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AN ECOLOGICAL RISK ASSESSMENT FOR GREAT HORNED OWLS AND EAGLES EXPOSED TO POLYCHLORINATED BIPHENYLS AND TOTAL DDT AT THE KALAMAZOO RIVER SUPERFUND SITE, MICHIGAN

By

Karl Daniel Strause

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ABSTRACT

AN ECOLOGICAL RISK ASSESSMENT FOR GREAT HORNED OWLS AND EAGLES EXPOSED TO POLYCHLORINATED BIPHENYLS AND TOTAL DDT AT THE KALAMAZOO RIVER SUPERFUND SITE, MICHIGAN

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Selection of receptors is a key element of effective risk and natural resource damage assessments. This is especially critical when site-specific field studies are employed. The great horned owl (*Bubo virginianus*; GHO) has advantages over other species as a key tertiary terrestrial receptor that can be used as an integrated measure of exposure to residues in a multiple-lines-of-evidence approach. The methods described herein exploit attributes of the GHO including its propensity to nest in artificial nesting platforms, which allows for better control of experimental conditions than normally experienced in studies of wildlife. The GHO 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) in Kalamazoo and Allegan Counties, Michigan. Over the course of five yrs, 48 artificial and six natural GHO nests, covering approximately 14 active territories along approximately 38 km of river floodplain, were monitored for activity at the KRSS. The study examined risks from total PCBs, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), and total DDTs (sum of DDT/DDE/DDD; ΣDDT) by measuring concentrations in eggs and nestling blood plasma. Dietary modeling was also completed to estimate potential ingested dose for the contaminants of concern (COCs) from site foraging activities. An ecological risk assessment compared concentrations of the (COCs) in eggs, plasma, or

diet to toxicity reference values to generate hazard quotient values descriptive of potential risk to resident raptor populations. Productivity/relative abundance measures for KRSS GHOs were compared with other GHO populations. Egg shell thickness was measured to assess effects of *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) on egg viability. Tissues data, dietary exposure estimates, and productivity/relative abundance measures indicated no population level effects were present at the Upper reach of the Kalamazoo River Superfund Site, closest to the sources of PCB contamination to the River. Bald eagles (*Haliaeetus leucocephalus*) residing at the Kalamazoo River Site were also studied using the multiple-lines-of-evidence approach. Observations of reduced productivity and elevated contaminant concentrations in eagle eggs and nestling plasma collected from the site indicated that contaminant exposures were likely at the threshold for adverse population effects for resident bald eagle populations. Additionally, data bases describing the concentrations of total PCBs in eggs and nestling plasma of great horned owls and total PCBs and *p,p'*-DDE in eggs and nestling plasma of bald eagles from the Great Lakes region were used to develop a relationship to predict concentrations of PCBs and DDE in eggs from measure concentration in nestling plasma. An accurate conversion factor to translate nondestructive plasma-based contaminant concentrations to comparable egg-based concentrations will prove valuable to risk assessors investigating the potential effects of chemical exposures to raptors.

For My Mother – Marie Theresa (Feairheller/Slawecki) Strause

she always knew I had it in me

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KEY TO ABREVIATIONS

APDD – average potential daily dose

APDDmeasured – average potential daily dose for a site-specific diet

APDDpredicted – average potential daily dose for a literature-based diet

bw – body weight

COC – contaminant of concern

CR – Ceresco reservoir

DDD – dichlorodiphenyldichloroethane

DDE – *p,p'*-dichlorodiphenyldichloroethylene

DDT – *p,p'*-dichlorodiphenyltrichloroethane

ΣDDT – DDD + DDE + DDT

dw – dry weight

EU – experimental unit

ERA – ecological risk assessment

FC – Fort Custer State Recreation Area

GHO – great horned owl

HQ – hazard quotient

HQ_{LOAEC} – hazard quotient calculated using the least observable adverse effect concentration toxicity reference value.

HQ_{NOAEC} – hazard quotient calculated using the no observable adverse effect concentration toxicity reference value.

IUPAC – international union of pure and applied chemists

KEY TO ABREVIATIONS (Cont'd)

KRAOC – Kalamazoo River area of concern

KRSS – Kalamazoo River superfund site

LKRSS – lower Kalamazoo River superfund site

LOAEC – lowest observable adverse effect concentration for tissue exposures

LOAEL – lowest observable adverse effect level for diet exposures

MDEQ – Michigan Department of Environmental Quality

MSU-ATL – Michigan State University Aquatic Toxicology Laboratory

NOAEC – no observable adverse effect concentration for tissue exposures

NOAEL – no observable adverse effect level for diet exposures

PCBs – polychlorinated biphenyls

PCDDs – polychlorinated-dibenzo-*p*-dioxins

PCDFs – polychlorinated-dibenzo-furans

PM/MR – Pere' Marquette/Manistee Rivers

RPD – relative percent difference

TB – Trowbridge (former) impoundment

TCDD – 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

$TEQ_{WHO\text{-}Avian}$ – 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents

TRV – toxicity reference value

UCL – upper confidence level

UKRSS – upper Kalamazoo River superfund site

USFWS – United States Fish and Wildlife Service

KEY TO ABREVIATIONS (Cont'd)

USFWS-ELFO – United States Fish and Wildlife Service, East Lansing Field Office

WHO – world health organization

ww – wet weight

Units of Measure

cm - centimeter

g – gram

h – hour

ha – hectare

in - inch

kg – kilogram

km – kilometer

L - liter

µg – microgram

µl – microliter

m - meter

min – minutes

mg - milligram

ml - milliliter

mm - millimeter

ng – nanogram

pg - picogram

wk - week

yr - year

Chapter 1

Site-Specific Assessments of Environmental Risk and Natural Resource Damage based on Great Horned Owls

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ABSTRACT

Selection of receptors is a key element of effective risk and natural resource damage assessments. This is especially critical when site-specific field studies are employed. The great horned owl (*Bubo virginianus*; GHO) has advantages over other species as a key tertiary terrestrial receptor that can be used as an integrated measure of exposure to residues in a multiple-lines-of-evidence approach. The methods described herein allow for minimization of uncertainty in assessment endpoints, while also minimizing the potential impact of the study on populations and maximizing the utility of data in testing of hypotheses. These methods exploit attributes of the GHO including its propensity to nest in artificial nesting platforms, which allows for better control of experimental conditions than normally experienced in studies of wildlife. The data collected are supportive of a multiple-lines-of-evidence approach including the elucidation of contaminant exposure by both predicted (dietary) and tissue-based methodologies. In addition, population-level measures of potential effects including productivity and abundance can be directly measured. Over the course of five yrs, 48 artificial and six natural GHO nests, covering approximately 14 active territories along approximately 38 km of river floodplain, were monitored for activity at the Kalamazoo River Superfund Site in Kalamazoo and Allegan Counties, Michigan. There were 25 nesting attempts observed in 20 active nests. Residue concentrations of polychlorinated biphenyls (PCBs) and otho- and para-substituted isomers of dichlorodiphenyltrichloroethane (DDT), including DDD and DDE (ΣDDTs) were measured in 24 eggs and 16 samples of nestling blood plasma. Exposure through the diet was predicted by determining a site-specific dietary composition (based on 285 dietary items) followed by sampling and quantifying

residue (PCBs) concentrations in 171 identified prey items that were collected from the locations where owls had taken the prey. Hazard assessments, based on measured concentrations in tissues and based also on predicted concentrations in the diet, produced similar results that indicated minimal risk to resident GHO populations (Hazard Quotients $\leq$ 1.5). The number of GHO present in an area was highly correlated with the number of attempted breeding events. The use of convergent lines of evidence resulted in greater confidence in the assessments of both exposure and potential effects. Repeated use of artificial nesting platforms by GHOs minimized temporal and spatial variability. The GHO was found to be a useful receptor for evaluating terrestrial contaminant exposures and associated risk utilizing a multiple-lines-of-evidence approach.

Keywords: ERA, receptor, raptor, great horned owl, exposure assessment, multiple-lines-of-evidence

INTRODUCTION

Raptor species have long been used as environmental monitors (IJC 1991; Sundlof et al. 1986; CEQ 1972) because they are sensitive to some of the more prevalent contaminants of concern (COCs), and have a high potential for exposure to those residues. Herein we describe direct, site-specific, field assessment methodologies that use the great horned owl (*Bubo virginianus*; GHO) as a sentinel or surrogate species for terrestrial-based organisms in assessing ecological hazards or natural resource damages as well as site-specific clean-up values for soils. The methodologies take advantage of useful attributes of the GHO in a multiple-lines-of-evidence approach to assess potential exposure to COCs and potential effects. Exposure was quantified both by predicting exposure through the diet and by measuring concentrations in blood plasma and eggs of GHOs. Both estimates of exposure were then compared to threshold concentrations for effects reported in the literature. Concurrent measures of GHO abundance and reproductive performance were used to assess consistency between predicted effects thresholds based on the risk assessments and observed effects in resident populations. Measurement endpoints from each line of evidence were combined in a weight of evidence approach to assess potential risks to resident GHO populations at the Kalamazoo River Superfund Site (KRSS). The methods were designed to minimize uncertainty in assessment endpoints (Fairbrother 2003), minimize the ecological impact of data collection, and maximize the utility of data in testing hypotheses.

Species Applicability

Guidelines promulgated by the United States Environmental Protection Agency (U.S. EPA) state that species-specific as well as site-specific factors dictate the applicability of an organism for use as a species of concern in risk assessments performed for "Comprehensive Environmental Response, Compensation, and Liability Act" (CERCLA)-based ecological field studies (USEPA 1994, 1997, 1999). The ultimate goal is to select specific populations or communities for which the collected data and resulting decisions can be extrapolated across the ecosystem of interest. Both the GHO and the specific methods described herein, have a broad applicability to key ecological components.

Comparisons of measurement endpoints for GHOs can be made across wide geographical regions and habitat types. The GHO is endemic throughout the temperate and sub-arctic regions of the Americas from Alaska to Argentina and has one of the largest ranges of all raptors (Houston et al. 1998; Burton 1984; AOU 1983). In addition, it is able to utilize more habitat types than any other American raptor species (Johnsgard 1988) while maintaining a foraging range and taxonomic dietary composition that is similar to a number of less adaptive medium and large terrestrial-based receptors (Austing and Holt 1966; Austing 1964; Craighead and Craighead 1956).

In addition to geographic applicability, a number of species-specific characteristics need to be considered when selecting organisms for study. These include intensity (concentration) and duration (time spent on-site) of exposure, appropriateness as a surrogate species, sensitivity to some of the primary contaminants of concern at many sites, including the KRSS, ecological function, relative ease of conducting field studies with the organism, and other recognized values (USEPA 1994). The GHO is a top food

web predator and year round resident throughout its range. Great horned owls are strict carnivores with large rates of ingestion, relative to their body weight (Tabaka et al. 1996) and have life spans known to exceed 28 yr (Nero 1992). These attributes, as well as the fact that GHOs have no known predators, makes the GHO a useful indicator of the magnitude and bioavailable fraction of contaminants in terrestrial ecosystems.

