributing to TEQs in addled eggs from the study area were 2,3,7,8-TCDF (58%) and 2,3,4,7,8-PeCDF (37%) (Figure 2.2).

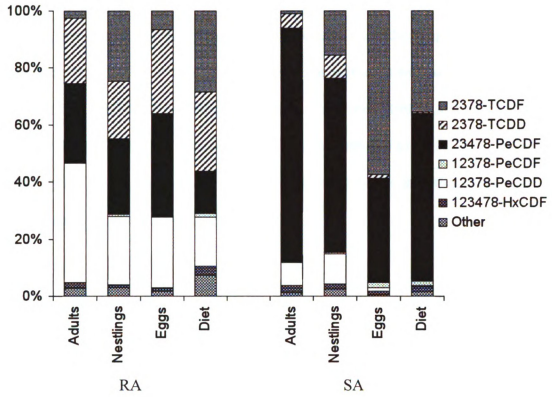

Figure 2.2. Congener contributions to TEQWHO-Avian concentrations in great horned owl (*Bubo virginianus*) nestling and adult plasma, addled eggs and site-specific diet in the Tittabawassee River floodplain in Midland, MI, USA.

Risk Assessment

The concentration of TEQWHO-Avian associated with the 95% UCL of the geometric mean of concentrations in blood plasma of nestlings were less than those observed in nestling bald eagles for which there was also no adverse effects observed [75]. Concentrations of TEQWHO-Avian in blood plasma of GHO nestlings were greater than concentrations in blood plasma of GHO nestlings exposed to TEQ from PCB in the vicinity of the Kalamazoo River that were not associated with adverse effects [25].

Values of HQ were near 1.0, but were greater than or less than 1.0, depending on the TRV or measure of exposure used. Hazard quotients for addled eggs from the study area were less than 1.0 when max $TEQ_{WHO\text{-}Avian}$ concentration was compared to the Hoffman (1998) and McLane (1980) NOAELs and the Nosek (1993) LOAEL. The HQ was greater than 1.0 when the max concentration was compared to the NOAEL from the Nosek study (HQ = 1.7) (Figure 2.4). Hazard quotients for eggs from the reference area were less than 1.0, regardless of the TRV used.

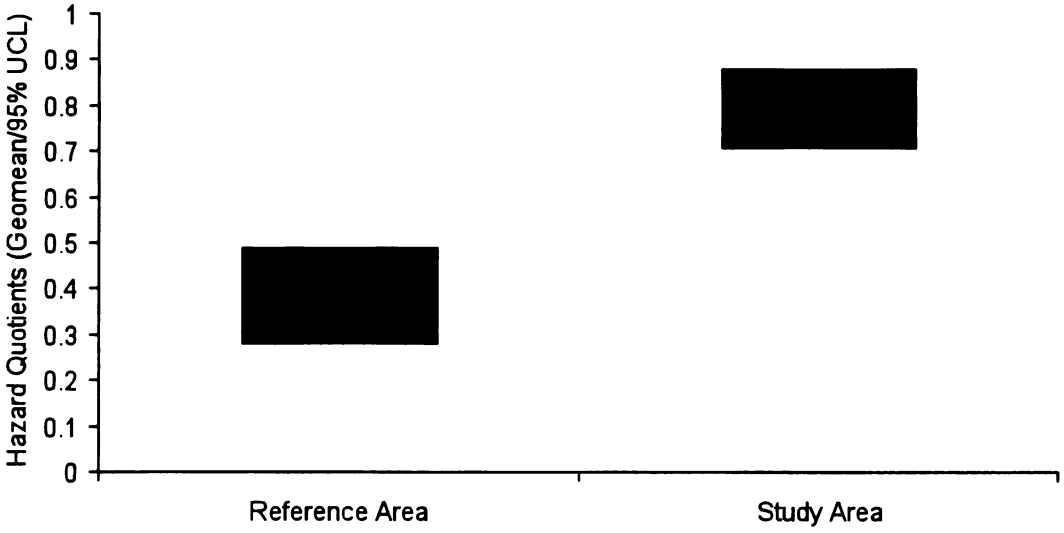

Figure 2.3. Hazard quotients for nestling great horned owl (*Bubo virginianus*) exposure to polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) in the Tittabawassee River floodplain in Midland, MI, USA.

