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RISK ASSESSMENT OF GREAT HORNED OWLS (*BUBO VIRGINIANUS*) EXPOSED TO POLYCHLORINATED BIPHENYLS AND DDT ALONG THE KALAMAZOO RIVER, MICHIGAN, USA

KARL D. STRAUSE,† MATTHEW J. ZWIERNIK,*† SOOK HYEON IM,‡ PATRICK W. BRADLEY,† PAMELA P. MOSELEY,† DENISE P. KAY,§ CYRUS S. PARK,† PAUL D. JONES,† ALAN L. BLANKENSHIP,†§ JOHN L. NEWSTED,†§ and JOHN P. GIESY†‖#

†Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48823, USA

‡DK Science Research & Development Center, DK B/D, 27-4 Gongduck-dong, Mapo-gu, Seoul, Korea

§ENTRIX, Okemos, Michigan 48823, USA

‖Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada

#Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, Special Administrative Region, China

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Abstract—The great horned owl (GHO; *Bubo virginianus*) was used in a multiple lines of evidence study of polychlorinated biphenyls (PCBs) and *p,p′*-dichlorodiphenyltrichloroethane (DDT) exposures at the Kalamazoo River Superfund Site (KRSS), Kalamazoo, Michigan, USA. The study examined risks from total PCBs, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{\text{World Health Organization [WHO]-Avian Toxicity Equivalency Factor [TEF]}}$), and total DDTs (sum of DDT, dichlorodiphenyldichloroethylene [DDE], and dichlorodiphenyldichloroethane [DDD]; ΣDDT) by measuring concentrations in eggs and nestling blood plasma in two regions of the KRSS (upper, lower) and an upstream reference area (RA). An ecological risk assessment compared concentrations of the contaminants of concern (COCs) in eggs or plasma to toxicity reference values. Productivity and relative abundance measures for KRSS GHOs were compared with other GHO populations. Egg shell thickness was measured to assess effects of *p,p′*-DDE. The concentrations of PCBs in eggs were as great as 4.7×10^2 and 4.0×10^4 ng PCB/g, wet weight at the RA and combined KRSS sites, respectively. Egg $TEQ_{\text{WHO-Avian}}$ calculated from aryl hydrocarbon receptor–active PCB congeners and WHO TEFs ranged to 8.0 and 1.9×10^2 pg $TEQ_{\text{WHO-Avian}}$/g, (wet wt) at the RA and combined KRSS, respectively. Egg ΣDDT concentrations were as great as 4.2×10^2 and 5.0×10^3 ng ΣDDT/g (wet wt) at the RA and combined KRSS, respectively. Hazard quotients (HQs) for the upper 95% confidence interval (UCI) (geometric mean) and least observable adverse effect concentration (LOAEC) for COCs in eggs were ≤1.0 for all sites. Hazard quotient values based on the no observable adverse effect concentration (NOAEC) 95% UCI in eggs were ≤1.0, except at the LKRSS (PCB HQ = 3.1; $TEQ_{\text{WHO-Avian}}$ HQ = 1.3). Productivity and relative abundance measures indicated no population level effects in the UKRSS.

Keywords—Raptors Bioaccumulation Terrestrial food chain 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin equivalents

INTRODUCTION

Due to the presence of elevated polychlorinated biphenyl (PCB) concentrations in fish, sediments, and floodplain soils, a portion of the lower Kalamazoo River was placed on the Superfund National Priorities List in August 1990 [1]. Polychlorinated biphenyls were used in the production of carbonless copy paper and paper inks for approximately 15 years [2]. During this period, recycling of paper, including some carbonless copy paper, resulted in releases of PCBs to the Kalamazoo River. The Kalamazoo River Superfund Site (KRSS) includes 123 km of river extending from the city of Kalamazoo, Michigan, USA, to Lake Michigan at Saugatuck, Michigan, USA. The primary contaminants of concern (COCs) are PCBs, including total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{\text{World Health Organization [WHO]-Avian Toxicity Equivalency Factor [TEF]}}$) from non-*ortho* (coplanar) and mono-*ortho* PCB congeners. However, other persistent polyhalogenated aromatic hydrocarbons such as *p,p′*-dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) (hereafter, ΣDDT) are also present. Each of these COCs has been linked to adverse reproductive effects in numerous mammals [3,4] and birds [5,6]. In addition to concerns about exposure through the aquatic food web, potential exposures of terrestrial-based receptors may also occur through the riparian floodplain soils that were former sediments in impoundments in the river. In 1986, three dams on the KRSS were partially dismantled, which exposed over 205 ha of PCB-contaminated former sediments, which now are floodplain soils. Concentrations of PCBs in surficial floodplain soils (0–25 cm) are generally greater than those of surficial sediments and range from <1 ng PCB/g (dry wt) to 8.5×10^4 ng PCB/g (dry wt) with a mean concentration of approximately 1.1×10^4 ng PCB/g (dry wt) [7–9].

The great horned owl (GHO; *Bubo virginianus*) was selected as a surrogate species to estimate risk to raptors in the terrestrial food chain. Raptors have long been used as environmental monitors [10,11]. Their sensitivity to the toxic effects of the types of COCs found at the KRSS, and their position at the top of the terrestrial food chain increases their potential for exposure to bioaccumulative contaminants. Great horned owls are highly territorial, year-round residents of the floodplain. Great horned owls also offer broad applicability as

* To whom correspondence may be addressed (zwiernik@msu.edu).

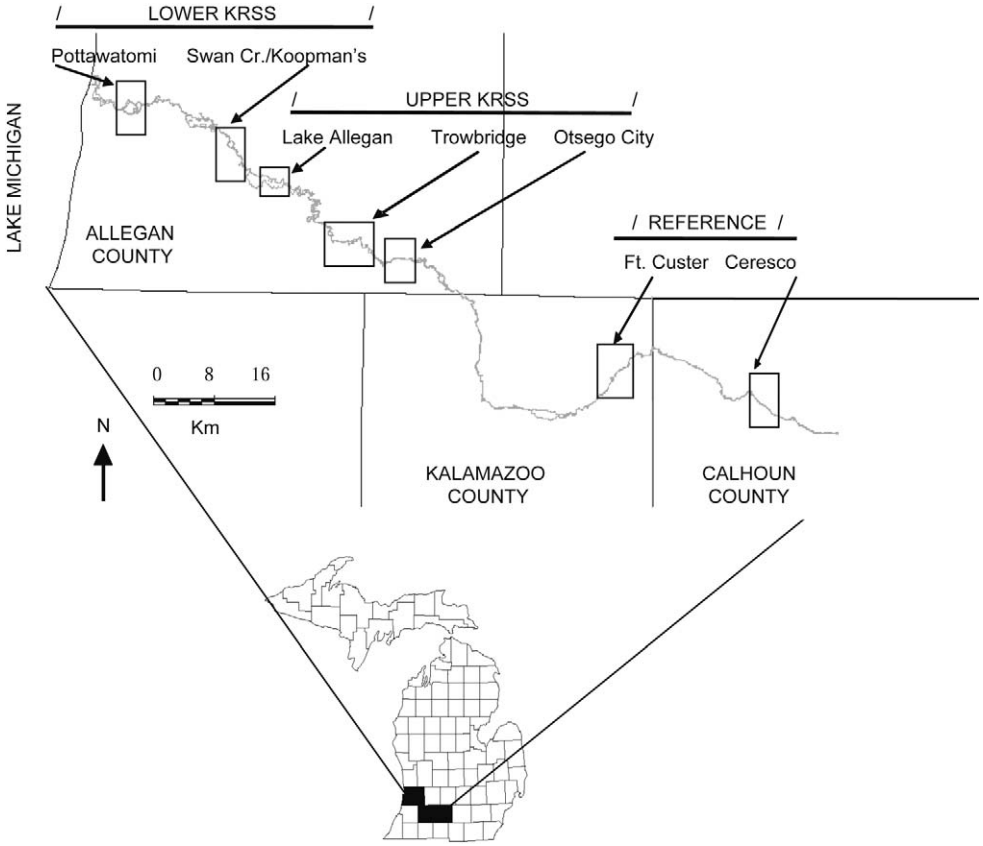

Fig. 1. Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, great horned owl (*Bubo virginianus*) study sites.

environmental monitors due to their longevity (up to 28 years in the wild) and stable rates of reproduction (normally one or two fledglings per year) [12]. The propensity of GHOs to use artificial nesting platforms also allows for better experimental control compared to wildlife studies that focus exclusively on natural nests. Great horned owl nestlings are sedentary and rely solely on prey collected by adults from areas proximal to the nest. Both eggs and nestlings are easily accessed, and GHO nestling exposure has been directly related to local contaminant concentrations (R.A. Frank, 1997, Master's thesis, University of Wisconsin, Madison, WI, USA). As a result, information on the GHO allowed site-specific estimation of the risk posed by PCBs, total $TEQ_{WHO\text{-}Avian}$, and ΣDDT to terrestrial raptors at the KRSS.