Great horned owls are considered to be among the most sensitive animals to some of the most common environmental contaminants that occur in terrestrial environments (Hoffman 1995). Great horned owls are susceptible to environmental contaminants because of their high dose potential (e.g., variety and mass of prey ingested) and inherent physiological sensitivity to chemical stressors. Dietary exposure of owls to small amounts of select contaminants such as organophosphates, organochlorines, and metals has been shown to cause lethal and sub-lethal effects including reproductive impairment or failure (Sheffield 1997). Because of these characteristics, the GHO is a useful sentinel or surrogate for other terrestrial species, or as a bio-indicator or bio-monitor for evaluating potential exposures of avian populations to contaminants (Sheffield 1997).

The nesting characteristics of GHOs provide advantages as indicators of contaminant bioavailability relative to raptors. In terms of both geographical location and habitat diversity, the GHO occupies the greatest range of nesting sites of any bird in the Americas (Baumgartner 1938). Great horned owls do not construct their own nests, but rather commandeer the nests of others, which are typically nests of squirrels, hawks or crows. For this reason, GHOs will utilize artificial nesting platforms (Bohn 1985; Holt 1996). Great horned owls will continue to use a nest as long as it remains successful and serviceable, but will not maintain a nest. Natural nests, especially usurped nests, are

rarely used for more than a single season (Frank 1997; Holt 1996). As a result, GHOs are almost always looking for a new nest within their territory and quickly move to constructed nesting platforms. The use of artificial nesting platforms obviates the need to locate and access natural nests and simplifies monitoring of GHOs. In addition, platforms allow for the dictation of foraging areas, consistency among years, and minimization of predation. Constructed platforms can be durable, placed in a wide range of locations, and maintained indefinitely. The resulting multi-year use of the same nest reduces variability in temporal and spatial exposure profiles.

Great horned owls offer advantages over other tertiary terrestrial receptors when assessing site-specific COC exposures and population health. As top predators, GHOs effectively integrate exposures to COCs from multiple trophic levels and habitats. Like most higher order terrestrial predators, GHOs are opportunistic feeders with a diet that includes a wide variety of small- and medium-sized mammals, birds, insects, amphibians, and invertebrates (Marti and Kochert 1996; Voous 1988; Marti 1974; Craighead and Craighead 1956). Exposures of GHO nestlings to residues have been shown to be directly related to local contaminant concentrations (Frank 1997) and their abundance has been shown to be directly related to available prey (Rohner 1996; Houston and Francis 1995; Rusch et al. 1972; Adamcik et al. 1978) and ultimately ecosystem health.

Concentrations of COCs in GHO can be directly assessed through the collection of tissues, eggs, or blood. Great horned owls have relatively high rates of reproduction, a factor that offers advantages in meeting sample size requirements. Great horned owls are relatively easy to capture and handle as compared to other terrestrial-based raptors. Nestlings between 5 and 6 wks of age can be easily accessed, banded, morphological

characteristics measured, blood sampled, and radio tagged (Austing and Holt 1966). Broods of pre-fledge nestlings (typically one to three individuals) are confined to the nest and rely solely on prey collected by adults from areas proximal to the nest. Parental foraging ranges of GHO are constrained during brood rearing due to nest defense and prey transport limitations. This ensures that exposures of both adult and nestling GHOs to COCs are directly linked to the immediate area surrounding the nest site.

Exposure of GHOs through the diet can be quantified by enumerating the composition of the diet and determining the concentrations of COCs in the prey items. These two parameters can then be combined to allow calculation of a weighted average concentration of COCs in the diet and an average potential daily intake (USEPA 1993). Methods to determine site-specific dietary composition are well described and include the combined examination of prey remains and regurgitated pellets (Marti 1987; Rusch et al. 1972; Errington 1930). Unlike other raptors, owls prefer to swallow their smaller prey items whole. The prey enters the glandular stomach where enzymes break it down. Undigested materials, including bones and hair are regurgitated in the form of a packed pellet within 2 to 24 h after consumption. These pellets and prey remains in and around the nest (associated with adult feeding perches) can be sampled over time.

The GHO as a Key Receptor (Case Study)

The studies and results reported here were part of a large group of studies in support of an ecological risk assessment of the KRSS (Blankenship et al. 2005; Kay et al. 2005; Millsap et al. 2004; Neigh et al. 2006a,b; Strause 2006). The KRSS was designated a Superfund site in 1990, and is comprised of nearly 100 km of the Kalamazoo River from

Portage Creek in the city of Kalamazoo to the downstream terminus at Lake Michigan. The primary COCs were identified as polychlorinated biphenyls (PCBs) with some evidence of elevated exposures of raptors to dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) (hereafter, ΣDDT) (Mehne 1993).

The GHO study was designed to determine bioavailability and accumulation of the COCs from a terrestrial food web in the contaminated floodplain of the Kalamazoo River. The potential for adverse effects to resident GHO populations was estimated using a hazard quotient (HQ) approach which was validated by comparisons to the abundances and reproductive productivity of GHOs in the target study areas, as well as reference areas, and information available in the literature about these population parameters at other uncontaminated locations.

METHODS

Study Site

The study area included sections of the Kalamazoo River, both upstream and downstream of known sources of contaminants. Four contiguous study areas of ≥7 km were utilized including two target areas, Lake Allegan State Game Area and the former Trowbridge impoundment, as well as two upstream reference sites at Fort Custer State Recreation Area and Ceresco Impoundment (Figure 1.1). Upstream or reference locations were selected based on habitat suitability and applicability to baseline watershed contaminant exposures, and included two areas encompassing 15 km of free flowing and impounded

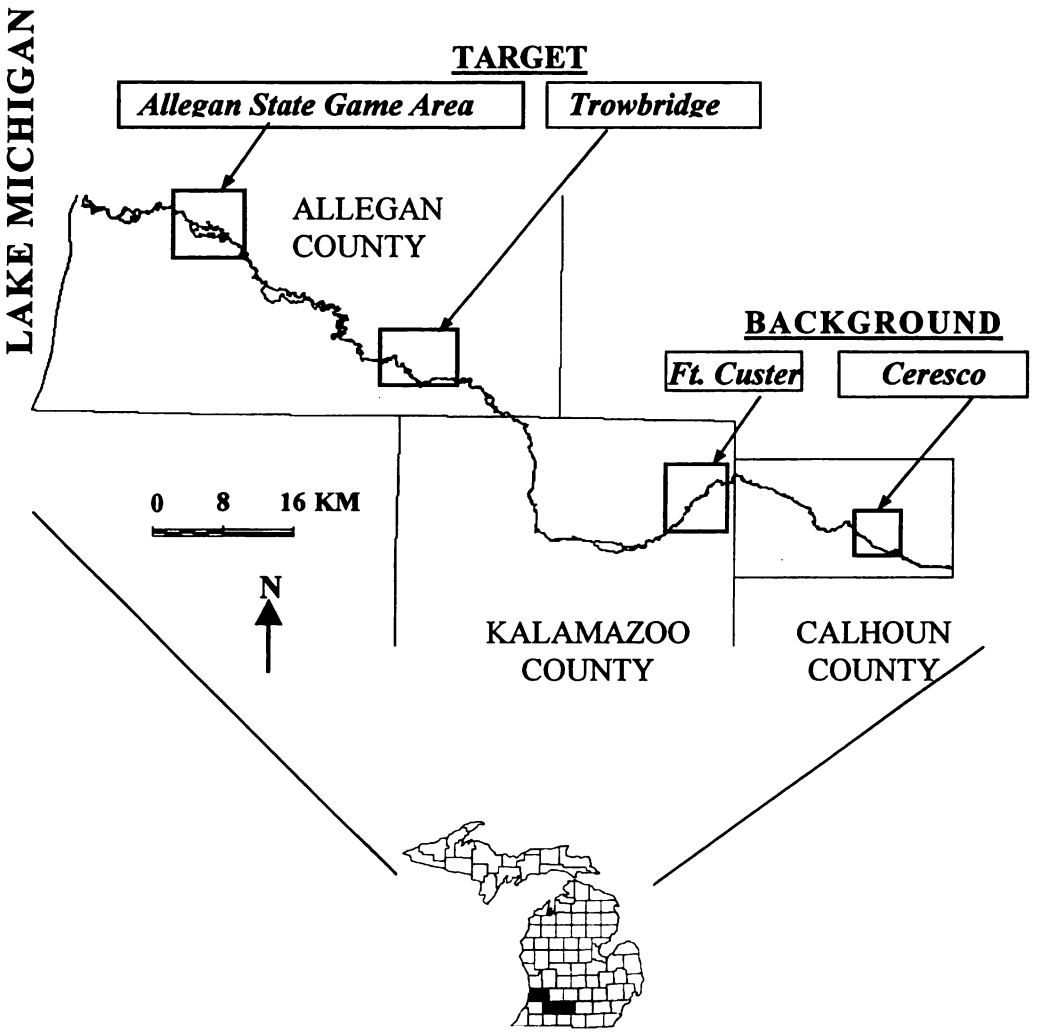

Figure 1.1. Map of sampling areas within Kalamazoo river floodplain. Superfund site extends 128 km from the city of Kalamazoo to its confluence with Lake Michigan. The sampling areas of Trowbridge and Allegan State Game Area lie 30 and 60 km downstream of Kalamazoo while the reference sampling areas of Fort Custer and Ceresco lie similar distances upstream of the start of the Superfund site, respectively.

areas of the river. Floodplain habitats included emergent marsh, wet meadow, emergent shrub and deciduous forested wetland. For the downstream and contaminated target areas, study locations included similar habitats of free flowing, impounded, and formally impounded sections of the river. Specific areas were selected based on a maximum potential for exposure of resident owls to the COCs from floodplain soils during foraging activities associated with nesting and subsequent rearing of offspring (Strause 2006).

Artificial Nesting Platforms

Nest platforms were constructed, with minor modifications, as described in Henderson (1992). A 3.5' x 3.5' piece of 1 in "chicken wire" mesh was cut into a circle and formed into a nesting cone by making one cut from the outer edge to the center and then overlapping the two cut edges until the cone was about 18 in deep. The cut ends of the chicken wire were bent around the overlapping ends to hold the cone together and to prevent sharp ends from protruding. A 3.5' x 3.5' section of dark gray Tyvek® was similarly cut, folded and placed into the wire cone. Tyvek® is strong, lightweight, and breathable and provides protection from weather, light, and moisture. A drainage hole was cut at the base of the Tyvek® cone and leaf litter was placed between the wire mesh and Tyvek®. Flexible 1/2" and smaller stems of willow and dogwood were woven, placed at the top edge, and spiraled downward around the inside of the nest. Stems were secured to the frame with light gauge stainless steel wire (Figure 1.2). Once installed, one to two L of shredded wood chips were added as nesting material and to level the inside of the nest cone.