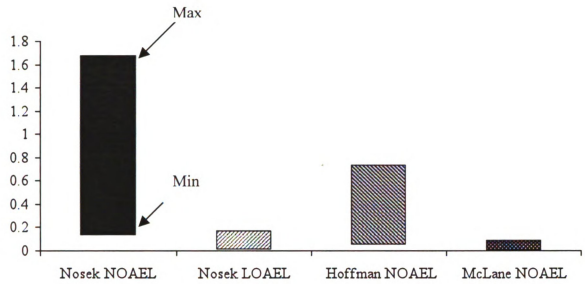

Figure 2.4. Study area hazard quotients (HQs) for polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) toxicity equivalents (TEQs) in great horned owl (*Bubo virginianus*) addled eggs in the Tittabawassee River floodplain in Midland, MI, USA. Hazard quotients are based on the max and min $TEQ_{WHO\text{-}Avian}$ concentrations in addled eggs collected from the floodplain.

Territory density

The mean relative density of GHO territories for the period 2004-2007 was significantly greater in the study area downstream of Midland, MI (0.24 territories/river km) than in the reference areas (0.09 territories/river km) (Mann-Whitney U, p=0.029) (Figure 2.5). More territories were identified in the study areas than reference areas for all years (Table 2.4) and the density of territories was greater in the study area than the reference area (Figure 2.5). Of those territories, a total of 30 were held by breeding pairs in the study area and 9 were held by breeding pairs in the reference areas. Since not all owls responded on every survey, the estimated rate of territory occupancy for both the reference and study areas was greater than call-response surveys indicated, (Figure 2.5).

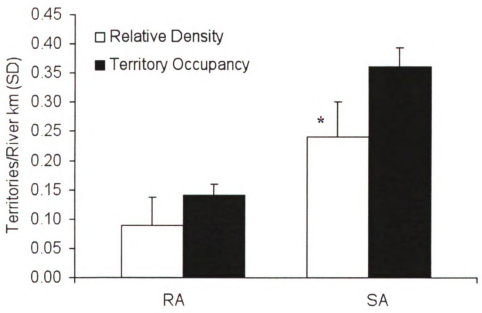

Figure 2.5. Relative density, as determined with call-response surveys, and territory occupancy of great horned owls (*Bubo virginianus*) in the reference (RA) and study areas (SA) in the Tittabawassee River floodplain in Midland, MI, USA.
* Relative density was significantly greater in the study area than in the reference area (Mann-Whitney U, p = 0.029)

Productivity

Overall productivity for the duration of the study, defined as number of fledglings/breeding pair, was greater in the study area (1.3) than in the reference area (0.89). A proportion of this difference was derived from a greater rate of nest failures (failed nests/breeding pair) in the reference area than in the study area. Productivity by year was also greater in the study area than in the reference area. Regardless of year, productivity on a spatial basis (nestlings fledged per river km) was greater in the study area (Mann-Whitney U, $p<0.05$) than in the reference area (Table 2.4).