The 5-year study used multiple lines of evidence to assess the potential effects of PCBs and ΣDDT on resident GHO populations in support of a baseline ecological risk assessment [13]. The specific objectives of this study were to measure concentrations of total PCBs, $TEQ_{WHO\text{-}Avian}$, and ΣDDT in eggs and blood plasma of GHO nestlings; conduct a site-specific risk assessment based on measured concentrations of these residues; evaluate whether egg shell thinning was occurring at this site from historical sources of DDT and its metabolites; and determine the relative abundance, site use, and productivity of GHO at the KRSS, relative to a reference location upstream of the KRSS PCB sources.

MATERIALS AND METHODS

Study sites

Study sites within the KRSS were chosen to provide maximum exposure of resident GHO to PCBs during normal foraging activities associated with nesting and rearing of offspring. Study sites included three segments of the Kalamazoo River between the cities of Marshall and Saugatuck, Michigan, USA, a distance of approximately 190 km by river (Fig. 1 and Table 1). The upstream reference location represented current regional background exposures in the watershed where PCB concentrations in river sediments and floodplain soils were less than those in the KRSS (less than 180 ng PCB/g [dry wt]). The reference location included two areas upstream of the KRSS, on the Ceresco reservoir (CR) and Fort Custer State Recreation Area (FC). The upper KRSS (UKRSS) was located closest to known point sources of PCBs, and included the three formerly impounded areas (Otsego, Plainwell, and Trowbridge) and two additional sites at existing impoundments created by the Otsego City Dam and Calkins Dam (Lake Allegan). The lower KRSS (LKRSS) included areas located downstream of Calkins Dam, which is the first in-stream dam inland from Lake Michigan, and extended to Lake Michigan at Saugatuck. This stretch of river is characterized by a free-flowing channel and frequently inundated wetland forest and marsh habitat. The FC and Trowbridge (TB) areas on the river were the sites of additional investigations that were used to make direct comparisons between GHO responses on a ''high potential exposure'' (TB) versus background ''no elevated exposure'' basis.

Artificial nesting platforms were placed within study sites based on surveys of the GHO population and habitat. Locations of actively defended GHO territories were determined using call–response and nest location surveys [14]. Nest trees were selected on the basis of a qualitative habitat inventory. Nest platforms were placed to provide for a maximum exposure

Table 1. Kalamazoo River great horned owl (*Bubo virginianus*) study sites (Kalamazoo River superfund site [KRSS], Kalamazoo, MI, USA) physical and chemical characterization

Study site	Habitat[a]	Length[b] (km)	Area of former sediments[c] (ha)	Mean surficial PCBs[d] (ng/g, dry wt)
Reference area				
Ceresco	UH, SS	11.5	NA[e]	170
Ft. Custer	FH	5.6	NA	9
Upper KRSS				
Plainwell	WM	2.5	24	15,000
Otsego City	M, FH	2.7	NA	1,138
Otsego	WM	3.0	31	12,000
Trowbridge	SS, WM, FH	7.6	132	15,000
Lake Allegan	M, FH	13.7	NA	NS[f]
Lower KRSS				
Koopman's Marsh	M, FH	2.1	NA	545
Swan Creek Highbanks	M	3.0	NA	396
Pottawatomi Marsh	M, WM	4.3	NA	567

[a] UH = upland hardwoods; SS = scrub/shrub wetlands; FH = deciduous forested wetlands; WM = emergent wetlands, seasonally flooded wet meadow; M = emergent wetlands, semipermanently flooded marsh.
[b] Run of river.
[c] Formerly impounded floodplain.
[d] Arithmetic mean polychlorinated biphenyl (PCB) concentration in 0- to 15-cm depth.
[e] NA = not applicable.
[f] NS = Not sampled.

due to foraging in the most expansive areas of the contaminated floodplain. The numbers of nest sites (including both artificial and natural nests) monitored at each site were as follows: reference area (RA)-26, UKRSS-22, LKRSS-6.

Field sampling

Specific details and rationale for the GHO study design and detailed descriptions of the field methods and sampling techniques employed are provided elsewhere [15]. A brief discussion of the sampling methods for each phase of the sample collection and analyses activities is provided below.

Fresh or addled egg sampling

Fresh eggs were collected as soon as possible following confirmed initiation of incubation. Addled eggs [16] were collected when blood was sampled from nestlings 4 to 6 weeks post-hatch or in instances where nest abandonment had occurred. Eggs were labeled, transported back to the laboratory, and stored at 4°C until processed. Length, width, whole-egg weight, and whole-egg water volume were measured. Egg contents were removed, weighed, and saved for subsequent residue analyses. Eggshells were rinsed, air-dried, and eggshell thickness measured (to the nearest 0.01 mm) at two to eight places by use of a Starrett Model 1010M micrometer (L.S. Starrett, Athol, MA, USA). Dry shell weight was measured and normalized to egg volume to calculate a Ratcliffe Index value [17]. All concentrations of residues in eggs were corrected for moisture loss [18].

Nestling blood plasma collections

Blood samples were taken using previously described methods [19] when nestlings were approximately 4 to 6 weeks of age and had attained a minimum body weight of 0.75 kg. A sample of 5 to 7 ml was withdrawn from the brachial vein with a 25-gauge hypodermic needle and syringe and sterile technique. Blood was transferred to a heparinized Vacutainer™ (BD, Franklin Lakes, NJ, USA) and labeled. Vacutainers containing whole blood were centrifuged at 1,200 rpm for 10 min within 48 h of field sampling. Plasma (supernatant) was transferred to a new Vacutainer appropriately labeled and stored upright at −20°C until measurement of PCBs and ΣDDT. The nestlings were banded with U.S. Fish and Wildlife Service leg bands and total body weight, bill depth, and length of the culman, foot pad, and eighth primary feather measured following standard techniques [20] (data not presented) after which the birds were returned to the nest unharmed.

Predicted egg concentrations

Concentrations of total PCBs in nestling plasma were used to calculate predicted concentrations in eggs. Great horned owl nesting territories in the KRSS and RA were closely monitored to allow fresh egg and nestling plasma samples to be collected from within the same nest or nesting territory [15]. Polychlorinated biphenyl concentrations of the colocated samples were compared using regression methods to develop a relationship from which concentrations in eggs could be predicted from those in blood plasma [21]. For comparative purposes, predicted egg concentrations also are discussed in the risk assessment.

TEQ computation

Concentrations of $TEQ_{WHO\text{-}Avian}$ in bird tissues were calculated by summing the products of concentrations of individual non-*ortho* and mono-*ortho* PCB congeners (77, 81, 105, 118, 126, 156, 157, 167, 169) and their respective bird-specific WHO TEFs [22]. Polychlorinated-dibenzo-*p*-dioxins and polychlorinated-dibenzo-furans were not measured and were not included in TEQ computation. Whenever a congener was not detected, a proxy value equal to one-half the limit of quantification was multiplied by the toxic equivalence factor to calculate the congener-specific TEQs. Co-eluting congeners were evaluated separately. Polychlorinated biphenyl congener 105 frequently co-eluted with congener 132, congener 156 frequently co-eluted with 171 and 202, congener 157 co-eluted with congener 200, and congener 167 co-eluted with congener 128. In order to report the maximum $TEQ_{WHO\text{-}Avian}$, the entire

concentration of the co-elution groups was assigned to the mono-*ortho* congener. Overall contributions to total $TEQ_{WHO\text{-}Avian}$ from congeners 105, 156, 157 and 167 ranged from 11 to 13%, 5 to 19%, 1 to 2%, and 1 to 2%, respectively.