Figure 1.2. (Left) Artificial nesting platform (Right) Platform installation, Note; stainless steel adjustable pipe clamps are not camouflage painted for demonstration purposes.

Placement of Nest Platforms

Nest platforms were placed in live trees of at least 25 cm in diameter at the base. Preferred sites included large trees on the leeward edge of shelterbelts or other areas somewhat protected from high winds. Effective nest placement was no less than 8 m from the ground and ideally at a height of 11.5 to 16.5 m. Pre-constructed nests were secured in a suitable crotch with camouflaged stainless steel adjustable pipe clamps. Because exposure to PCB contaminated floodplain soils was being evaluated, nests were located within 100 m of preferred foraging habitat and offered GHOs a combination of concealment, easy flight access, and proximity to selected foraging grounds. Ten to 15 nests were deployed per study area resulting in a density of one to three artificial nesting

platforms per breeding territory. In all, a total of 54 nests were monitored including six natural nests.

Exposure Based on Measured Concentrations

The first measure of exposure included concentrations of PCBs and ΣDDT in eggs and nestling blood plasma. Egg samples were collected as soon as incubation activity was confirmed. Individual eggs were placed in pre-labeled, shock-absorbing, crush-proof transport containers placed in a pack and carried to the ground. Eggs were labeled, transported back to the laboratory and stored at 4 °C until processing. Weight, volume and eggshell thickness of eggs were determined (Stickel et al. 1973). The contents of the eggs were then homogenized. Blood was collected from nestlings by use of sterile technique (Henny 1997; Henny and Meeker 1981; Mauro 1987) when they were 5 to 6 wk of age and weights were $\geq$ 0.75 kg. Owlets at this stage were relatively easy to capture, tolerated handling and could be returned unharmed to the nest. Blood was drawn with 25-gauge hypodermic needles into 10 ml syringes containing sodium heparin solution and then transferred to pre-labeled heparinized Vacutainers™ and placed on cold packs in an insulated cooler. During the collection of nestling blood samples, individual nestlings were identified by attaching leg bands (United States Fish and Wildlife Service (USFWS) #9 rivet) following standard USFWS protocols. Addled eggs and egg shell fragments also were collected at the time of banding. Vacutainers™ containing whole blood were transported to the laboratory and centrifuged at 1200 rpm for 10 min and the plasma (supernatant) was transferred into a new Vacutainer™ appropriately labeled and stored upright at –20 °C.

Quantification of select COCs was performed at the Michigan State University (MSU) Aquatic Toxicology Laboratory (ATL) based on project-specific data quality objectives. Total concentrations of PCBs (congener-specific analysis) and ΣDDT were determined using EPA method 3540 (SW846), soxhlet extraction, as described elsewhere (Neigh et al, 2006b). Briefly, concentrations of PCBs, including di- and mono-ortho-substituted congeners were determined by use of a gas chromatograph equipped with a ^{63}Ni electron capture detector (GC-ECD). Concentrations of non-ortho-substituted PCB congeners and ΣDDT were determined by gas chromatograph mass selective detector (GC-MS). The limit of quantification for di- and mono-ortho-substituted PCBs was conservatively estimated to be 1.0 ng PCB/g, ww. For coplanar PCB congeners and ΣDDT analytes, method detection limits varied among samples but were maintained for all samples at <0.1 ng/g, ww. Either TurboChrom (Perkin Elmer, Wellesley, MA, USA) or GC Chemstation software (Agilent Technologies, Wilmington, DE, USA) was used to identify and integrate the individual PCB congener peaks. Total concentrations of PCBs were calculated as the sum of all resolved PCB congeners.

Dietary Exposure

The site-specific exposure to PCBs via the diet was predicted by determining the relative proportions of prey items in the diet followed by measurement of PCBs in representative samples of those items collected from the reference and target floodplain study locations. Site-specific dietary composition for resident owls was determined from prey of actively nesting GHOs. Prey items included regurgitated pellets and any uneaten remains of prey such as bones, feathers, scales, and fur (hereafter referred to together as prey remains).

All prey remains were collected from around the nest tree and beneath feeding perches prior to egg drop and incubation. Prey remains were again collected from within the nest, around the base of the nest tree, and below any associated feeding perches at time of banding and subsequently at 10-d intervals. Use of this method ensured minimal nest disturbance while insuring that fresh prey remains were being collected. The systematic and complete removal of prey remains was done to reduce the chance of overestimating the frequency of occurrence of large prey species because of their tendency to be represented in more than one pellet or prey sample (Marti 1974). Prey remains were placed into containers and individually labeled as to collection time and relation to nest. Prey remains collected from within the nest were limited to those items that were fully consumed. Partially consumed prey items were not collected and instead were noted as to species and size.

Relative proportions of prey items in the diet were determined by examining unconsumed prey remains as well as skeletal remains in regurgitated pellets (Hayward et al. 1993). Prey items were identified down to the lowest practical taxonomic classification and grouped by species, family or order. Pellet contents were quantified as to the minimum number of individuals from each taxon necessary to account for the assemblage of remains. For prey items too large to swallow whole (> 100 g), individual time points and collection sties were examined together to reconcile the frequency of occurrence of larger prey species when remains of the same prey item were present in multiple samples. Multiple prey item identification keys were utilized for comparative identification including owl pellet identification keys (Carolina Biological Supply Company, Burlington, NC, USA) and the vertebrate skeletal collection from the MSU

museum. Avian remains were identified with the aid of MSU Kellogg Biological Station bird sanctuary personnel. Dietary composition was based on the frequency of occurrence of all identifiable prey items and compiled on the basis of absolute (%) frequency of occurrence and relative (%) composition of biomass.

Prey Item Sampling

Once identified as a principal component of owl diet, prey species were collected from the most contaminated GHO foraging areas and a reference location. Species selection and sample sizes were determined based on sensitivity and power analyses of preliminary data and expected contribution to GHO dietary exposures. For this study, a total of 171 small mammals including meadow voles (*Microtus pennsylvanicus*), white-footed mice (*Peromyscus leucopus*), deer mice (*Peromyscus maniculatus*), meadow jumping mice (*Zapus hudsonius*), eastern chipmunks (*Tamias striatus*), short tail shrew (*Blarina brevicauda*) and masked shrews (*Sorex cinereus*) were sampled from six locations at two time points. Also sampled from these locations were arthropods, including four orders each of terrestrial and aquatic invertebrates. Larger mammals such as red squirrels (*Tamiasciurus hudsonicus*), fox squirrels (*Sciurus niger*), eastern cottontails (*Sylvilagus foridanus*), muskrats (*Ondatra zibethicus*), and mink (*Mustella vison*) as well as passerine species including the American robin (*Turdus migratorius*), house wren (*Troglodytes aedon*) and tree swallows (*Tachycineta bicolor*) were sampled opportunistically throughout the study area. Sampling techniques varied depending on target species.

Hazard Evaluation

Here we provide methodologies for site-specific assessment of the hazard of chemicals in soils to GHOs based on a multiple-lines-of-evidence approach that could enable well-informed decisions regarding potential remedial actions and determination of natural resource damages (EPA 1997; Fairbrother 2003). Such an approach has been applied at other contaminated sites for wildlife species such as mink (Bursian et al. 2003) or tree swallows (Custer et al. 2005). However, to our knowledge, this is the first case in which field studies and multiple-lines-of-evidence have been utilized to assess potential risks of PCBs and ΣDDT to GHOs. The multiple-lines-of-evidence included several methods of estimating exposure. Direct observations of population densities and reproductive success were made and compared to the results of the hazard assessment. Exposure of GHOs to these compounds was characterized in two ways. Concentrations of PCBs in the diet were calculated from the site-specific dietary composition and concentrations in prey items, as well as measured concentrations of PCBs and ΣDDT in eggs and blood plasma of nestling GHOs.

Each measure of exposure was compared to the threshold for a toxic effect determined from the literature and expressed as a toxicity reference value (TRV) (USEPA 1998). An ecological risk assessment (ERA) was conducted by calculating hazard quotients (HQs). Hazard quotients were determined by dividing the measured or predicted concentration in the diet, egg or nestling blood plasma by the appropriate TRV. Toxicity reference values for the GHO hazard assessment were selected following criteria outlined by the USEPA (1995) and were derived from chronic toxicity studies in which a dose-response relationship was observed in the species of concern, or alternatively a

closely related species (Strause et al. 2007a,b). Toxicity reference values were selected from studies that examined effects of PCBs and ΣDDT in owls and eagles, however, there were no suitable studies that used the GHO as the test species. The PCB TRVs were based on a feeding study with screech owls (*Otus asio*) that examined productivity endpoints (McLane and Hughes 1980). The ΣDDT TRVs were based on field studies that examined productivity and eggshell thinning endpoints in bald eagles (*Haliaeetus leucocephalus*) (Elliott and Harris 2002), and shell thinning endpoints in the barn owl (*Tyto alba*) (Klaas et al. 1978). Aside from applying an uncertainty factor of 3 to derive the PCB lowest observable adverse effect concentration from a validly determined no observable adverse effect concentration, application of additional uncertainty or extrapolation factors to our selected TRVs was not necessary. Comparisons were made between, within, and among, sites, individuals and prey species. The multiple-lines-of-evidence approach can be optimized, based on information needed, level of effort available, and site-specific criteria and characteristics.

Population Density and Reproductive Success

The final line of evidence included measurements of population health. Health of the GHO population was assessed through the evaluation of productivity including nest success, number of nestlings per nest, fledging success, and nestling age and growth measurements as well as GHO abundance. Much of the information on population dynamics was acquired in conjunction with the owl banding and nest monitoring tasks described above. However, an additional effort was made to evaluate GHO population health using vocalization surveys.

Vocalization surveys

The results of vocalization surveys and triangulation were used to identify active breeding territories, locations of nests, site use, relative abundance and confirmation of fledging success. A combination of vocalization survey methods were used including an active method in which GHO hoots were broadcast to provoke responses (Frank 1997; Brenner and Karwoski 1985), and a passive or silent survey method during sensitive lifestage events and the periods when GHOs were most active (e.g., just before and during mating) (Rohner and Doyle 1992). Relative abundance determinations were made based on the number of individuals responding on a per survey basis. Pair vocalization responses and post survey observations were evaluated and referenced to literature-based foraging areas to delineate active territories. Nestling fledge success was determined by nestling vocalizations post banding and/or subsequent visual confirmation. All positive responses and non-responses were recorded. For the positive responses, sex and age (adult or juvenile) and global positioning system coordinates of river location, and approximate azimuth values (compass readings) of response origin were recorded for the purpose of location by triangulation. Post surveys, targeted areas of 150 m radius were searched systematically by foot for signs of GHO activity (whitewash and castings) to determine roost sites and to locate nests. Active or potentially active roost sites and nest locations were recorded using a GPS receiver. To minimize disturbance to incubating birds, ground activity during the months of February and March was limited to occupancy identification of previously located nests and known nesting platforms. Nesting activity was confirmed visually by spotting scope from predetermined

monitoring locations no less than 50 m from the nest or by overhead flights using fixed wing aircraft.

