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GREAT HORNED OWL (*BUBO VIRGINIANUS*) DIETARY EXPOSURE TO PCDD/DF IN THE TITTABAWASSEE RIVER FLOODPLAIN IN MIDLAND, MICHIGAN, USA

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Abstract—Soils and sediments in the floodplain of the Tittabawassee River downstream of Midland, Michigan, USA contain elevated concentrations of polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-*p*-dioxins (PCDD). As a long-lived, resident top predator, the great horned owl (*Bubo virginianus*; GHO) has the potential to be exposed to bioaccumulative compounds such as PCDD/DF. Site-specific components of the GHO diet were collected along 115 km of the Tittabawassee, Pine, Chippewa, and Saginaw Rivers during 2005 and 2006. The site-specific GHO biomass-based diet was dominated by cottontail rabbits (*Sylvilagus floridanus)* and muskrats (*Ondatra zibethicus*). Incidental soil ingestion and cottontail rabbits were the primary contributors of PCDD/DF to the GHO diet. The great horned owl daily dietary exposure estimates were greater in the study area (SA) (3.3 to 5.0 ng 2,3,7,8-TCDD equivalents (TEQ$_{WHO-avian}$)/kg body wt/d) than the reference area (RA) (0.07 ng TEQ$_{WHO-Avian}$/kg body wt/d). Hazard quotients (HQs) based on central tendency estimates of the average daily dose and no-observable-adverse effect level (NOAEL) for the screech owl and uncertainty factors were <1.0 for both the RA and the SA. Hazard quotients based on upper end estimates of the average daily dose and NOAEL were <1.0 in the RA and up to 3.4 in the SA. Environ. Toxicol. Chem. 2010;29:2350–2362. © 2010 SETAC

Keywords—Dioxin   Furan   Risk   Raptor   Equivalents

INTRODUCTION

Soils and sediments of the Tittabawassee River floodplain in mid-Michigan, USA 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 (Midland, Michigan, USA) [2]. 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 [3]. 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) [4–12].

To determine if the PCDD and PCDF in the soils and sediments 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 status and conditions including reproductive productivity. The convergence or divergence of the three lines of evidence was used to help determine the potential for adverse effects of PCDD/DF [13].

As top terrestrial predators in the Tittabawassee River floodplain, GHOs 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) [14] and relatively great ingestion rate [15], the GHO could be exposed to significant concentrations of PCDD/DF 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 [16–20].

Herein are reported the results of a site-specific dietary exposure assessment for resident GHOs exposed to PCDD/DF 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, and 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 (Fig. 1). The upstream and downstream boundaries of the SA are Midland 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 concentrations of PCDD/DF in the region [1].

Nests

Great horned owls will readily occupy artificial nesting structures [21,19]. By taking advantage of this behavior researchers can encourage owls to nest in accessible locations and trees that can be safely climbed. For the present study, 75 cone-shaped nests were constructed by weaving dogwood and willow through chicken wire following previously described methods [21]. Two to three nests were deployed in each of nine RA and 13 SA GHO territories that were identified through call-response surveys and incidental observations [22]. Additional nests were installed in areas with good habitat but unconfirmed GHO occupation. In total, 28 nests were placed in the reference areas over approximately 35 km of river, while 47 nests were placed in the SA spanning approximately 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 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 to 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 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 [23]. The pellets can be used to identify the owls prey. 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 to allow 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, prey remains were transferred into
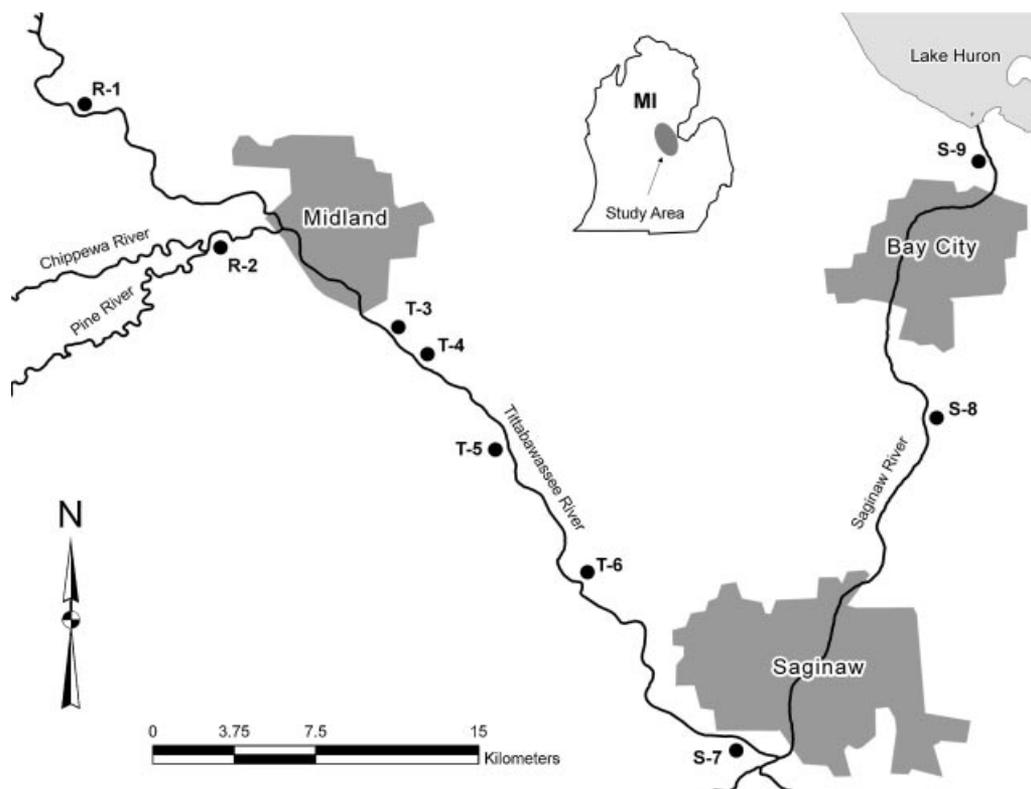

Fig. 1. Study locations within the Chippewa, Tittabawassee, and Saginaw River floodplains, Michigan, USA. Reference areas (R-1 and R-2); Tittabawassee River study areas (T-3 to T-6); and Saginaw River study areas (S-7 to S-9).