Relative abundance and site use (vocalization surveys)

Vocalization surveys consisted of an active method in which GHO hoots were broadcast to provoke responses (call–response method) from adult and juvenile owls [14,15]. Great horned owl relative abundance was monitored over three years (2000–2002) at the FC (reference) and TB (UKRSS) locations. Abundance and site use investigations were not conducted at the LKRSS. Hoot call–response surveys were conducted from late April through early January (up to two surveys per location per month) to determine the relative abundance and site use characteristics for juvenile, nonterritorial individuals (foraging adults) and territorial nesting pairs of owls. Surveys were completed under dry, windless conditions during crepuscular hours, beginning approximately 60 min prior to sunrise or approximately 30 min after sunset. Calls were broadcast at 0.5-km intervals within the river corridor at predetermined locations using a global positioning system to locate the exact coordinates.

Productivity monitoring

Active nests at the FC and TB locations were monitored to confirm fledgling success either visually and/or audibly during vocalization surveys (based on begging call responses of juveniles to broadcasts of adult GHO hoot calls). Productivity measurements were not conducted in the LKRSS.

Chemical analysis—Extraction and clean-up

Total concentrations of PCBs (congener-specific analysis) and ΣDDT were determined using U.S. Environmental Protection Agency method 3540 (SW846), Soxhlet extraction, as described elsewhere [23]. Measured quantities of plasma and egg were homogenized with anhydrous sodium sulfate (EM Science, Gibbstown, NJ, USA) using a mortar and pestle. All samples, blanks, and matrix spikes included PCB 30 and PCB 204 as surrogate standards (AccuStandard, New Haven, CT, USA). Extraction blanks were included with each set of samples. Quality assurance and quality control sets composed of similar tissues were included with each group of 20 samples. Concentrations of PCBs, including di-*ortho*- and mono-*ortho*-substituted congeners (coplanar) were determined by gas chromatography (PerkinElmer AutoSystem, Waltham, MA, USA, and Hewlett Packard, Palo Alto, CA, USA, 5890 series II) equipped with a ^{63}Ni electron capture detector. Concentrations of non-*ortho*-substituted PCB congeners and ΣDDT were determined by gas chromatograph mass selective detector (Hewlett Packard 5890 series II gas chromatograph interfaced to a HP 5972 series detector). Concentrations of the COCs were reported on a volumetric (plasma) and mass (egg) wet weight (wet wt) basis. A solution containing 100 individual PCB congeners was used as a standard. Individual PCB congeners were identified by comparing sample peak retention times to those of the known standard, and congener concentrations were determined by comparing the peak area to that of the appropriate peak in the standard mixture. Coplanar PCB congeners and ΣDDT were detected by selected ion monitoring of the two most abundant ions of the molecular cluster. The limit of quantification for di-*ortho*- and mono-*ortho*-substituted PCB congeners was conservatively estimated (minimum surface to noise ratio of 10.0) to be 1.0 ng PCB/g (wet wt), using an extraction mass of 20 g, a 25 pg/μl standard congener mix, and 1-μl injection volume. For coplanar PCB congeners and ΣDDT analytes, method detection limits varied among samples. This was achieved using sample-specific extraction mass and a minimum surface to noise ratio of 3.0 to maintain the method detection limit for all samples at <0.1 ng/g (wet wt). Either TurboChrom (PerkinElmer) or GC Chemstation software (Agilent Technologies, Wilmington, DE, USA) was used to identify and integrate the peaks. Total concentrations of PCBs were calculated as the sum of all resolved PCB congeners.

Table 2. Toxicity reference values (TRVs) for total polychlorinated biphenyls (PCBs), 2,3,7,8- tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), and total dichlorodiphenyltrichloroethane (ΣDDT) concentrations in great horned owl (*Bubo virginianus*) eggs. Reference number is located next to each value

	Tissue based TRV	Response endpoint[a]	Reference
Total PCBs (ng/g, wet wt)			
NOAEC[b]	7,000	EST, CS, EV, FS	[24]
LOAEC[c]	21,000	EST, CS, EV, FS	[24]
Total TEQs (pg/g, wet wt)			
NOAEC	135	EI, EV	[26–28]
LOAEC	400	EI	[26]
Total DDT (ng/g, wet wt)			
NOAEC	3,600	EST, FS	[29,30]
LOAEC	12,000	EST, EV, FS	[31,32]

[a] EST = egg shell thickness; CS = clutch size; EV = egg viability; FS = fledgling success; EI = enzyme induction.
[b] NOAEC = no observable adverse effect concentration.
[c] LOAEC = lowest observable adverse effect concentration.

Toxicity reference values

In the present study, tissue-based toxicity reference values (TRVs) were used to evaluate the potential for adverse effects due to PCBs, $TEQ_{WHO\text{-}Avian}$, and ΣDDT at each study site. Ideally, TRVs are derived from chronic toxicity studies in which a dose–response relationship has been observed for ecologically relevant endpoints in the species of concern, or a closely related species (e.g., other raptor species). Chronic studies must include sensitive lifestages to evaluate potential developmental and reproductive effects, and there must be minimal impact from cocontaminants on the measured effects. Toxicity reference values used in this assessment were based on values reported in the literature for no observable adverse effect concentrations (NOAECs) and lowest observable adverse effect concentrations (LOAECs) for total PCBs, $TEQ_{WHO\text{-}Avian}$, and ΣDDT in eggs of owls or similar raptor species (eagles, ospreys).

For PCBs in GHO eggs, TRVs based on the NOAEC and LOAEC were determined to be 7×10^3 and 21×10^3 ng PCB/g egg (wet wt). These TRV values are based on a feeding study with screech owls (*Otus asio*) exposed via diet to Aroclor 1248 in which no effects were seen at mean egg concentrations of 7.0×10^3 ng/g (wet wt) and a maximum concentration of 1.8×10^4 ng/g (wet wt) [24] (Table 2). Since a LOAEC was not determined in that study, the LOAEC was estimated by multiplying the NOAEC by an uncertainty factor of 3 [25].

No relevant studies on effects of $TEQ_{WHO\text{-}Avian}$ in the eggs of owl species were available from which to derive a TRV.

Thus, a tissue-based NOAEC for $TEQ_{WHO\text{-}Avian}$ in GHO eggs was estimated to be greater than 1.4×10^2 pg $TEQ_{WHO\text{-}Avian}$/g egg (wet wt) from the no observable effect concentration observed in bald eagle (*Haliaeetus leucocephalus*) chicks (presented on an egg basis) [26] with additional supporting evidence from studies of osprey (*Pandion heliaetus*) egg exposures [27,28]. A LOAEC concentration of 4.0×10^2 pg $TEQ_{WHO\text{-}Avian}$/g egg (wet wt), based on CYP1A induction, was also adopted from the lowest observable effect concentration determined in the same eagle study [26] (Table 2). It should be noted that no adverse effects on developmental or any other ecologically relevant endpoints were observed at these concentrations. Thus, these TRVs would be expected to be conservative and protective of GHOs.

A TRV based on the NOAEC for ΣDDT in GHO eggs was estimated to be greater than 3.6×10^3 ng ΣDDT/g egg (wet wt). The selection of this value as a conservative estimate of the TRV is supported by the analyses of effects presented by Wiemeyer et al. [29] for bald eagles and reinforced by Elliott and Harris [30], who identified 6.0×10^3 ng DDE/g egg (wet wt) as a LOAEC threshold for bald eagles. A LOAEC concentration of 1.2×10^4 ng ΣDDT/g egg (wet wt) was selected from the study of effects in the barn owl (*Tyto alba*) [31] and is supported by recommendations for bald eagles [32] (Table 2).

Risk assessment

Potential risk was assessed by calculation of hazard quotients (HQs) by dividing concentrations of PCBs, total $TEQ_{WHO\text{-}Avian}$, and ΣDDT measured in GHO eggs by tissue-based (egg) NOAEC and LOAEC TRVs identified for these chemicals (Table 2). Concentrations of total PCBs, total $TEQ_{WHO\text{-}Avian}$, and ΣDDT in eggs were considered to be the most sensitive measures of exposure with which to assess the potential effects of these COCs. When compared to the selected TRVs, this measure of exposure was considered to be a conservative estimate of risk at all life stages [5]. The HQs were calculated by dividing concentrations of each COC in egg (using both the lower and upper 95% confidence interval [CI] of the geometric mean) by the egg-based TRV. The shell-thinning effects of DDE were evaluated by comparing current measurements of eggshell thickness and shell weight (Ratcliffe Index) to the pre-1947 benchmark values reported for GHOs [33].