RESULTS

Over the course of five years, monitoring efforts were completed at 48 artificial and six natural nests covering approximately 14 active territories and approximately 38 km of river. Nesting activity was observed at 20 individual nests and resulted in 25 nesting attempts. Of the 20 nests utilized by GHO, five were natural nests, including four appropriated nests of other avian species and a tree cavity nest. Artificial nesting platforms were successful in attracting GHOs to preferred study areas in the floodplain. In fact, nesting activity did not occur in natural nests in those territories for which artificial nesting platforms were in place. Reuse of artificial nesting platforms over multiple seasons allowed for the minimization of temporal and spatial variability and allowed easy access for researchers. The robust owl population was ideal for evaluating the multiple-lines-of-evidence at both the target and reference sites.

Detailed methods and results for contaminant analysis and exposure assessments are provided in separate papers (Strause et al. 2007a,b). The results are summarized here to illustrate the effectiveness of the methods and as an example of sample sizes that may be necessary to detect differences between the study and reference locations. Great horned owl exposures were assessed by collecting both fresh eggs (destructive) and nestling blood plasma (non-destructive). Sample availability varied among years and locations (Table 1.1).

Table 1.1. Sampling scope and blood plasma and egg summary. Description of sampling effort by year and location. Note that for 2000 - 2002 the Fort Custer and Trowbridge sampling areas were monitored for productivity, thus only addled eggs were collected.

		Reference Sample Sites		Target Sample Sites	
Year		Ceresco	Fort Custer	Trowbridge Impoundments	Allegan SGA
2000	Active Nests		0	1	2
	Plasma		0	1	0
	Eggs		0	0	3
	Data Targeted[1]	NM	P, NP, RA	P, NP, RA	E
2001	Active Nests	1	1	2	1
	Plasma	0	1	4	0
	Eggs	1	0	0	2
	Data Targeted[1]	E	P, E, NP, RA	P, E, NP, RA	E
2002	Active Nests	2	0	4	2
	Plasma	1	0	3	2
	Eggs	2	0	1	5
	Data Targeted[1]	E, NP	P, E, NP, RA	P, E, NP, RA	E, NP
2003	Active Nests	1	1	2	1
	Plasma	1	0	1	2
	Eggs	1	1	3	0
	Data Targeted[1]	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
	Data Targeted[1]	E, NP	E, NP	E, NP	E, NP

[1] NM=not monitored; P=productivity; E=egg sampling; NP=nestling plasma sampling; RA=relative abundance

A total of 40 egg and blood plasma samples were collected. Of the 24 eggs collected during the study, five were from the reference areas and 19 were from the target areas. Blood plasma was collected from 16 individual nestlings, this included four samples from the reference areas, and 12 samples from the target areas. Statistically significant differences in concentrations of total PCBs were observed among locations (reference versus target) for the predicted dietary exposure and for total PCB and ΣDDT concentrations in GHO eggs and blood plasma (Strause et al. 2007a,b). PCB concentrations in eggs were significantly greater at the Allegan SGA compared to the Trowbridge and reference sites ($p<0.05$), and total DDT concentrations were significantly different among each of the Allegan SGA, Trowbridge and reference sites ($p<0.03$).

These differences were the result of exposures to mean PCB concentrations in floodplain soil of approximately 0.17 mg PCBs/kg, dw (dry weight) in reference areas and approximately 15 mg PCBs/kg, dw in the target areas. Differences in dietary composition between the reference and target areas also were observed (Figure 1.3). Differences between predicted dietary exposures (average potential daily dose) were largely the result of significant differences in concentrations in the prey items (Table 1.2), and were not a product of differences in dietary prey item composition. Concentrations of PCBs and ΣDDT in eggs were significantly different between reference and target areas (Figure 1.4). Diet-based HQ values calculated from geometric mean total PCB concentrations in prey animals collected from the most contaminated areas of the KRSS floodplain were less than 1.0 at the target locations. Tissue-based HQs calculated from the geometric mean concentrations of total PCBs and ΣDDT in eggs were ≤ 1.5 at all target locations (Strause et al. 2007a). In addition, a well defined relationship was

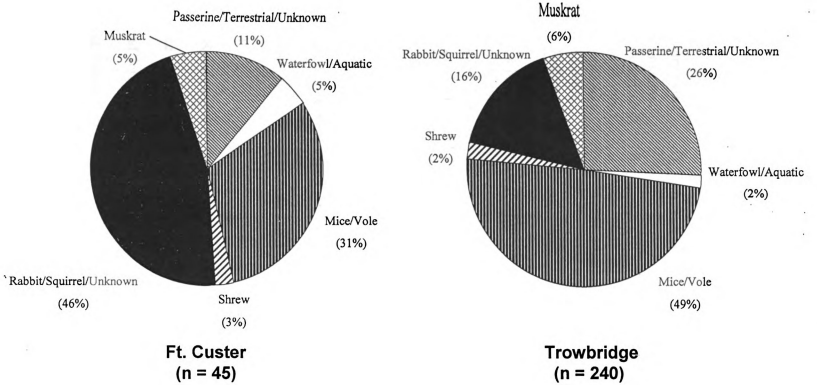

Figure 1.3. Dietary composition of GHO as determined by pellet and prey remains analysis. Data presented as percent frequency of occurrence from active nests within sampling area 2000-2002.

Table 1.2. PCB concentrations in GHO dietary items sampled from proximal foraging areas. Waterfowl were not sampled based on sensitivity analysis.

Dietary items	N	Trowbridge Mean total PCBs (± std dev) (mg/kg)[2]	N	Fort Custer Mean total PCBs (± std dev) (mg/kg)[2]
Small mammal[1]	21	*0.13 ± 0.16	18	0.021 ± 0.042
House wren adult	6	*3.57 ± 2.30	5	0.09 ± 0.032
American robin	8	*1.14 ± 1.44	4	0.091 ± 0.65
Tree swallow	5	*11.46 ± 11.90	2	1.49 ± 0.15
Shrew	17	*1.31 ± 0.94	16	0.009 ± 0.005
Muskrat	7	*0.07 ± 0.03	4	0.01 ± 0.01

[1] Includes; white-footed mouse, deer mouse, jumping mouse, meadow vole, red squirrel, and eastern chipmunk.
[2] On a wet-weight basis.
* Indicates a significant difference between sites at $p<0.05$.

established for total concentrations of PCB in eggs and those in nestling blood plasma (Strause et al. 2007c). The statistical power of the tests were such that statistically significant differences (Type I error (α) of 0.05 and Type II error (β) < 0.20) in exposure could have been detected with as few as 4 eggs or 12 samples of nestling blood plasma per area.

Relative abundance of GHOs per river km was significantly different between the reference and target areas of the Kalamazoo River, but reproductive productivity per defended territory (number of nestlings fledged per active nest) was not significantly different between study sites. During the three-year period (2000 – 2002) in which abundance measurements were completed at the KRSS, significant differences in the

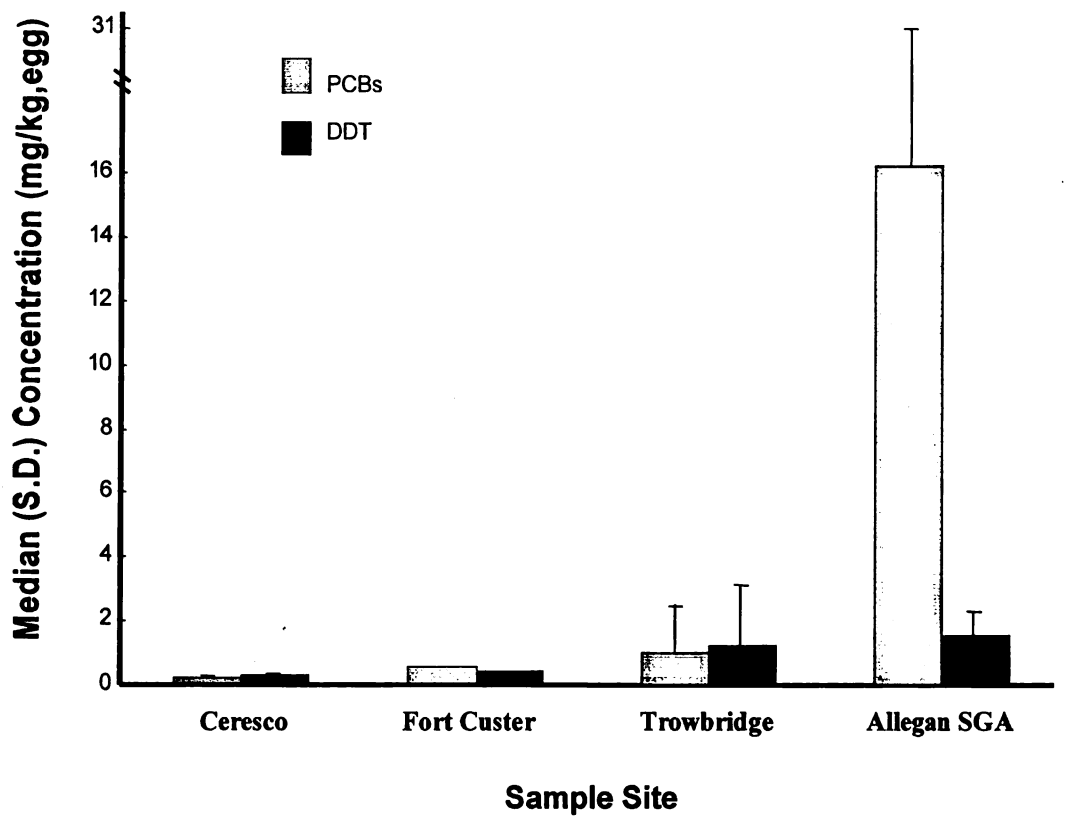

Figure 1.4. Concentrations of PCBs and ΣDDTs in GHO tissues (egg). Median concentrations and associated one standard deviation of samples collected at four locations. Sampling locations presented from upstream to downstream (left to right) with the two reference sites upstream of point sources (Ceresco and Fort Custer), and two target sites downstream of point sources (Trowbridge and Allegan State Game Area).

number of adult, juvenile and paired responses of GHOs were observed, with the Trowbridge impoundment (target area) having greater numbers of each response compared to Ft. Custer (reference) (Table 1.3). The Trowbridge impoundment had a greater number of active nests (6 versus 1) and greater overall recruitment to floodplain populations with six successful fledglings compared to one successful fledgling at Ft. Custer, however, the mean rates of productivity for the two sites were identical at 1.0 fledgling per active nest (Table 1.3).