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 [23]. 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 [23–26]. 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 [26] using appropriate mammal and bird guide books [27,28] and a reference for Michigan crayfish [29]. Since the distribution of sizes of individuals in the diet was unknown, the average size of adults was applied as a conservative measure of 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. [30] and Fredricks et al. [31]. Dietary items were sampled from two locations in the RA (R-1, R-2) and five locations in the SA (T-3 to T-6 and S-7) (Fig. 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. This was done to remove their stomach contents and avoid double counting since incidental soils were also included in the diet. 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 below the tibiotarsal articulation and feathers.

Based on historical data [1] polychlorinated biphenyls (PCBs) were not considered contaminants of concern in the Tittabawassee River floodplain, and therefore were not evaluated in detail in the present study. However, analysis for

dioxin-like PCBs (dl-PCBs) was conducted on a subset of samples, and the relative importance of dl-PCBs to PCDD/DF can be evaluated.

The PCDD/DF and dl-PCBs were quantified in accordance with EPA Method 8290 with minor modifications [32]. Briefly, samples were homogenized with anhydrous sodium sulfate and Soxhlet extracted in hexane:dichloromethane (1:1) for 18 h. Before extraction, known amounts of ^{13}C-labeled 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 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 PCDD and PCDF. 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/DF. Components were analyzed using high-resolution gas chromatography/high-resolution mass spectrometry. Hewlett-Packard 6890 GC (Agilent Technologies, Wilmington, DE, USA) was connected to a MicroMass$^{®}$ high resolution mass spectrometer (Waters). PCDF/DD congeners were separated on a DB-5 capillary column (Agilent Technologies) (60 m × 0.25 mm inner diameter [i.d.]). The mass spectrometer was operated at an EI energy of 60 eV with a 0.25-μm film thickness and an ion current of 600 μA. Congeners were identified and quantified by use of single ion monitoring 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 × 0.25 mm i.d., 0.25 μm film thickness) column (Agilent Technologies). Chemical analyses included pertinent quality assurance practices, including matrix spikes, blanks, and duplicates. Concentrations of TEQs$_{WHO-Avian}$ (ng/kg wet wt) were calculated for PCDD/DF and dl-PCBs where data were available by summing the product of the concentration of each congener, multiplied by its WHO avian 2,3,7,8-TCDD equivalency factor (TEF) [33].

Potential average daily dose

The potential average daily dose (ADD$_{pot}$) for GHO was calculated using the equation for wildlife dose from the Wildlife Exposure Factors Handbook [34] and the site-specific, biomass-based GHO diet (Eqn. 1).

$$\text{ADD}_{pot} = \Sigma(C_k \text{FR}_k \text{NIR}_k) \tag{1}$$

where C_k = concentration of TEQ$_{WHO-Avian}$ (wet wt) 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, wet wt)/bw (g, wet wt)/d).

Two approaches using Equation 1 were used to estimate the ADD$_{pot}$ from the measured tissue residue concentrations in prey items. 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. 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 (Ver. 2.10.0, R Foundation for Statistical Computing, Vienna, Austria, 2009, www.R-project.org) the ADD$_{pot}$ was repeatedly calculated (Eqn. 1), each time drawing a single randomly sampled dietary item (C_k) 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/DF in the Tittabawassee River floodplain [30].

A site-use factor of 1.0 was applied based on the conservative assumption that GHOs obtain 100% of their diet from within the 100-year floodplain. The normalized ingestion rate (0.072 g-ingested/g bw/d) was calculated from ingestion rates reported by Craighead and Craighead [35] for the GHO.

To the extent possible, concentrations in site-collected prey items 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 (*Vulpes vulpes*) (2.8%) [33], but more than the 0% suggested for red tail hawks (*Buteo jamaicensis*) [36]. Congeners of PCDD/DF in soil were assumed to be 80% bioavailable [37].

Statistical analyses

All statistical analyses were performed using Systat 11 software (Systat, 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 TEQ$_{WHO-Avian}$.

Hazard assessment

Hazard quotients (HQs) were used to determine potential for adverse effects from GHO dietary exposure to PCDD/DF. The HQs were calculated by dividing ADD$_{pot}$ dietary TEQ$_{WHO-Avian}$ by no-observed-adverse-effect-level (NOAEL) TRVs derived

Table 1. Dietary-based toxicity reference values (TRVs) for 2,3,7,8- tetrachlorodibenzo-*p*-dioxin equivalents (TEQ$_{WHO-Avian}$) for great horned owl (*Bubo virginianus*) potential average daily dose (ADD$_{pot}$). NOAEL = no observable adverse effect level; (LOAEL) =lowest observable adverse effect level.