Statistical analyses

Data sets for each of the variables were analyzed for normality by use of the Kolmogorov–Smirnov, one-sample test with Lilliefors transformation, and for homogeneity of variance by Levene's test. Concentrations of COCs were generally log-normally distributed, and therefore all concentrations were log-transformed to more closely approximate the normal distribution. Variables that satisfied assumptions of normality and homogeneity (log-transformed values for ΣDDT in plasma, $TEQ_{WHO\text{-}Avian}$ in eggs, shell thickness, and Ratcliffe Index) were analyzed using parametric methods, including one-factor analysis of variance (ANOVA) with Tukey's honestly significant difference (multiple comparisons) and *t* test for simple pairwise comparisons. When parameters did not satisfy either or both assumptions of normality and homogeneity (log-transformed values for PCBs in eggs and plasma and ΣDDT in eggs), nonparametric statistical methods were used, including Kruskal–Wallis ANOVA and Median Test (multiple comparisons) and the Mann–Whitney *U* test. Associations between parameters were made with Pearson Product Correlations. Results of the vocalization survey expressed as relative abundance or site use were made with the chi-square test (χ^2). Tests for normality, homogeneity of variance and treatment effects (spatial trends) were completed using the Statistica (Version 6.1) statistical package (Statsoft, Tulsa, OK, USA). The criterion for significance used in all tests was $p < 0.05$.

For eggs and plasma, the experimental unit for concentrations of PCBs, ΣDDT, $TEQ_{WHO\text{-}Avian}$, and egg measurements (e.g., shell thickness, Ratcliffe index) was the nest. Where multiple samples were analyzed from the same clutch of eggs or brood of nestlings, analytical results for the associated samples were reported as the arithmetic mean for each nest.

RESULTS

Between 2000 and 2004, a total of 54 nesting sites (48 artificial and six natural) were sited and/or identified, and monitored for GHO occupancy with samples of eggs and/or blood plasma collected from some of these sites (Table 3). Total PCB and ΣDDT concentrations were measured in a total of 24 eggs and 16 nestling blood plasma samples that were collected from 25 active nests. Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were calculated only for eggs. After consolidating multiple egg collections, egg sample sizes for each COC were: total PCBs, $n = 17$; $TEQ_{WHO\text{-}Avian}$, $n = 15$; ΣDDT, $n = 17$. Relative abundance and site use estimates at FC and TB are based on the completion of 46 successful call–response surveys. Productivity measurements for FC and TB are based on observations of seven active nests that produced a total of seven fledglings.

Total PCB concentrations

Geometric mean concentrations of total PCBs in eggs of GHOs inhabiting the Kalamazoo River floodplain were progressively greater downstream than upstream. The least PCB concentrations were measured in samples from the upstream RA and the greatest concentrations occurred in eggs from the LKRSS (Table 4, Fig. 2). Geometric mean egg PCB concentrations at the RA and UKRSS sites were not significantly different from each other (Kruskal–Wallis, $p = 0.157$), but the geometric mean concentrations at both of these sites were significantly less than concentrations at the LKRSS (Kruskal–Wallis, $p < 0.05$). Concentrations of PCBs in blood plasma exhibited the same spatial distributions as eggs. The results of statistical testing reflect the limited sample sizes for RA ($n = 3$) and LKRSS ($n = 2$) plasma samples. Geometric mean concentrations of PCBs in blood plasma at both the RA and LKRSS sites were not significantly different from each other (Kruskal–Wallis, $p = 1.36$), and the geometric mean concentrations at both of these sites were significantly less than concentrations at the UKRSS (Kruskal–Wallis, $p < 0.01$) (Table 4).

Temporal trends in PCB concentrations in eggs or plasma were examined to identify potential confounding influences on GHO exposure to PCBs at the site. No trends were evident in PCB concentrations of eggs or plasma between 2000 and 2004 at any of the study sites where samples were collected in at least three of the five years of sampling (Kruskal–Wallis, $p > 0.38$).

Predicted egg concentrations

A total of 14 paired GHO nestling plasma and egg samples were obtained in this study. Log-normalized GHO PCB data

Table 3. Numbers of active great horned owl (*Bubo virginianus*) nests and samples collected by year (2000–2004)

	Sample site[a]			
	Reference area		Kalamazoo River Superfund Site (KRSS)	
	Ceresco	Ft. Custer	Upper KRSS	Lower KRSS
2000				
Active nests	0	0	1	2
Plasma	0	0	1	0
Eggs	0	0	0	3
Sampling scope	NS	RA, P, NP	RA, P, NP	E
2001				
Active nests	1	1	2	1
Plasma	0	1	4	0
Eggs	1	0	0	2
Sampling scope	E	RA, P, NP, E	RA, P, NP, E	E
2002				
Active nests	2	0	4	2
Plasma	1	0	3	2
Eggs	2	0	1	5
Sampling scope	E, NP	RA, P, NP, E	RA, P, NP, E	E, NP
2003				
Active nests	1	1	2	1
Plasma	1	0	1	2
Eggs	1	1	3	0
Sampling scope	E, NP	E, NP	E, NP	E, NP
2004				
Active nests	0	0	2	2
Plasma	0	0	0	0
Eggs	0	0	3	2
Sampling scope	E, NP	E, NP	E, NP	E, NP

[a] Kalamazoo River Superfund Site, Kalamazoo, Michigan, USA. NS = not sampled; E = egg; NP = nestling plasma; RA = relative abundance; P = productivity.

are plotted in Figure 3. The formula describing the egg-to-plasma relationship (conversion factor equation) for PCBs in GHOs is expressed on a log-basis as log (PCB_{egg} μg/g) = 1.647[log (PCB_{plasma} ng/ml)] − 2.578 ($r^2 = 0.666$, $p < 0.001$). Predicted mean concentrations of PCBs in eggs (μg PCB/g egg, wet wt) and ranges are given along with corresponding values measured in eggs for each sample site (Table 5). Predicted and measured geometric mean concentrations of PCBs in eggs from the RA and UKRSS were not significantly different (Mann–Whitney *U* test, $p = 0.65$ [RA], $p > 0.9$ [UKRSS]). The predicted geometric mean PCB concentration in eggs from the LKRSS was approximately one-third that measured in eggs. However, the difference between the predicted and measured concentrations was not statistically significant (Mann–Whitney *U* test, $p = 0.24$).

$TEQ_{WHO\text{-}Avian}$ concentrations

Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were calculated solely from GHO egg samples since minimum achievable method detection limits for individual coplanar PCB congeners in GHO plasma were limited by sample volume. Concentrations of $TEQ_{WHO\text{-}Avian}$ in GHO eggs at the RA and both KRSS sites were significantly correlated with concentrations of total PCBs ($r = 0.89$, $p < 0.001$). Concentrations of $TEQ_{WHO\text{-}Avian}$ were greater downstream than upstream with the greatest concentrations calculated for eggs from LKRSS. Geometric mean concentrations of $TEQ_{WHO\text{-}Avian}$ were significantly different among all three sites (ANOVA with Tukey's, $p < 0.005$) (Table 4, Fig. 2). All four non-*ortho*-substituted PCBs (International Union of Pure and Applied Chemistry [IUPAC] congener numbers 77, 81, 126, 169) and five of the eight mono-*ortho*-substituted PCBs (IUPAC numbers 105, 118, 156, 157, 167) were regularly detected in egg samples from the three study sites. Mono-*ortho*-substituted congeners 114, 123, and 189 were not detected. Together, the non-*ortho*-substituted PCB congeners contributed 73.4%, 67.4%, and 54.2% of total $TEQ_{WHO\text{-}Avian}$ at the RA, UKRSS, and LKRSS, respectively (Fig. 4). At least one of the non-*ortho*-substituted congeners monitored in the study was not present at concentrations greater than the detection limit in 80% of the samples from the RA site, 60% of the samples from the UKRSS, and 20% of the samples from the LKRSS. The rank order of the frequency of detection for both non-*ortho*- and mono-*ortho*-substituted PCBs in eggs was: RA, detected in 100% of samples (105, 118, 167, 169), 80% of samples (126, 157), 60% of samples (156), 40% of samples (77, 81); UKRSS, detected in 100% of samples (118, 126, 167, 169), 80% of samples (77, 105, 156, 157), 40% of samples (81); LKRSS, detected in 100% of samples (77, 105, 118, 126, 156, 157, 167, 169), 80% of samples (81).