DISCUSSION

Use of the GHO as a key receptor species in ERAs is predicated on its relatively great exposure potential, broad applicability among geographic regions and ecosystems, and ease of study. While the first two characteristics have been well documented for the GHO, its nocturnal nature and aggressive disposition may have previously dissuaded researchers from using the species in previous ERAs. For this study, the GHO proved to be a relatively easy and effective receptor species with applicability to both screening level and site-specific baseline ERAs. The single most important outcome of this study was our ability to induce breeding pairs of GHOs to occupy nesting sites centrally located within areas of interest and reuse those nesting sites over multiple years. This provided for conservative and worst case exposure assessment evaluations and risk characterizations. These behavioral attributes of the GHO offered significant advantages over other top terrestrial food web receptors including all other large resident raptors.

Table 1.3. Relative abundance and reproductive productivity of GHOs. Abundance based on adult, juvenile and pair responses to great horned owl calls broadcast from predetermined locations throughout sampling areas.

	Ft. Custer	*Trowbridge*
Relative Abundance[1]	N^2=24	N^2=22
Adults	Mean Response Rate[3]	
Total[4]	1.31	2.76
Foraging[5]	0.85	1.64
Paired[6]	0.47	1.13
Juveniles	Response Frequency[7] n, (%)	
Fledgling	1 (4%)	8 (36%)
Productivity		
Active Nests	1	6
Fledglings	1	6
Fledglings/Nest	1	1

[1] Derived from hoot call/response surveys completed at dawn and dusk.

[2] N=number of complete surveys.

[3] Mean response rate is averaged across N completed surveys.

[4] Includes discrete responses from both individual and paired owls.

[5] Includes responses from unpaired individuals only.

[6] Includes responses from paired (male + female) owls only.

[7] Response frequency of fledgling owls, n=number of surveys with at least one fledgling begging call response, (%)= (n) / number of surveys (N^2).

The strategy of conducting initial surveys to identify occupied GHO territories, followed by reconnaissance of active owl territories within the areas of interest was effective for locating existing owl territories. However, successful location of optimally located natural nests (in relation to contaminated floodplain foraging habitats) was rare. Site-specific characteristics indicate that this may have been due to an absence of available nests in the floodplain of the study area because other nest building species are more limited in nesting habitat and prefer upland areas. Artificial nesting platforms were placed inside the perimeter of defended territories and centrally located within the areas

of interest. The density of nesting platform placement ranged from one to three nests per territory at 500 to 1000 m intervals. Over the five-year study period, nesting activity was identified in 81% of the territories containing nesting platforms. Nesting activity occurred in 90% of territories in which paired owls were identified. Both relative abundance and pair response were useful predictors of nesting potential. Nesting activity in natural nests was never observed in those territories in which artificial nesting platforms were placed at least 6 mo prior to expected egg drop. Platforms were utilized preferentially even in instances where appropriate natural nests were available and/or when natural nests were utilized the year prior to artificial nest placement. Breeding and reproductive success of nesting pairs utilizing artificial nesting platforms was comparable to natural nest-based reproductive studies. Of the territories in which a platform had been placed, GHOs initiated incubation 65% of the time. In a 28-yr study, in a proximal geographical area of similar characteristics, it was found that 62% of owls in occupied territories initiated incubation. For that study, the resulting annual mean productivity expressed as the number of young/occupied territory varied moderately from 0.5 to 1.1 and the number of fledglings/successful nest was a consistent value of 1.7 (Holt 1996). In this study, reproductive productivity in both the reference and target was similar to the Holt study. The annual mean number of juveniles fledged per occupied territory ranged from 0.5 to 1.6 and the number of fledglings/successful nest was 1.4. Post-fledge survival was successfully monitored in all territories in which active surveys were systematically performed and nestlings were banded. Monitoring of survival of juveniles by their begging responses to broadcasted adult hoot calls was possible as late as 24 wk

post-banding. An option for longer-term monitoring of juvenile survivorship includes the temporary attachment of a radio transmitter prior to fledging from the nest.

In this study, several methods of estimating exposure were applied. For select nests, fresh eggs were sampled shortly after identification of incubation activity, while other territories were monitored for productivity. Nestlings from these territories as well as nestlings from egg sampling territories (re-nesters or completed incubation of partial clutch sampling) were banded, 7 ml of blood collected, general health determined, and select morphological measurements taken. Prey remains (including pellets) were collected from active nests, the base of the nest tree, and beneath nearby feeding perches for all nests in which fledgling productivity was monitored. Pellet and prey remains analyses identified 285 individual prey items.

In order to determine which type of egg sampling would have the least effect on territorial and site-wide productivity, fresh eggs were collected using two different sampling approaches. Either the entire clutch was collected to induce re-nesting or a single egg was left to induce continued incubation of the remaining egg(s). When the total clutch sampling approach was used, two of four pairs re-nested and produced three young. For nests in which the most recently laid eggs were left for continued incubation, three of seven pairs continued incubation. Two of the nests each produced one nestling and the third nest was destroyed by severe weather. In all, 24 eggs were sampled from 12 different territories.

Over the five-year study, the territories targeted for egg collection varied. Overall, the egg sampling effort targeted 24 territory-years in which 15 territory-years contained incubation activity. A territory-year is a level of effort term defined as any one

territory monitored over one year. Thus, a single territory monitored over four years or four territories monitored over a single year both involve the same level of effort, 4 territory-years. The cumulative number of sampled eggs would have increased to 30 eggs had the entire clutch been collected for all territories targeted for fresh egg collections.

Conclusions resulting from each of the lines of evidence examined in this study were consistent between and among sites. Contaminant exposure based on both dietary- and tissue-based methodologies produced similar results of significantly different COC exposures for the downstream target vs. the upstream reference area. However, the results of the hazard assessments indicated that GHO populations residing in the floodplain were not at risk for effects induced by total PCBs or ΣDDT in contaminated soils. Maximum HQ values of <1.0 (diet exposures) and <1.5 (egg exposures) indicated that exposure of GHOs to the COCs in Kalamazoo River were below or near the threshold for effects (Strause et al. 2007a,b). Confidence in the risk conclusions was further strengthened by site-specific measurements of productivity, abundance and nestling growth and success. Population parameters for target area owls were not significantly different from the upstream reference areas, and were similar to those expected in a healthy environment. The mean rate of 1.0 fledgling per active nest observed at both locations was consistent with productivity measures for healthy mid-western GHO populations residing in upland habitats (Holt 1996). Additionally, measures of site-use (abundance) indicated the target area populations at Trowbridge were near the carrying capacity for undisturbed GHO habitats (Houston et al. 1998). This consistency across each of the multiple lines of evidence for both measurement and

assessment endpoints combined with the relative certainty of each measurement, the minimal impact on the receptor and environment, and the level of effort expended, highlights the utility of the GHO as a receptor in this and possibly other ERAs and natural resource damage assessment investigations.

Here we have provided an overview of the advantages of the GHO as a site-specific surrogate species for the determination of potential risk of contaminants in terrestrial ecosystems. We have given a short overview of a case history. The space available here was limited. For that reason, neither the methods nor the results could be fully described. Detailed methods in the form of standard operating procedures are available from the authors. In addition, detailed results of the assessments are published elsewhere (Strause et al. 2007a, b, c).

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Animal Use

All aspects of the study that involved the use of animals were conducted in the most human way possible. Toward this end, all aspects of the study were conducted using standard operating procedures that had been approved (AUF #^{s} 02/10-030-00; 03 /04-044-00; 08/03-105-00) by the MSU All University Committee on Animal Use and Care (AUCAUC). All of the necessary state and federal approvals and permits obtained for the project (Michigan Department of Natural Resources (MDNR) Land Use Permit #A-02-09; MDNR Scientific Collecting Permit #SC1220; USFWS Migratory Bird Scientific Collection Permit #MB081272-1) are on file at the MSU-ATL.

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Chapter 2

Plasma to egg conversion factor for evaluating PCB and DDT exposures in great horned owls and bald eagles.

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ABSTRACT

The benefits of nondestructive sampling techniques, such as plasma sampling, to directly measure contaminant exposure levels in at-risk or protected raptor populations are many. However, such assays are generally inconsistent with the most certain source of toxicity reference values that are based on feeding studies and quantified as dietary or in ovo (egg-based) concentrations. An accurate conversion factor to translate nondestructive plasma-based contaminant concentrations to comparable egg-based concentrations will prove valuable to risk assessors investigating the potential effects of chemical exposures to raptors. We used data bases describing the concentrations of total polychlorinated biphenyls (PCBs) in great horned owls (GHO; *Bubo virginianus*) and total PCBs and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) in bald eagles (*Haliaeetus leucocephalus*) from the Great Lakes region to develop a relationship to predict concentrations of PCBs and DDE in eggs. To develop a robust predictive relationship, all of the source data included concentrations of both total PCBs and/or DDE for nestling blood plasma and egg samples collected from within discrete active nesting territories and, in most instances, the same nest. The key characteristics (slope and elevation) of each relationship were tested for differences related to species and geographic region. Predicted variability of relationships were examined and compared to variability associated with natural systems. The results of statistical testing indicate that applying the conversion factors between species (GHO to bald eagle) and among geographic regions yields predicted egg concentrations that are not statistically dissimilar and are within the

natural variability observed for residue concentrations among eggs of raptors within species and region.

Keywords: Raptors, PCBs, Plasma, Egg, Non-destructive sampling

INTRODUCTION

Because raptors are at the top of the food chain, they are maximally exposed to many persistent and bioaccumulative residues [1]. This, combined with the fact that they are susceptible to the toxic effects of many contaminants of concern, means that raptors can be used as effective and sensitive biological monitors for contaminant exposures and assessment of environmental effects [2]. Raptors also are often used as environmental sentinels for monitoring of contaminants [3] or as primary or surrogate receptor species in ecological risk assessments [4]. Raptors are particularly useful, because they are often territorial and long lived, reproducing in the same territory over long periods of time. Thus, extensive data bases of historical contaminant exposures are often available.