	Dietary-based TRV (ng/kg body wt/d)	Study type	References
NOAEL	14.5	Feeding	[38]
LOAEL	14	i.p injection	[8,40]

from two avian feeding studies (Table 1), (Eqn. 2).

$$HQ = \frac{ADD_{pot}(ng\ TEQ_{WHO-Avian}/kg\ body\ weight/d)}{dietary\ TRV(ng\ TEQ_{WHO-Avian}/kg\ body\ weight/d)} \quad (2)$$

Because there is no single definitive TRV for GHO exposure to PCDD/DF, 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 wet weight Aroclor 1248 in their diet [38]. Aroclor 1248 is a PCB mixture with a dioxin toxicity equivalency factor of 2.57×10^{-4} kg/kg in birds [39]. 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, wet weight Aroclor 1248. By applying this factor to the NOAEL, a maximum NOAEL of 105 ng TEQ$_{WHO-Avian}$/kg body wt/d was attained. Using the allometric relationships, body weight and food consumption rates given by Sample et al. [40] the TRV was a daily dose of 14.5 ng TEQ$_{WHO-Avian}$ /kg body wt/d.

When ring-necked pheasants (*Phasianus colchicus*) were injected weekly intraperitoneally with 2,3,7,8-TCDD for 10 weeks [8] egg production and hatchability was less at 1 μg/kg/week (140 ng TEQ$_{WHO-Avian}$ /kg bw/d) [40]. No effects were observed at 0.1 μg/kg/week (14 ng/kg/d). Thus, NOAEL and LOAEL values of 14 ng TEQ$_{WHO-Avian}$ /kg bw/d and 140 ng TEQ$_{WHO-Avian}$ /kg bw/d were used as TRVs in the present study.

RESULTS

Diet reconstruction

Egested pellets and prey remains of GHO were collected from 13 nests during 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 omnivorous small mammals (e.g., white-footed and deer mice) dominated the GHO diet (36 and 27%, respectively). On a biomass basis, however, cottontail rabbits and muskrats represented the bulk of GHO dietary intake (57 and 22%, respectively). The GHO biomass-based diet also included meadow voles (6.5%), waterfowl (5.2%), passerine birds (5.5%), herbivorous small mammals (2.0%), star-nosed moles (0.09%), short-tail shrews (0.04%), and crayfish (0.04%) (Fig. 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
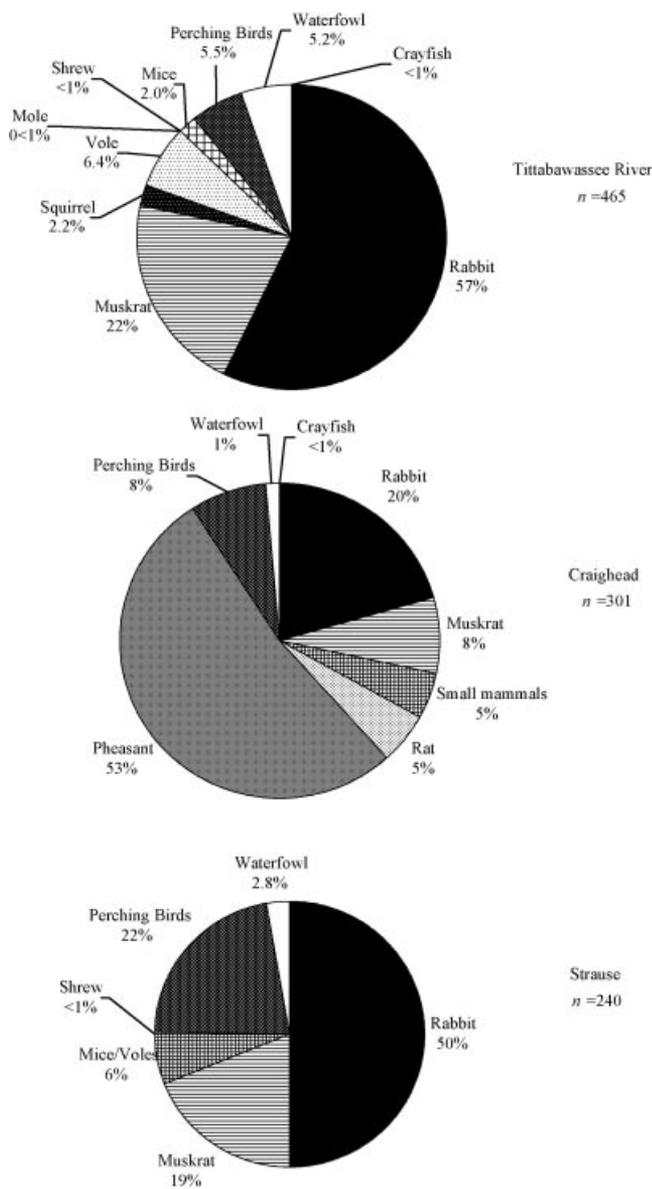
Fig. 2. Great horned owl (*Bubo virginianus*) mass-based site-specific and literature-based dietary compositions. Literature-based diets are from Strause et al. [17] and Craighead and Craighead [35].