Table 2.4. Great horned owl (*Bubo virginianus*) productivity in the Tittabawassee River floodplain reference and study areas in Midland, MI, USA, 2005-2007

	2005		2006		2007		All years	
	Ref	Study	Ref	Study	Ref	Study	Ref	Study
Occupied territories	7	10	9	13	8	11	24	34
Territorial pairs	4	9	6	13	5	11	15	33
Breeding pairs	2	9	3	11	4	10	9	30
Eggs hatched	2	10	5	17	3	12	10	39
Addled eggs	1	3	0	1	1	2	2	6
Nestlings predated	0	0	0	0	1	0	1	0
Nestlings out of nest	0	0	0	0	0	0	0	0
Nestlings missing	0	0	1	0	0	1	1	1
Failed nests	N/A	0	0	0	2	3	2	3
Fledglings	2	11	4	17	2	11	8	39
#Fledglings/Breeding pair	1.00	1.22	1.33	1.55	0.50	1.10	0.89	1.30
#Fledglings/Territorial pair	0.50	1.22	0.67	1.31	0.40	1.00	0.53	1.18
#Fedglings/River km[a]	0.02	0.3	0.1	0.5	0	0.3	0.2	1.2

[a]Only includes fledglings along survey routes.

DISCUSSION

Concentrations of PCDD/DF

The lesser concentrations of PCDD/DF measured in blood plasma of nestling GHO and addled eggs are consistent with the results of a site-specific dietary study that predicts great horned owls in the Tittabawassee River floodplain study area have a potential average daily dose of 3.3 ng $TEQ_{sWHO\text{-}Avian}$/kg bw/d (Chapter 1). The study described the GHO diet and concentrations of PCDD/DFs in prey items, such as rabbits, muskrats and meadow voles, which make up 85% of the GHO biomass-based diet from the Tittabawassee River floodplain. Concentrations of PCDD/DF were greater in the diet from the study area than in the reference area. Rabbits, muskrats and meadow voles eat plants, which do not readily take up dioxin-like chemicals from soil. When plants do take up the chemicals, they are not readily translocated to the leaves [58,59]. The resulting small concentrations in plants lead directly to lesser concentrations of PCDD/DF in the GHO's herbivorous prey, and concentrations in GHO are proportional to the concentrations of prey.

The fact that concentrations of $TEQ_{WHO\text{-}Avian}$ in plasma of nestlings were less than those in adult plasma concentrations in both the reference and study areas was most likely due to growth dilution. their bodyweight from less than 50 g to greater than 700 g in 6-8 wk. Also, adult GHOs are long-lived and occupy their territories year-round, which results in a bioaccumulation over time.

Congener Profiles

The congener profiles for GHO eggs and plasma samples were in agreement with congener profiles for other birds and mammals in the reference and study area floodplains (Chapter 1, [38]. In addition, profiles of congener concentrations in GHO from both the reference and study areas are consistent with the congener patterns in the site-specific diet (Figure 2.2) (Chapter 1). This result is consistent with GHOs being exposed to PCDD/DFs through prey from within the floodplain. While the predominant congeners in the soil and sediment congener profiles were 2,3,7,8-TCDF and 2,3,7,8-TCDD (Chapter 1), wildlife in the floodplain preferentially retained 2,3,4,7,8-PeCDF and appeared to quickly metabolize 2,3,7,8-TCDF, which results in a different congener profile in the tissues of the animals than in the soils and sediments. Previous studies have documented swift metabolism of 2,3,7,8-TCDF in mallards [76] and gulls [77]. Retention of 2,3,4,7,8-PeCDF has been observed in bald eagles in Canada [78], cormorants in the Great Lakes [79], terns in the Netherlands [80], and barn owls in Germany [81]. While little work has been done with birds to examine this retention, kinetics studies with mink have shown 2,3,4,7,8-PeCDF is preferentially sequestered in the liver [82]. Retention of 2,3,4,7,8-PeCDF in birds has been confirmed in controlled laboratory studies in which 2,3,7,8-TCDF was injected into eggs of white leghorn chickens, quail or pheasants (Zwiernik, personal communication). The relative contribution of 2,3,7,8-TCDF to addled egg $TEQ_{sWHO\text{-}Avian}$ in the study area was greater than expected based on plasma results and avian metabolism of the congener. However, this congener pattern is likely a direct result of a single nest, in which eggs contained the greatest concentrations of $TEQ_{sWHO\text{-}Avian}$ observed in GHO eggs from the

study area. Nearly 80% of the $TEQ_{sWHO\text{-}Avian}$ in the addled egg were contributed by 2,3,7,8-TCDF. Thus, the greater concentration of 2,3,7,8-TCDF in that addled eggs could have resulted from maternal exposure via prey items and immediate yolk deposition. This type of deposition of residues has been previously observed for bald eagles [23].