Historically, contaminant monitoring programs utilizing raptors have primarily used eggs because of the several advantages of using them for assessing contaminant exposure and effects. These include ease of collection and the fact that the proximal exposure of the developing embryo to the chemicals gives a direct measure of one of the most sensitive endpoints, embryo lethality [5]. Eggs are relatively easy to transport and store, and egg samples from wild bird populations are available independent of egg fertility. In addition, since lipophilic compounds tend to accumulate in lipids of eggs, quantification of residues is facilitated. Furthermore, controlled laboratory studies, including feeding and egg-injection studies, offer direct comparisons of concentrations of residues in eggs with effects such as survival, eggshell integrity and developmental deformities [6-9]. Egg-based contaminant exposure measurements have also been correlated with temporal and spatial effects. Nevertheless, egg sampling has some serious limitations when used in site-specific and long-term investigations of potential

ecological risk. These include the destructive nature of the sample, a high level of nest disturbance that significantly increases the frequency of nest abandonment, high levels of uncertainty for assigning spatial origin to the observed exposure concentration, and narrow temporal limits to the "window" of monitored exposure [10,11,(Frank, 1997, Master's thesis, University of Wisconsin, Madison, WI, USA]. Egg sampling efforts also may be limited by the gender-restricted nature of the sample.

When the disadvantages of determining egg-based exposure data outweigh the advantages, residues are measured in blood plasma [12-14]. This approach also has several advantages. These include the ability to collect blood without destroying the individual, the ability to collect samples from the same individual over time and the ability to collect samples from nestlings [14]. Because nestlings are sedentary and most residues in their blood are accumulated from food; nestling blood plasma is an integrated measure of concentrations of residues in the area proximal to the nest site, much more so than are concentrations of residues in eggs or adult plasma samples [11,15]. For most raptor species, collection of blood plasma from nestlings reduces the risk of injury to the bird and minimizes abandonment or nest relocation by adult birds. Also, blood samples need not be gender- or age-specific. Use of blood plasma has been further advanced by development of more sensitive methods of residue analyses that has lessened the mass of analyte required for quantification.

Limitations of plasma contaminant data are primarily in interpreting the effects of residues in blood plasma. There is relatively little information relating the concentrations of specific residues in blood plasma of nestling raptors to adverse outcomes, while there is more information on the effects of concentrations of residues in eggs on effects on both

individuals and populations. Long term monitoring of residues in eggs, blood and raptor populations have demonstrated that trends in concentrations of residues are similar for eggs and blood plasma [15]. Thus, the use of blood plasma for monitoring populations for adverse effects would be facilitated by predicting concentrations of residues in blood to concentrations in eggs.

We used synoptic sampling of blood plasma from nestling great horned owls (*Bubo virginianus*) and bald eagles (*Haliaeetus leucocephalus*) from the Great Lakes region to develop a relationship to predict concentrations of polychlorinated biphenyls (PCBs) and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) in eggs. We compared these relationships to those previously published for bald eagles from other regional sub-populations and assessed the variability of predicting total PCB concentrations in eggs from those in blood plasma.

MATERIALS AND METHODS

Collection of Great Horned Owl Blood Plasma and Eggs

Blood plasma from nestlings and fresh or addled eggs of great horned owls (GHO) were collected from the Kalamazoo River Superfund Site (KRSS). Collections were made between April 2000 and April 2004 along a 190 km stretch of the river's floodplain between the cities of Marshall and Saugatuck, MI (Figure 2.1). Collections were made from both naturally occurring nests and artificial nesting platforms. This location represented a gradient of concentrations of both PCBs and ΣDDT (dichlorodiphenltrichloroethane (DDT) and its metabolites

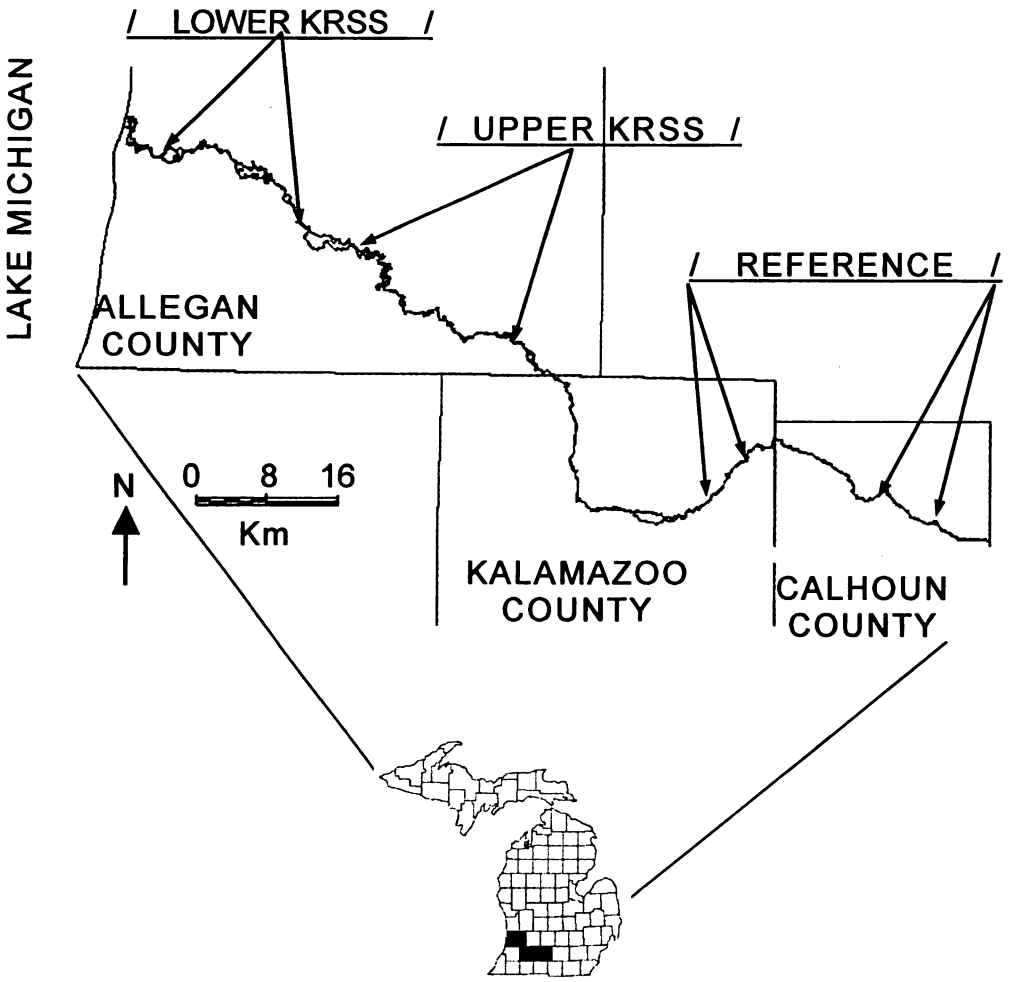

Figure 2.1. Map of area of the Kalamazoo River, indicating the location in southern Michigan as well as the three reaches across which a gradient of polychlorinated biphenyl (PCB) concentrations was observed.

dichlorodiphenyldichloroethylene (DDE)/dichlorodiphenyldichloroethane (DDD)) that ranged from local "background concentrations" to relatively great concentrations of PCBs and ΣDDT [16]. Matched egg and nestling plasma samples were collected from nests of the same mated pair occupying the same nest in the same reproductive year. In other instances, matched egg and nestling plasma were collected from the same nest over a period of two or more years. In cases where nesting pairs selected a new nest site, samples were collected from alternate nests within the same territory over two or more consecutive years. To encourage re-nesting after collection of eggs, fresh eggs were collected as soon as possible following confirmation of incubation. Addled eggs were obtained when nests were abandoned.

Eggs were labeled, transported back to the laboratory, and stored at 4 °C until processing. Length, width, and whole-egg weight and water volume were measured prior to removal of contents. Egg contents were stored in solvent-rinsed glass jars at -20 °C until measurement of PCBs and ΣDDT.

Nestling blood samples were collected when nestlings were approximately 4 to 6 wk 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 heparinized disposable syringe (25-gauge hypodermic needle) and sterile technique. Blood was transferred to a heparinized Vacutainer™ tubes and labeled. Vacutainers™ containing whole blood were centrifuged at 1200 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.

Whole egg homogenates and nestling plasma samples were processed and analyzed for congener-specific total PCBs and ΣDDT using methods described previously [17]. All chemical concentrations in eggs were corrected for moisture loss [18].

Collection of Bald Eagle Blood Plasma and Eggs

The values used to develop the egg to plasma relationships for bald eagles in the Great Lakes region were compiled from studies conducted by several State and Federal agencies as well as public and private research institutes, the majority of which were completed between 1996 and 2002. With a few exceptions, most egg samples included in this data base originated from the United States Fish and Wildlife Service (USFWS) environmental contaminants program using addled egg collection [19]. Most measurements of residues in blood plasma were from the Michigan wildlife contaminant trend monitoring program administered by the Michigan Department of Environmental Quality (MDEQ), Office of Surface Water Quality [20-23]. Additional data were also used [17,24,25, (Bowerman, 1991, Master's thesis, Northern Michigan University, Marquette, MI, USA)].

All studies reported concentrations of total PCB, and/or *p,p'*-DDE for blood plasma of nestlings and/or egg samples collected from discrete active eagle nesting territories. From these reports, concentrations of individually paired blood plasma and egg samples were assembled according to the following three general guidelines: 1) Plasma samples collected from 1996 forward were paired with egg samples collected within a 5-year window of sampling for the two media (eg., egg ('97) paired with plasma

('01)); 2) Samples collected prior to 1996 were paired only for the same or two consecutive collection years; 3) For either grouping, a third or fourth sample was included in instances where two consecutive collections of plasma or egg were made (eg., egg('86/'87), plasma('87/'88)), in which case the geometric mean concentration of the two combined samples was used. The selection of a 5-year maximum window for pairing the most recent samples was based on the trend monitoring data for PCB and *p,p'*-DDE concentrations in eggs that were not significantly different within sub-populations, between years from 1996 onward [19].

Sample collection and processing for these studies are consistent with the methods described for Kalamazoo River GHOs, but methods of chemical analyses varied to some degree. USFWS analyses of *p,p'*-DDE and total PCBs in addled eggs were completed by the USFWS Patuxent Analytical Control Facility using gas-liquid chromatography. Nominal lower limits of detection were 10 ng/g, ww for DDE and 50 ng/g for total PCBs. Egg concentrations were corrected for moisture loss [19]. MDEQ analyses of *p,p'*-DDE and total PCBs (sum of 20 PCB congeners) in nestling plasma were completed at the Clemson Institute of Environmental Toxicology using capillary gas chromatography with electron capture device following U. S. Environmental Protection Agency approved methods. All reported results were confirmed by dual column analyses. Quantification levels for both compounds were 2 ng/g [20-23]. For use in this assessment, the MDEQ PCB plasma concentrations for the 20 quantified PCB congeners were converted to a total PCB equivalent using the relationship: Total PCBs = 4.57(sum 20 PCB congeners, ng/g ww) + 0.98 [24]. Analyses of *p,p'*-DDE and total PCBs in nestling plasma for the Green Bay and Fox River samples [25] were completed

at Michigan State University and included the use of gas chromatography with electron capture detection and confirmation with mass spectrometry. Detection limits were 2.5 ng/g for DDE and 5 ng/g for total PCBs. Analytical methods for additional egg and plasma samples from Green Bay and Fox River [26] are provided by the authors.