for which concentrations of PCDD/DF were available composed 93% of the biomass of the GHO diet (Table 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 concentrations by weight. Masked (*Sorex cinereus*) and short-tailed shrew (*Blarina brevicauda*) 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 (*Sciurus niger*) and waterfowl, data from separate Tittabawassee River floodplain studies, where wild game species were collected and edible portions analyzed for PCDD/DF [41,42] were used. In those studies there was not a significant difference in PCDD/DF concentration in cottontail rabbit (*Sylvilagus floridanus*) 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 [41,42] wood duck (*Aix sponsa*) muscle had six-fold greater PCDD/DF concentrations than turkey muscle, and approximately 30-fold greater PCDD/DF concentrations than muscle from Canada geese (*Branta canadensis*) collected in the vicinity of S-7 (Fig. 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 ($TEQ_{WHO-Avian\ TEFs}$) ranged from 8 ng/kg for the meadow vole to 610 ng/kg for combined masked and short-tailed shrews (surrogates for the star-nosed mole) in the study area. Geometric mean $TEQ_{WHO-Avian}$ 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 $TEQ_{WHO-Avian}$ concentrations of the sampled prey items contributed by dl-PCB concentrations in the SA ranged from 2.8 ng/kg for small mammals to 11 ng/kg for passerine nestlings (Table 3). Geometric mean $TEQ_{WHO-Avian}$ concentrations of the sampled prey items contributed by dl-PCB concentrations in the SA ranged from 2.8 ng/kg for small mammals to 10.6 ng/kg for passerine nestlings, and in the RA ranged from 3.2 ng/kg for small mammals to 12.3 ng/kg for passerine nestlings (Table 3). In general, the lesser PCB concentrations reflect the limit of detection for PCB congeners.

Potential average daily dose

Using the traditional approach to estimating ADD_{pot}, the geometric mean GHO ADD_{pot} of $TEQ_{WHO-Avian}$ from PCDD/DF was 47-fold greater in the study area than the reference area, and the upper estimate based on the 95 UCL_{gm} was approximately 90-fold greater in the study area than the reference area (Table 4). Using the resampling method, the median and 95th centile of the GHO ADD_{pot} of $TEQ_{WHO-Avian}$ from PCDD/DF were 76- and 200-fold greater in the study area than the reference area, respectively (Table 4). Cottontail rabbits and incidental soil ingestion contributed the majority of the GHO ADD_{pot} (Fig. 3).

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 $TEQ_{WHO-Avian}$ in the reference area (Fig. 4, Table S1 and S2). With the addition of soil ingestion, 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF contributed 99% of the PCDD/DF $TEQ_{WHO-Avian}$ 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%) (Fig. 4, Table S1 and S2).

The data set for dl-PCBs was limited and because many PCB congeners were at or below detection limits, it was not possible

Table 2. Geometric mean and 95% upper confidence level (UCL) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent (TEQ$_{WHO-Avian}$) concentrations from polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzo furans (PCDD/DF) in great horned owl (*Bubo virginianus*) prey items collected from the reference area (RA) and study area (SA) in the Tittabawassee River (MI, USA).

PCDD/DF	Reference Area (RA)			Study Area (SA)		
	n	Geomean	95% UCL	n	Geomean	95% UCL
Dietary component concentrations ng PCDD/DF TEQ$_{WHO-Avian}$/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]	66	7.90	10	127	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 3. Geometric mean and 95% upper confidence level (UCL) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent (TEQ$_{WHO-Avian}$) concentrations from polychlorinated biphenyls (PCBs) in great horned owl (*Bubo virginianus*) prey items collected from the reference (RA) and study areas (SA) in the Tittabawassee River (MI, USA). NA = not available.

PCB	Reference Area (RA)			Study Area (SA)		
	n	Geomean	Max	n	Geomean	Max
Dietary component concentrations ng PCB TEQ$_{WHO-Avian}$/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	4.0	4.0
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.

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$_{WHO-Avian}$. In contrast, in the SA, dl-PCB concentrations appear at background, or only slightly above background, and the relative contribution of dl-PCB is inversely related to PCDD/DF concentrations. For example, in other small mammals and passerines, TEQ$_{WHO-Avian}$ from PCBs contributes about 3 to 5% of that

of TEQ$_{WHO-Avian}$ from PCDD/DF, whereas in meadow voles TEQ$_{WHO-Avian}$ from PCBs is approximately 60% of that of TEQ$_{WHO-Avian}$ from PCDD/DF. The proportion of the $\sum$TEQ in the diet of GHO that was contributed by dioxin-like PCB congeners was approximately 3% in the study area.

Hazard assessment

Hazard quotients were calculated based on the geometric mean and 95% UCL$_{gm}$ (traditional approach) for the site-specific diet. The HQs were less than 1.0 for the site-specific

Table 4. Potential average daily dose (ADD$_{pot}$) (ng TEQ$_{WHO-Avian}$/kg bw/d) from polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzo furans (PCDD/DF) for great horned owls (*Bubo virginianus*) in the Tittabawassee River (MI, USA) floodplain from the site-specific and two literature-based diets. 95% UCL$_{gm}$= upper confidence limit of the geometric mean.