Polychlorinated biphenyl congeners 81 and 126 have the greatest $TEF_{WHO\text{-}Avian}$ values relative to other congeners. Congeners 81 and 126 were detected in 53% and 93%, respectively, of all egg samples. Together they comprised 63.1% of the total concentration of $TEQ_{WHO\text{-}Avian}$ in eggs from the RA location, 61.8% at the UKRSS, and 42.1% at the LKRSS.

Relative potency

The relative contributions of non-*ortho*- and mono-*ortho*-substituted congeners can be evaluated by standardizing the

Table 4. Geometric mean, wet weight (range), total polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent ($TEQ_{WHO\text{-}Avian}$) concentrations, relative potency, and lipid concentrations in great horned owl (*Bubo virginianus*) eggs and plasma (total PCBs only) from the Kalamazoo River Superfund Site (KRSS), Kalamazoo, Michigan, USA

	Plasma PCB			Egg PCB[b]			$TEQ_{WHO\text{-}Avian}$[c]		Relative potency[cd]			Lipid[e]		
Study site[a]	ng/ml	Range	*n*	ng/g	Range	*n*	pg/g	Range	*n*	μg/g	Range	*n*	%	Range	*n*
Reference	14	7.9–25.9	3	258	165–474	5	3.19	1.52–8.37	5	12.40	7.78–17.65	5	6.31	5.74–7.3	5
Upper KRSS	46	25.2–80.4	6	1,441	530–4,408	5	14.38	7.11–28.67	5	9.98	5.18–24.29	5	6.25	3.78–8.51	5
Lower KRSS	68	31.1–147	2	7,897	1,305–39,722	7	137	85–192	5	13.17	4.85–46.7	5	6.52	5.26–8.01	7

[a] Reference sample site includes samples from the Ceresco and Ft. Custer locations, Kalamazoo, Michigan, USA. Upper KRSS sample site includes samples from the Trowbridge, Otsego City Dam, and Lake Allegan impoundments. Lower KRSS sample site includes samples from Koopman's Marsh, Swan Creek Highbanks, and Pottawatomi Marsh.
[b] PCB concentrations include fresh eggs and addled eggs.
[c] TEQ, relative potency, and lipid concentrations include fresh and addled eggs.
[d] Relative potency = TEQ(pg/g)/PCB(μg/g).

$TEQ_{WHO\text{-}Avian}$ to the total PCB concentration to obtain a relative potency value [34]. Relative potency values can be used to assess the degree of weathering and to evaluate exposure and bioaccumulation between trophic levels of an impacted food web and resulting changes in toxic potency of the weathered mixture. Geometric mean relative potency values for $TEQ_{WHO\text{-}Avian}$ and total PCBs in GHO eggs are similar among the KRSS and RA sites. The greatest geometric mean concentration, (1.3×10^1 μg/g, wet wt) was observed for eggs collected at LKRSS (Table 4).

ΣDDT concentrations and eggshell measurements

Total DDT was detected in all egg and plasma samples analyzed. *p,p′*-Dichlorodiphenyldichloroethylene occurred at the greatest concentration of the measured DDT analytes and contributed roughly 98% and 95% to the ΣDDT in all egg and plasma samples, respectively. Total DDT concentrations in eggs were greater from the KRSS than were those from the RA location, and concentrations in the UKRSS and LKRSS were approximately equal. Geometric mean concentrations of ΣDDT were significantly different between the RA site and both UKRSS and LKRSS (Kruskal–Wallis, $p < 0.03$). Geometric mean ΣDDT concentrations in eggs were not significantly different between UKRSS and LKRSS (Kruskal–Wallis, $p = 0.95$) (Table 6 and Fig. 5). The spatial distribution of ΣDDT concentrations in blood plasma was similar to that of concentrations in eggs, but there was no statistically significant difference among the three sites (ANOVA with Tukey's $p = 0.22$). Egg shell thickness and Ratcliffe Index measurements displayed similar trends among the RA site and KRSS (Table 6 and Fig. 5). The mean Ratcliffe Index at LKRSS was slightly less than values observed at the UKRSS and RA site. However, this difference was not statistically significant and neither egg shell thickness, nor the Ratcliffe Index were significantly different among the three sampling locations. (ANOVA with Tukey's, $p = 0.27$, $p = 0.44$, respectively). Additionally, eggshell thickness and Ratcliffe Index were not significantly correlated with ΣDDT concentrations in eggs ($r = 0.35$, $p = 0.17$ and $r = 0.04$, $p = 0.8$ respectively).

Relative abundance and site use

Rates of hoot call responses for individual, pairs, and juvenile birds did not vary by time of survey (AM vs PM) or season (Table 7). Significant differences in the distribution and frequency of responses of individual ($\chi^2 = 16.79$, $df = 2$, $p = 0.001$; $\chi^2 = 16.6$, $df = 1$, $p = 0.001$) and the frequency of responses of paired owls ($\chi^2 = 7.0$, $df = 1$, $p = 0.01$) were observed between FC and TB, with TB having a greater relative abundance of both resident classes. Juvenile response frequencies were also significantly greater ($\chi^2 = 7.57$, $df = 1$, $p = 0.01$) at TB.

Productivity

Over the period of 2000 to 2002, there was no discernible difference in productivity (fledglings/active nest) between the nest sites in the upstream (FC) and downstream (TB) study areas where fledgling success was monitored (active nests $n = 1$, FC and $n = 6$, TB; no statistical testing performed due to the small sample size at FC). For the 3-year study period, the arithmetic mean rate of productivity was 1.0 successful fledglings per active nest at both locations (Table 7). There were more active nests (six vs one) and fledglings at TB (six vs one) than FC. At TB there were two nests that each produced

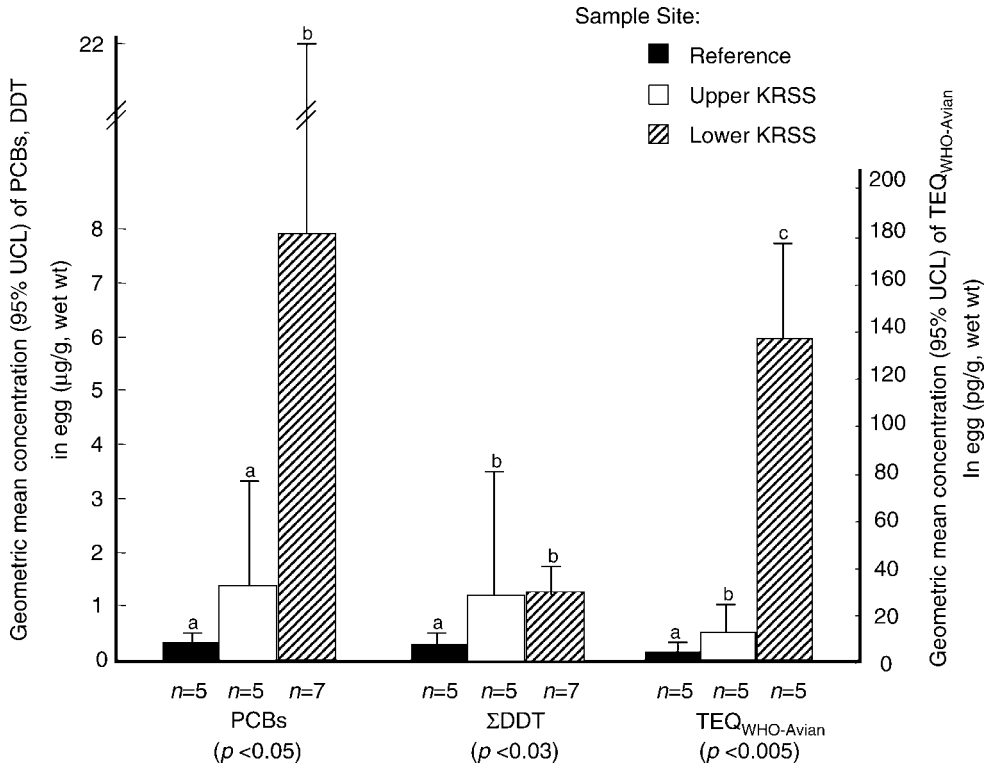

Fig. 2. Geometric mean (wet wt) total polychlorinated biphenyls (PCBs), total dichlorodiphenyltrichloroethane (ΣDDT), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*Bubo virginianus*) eggs collected from the Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, error bars show the 95% upper confidence interval (UCI).

two fledglings, and also two failed nesting attempts (both nests abandoned during incubation). The observed number of active nests at TB compared to FC was very similar to the results of the call–response surveys. Measures of relative abundance and site use obtained with the call–response surveys also indicated that there was a greater number of actively defended territories (or resident owls) in the floodplain at TB.