Statistical Analyses

Sample sets were analyzed for normality by the Kolmogrov-Smirnov, one-sample test with Lilliefors transformation. Concentration data were log-normally distributed and after log-transformation satisfied assumptions of normality. To evaluate the plasma to egg relationship for each PCB and *p,p'*-DDE data base, a Pearson product-moment correlation analysis was performed on the log-transformed values. Paired blood plasma and egg concentrations of residues were plotted as a function of the blood plasma values and the line of best fit for each sample set was derived through simple regression and residuals analyses. Normality and correlation analyses were completed using the Statistica (Version 6.1) statistical package (Statsoft, Tulsa, OK, USA.). Regression residuals were calculated using EXCEL (Microsoft® Windows PE, 2002; Microsoft, Redmond, WA, USA).

To assess the robustness of the relationships developed for the GHO, predictions were compared with measured values for bald eagles at other locations available in the literature. Tests for homogeneity of regression coefficients and elevation used analysis of covariance methods (ANCOVA) [27]. Multiple comparisons among elevations were made by use of Tukey's HSD for unequal sample size [27]. The criterion for significance

used in all tests was $p<0.05$. Comparisons of conversion factor predictive variability were made by calculating the relative percent difference (RPD).

To test the similarity between egg to nestling plasma relationships between bald eagles of the Pacific Coast versus bald eagles of the Great Lakes, an ANCOVA was used to test for equality of the population regression coefficients (slope) and elevation. Tests of elevation may be considered to be the same as asking whether the two population Y intercepts are different. However, one must be cautious of misleading interpretations from such a characterization if discussion of the Y intercepts would require a risky extrapolation of the regression lines far below the range of X for which data were obtained [27,28]. The use of elevations instead of Y intercepts also assures comparison of relationships over only the range of plasma/egg concentrations measured in each study and eliminates the potentially confounding effects that analytical detection limits may contribute to a test of Y intercepts. If either test identified a statistically significant difference within the pool of data being evaluated, additional pair-wise comparisons were completed using Tukeys HSD to identify which of the population slopes/elevations differed from one another.

RESULTS

Great Horned Owls

The fidelity of GHO to established home territories and preferred nesting sites resulted in multiple instances of re-nesting that provided samples of both nestling blood plasma and eggs for the same breeding pair in the same nesting territory. A total of 14 paired GHO nestling blood plasma and egg samples were assembled from a total of 16 blood plasma and 17 egg samples. This included four nests where the samples of blood plasma and eggs were collected in the same nest during the same year and three nests where the paired samples were collected from the same nest but during different years. In seven instances, samples were collected from two different nests within an active nesting territory but during different years.

Relationships between concentrations of both total PCBs and ΣDDT were investigated. Total PCB concentrations for these 14 data points (Table 2.1) exhibited a gradient among the three reaches of the KRSS and there was a significant positive correlation (r=0.766, p=0.001) between log-normalized nestling blood plasma and egg PCB concentrations (Figure 2.2). The narrow range of ΣDDT concentrations detected in KRSS GHO plasma and egg did not exhibit such a gradient and concentrations of ΣDDT in eggs were negatively correlated with those in blood plasma (r=-0.735, p=0.003). Thus, a ΣDDT egg to blood plasma predictive relationship was not developed for GHOs.

Table 2.1. Great Lakes (Kalamazoo River) great horned owl plasma to egg polychlorinated biphenyl (PCB) conversion factor sample pairing.

PCB Sample Location (sample year: plasma/egg)	Plasma (ng PCB/ml)	Egg (μg PCB/g ww)
Lower KRSS (02/00)	147	12.2
Lower KRSS (02/01)	147	19.8
Lower KRSS (02/02)	147	25.7
Lower KRSS (02/04)	147	2.09
Upper KRSS (02/03)	80.4	1.61
Upper KRSS (00/02)	60.2	2.74
Upper KRSS (01/03)	43.7	1.61
Upper KRSS (03/03)	31.3	0.61
Lower KRSS (03/04)	31.0	2.78
Reference (03/01)	25.9	0.31
Reference (03/02)	25.9	0.17
Reference (03/03)	25.9	0.22
Upper KRSS (01/02)	24.1	2.74
Reference (02/02)	14.0	0.21

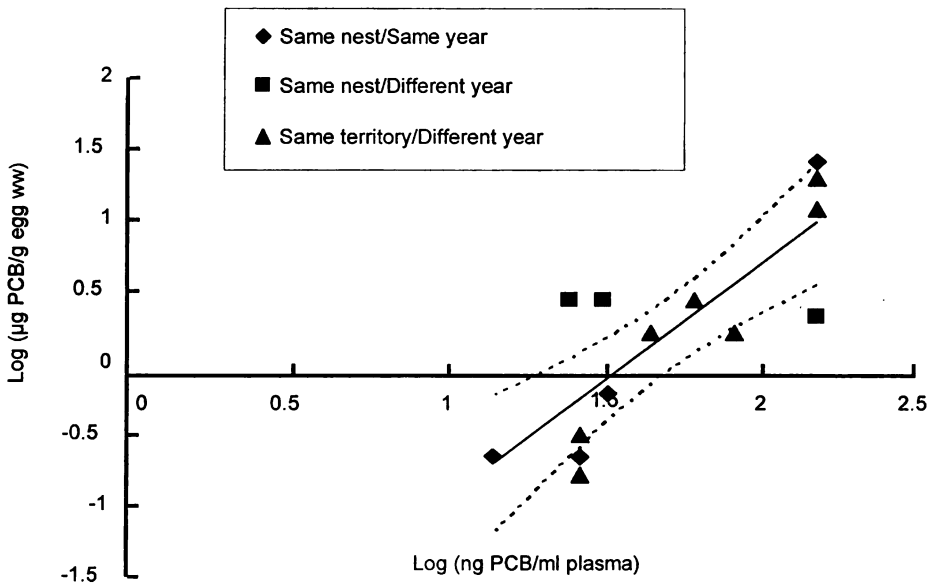

Figure 2.2. Concentration of total polychlorinated biphenyls (PCBs) in eggs of Great Horned Owls as a function of PCBs in blood plasma of nestling Great Horned Owls along the Kalamazoo River, Michigan. Regression line with 95% confidence limits of the predicted line ($\log_{10}$ (µg PCB $_{egg}$/g, ww) = 1.647[$\log_{10}$ ng PCB_{plasma}/ml)] – 2.578) ($p<0.001$, $r^2=0.666$).

Great Lakes Bald Eagles

A total of 30 (total PCBs) and 31 (*p,p'*-DDE) paired nestling plasma and egg samples were assembled from the available individual samples from the Great Lakes region (Table 2.2). Pairings originate from a single nesting territory and are combined following the pairing guidelines discussed previously. Great Lakes bald eagles exhibited significant positive correlations between total PCB and *p,p'*-DDE concentrations in nestling blood plasma and egg samples (PCBs: r=0.789, p<0.001; *p,p'*-DDE: r=0.569, p=0.001). Log-normalized PCB and *p,p'*-DDE concentrations were plotted along with the line of best fit and 95% confidence interval for the regression line (Figure 2.3, Figure 2.4).

Table 2.2. Great Lakes bald eagle plasma to egg polychlorinated biphenyl (PCB) and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) conversion factor sample pairing.

PCB Sample Location (sample year: plasma/egg)	Plasma (ng PCB/ml)	Egg (µg PCB/g) ww	Plasma (ng DDE/ml)	Egg (µg DDE/g) ww	*p,p'*-DDE Sample Location (sample year: plasma/egg)
Peshtigo River (92/92)[a,b]	901	66.6	361	14.7	Peshtigo River (92/92)[a,b]
10 Mile Creek (99/97)[a,c,d]	866	8.0	83.0	2.90	10 Mile Creek (99/97)[a,c]
10 Mile Creek (99/99)[a,c,d]	866	9.0	83.0	2.60	10 Mile Creek (99/99)[a,c]
N. Lk./No-See-Um Cr. (99-00/99)[a,e,d]	856	3.30	114	5.20	North Lk./No-See-Um Cr. (99-00/99)[a,f]
Kalamazoo R. Ottawa Marsh (00/00)[g]	773	42.2	27.0	8.10	Kalamazoo R. Ottawa Marsh (00/00)[a,e]
Skull/Stoney Is. (99/96)[a,c,d]	394	12.0	15.0	4.90	Skull/Stoney Island (99/96)[a,c]
Kalamazoo R. Swan Cr. (99/96)[a,c,d]	367	41.2	14.0	8.19	Kalamazoo R. Swan Cr. (99/96)[a,c]
Boutlier Lake (87/86)[a,h]	319	55.3	235	29.9	Boutlier Lake (87/86)[a,h]
Dinsmoore (02/99)[a,i,d]	281	6.16	39.0	4.0	Dinsmoore (01/99)[a,i]
Pere Marquette R. (00-01/02)[g]	235	23.9	31.0	14.9	Pere Marquette R. (00/02)[e,g]
Pere Marquette R. (00-01/98)[a,g]	235	34.0	31.0	0.29	Pere Marquette R. (00/98)[a,e]
UP Vulcan (02/99)[a,i,d]	206	13.0	5.0	15.0	UP Vulcan (02/99)[a,i]
Oxbox Lake (00/00)[a,e,d]	176	3.40	4.0	0.72	Oxbox Lake (00/00)[a,e]
Badwater Lake (02/00)[a,i,d]	175	1.50	7.0	0.30	Badwater Lake (02/00)[a,i]
Big Charity Island (99/96)[a,c,d]	162	18.9	9.0	3.74	Big Charity Island (99/96)[a,c]
UP Vulcan (99/99)[a,c,d]	146	13.0	5.0	15.0	UP Vulcan (99/99)[a,c]
Copper Peak (00/96)[a,d,e]	106	4.30	14.0	1.30	Copper Peak (00/96)[a,e]
Badwater Lake (99-00/00)[a,d,e]	52.0	1.50	4.0	0.30	Badwater Lake (99-00/00)[a,e]

Peavy Pond East (99-00/00)[a,d,e]	41.0	2.20	12.0	1.30	Peavy Pond East (99-00/00)[a,e]
Iron Lake (02/00)[a,d,i]	34.0	0.65	9.0	0.62	Iron Lake (02/00)[a,i]
Fortune Lake Island (00/00)[a,d,e]	28.0	0.47	10.0	0.51	Fortune Lake Island (00/00)[a,e]
Paint Lake (02/00)[a,d,i]	23.0	0.48	18.0	0.26	Paint Lake (02/00)[a,i]
Rock Lake/Carney Outlet (02/99)[a,d,i]	22.0	1.10	10.0	0.10	Rock Lake/Carney Outlet (02/99)[a,i]
Boney Falls (00-01/99)[a,d,e,f]	16.0	1.90	3.0	0.60	Boney Falls (00-01/99)[a,e,f]
Boney Falls (00-01/00)[a,d,e,f]	16.0	2.20	3.0	0.93	Boney Falls (00-01/00)[a,e,f]
Buck/Armstrong Lake (01/97)[a,d,f]	14.0	0.55	5.0	0.62	Iron Lake (99-00/00)[a,e]
Fox River (91-92/90)[j]	195	36.0	4.0	0.38	Thousand Island Lake (99-00/00)[a,e]
Big Creek (00/01)[g]	43.0	0.98	3.0	0.62	Black Creek Flooding (99/97)[a,c]
Otter Lake (00-01/00)[a,d,e,f]	13.0	0.49	3.0	0.10	Michigamme River (99/98)[a,c]
			3.0	0.44	Blue Lakes (99/97)[a,c]

[a] Egg concentrations from USFWS [19].