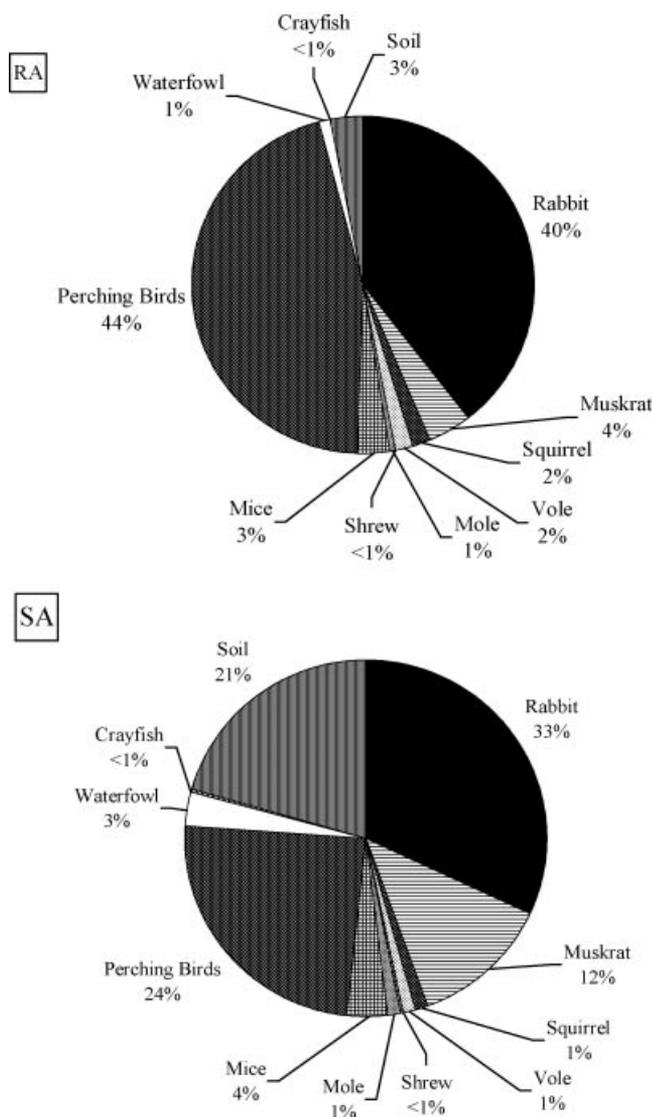| Diet	Traditional Approach ADD$_{pot}$ Geometric mean (95% UCL$_{gm}$) ng TEQ$_{WHO-Avian}$/kg body weight/d		Resampling Approach ADD$_{pot}$ 50th centile (95th centile) ng TEQ$_{WHO-Avian}$/kg body weight /d	
	Reference Area (RA)	Study Area (SA)	Reference Area (RA)	Study Area (SA)
Site-specific	0.07 (0.09)	3.3 (7.1)	0.07 (0.23)	5.0 (47)
1940s Michigan [34]	0.09 (0.13)	5.8 (9.6)	0.06 (0.23)	7.2 (22)
Kalamazoo [17]	0.16 (0.20)	5.7 (9.5)	0.14 (0.81)	7.6 (45)

Fig. 3. Prey item contributions to great horned owl (GHO) geometric mean, potential average daily dose (ADD_{pot}) in the reference (RA) and study (SA) areas in the Tittabawassee River floodplain in Midland, MI, USA.

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 RA was 0.005 (Nosek NOAEL and 95% UCL_{gm} ADD_{pot}). 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 (Fig. 5).

Hazard quotients were calculated using the median and 95th centile (resampling method) of the GHO ADD_{pot}. HQs based on median and 95th centile ADD_{pot} in the RA were 0.005 and 0.016, respectively. In the SA, HQs for the median and 95th centile ADD_{pot} were 0.36 and 3.4, respectively, based on the NOAEL [7]. Similarly, using the resampling approach to estimate GHO ADD_{pot} with the dietary item concentrations from the present study but using literature-based diets [17,34], the HQs based on median estimates of ADD_{pot} were less than 1.0, whereas HQs based on 95th centile estimates of the ADD_{pot} were 1.3 and 3.2 (Fig. 5). As in the traditional method, all

estimates of ADD_{pot} for $TEQ_{WHO-Avian}$ were at least three-fold less than the TRV based on the LOAEL.

DISCUSSION

Site-specific diet

The GHO diet varies in both composition and integration of trophic levels. The GHO diet has been well-characterized [26,35,44–51], 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, USA [45]. 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 [19].

The diet of GHOs in the Tittabawassee River floodplain differs from other reported Michigan GHO diets. Pheasants dominate the Craighead [35] diet (52.5% of diet by biomass) (Fig. 2). The Craigheads studied GHOs in Michigan in 1942 and 1948, 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 [52]. 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 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 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. [17] diet shows a preponderance of passerines (Fig. 2). Because passerine nestlings in the SA had greater $TEQ_{WHO-Avian}$ concentrations than most other GHO prey items collected from the floodplain (Table 2), an ADD_{pot} based on the diet observed by Strause et al. [17], like the Craighead diet, would overestimate GHO exposure to PCDD/DF (Table 4), although the upper estimates from the resampling approach were similar between the Strause et al. 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 contributed 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 greatest proportion of the geometric mean GHO dietary exposure (33%) (Fig. 3).