Risk Assessment

Values of HQ, based on concentrations of $TEQ_{WHO\text{-}Avian}$ in blood plasma of nestlings were dependant on which TRV was used. The more conservative TRV suggested by Strause et al (2007) (0.80 ng $TEQ_{WHO\text{-}Avian}$/kg plasma) resulted in HQs greater than 1.0 for both the reference and study areas. Because no effects were observed in the study by Strause et al (2007) it is not possible to determine a threshold for effects from that study. A study of the relationship between concentrations of $TEQ_{WHO\text{-}Avian}$ and effects on bald eagles [75] has a similar limitation. However, the NOAEL derived for bald eagles was greater than concentrations of $TEQ_{WHO\text{-}Avian}$ in blood plasma of nestling GHO in both the study and reference areas, thus it is unlikely that GHO nestling plasma concentrations exceed threshold effect concentrations for Bald Eagles (Figure 2.3).

No studies have related concentrations of $TEQ_{WHO\text{-}Avian}$ in blood plasma of adult raptors with responses. Therefore, HQs were not calculated based on concentrations of for adult GHOs. If the adult plasma $TEQ_{WHO\text{-}Avian}$ concentrations are directly compared to the plasma-based TRV derived for nestlings, the HQs are greater than 1.0 in both the reference and study areas. However, comparing concentrations of $TEQ_{WHO\text{-}Avian}$ in blood plasma of adults to a TRV based on responses of nestlings is considered to be

overly conservative. This is because the plasma $TEQ_{WHO\text{-}Avian}$ concentrations are greater in the parenting adults as compared their nestlings which are most sensitive to AhR-mediated effects [83]. While trapping adult owls is more labor intensive than collecting blood from nestlings, the data from adults could prove useful in future assessments of the efficacy or remedial actions. Adult GHOs have been known to live up to 28 years in the wild [16] and occupy their territories year-round. Monitoring their exposure levels and productivity could provide valuable data for understanding how dioxin-like contaminants accumulate or are metabolized over an owl's lifetime and what effects, if any, the chronic exposure may have on their productivity.

The maximum concentration of $TEQ_{WHO\text{-}Avian}$ in addled eggs from the study area (Table 2.3) was less than the LOAEL from the Nosek study [43] and the NOAELs based on the studies of McLane [14] and Hoffman [84] (Figure 2.4). These results further suggest that concentrations of $TEQ_{WHO\text{-}Avian}$ in soils and sediments the Tittabawassee River floodplain would be unlikely to result in population-level effects on GHOs inhabiting that habitat.

Productivity and Abundance

Great horned owl productivity (# fledglings/breeding pair) was slightly greater in the study area than in the reference (Table 2.4). Productivity was similar to that reported for less contaminated areas and considered normal for the owls in the temperate forest. In a survey of 1,236 nesting attempts over 28 years, Holt observed a mean productivity of 1.3 young/breeding attempt in the Cincinnati, Ohio, USA, area [12]. It has been suggested

#fledged/territorial pair is a more conservative measure of raptor productivity [12], as failure to breed is a measure of population health. However, GHOs are known to skip breeding about every third year in temperature regions. Using territorial pairs, rather than breeding attempts, as a basis for productivity would group a natural predilection to skip breeding with failed breeding attempts, and thereby underestimate the population's productivity.