Risk assessment

Measured 95% UCI (geometric mean) concentrations of PCBs in eggs collected from the UKRSS did not exceed the egg-based NOAEC TRV. The maximum HQ_{NOAEC} in the UKRSS was <1.0 (HQ = 0.5). The PCB concentrations in eggs from LKRSS included the four greatest individual PCB concentrations out of 12 eggs for the entire KRSS. In the LKRSS, the 95% UCI (geometric mean) concentration of PCBs in eggs, resulted in HQs of 1.0 and 3.1 when compared to the LOAEC and NOAEC, respectively (Fig. 6).

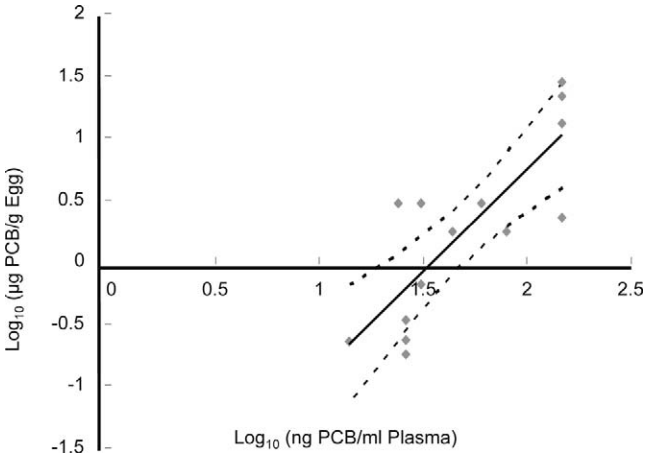

Fig. 3. Egg to plasma polychlorinated biphenyl (PCB) relationship for Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, great horned owls (*Bubo virginianus*), including the 95% confidence interval on the line of best fit.

Hazard quotient values for $TEQ_{WHO\text{-}Avian}$, based on both the LOAEC and NOAEC, were <1.0 for all individual egg samples from the RA and UKRSS locations. The greatest HQ_{NOAEC} was 0.2 for the UKRSS 95% UCI (geometric mean). Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) in the LKRSS, which included the five greatest concentrations out of 10 eggs in the KRSS, resulted in HQs of 0.5 and 1.3, respectively, when the 95% UCI (geometric mean) concentration was compared with the LOAEC and NOAEC (Fig. 6).

Hazard quotient values for the 95% UCI (geometric mean) ΣDDT concentrations, based on both the NOAEC and LOAEC were ≤1.0 for all three sites with a maximum HQ_{NOAEC} value of 1.0 at the UKRSS (Fig. 6). Geometric mean eggshell thickness values at the RA and UKRSS were not significantly different and ≤1% below the pre-1947 benchmark for GHO, but mean thickness at the LKRSS was 4% less than the pre-1947 value. Values for the Ratcliffe Index were 6% less at the RA location than the pre-1947 values while values were 4% and 7% less at UKRSS and LKRSS, respectively.

Hazard quotient values for predicted 95% UCI (geometric mean) concentrations of total PCBs in eggs at all three sites are less than values based on measured concentrations in eggs (Table 5). Use of the predicted concentrations of PCBs in eggs at LKRSS resulted in a geometric mean HQ_{NOAEC} of 0.37 compared to a value of HQ_{NOAEC} of 1.3 based on the comparable measured geometric mean concentration in eggs.

Table 5. Predicted egg (from plasma)[a] and measured egg total polychnorinated biphenyl (PCB) concentrations (ng/g) and calculated geometric mean no-observable-adverse effect concentration (NOAEC) hazard quotients (HQs) in resident great horned owls (*Bubo virginianus*) from the Kalamazoo River Superfund Site (KRSS), Kalamazoo, Michigan, USA

	Reference		Upper KRSS		Lower KRSS	
	Predicted egg $n = 3$	Measured egg $n = 5$	Predicted egg $n = 6$	Measured egg $n = 5$	Predicted egg $n = 2$	Measured egg $n = 7$
Range	80–562	165–474	558–3,630	530–4,408	760–10,299	1,305–39,722
Geometric mean	209	258	1,462	1,441	2,798	7,897
Arithmetic mean	282	277	1,760	1,978	5,530	14,867
Standard deviation (SD)	250	123	1,116	1,628	6,745	14,527
Arithmetic mean ± 1 SD	32–532	154–400	644–2,876	350–3,606	0–12,275	340–29,394
NOAEC HQ (geometric mean)	0.03	0.04	0.21	0.21	0.40	1.13

[a] Predicted egg concentrations calculated using the great horned owl plasma to egg conversion factor [21]: log (egg, μg/g) = 1.674[log (plasma, ng/ml)] − 2.578.

DISCUSSION

A behavioral attribute that favors use of GHOs in ecological studies is their preference to use nests built by other bird species. To our knowledge, the present study is the first to successfully incorporate this behavior into a study designed to induce GHOs to occupy areas of maximum exposure potential, and provide for conservative and worst-case exposure assessment evaluations of the terrestrial food web in a site-specific baseline ecological risk assessment. Previously, Strigiformes have been used to determine ambient or baseline environmental conditions of avian exposure to DDE and other chlorinated hydrocarbon COCs [35]. A second class of investigations focused on local and acute poisoning episodes stemming from use of the acetylcholinesterase-inhibiting organophosphate and carbamate pesticides [36].

Comparison of total PCB concentrations to other locations

Few studies have measured concentrations of PCBs in eggs of wild GHO ([37]; B.G. Rosenberg, 1990, Master's thesis, University of Manitoba, Winnipeg, MB, Canada), and in some instances the analytical results (e.g., PCB quantification on an Aroclor-basis) are not directly comparable to PCB concentrations generated from congener-specific analyses. Surveys of healthy GHO populations in Ohio, USA [37] and Saskatchewan, Canada (B.G. Rosenberg, 1990, Master's thesis) found arithmetic mean PCB concentrations of 3.1×10^3 ng/g (wet wt) and 3.3×10^3 ng/g (wet wt), in eggs, respectively. These concentrations are greater than the arithmetic mean PCB concentration observed in GHO eggs from the UKRSS (2.0×10^3 ng/g egg, wet wt). Although these studies were limited in scope, the results support the conclusions of the risk assessment which suggest that GHOs in the UKRSS are unlikely to be affected by exposure to PCB.

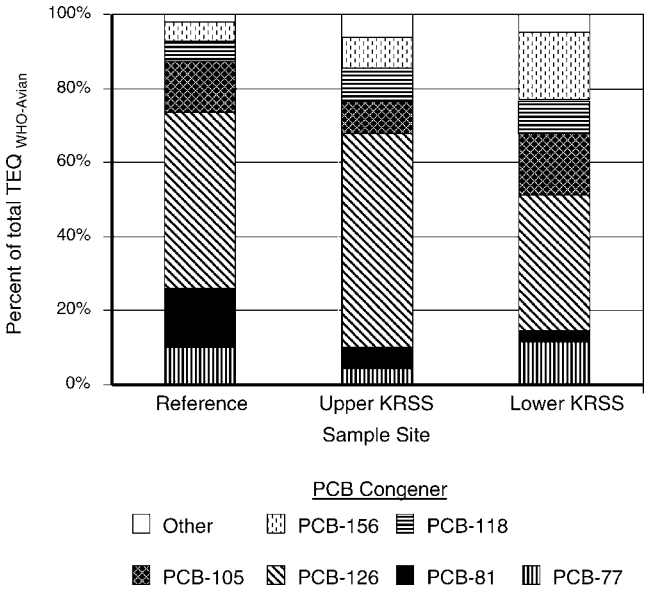

Fig. 4. Percent contribution of polychlorinated biphenyl (PCB) coplanar and mono-*ortho*-substituted congeners to total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{\text{World Health Organization [WHO]-Avian Toxicity Equivalency Factor [TEF]}}$) in great horned owl (*Bubo virginianus*) egg samples at the Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA.