The prey items with the least tissue concentrations of PCDD/DF (rabbits, muskrats, and meadow vole) 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,
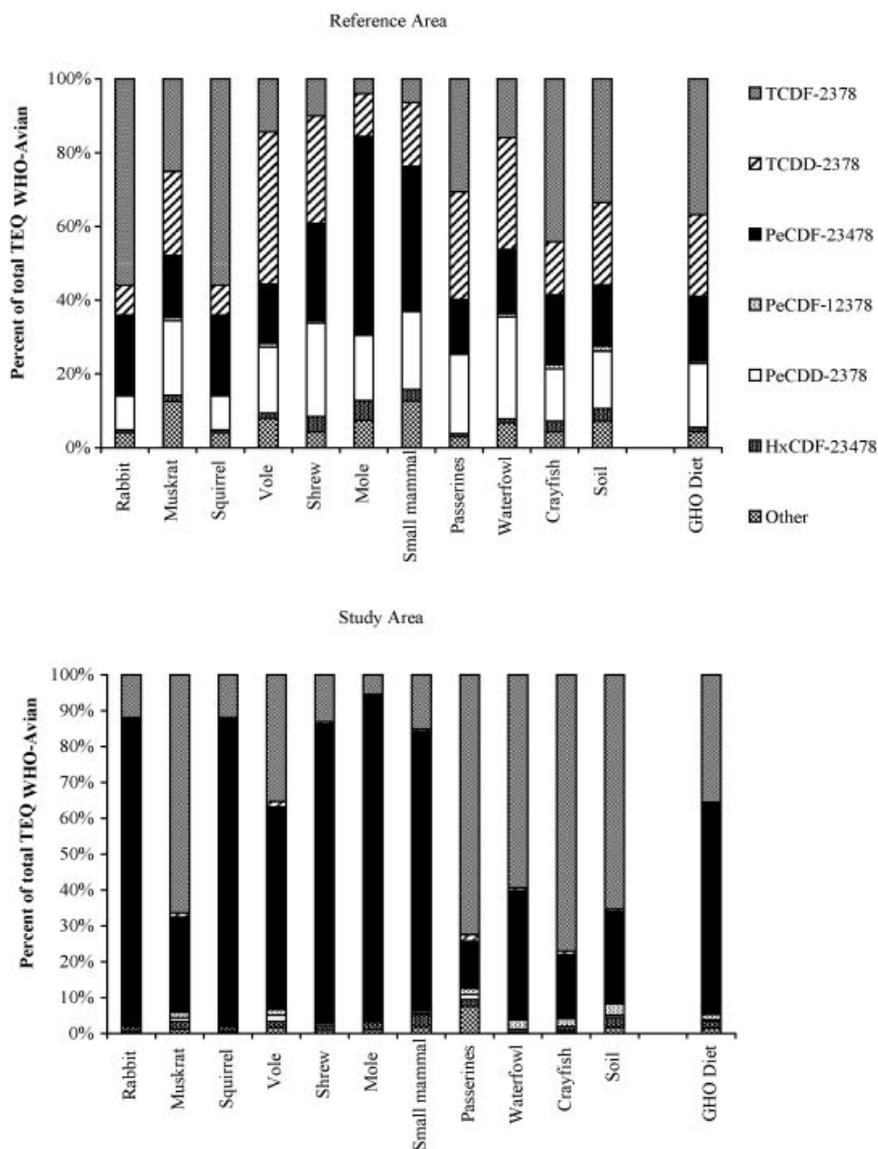
Fig. 4. Prey item contributions to 2,3,7,8-TCDD equivalents (TEQ$_{WHO-Avian}$) calculated based on World Health Organization (WHO) toxicity equivalence values (TEFs) in the great horned owl (GHO, *Bubo virginianus*) diet in the Tittabawassee River (MI, USA) reference (RA) and study areas (SA). TCDF = tetrachlorodibenzofuran; TCDD = tetrachlorodibenzodioxin; PeCDF = pentachlorodibenzofuran; PeCDD = pentachlorodibenzodioxin; HxCDF = hexachlorodibenzofuran.
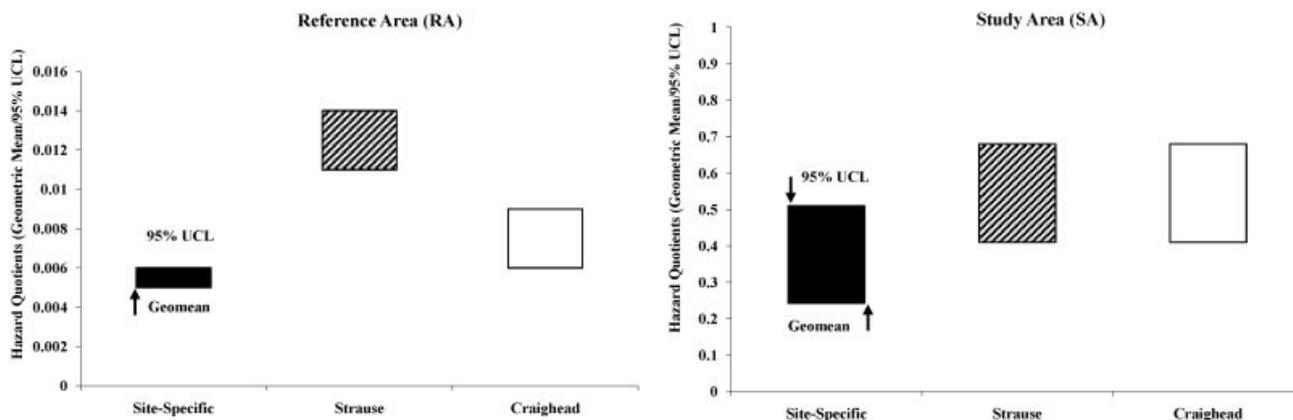

Fig. 5. Hazard quotients (HQ) for great horned owl (*Bubo virginianus*) based on dietary exposure to polychlorinated dibenzofurans and polychlorinated dibenzo-*p*-dioxins in the Tittabawassee River floodplain in Midland (MI, USA), predicted from dietary compositions that were based on a site-specific survey at the study area (SA) and two literature-based diets developed by Strause et al. [17] and Craighead and Craighead [35]. The range represented by the HQ values equivalent to the geometric mean (bottom of bar) and U95% CI (top of bar. UCL = upper confidence limit.