The mean GHO population density in the Tittabawassee River floodplain (0.36 pairs/river km in the study area) (Table 2.4) is considerably greater than the 0.10-0.20 pairs/km^2 identified in other studies of GHO density in prime habitat and the territory density in the reference areas (0.14 pairs/river km). The greater GHO abundance in the study area may be due to land use and microhabitat differences.

Territory occupancy was determined for GHOs inhabiting the reference and study areas by repeatedly surveying the rivers and checking nests over the period of 2005-2007 (Figure 2.4). The study area GHO territory density (0.36 territories/river km) was greater than the relative abundance estimate suggests (0.24 territories/river km). The relative abundance also underestimated GHO territory occupancy in the reference area (occupancy: 0.14 territories/ river km; estimated: 0.09 territories/ river km). This is expected since nest visits identified occupied territories of owls that did not always respond during surveys. Owls that were out of human earshot during surveys would lead to an underestimate of GHO density. The GHO occupancy determined in 2005-2007 would not have been possible without the call-response surveys, but the disparity between the occupancy and relative abundance measurements underscores the usefulness of reconnaissance to confirm territory occupancy status.

Multiple Lines of Evidence

This study was part of a three-pronged multiple lines of evidence risk assessment that examined great horned owl population health, dietary exposure to PCDD/DFs and tissue concentrations of PCDD/DFs in the Tittabawassee River floodplain. GHOs in the Tittabawassee River floodplain downstream of Midland, MI were exposed greater concentrations of PCDD/DFs and have some of the greatest plasma $TEQ_{WHO\text{-}Avian}$ concentrations observed in raptors in the wild. However, nestling blood plasma $TEQ_{WHO\text{-}Avian}$ concentrations were not sufficient to cause adverse effects. The concentration of $TEQ_{WHO\text{-}Avian}$ in the addled eggs was less than conservative, lab-based TRVs. In addition, GHO productivity and abundance in the study area were greater than the upstream reference areas, suggesting that despite their exposure to PCDD/DFs, great horned owls in the study area are not experiencing adverse population effects. Furthermore, the exposure and population health measure results described herein are in agreement with the results from the site-specific dietary exposure assessment (Chapter 1), which found that GHOs in the study area are not at risk for adverse effects from dietary exposure to PCDD/DFs. The concordance among the three lines of evidence are consistent with the conclusion that, while exposed to significant concentrations of PCDD/DFs from the soils, sediments and prey items, great horned owls are not at risk from that exposure, and populations of GHOs in the Tittabawassee River floodplain are not being adversely affected.

CONCLUSION

Great horned owls (*Bubo virginianus*; GHO) inhabiting the Tittabawassee River floodplain downstream of Midland, MI, USA, are not at risk for adverse effects from their exposure to the polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-*p*-dioxins (PCDD) in the floodplain soils and sediments. This conclusion is supported by the convergence of multiple lines of evidence, including dietary exposure, tissue concentrations and population health. Great horned owl dietary exposure to PCDD/DFs in the study area was greater than in the reference area, but lesser than no-observed-adverse-effect-levels from laboratory-based exposures. The primary prey items of Tittabawassee River basin GHOs based on biomass and caloric intake were E. cottontail rabbits and muskrats, both of which are herbivores with relatively low body burdens of PCDD/DFs. The PCDD/DF concentrations in GHO adult and nestling plasma and addled eggs from the study area reflect this low dietary exposure. While tissue concentrations of PCDD/DFs were greater in the study area than in the reference area, the concentrations were lesser than concentrations at which adverse effects could be expected. Finally, there is no evidence that the GHO population downstream of Midland, MI, is adversely impacted by its exposure to PCDD/DFs. The owls' reproductive success (#fledglings/breeding attempt) was greater for owls residing in the study area as compared to the reference area, and reproductive parameters were within the normal range for the region. In addition to their slightly greater reproductive success, GHOs were more abundant in the study area and had a greater territory density than the reference area population.