PCB congener profiles

The relative concentrations of PCB congeners used to calculate $TEQ_{\text{WHO-Avian}}$ in KRSS GHO eggs were similar to those observed in eggs of barn owls [38] and eagles [39,40] in North America and Europe. Similarities among the patterns include the predominance of PCB126 as the maximum detected concentration expressed on a wet weight basis (ratio of PCBs 126: 77 ~2:1 or greater) among coplanar congeners, and as the greatest relative contributor to total $TEQ_{\text{WHO-Avian}}$. This is consistent with observations that PCB 77 and 81 are more susceptible to metabolism than PCB 126 and 169 [41]. Among mono-*ortho*-substituted congeners, PCB 118 occurred at the greatest concentrations and PCBs 105 and 156 contributed the greatest relative proportion to $TEQ_{\text{WHO-Avian.}}$

ΣDDT in eggshell measurements

The ΣDDT concentrations in GHO eggs from all regions of the KRSS were within the range of ΣDDT concentrations reported for investigations of healthy GHO populations associated with nonpoint source exposures to ΣDDT ([37,42]; B.G. Rosenberg, 1990, Master's thesis). The fact that the relatively small concentrations of ΣDDT measured in GHO eggs from the KRSS were similar to those measured in eggs from other healthy GHO populations indicates that ΣDDT concentrations in KRSS GHO eggs were not having an adverse effect on GHO in the KRSS. The spatial distributions of ΣDDT concentrations observed in this study indicate that there was relatively little historical use of this pesticide in the RA. Greater concentrations of ΣDDT in the UKRSS and LKRSS may be related to historical agricultural use since both of these sites receive significant inflow from tributaries with drainage basins that contain agricultural development, including fruit production. Additionally for the LKRSS, the greater concentrations

Table 6. Geometric mean, wet weight (range), total dichlorodiphenyltrichloroethane (ΣDDT)[a] concentrations, eggshell thickness, and Ratcliffe Index values for great horned owl (*Bubo virginianus*) eggs and plasma (ΣDDT only) from the Kalamazoo River Superfund Site (KRSS), Kalamazoo, Michigan, USA

Study site[b]	Plasma ΣDDT (ng/ml)	*n*	Egg ΣDDT (ng/g)	*n*	Eggshell thickness (mm)	*n*	Ratcliffe Index No.	*n*
Reference area	47 (14–168)	3	314 (246–417)	5	0.376 (0.346–0.393)	5	1.9 (1.81–1.96)	5
Upper KRSS	107 (60–169)	6	1,269 (306–4,987)	5	0.377 (0.363–0.412)	5	1.93 (1.89–2)	5
Lower KRSS	94 (59–152)	2	1,305 (618–2,013)	7	0.363 (0.347–0.384)	7	1.88 (1.76–1.99)	7

[a] Total DDT concentrations include dichlorodiphenyltrichloroethane (DDT) and metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD).

[b] Reference area sample sites include the Ceresco and Ft. Custer locations, Kalamazoo, Michigan, USA. Upper KRSS sample sites include the Trowbridge, Otsego City Dam, and Lake Allegan impoundments. Lower KRSS sample sites include Koopman's Marsh, Swan Creek Highbanks, and Pottawatomi Marsh.

of ΣDDT bioavailability may be associated with exposures in Great Lakes–influenced habitats where fish are known to have greater concentrations of ΣDDT [43].

Toxicity reference values

Few studies meet all of our criteria for deriving a TRV for PCBs in GHOs. While birds, especially raptors, are generally considered to be some of the most exposed and sensitive animals to the effects of chlorinated hydrocarbons, there is a wide range of sensitivities to PCB and other aryl hydrocarbon receptor–active chemicals among species [44,45]. Application of laboratory or field-derived TRVs among dissimilar avian orders, such as Galliformes and Strigiformes, introduces uncertainty and associated conservative bias that can result in protective but unrealistic TRV values. Additionally, values for thresholds of effect, based on the results of acute studies are of little use when trying to establish TRVs for chronic effects in wildlife. Cocontaminants in test diets or from field studies can substantially confound the toxicity results relative to a single chemical or class of chemicals. In particular, assignment of causality can be problematic when elevated levels of cocontaminants are present. Similarly, complex mixtures such as PCBs, which are subject to environmental weathering, are dynamically changing in relative congener composition and toxic potency depending on the environment to which they are exposed. Quantifying the toxicity of neat mixtures or even weathered mixtures from different systems may not reflect the actual toxicity of the mixture of concern. To address any one of these uncertainties, risk assessors frequently apply an uncertainty factor to the published toxicological benchmark. Aside from applying an uncertainty factor of 3 to derive the PCB LOAEC from a validly determined NOAEC, application of additional uncertainty or extrapolation factors to our selected TRVs was not necessary. This is because the selected studies meet the key requirements as described above. Most specifically, the test species used were closely related wildlife species (a specific preference stated in the Great Lakes Water Quality Criteria documents) [25]. The selected studies also employed chronic exposures over sensitive life stages and measured ecologically relevant endpoints with minimal impact from cocontaminants.

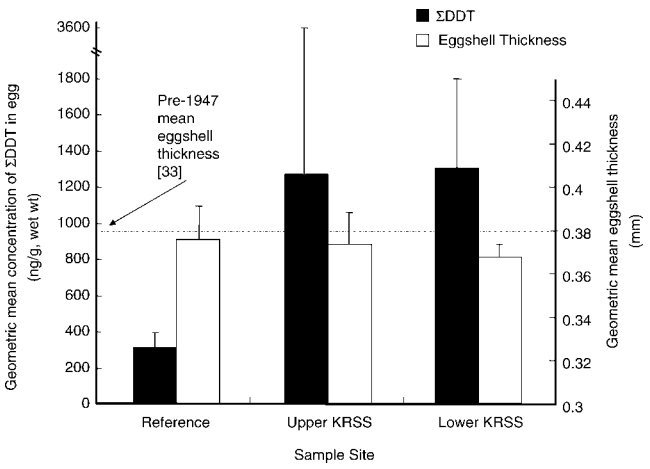

Fig. 5. Geometric mean (wet wt) total dichlorodiphenyltrichloroethane (ΣDDT) concentrations in great horned owl (*Bubo virginianus*) eggs and eggshell thickness at the Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, error bars show the 95% upper confidence interval (UCI).

Risk assessment

Studies of PCB accumulation patterns in the terrestrial food web at the KRSS [46] have found considerable variation in site-specific patterns of bioavailability, bioaccumulation, and biomagnification. Site-specific exposure potentials are altered by habitat and hydrologic conditions. Site characterization studies have shown the mean PCB concentration in floodplain soils to be greatest in UKRSS and more than twice the concentration observed in floodplain soils of the LKRSS (Table 1). Our measures of PCBs in GHO eggs and GHO nestling blood plasma did not parallel these spatial patterns of PCB exposure potential. Inconsistencies of this type underscore the importance of site-specific studies at sites as large and diverse as the Kalamazoo River. The greater exposure of GHOs to PCBs at the LKRSS may be due to exposure through trophic pathways that include both terrestrial and aquatic pathways including fish from Lake Michigan. For example, diet studies of GHO in this stretch of the KRSS may show that a large portion of the diet is composed of Anseriform (waterfowl) or Charadriform (gulls) prey.

In the present study, use of either total concentrations of PCBs or $TEQ_{WHO\text{-}Avian}$ as measures of exposure in GHO eggs resulted in similar estimates of risk. A review of the 95% UCI (geometric mean) concentration ranges of HQ for total PCBs and total $TEQ_{WHO\text{-}Avian}$ indicates a high degree of concordance between these two measures of exposure. Ranges of HQ_{NOAEC} and HQ_{LOAEC} based on either total PCBs or $TEQ_{WHO\text{-}Avian}$ almost completely overlap and HQ_{LOAEC} were consistently ≤ 1.0 at each of the three study sites (Fig. 6).