which do not readily take up dioxin-like compounds from soil. When plants do take up the chemicals, they are not readily translocated to the leaves [53,54]. 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 (Figs. 5 and 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 (Fig. 6). This is consistent with 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 $TEQ_{WHO-avian}$ for the RA and SA respectively, but there is uncertainty surrounding raptor soil ingestion and the bioavailability of PCDD/DF in floodplain soil. It has been suggested that incidental soil ingestion by terrestrial predators is approximately 2.8% of its diet (dw), based on red fox stomach content analysis [55]. 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 [36]. This is likely an underestimate, however, because the small mammals the owls prey on live in contact with soil and can contain soil in their gastro-intestinal tract, 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 GHO 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 $TEQ_{WHO-avian}$ /kg body weight/d, respectively, whereas with soil at 2.8% the median and 95th centile ADD_{pot} were and 6.4 and 48 ng $TEQ_{WHO-avian}$ /kg body weight/ 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 4. Uncertainty due to soil ingestion has a negligible effect on the upper end of the estimate.
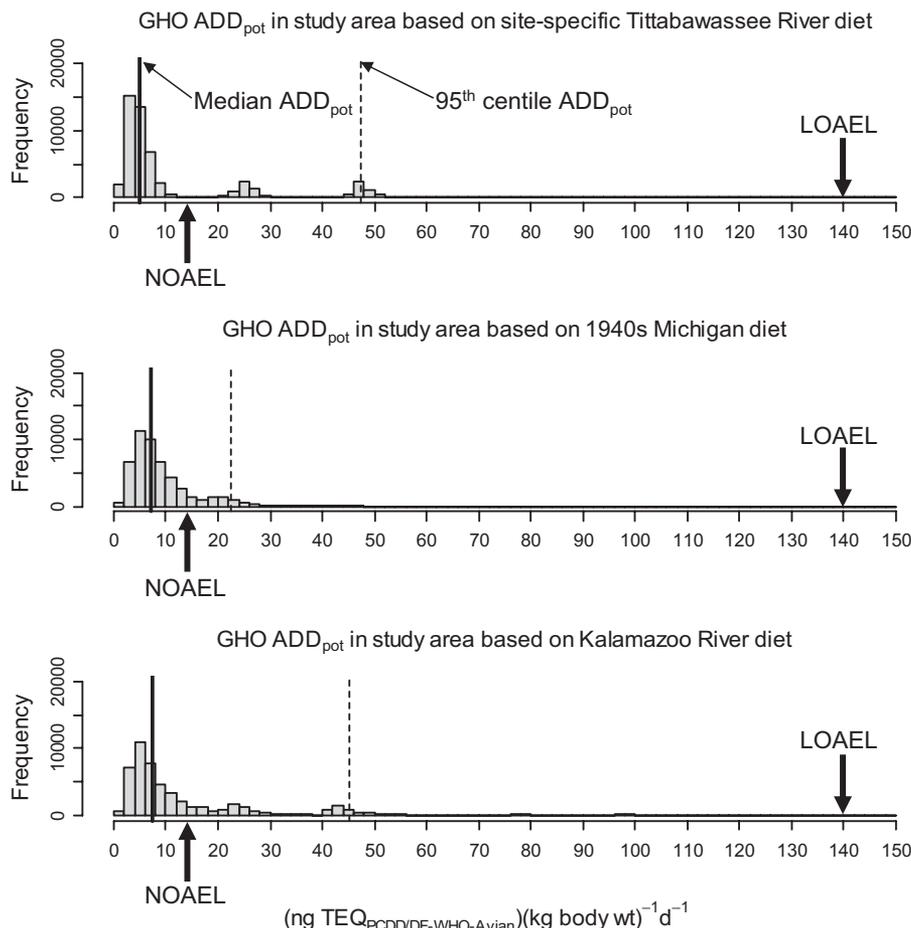

Fig. 6. Histograms of GHO (great horned owl, *Bubo virginianus*) daily dietary exposure estimates in the Tittabawassee River (MI, USA) study area based on the site-specific diet, a historical Michigan diet [35] and a Kalamazoo River diet [17]. Distributions were created by randomly sampling one item (measured concentration) from each dietary category to create 50,000 iterations of possible potential average daily dose (ADD_{pot}) results for Equation 1. The estimated NOAEL [8,38] and LOAEL [8] for dietary exposure are noted on each histogram. TEQ = 2,3,7,8-TCDD equivalents.

Along with soil ingestion rate, there is uncertainty surrounding the bioavailability of PCDD/DF from soil. Bioavailability can differ between soil type and species [56]. An estimate of the bioavailable fraction of 0.8 was used. In a feeding study conducted with White Leghorn chickens (*Gallus domesticus*), it was found that congeners with fewer chlorines were more bioavailable from sandy loam [37]. For example, tetra-substituted congeners were from 70 to 80% bioavailable. Tetrachlorinated congeners dominate the TEQ$_{WHO-Avian}$ 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 [57]. 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 greater than the bioavailability observed for PCDD/DF in Tittabawassee River soil samples fed to rats and pigs [56].

Because concentrations of PCDD/DF were not available for some prey items, surrogate values were used. These substitutions are unlikely to result in an underestimation of GHO exposure to PCDD/DF. The nestling passerine TEQ$_{WHO-Avian}$ 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 concentrations in squirrel muscle were available from a wild game study, the whole-body rabbit TEQ$_{WHO-Avian}$ concentrations was substituted because they were 70-fold greater than the concentrations in squirrel muscle and because they included fatty tissues and livers they are likely to be more representative of the tissues to which GHOs would be exposed. Waterfowl data were not available in most Tittabawassee River (TR) locations, so we assigned turkey muscle with skin to waterfowl. Although wood duck at S-7 (the only location where data were available) had greater TEQ concentrations than turkey at S-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. Since whole body waterfowl concentrations were not available, to consider the potential effect, the ADD$_{pot}$ was calculated with waterfowl data increased 6.3-fold to approximate a diet of wood duck, and 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, in the estimate of ADD$_{pot}$.