Closing Statement

While a comprehensive five-year study may not always be feasible, it is recommended that researchers seek a thorough grounding in the populations they study. The extra effort involved in constructing the site-specific GHO diet and potential average daily dose, trapping adults, performing extra reconnaissance in known territories and surveying the study and reference areas multiple times substantially reduced uncertainties and provided a thorough portrait of the GHO population in the Tittabawassee River floodplain.

This study underscores the importance of incorporating real, measured data in risk assessments. Risk managers often rely on models laden with conservative uncertainty factors that, in the case of the Tittabawassee River floodplain owls, would dramatically overestimate the owls' exposures to PCDD/DFs. Remediation efforts based off protective models could be unnecessary and invasive remedial actions would likely lead to the destruction of the owls' habitat. Decisions made in ignorance could dismantle a healthy population – all in the name of its survival.

APPENDIX

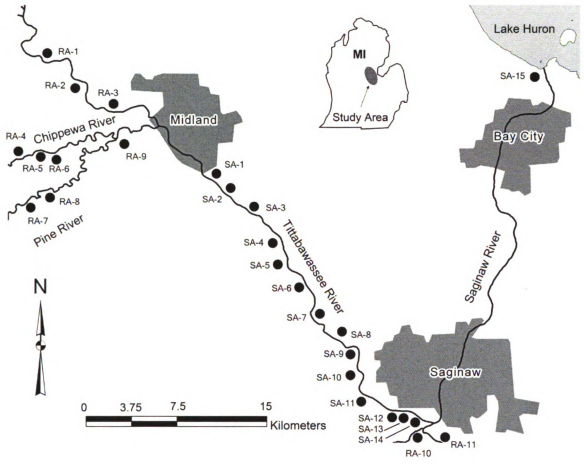

Figure A.1. Great horned owl (*Bubo virginianus*) territories in the Tittabawassee River reference and study area floodplains in Midland, MI, USA, 2005-2008.

Table A.1 Productivity data collected for great horned owl (*Bubo virginianus*) territories in the Tittabawassee River floodplain downstream of Midland, MI USA, 2005-2007

	Year		
Territory	2005	2006	2007
SA-1	F(1)	F(2)	F(2)
SA-2	U	U	U
SA-3	S	P	U
SA-4	F(1), AE	F(1)	F(2)
SA-5	F(1)	F(2)	FN, AE
SA-6	S	F(1)	U
SA-7	F(2)	F(1), AE	FN, AE
SA-8	F(1)	F(1)	F(1), NM(1)
SA-9	U	P	F(2)
SA-10	U	1F	P
SA-11	F(1)	F(2)	FN
SA-12	F(1)	F(2)	U
SA-13	F(1)	F(2)	F(1)
SA-14	U	U	F(1)
SA-15	F(2)	F(2)	F(2)

U=unoccupied
S=occupied by a single adult
P=territorial pair, no evidence of breeding activity
F(#)=breeding pair, # nestlings fledged
NM(#)=breeding pair, # nestling mortality
AE=breeding pair, addled egg collected or observed
FN=breeding pair, failed nest

Table A.2 Productivity data collected for great horned owl (*Bubo virginianus*) territories in the reference areas upstream of the Tittabawassee River floodplain in Midland, MI USA, 2005-2007

	Year		
Territory	2005	2006	2007
RA-1	F(1)	F(1)	F(1)
RA-2	U	P	S
RA-3	P	U	AE
RA-4	N/A	S	N/A
RA-5	P	U	U
RA-6	S	S	U
RA-7	S	P	NM(1)
RA-8	U	P	P
RA-9	S	F(2)	S
RA-10	N/A	N/A	F(1)
RA-11	F(1), AE	F(1), NM(1)	S

U=unoccupied
S=occupied by a single adult
P=territorial pair, no evidence of breeding activity
F(#)=breeding pair, # nestlings fledged
NM(#)=breeding pair, # nestling mortality
AE=breeding pair, addled egg collected or observed
FN=breeding pair, failed nest
N/A=data not collected

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