Concentrations of ΣDDT in eggs of GHO at the KRSS were correlated with neither eggshell thickness (mm) nor Ratcliffe Index. Changes in values relative to the pre-1947 values for mean eggshell thickness (maximum −4%) and Ratcliffe Index

Table 7. Relative abundance and productivity of resident great horned owls (*Bubo virginianus*) at Ft. Custer (reference area) and Trowbridge (upper KRSS[a]) from 2000 to 2002

	2000		2001		2002		All years (2000–2002)	
	Ft. Custer	Trowbridge	Ft. Custer	Trowbridge	Ft. Custer	Trowbridge	Ft. Custer	Trowbridge
Relative abundance[b]	CS = 4[c]	CS = 7	CS = 9	CS = 7	CS = 11	CS = 8	CS = 24	CS = 22
Adults				Mean response rate[d]				
Total[e]	2.5	2.57	0.89	2.71	0.55	3	1.31	2.76
Foraging[f]	1.5	1.43	0.67	1.86	0.37	1.63	0.85	1.64
Paired[g]	1	1.14	0.22	0.86	0.18	1.38	0.47	1.13
Juveniles				Response frequency no. (%)[h]				
Fledgling[i]	0 (0)	0 (0)	1 (11)	7 (100)	0 (0)	1 (12)	1 (04)	8 (36)
Productivity[j]	$n = 0$[k]	$n = 1$	$n = 1$	$n = 2$	$n = 0$	$n = 3$	$n = 1$	$n = 6$
Fledglings	0	1	1	4	0	1	1	6
Fledglings/nest	0	1	1	2	0	0.3	1	1

[a] Kalamazoo River Superfund Site, Kalamazoo, Michigan, USA.
[b] Relative abundance estimates derived from hoot call/response surveys completed at dawn and dusk.
[c] CS = number of complete surveys.
[d] Mean response rate is averaged across number of completed surveys for each year. All years mean is the mean of the means.
[e] Includes discrete responses from both individual and paired (male + female) owls.
[f] Includes responses from unpaired individuals only.
[g] Includes responses from paired (male + female) owls only.
[h] Average response frequency of fledgling owls (No. = number [percent] of completed surveys with at least one fledgling begging call response) expressed on a yearly basis, and averaged over all years.
[i] A measure of current and successful breeding activity.
[j] The total number of successful fledglings from all active nests (no. fledglings/no. active nests) per year within each sampling area, and averaged over all years/sum total of all active nests.
[k] n = number of active nests.

(maximum −7%) in the KRSS were not close to threshold values of 15 to 20% associated with adverse effects on successful raptor reproduction and population maintenance [17,33].

The results of the hazard assessment suggest that GHO populations residing in the RA and the UKRSS are not at risk for effects induced by PCBs, $TEQ_{WHO\text{-}Avian}$, or ΣDDT in floodplain soil. This conclusion is consistent with measurements of fledgling productivity. At the LKRSS, the HQ_{NOAEC} values for eggs are slightly greater than 1.0, with values as great as 3.1 and 1.3 for 95% UCI (geometric mean) concentrations of total PCBs and $TEQ_{WHO\text{-}Avian}$, respectively. These HQ_{NOAEC} values indicate that exposure of GHO to PCBs in this reach of the Kalamazoo River were near the threshold for effects. It is important to note that the true effect level for individuals lies somewhere between the NOAEC and LOAEC and, even conservatively, population effects have not been expected at a HQ of 10.0. The HQ_{NOAEC} values near 1.0 are not likely to be associated with adverse reproductive effects in individual resident GHOs in the LKRSS.

Multiple lines of evidence and assessment of population-level effects

This assessment employed a multiple lines of evidence approach to minimize uncertainty in assessment endpoints and to provide the best available information for remedial decision-making for later stages of site clean-up efforts. Included in the tissue-based ''top-down'' HQ approach [13], on which we report here, the potential effects of PCBs and ΣDDT on GHO productivity and relative abundance and site use were monitored in the FC and TB areas of the RA and UKRSS sites.

Mean productivity rates were similar among locations where exposures to PCBs were much different, with 1.0 successful fledglings per active nest at the two locations where reproductive success was monitored. The mean rate of 1.0 fledgling per active nest observed at both locations is consistent with productivity measures for healthy midwestern [47] GHO populations residing in varied upland habitats. Measures of site use indicate TB populations, as determined by territory-holding nesting pairs, were near the carrying capacity (roughly one pair per 1,600 ha and a total of three pairs in the TB floodplain) [12]. Furthermore, nest acceptance rates and nest fidelity of actively breeding GHOs across all nesting seasons included in the study were consistent with previous studies of artificial nest acceptance and habitat usage by Strigiformes in midwestern forests [11,47,48]. For unknown reasons, the observed number of active nests at FC (1.0 active pair in any study year) was less than the carrying capacity of this study location. Adult mortality may have been a prime factor in the low density of active nesting pairs at FC, as we are aware of four confirmed adult deaths (three owl–car collisions, one owl–train collision) during the study period from 2000 to 2004. Unfortunately, we were not able to identify the sex of these dead owls. Owl population health data support the conclusion that TB populations are not suffering adverse effects to population maintenance.

The top-down assessment of potential hazards to resident GHO populations completed in this investigation has employed intensive sampling effort of maximum exposure, state of the art analytical techniques, multiple methods to estimate exposure to PCBs, an assessment of potentially confounding chemical stressors, valid statistical methods, and conservative benchmarks of toxicity. The results of these studies suggest that current concentrations of PCBs, expressed either as total PCB concentrations or as $TEQ_{WHO\text{-}Avian}$ are not sufficient to pose a significant risk to GHO populations in the UKRSS where large areas of former sediments are now exposed as floodplain soils. The data also indicate that risk in the LKRSS, while greater, is unlikely to be sufficient to cause adverse population-level effects.

Results from the present study concur with earlier investigations at the KRSS that indicated GHOs would effectively

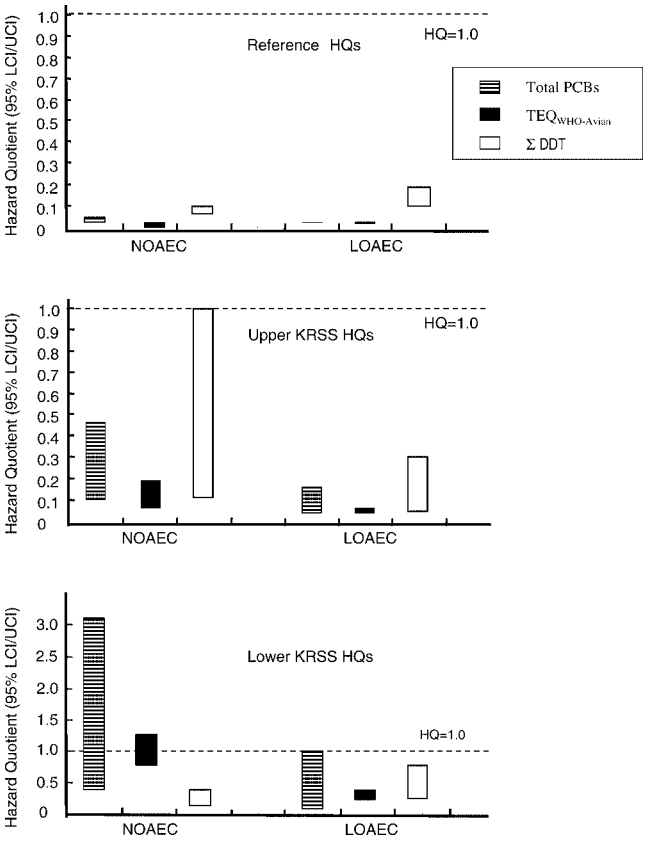

Fig. 6. Hazard quotients (HQ) for the effects of total polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{\text{World Health Organization [WHO]-Avian Toxicity Equivalency Factor [TEF]}}$), and total dichlorodiphenyltrichloroethane (ΣDDT) for great horned owl (*Bubo virginianus*) eggs at the Kalamazoo River superfund site (KRSS), Kalamazoo, Michigan, USA, based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). Each box encompasses the 95% upper and lower confidence interval (UCI/LCI) about the geometric mean concentration.

integrate exposures from primary environmental media through multiple trophic levels [46]. The present study confirms that GHOs are a useful sentinel species for site-specific baseline ecological risk assessments employing a multiple lines of evidence approach that includes using top-down methodology to combine measured residues of COCs in tissues and counts of population and productivity. The present study's successful use of GHO provides a workable model that can be applied to other large sites with extensive areas of contaminated soils that require ecological investigations of risk or long-term monitoring for potentially impacted terrestrial communities. In instances where elevated levels of contaminants cause concern for potential environmental effects, measures of owl chemical exposure, productivity, and abundance can serve as an index of overall ecosystem health.

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