Another significant uncertainty in interpretation of these results is that of spatial resolution. The GHO ADD$_{pot}$ presented here assumes 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 discrete 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 TEQ$_{WHO-Avian}$ 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$_{WHO-Avian}$ the HQ could be underestimates. While concen-

trations of PCBs were not measured in all samples, concentrations of the dl-PCBs were measured in a few representative samples (Table 3). The PCB concentrations were at or near detection limits in many samples, and PCB concentrations from RA and SA were similar. An estimate of the ADD$_{pot}$ obtained for GHO at the SA using only the dietary categories available in Table 3, suggests including dl-PCBs in the ADD$_{pot}$ calculation would increase the estimate by approximately 8% in the SA. Based on this and the fact that in this area the contribution of PCNs is less than that of PCBs [58] and the relative potencies of PCNs [59] 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 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 potential dietary concentrations or doses. In contrast, the traditional method uses the equation to combine summary statistics (mean, geometic mean, median, and confidence limits) for each dietary category, but the combined summary statistics are not necessarily an accurate estimate of the summary statistics for the resultant distribution.

The median GHO dietary exposure estimate obtained from resampling and the geometric mean ADD$_{pot}$ determined with the traditional method are generally similar (Table 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 estimate than does the 95th centile in the resampling approach. The resampling approach identifies two outlying or extreme clumps in the distribution of GHO exposure estimates (Fig. 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$_{WHO-Avian}$ in these cottontail rabbits, both sampled at T-6 (Fig. 1), were 534 ng TEQ$_{WHO-Avian}$/kg and 1074 ng TEQ$_{WHO-Avian}$/kg. Without these two rabbits, the geometric mean TEQ$_{WHO-Avian}$ concentration for rabbits in the study area would have been 12 ng TEQ$_{WHO-Avian}$/kg, wet weight, and the SA GHO ADD$_{pot}$, based on the traditional method, would have been 2.7 ng TEQ$_{WHO-Avian}$/kg body weight/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 of ADD$_{pot}$ better illustrates the range of possible exposure. Furthermore, the variation evident in the distribution of the GHO ADD$_{pot}$ addresses the uncertainty discussed above regarding spatial variation in exposure within the Tittabawassee River floodplain. This is variation that may be overlooked using the traditional method of estimating the ADD$_{pot}$.

The greatest uncertainty in the risk characterization presented here is most likely in the denominator of Equation 2.
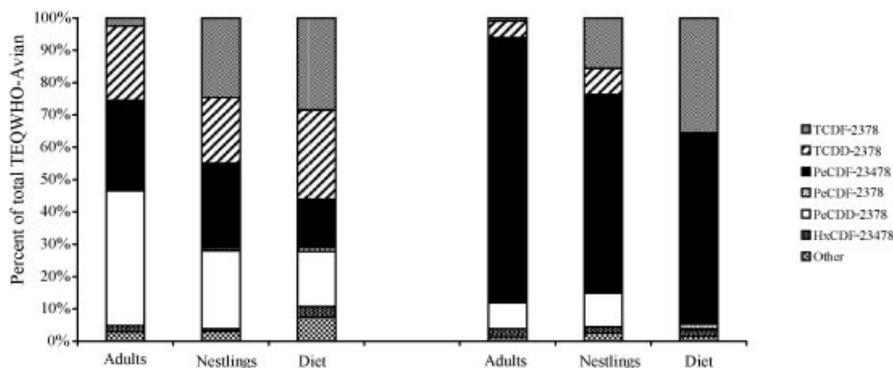
Fig. 7. Congener contributions to 2,3,7,8-TCDD equivalents (TEQ$_{WHO\text{-}Avian}$) based on the toxicity equivalency factors (TEFs) suggested by the World Health Organization (WHO). TEQ$_{WHO\text{-}Avian}$ concentrations in great horned owl (*Bubo virginianus*) nestling and adult plasma and diets in the Tittabawassee River floodplain in Midland, Michigan, USA.

Although there is reassurance in the convergence of the screech owl Aroclor feeding study and the pheasant dioxin intraparti-neal injection study to a single NOAEL of 14, there remains considerable uncertainty in comparison of these no-effect concentrations to the predominately furan exposure of 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. The TRV values used in this study were for pheasants and screech owls; thus, 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 [60]. 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 congener profiles of the GHO diet were determined by summing the weighted congener profiles of GHO prey items (the average congener contribution for each prey item was multiplied by that prey item's contribution to the GHO bio-mass-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 congener profile of the GHO diet. This is consistent with the congener profiles from adult and nestling GHO plasma, thereby increasing confidence in the dietary exposure assessment (Fig. 7, Table S3).

Hazard assessment

Great horned owl dietary exposure estimates in the present study indicate that adverse effects are unlikely. All median and geometric mean exposure estimates were at least two-fold less than the NOAEL TRV. Although upper estimates in some cases exceeded the NOAEL TRV by three-fold, the greatest upper exposure estimates were about three-fold less than the LOAEL TRV [8]. Even allowing for a 10% increase due to potential dl-PCB contribution and a 10% increase that might result from using surrogate concentrations in some prey item categories, this result 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

The present 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 the present study, that GHOs in the Tittaba-wassee 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 studies, which found that GHO reproduction, survival and abundance are not negatively impacted (Coefield et al., this issue) [22].

SUPPLEMENTAL DATA

Animal use and protocol. (20.5 KB DOC).

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