[b] Plasma and egg concentrations from Dykstra et.al.[25]

[c] Plasma concentrations from MDEQ [23].

[d] PCB plasma concentrations for 20 PCB congeners are converted to a total PCB equivalent using the relationship: Total PCBs=4.57(sum 20 PCB congeners, ng/g)+0.98 [26].

[e] Plasma concentrations from MDEQ [22].

[f] Plasma concentrations from MDEQ [21].

[g] Plasma and/or egg concentrations from Strause K. [17] .

[h] Plasma concentrations from (Bowerman, 1991, Master's thesis, Northern Michigan University, Marquette, MI, USA)

[i] Plasma concentrations from MDEQ [20].

[j] Plasma and egg concentrations from Dykstra and Meyer [24].

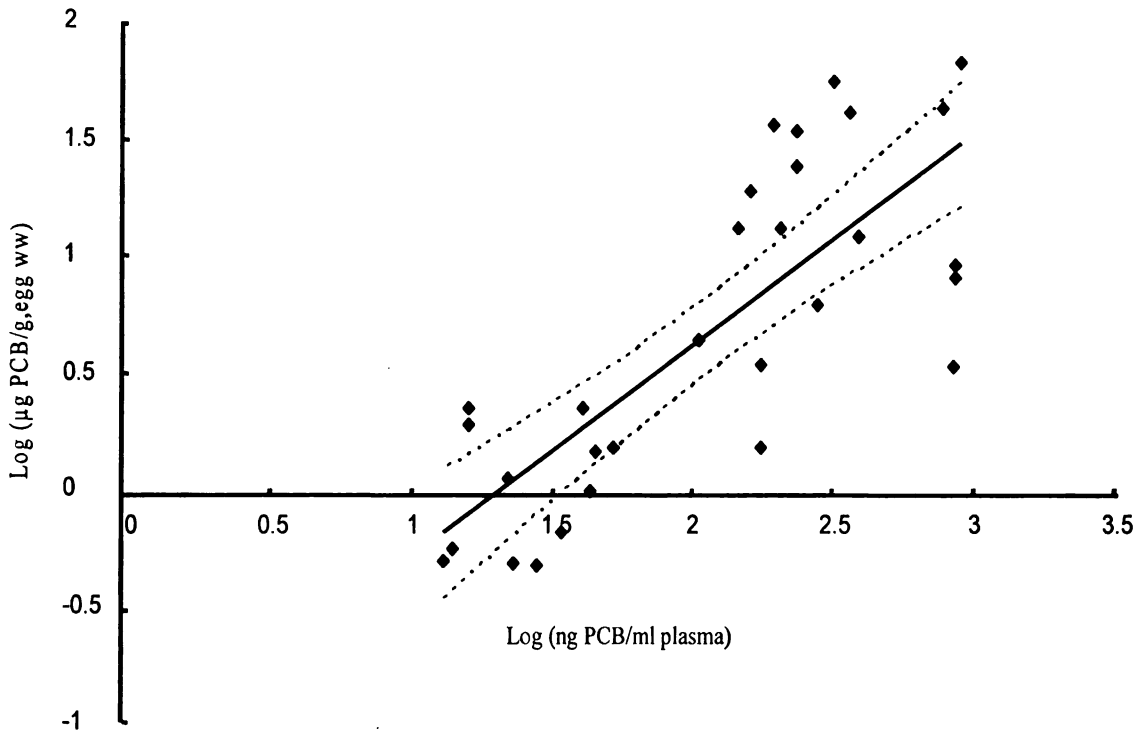

Figure 2.3. Concentration of total polychlorinated biphenyls (PCBs) in eggs of bald eagles as a function of PCBs in blood plasma of nestling bald eagles in the Great Lakes region. Regression line with 95% confidence limits of the predicted line ($\log_{10}$ µg PCB_{egg}/g, ww) = 0.905[$\log_{10}$ ng PCB_{plasma}/ml)] – 1.193) ($p<0.001$, $r^2=0.623$).

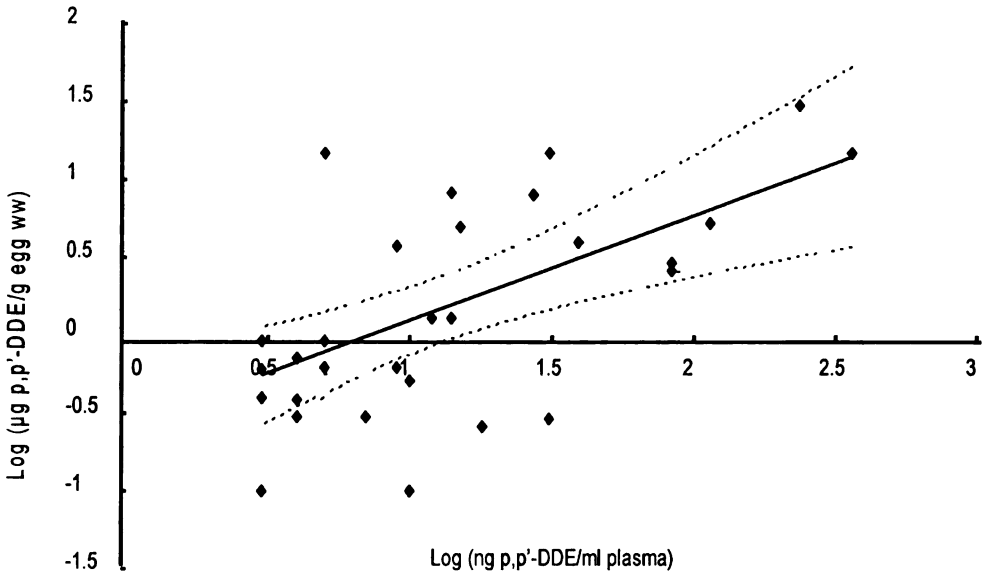

Figure 2.4. Concentration of total *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) in eggs of bald eagles as a function of *p,p'*-DDE in blood plasma of nestling bald eagles in the Great Lakes region. Regression line with 95% confidence limits of the predicted line ($\log_{10}$ µg *p,p'*-DDE_{egg}/g, ww) = 0.676[$\log_{10}$ ng *p,p'*-DDE_{plasma}/ml)] – 0.578) ($p<0.001$, $r^2=0.324$).

Confirmation of Concept

To test if the generality of relationships for the prediction of concentrations of residues in eggs from those in blood plasma of nestlings are consistent between owls and eagles, and among geographically distinct sub-populations, ANCOVA for slope and elevation were conducted. The relationship between residue concentrations in nestling blood plasma and egg has not been previously investigated for GHOs, but Elliott and Norstrom [13] and Elliot and Harris [29] have examined the distribution of PCBs and *p,p'*-DDE in bald eagle nestling plasma and egg samples from the Pacific Coast of Canada and the U.S.. We have presented a comparison of the key characteristics of each of the four PCB data bases (one GHO, three bald eagle) and three bald eagle *p,p'*-DDE data bases for which nestling plasma and egg contaminant relationships are now available (Table 2.3, Table 2.4). A more robust egg to blood plasma relationship is obtained by using individual samples from the same nest or a much larger number of summary mean concentrations [29].

Table 2.3. Plasma to egg conversion factors (CF) for total polychlorinated biphenyls (PCBs), Orders Strigiformes/Falconiformes.

PCB Plasma to Egg Relationships

Species (Subpopulation) [Reference]	Sample Pairing (N)	Strength[a]	Plasma Sample Range (ng PCB/ml)	Egg Sample Range (µg PCB/g ww)	Slope[b]	Intercept[b]
Great Horned Owl (Great Lakes) [17]	Individual Pairs[c] (14)	R^2=0.666 p<0.001	(14 – 147)	(0.2 – 25.7)	1.647	-2.578
Bald Eagle (Great Lakes) [17]	Individual Pairs[c] (30)	R^2=0.623 p<0.001	(13 – 901)	(0.47 – 66.6)	0.905	-1.193
Bald Eagle (Pacific Coast) [13]	Geometric Mean Pairs[d] (6)	R^2=0.869 p<0.03	(14 – 80)	(2.6 – 12.7)	0.734	-0.409
Bald Eagle (Pacific Coast/Great Lakes) [29]	Geometric Mean Pairs[d] (9)	R^2=0.785 p=0.001	(14 – 207)	(2.4 – 31.3)	0.824	-0.583

Table 2.3. (Cont'd)

a. R^2 and significance values for the line of best fit. Values for CF Equations [13,29] describe the data base as it is presented in each respective manuscript. All remaining values are derived from the data bases and analyses included in this assessment.

b. Regression slope and y-intercept values presented in Table 3 describe a log:log relationship between nestling plasma total PCB concentration (x = ng/ml, ww) and fresh/addled whole egg total PCB concentration (y = µg/g, ww).

c. Individual nestling plasma and egg sample pairings representative of exposure conditions within an active raptor nesting territory.

d. Geometric mean nestling plasma and egg sample pairings representative of exposure conditions within a regional raptor breeding area.

Table 2.4. Plasma to egg conversion factors (CF) for *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), Order Falconiformes.

p,p'-DDE Plasma to Egg Relationships

Species (Subpopulation) [Reference]	Sample Pairing (N)	Strength[a]	Plasma Sample Range (ng DDE/ml)	Egg Sample Range (µg DDE/g ww)	Slope[b]	Intercept[b]
Bald Eagle (Great Lakes) [17]	Individual Pairs[c] (31)	R^2=0.324 p<0.001	(3 – 361)	(0.1 – 29.9)	0.676	-0.578
Bald Eagle (Pacific Coast) [13]	Geometric Mean Pairs[d] (6)	R^2=0.912 p<0.01	(7 – 100)	(2.2 – 9.7)	0.637	-0.220
Bald Eagle (Pacific Coast/Great Lakes) [29]	Geometric Mean Pairs[d] (9)	R^2=0.918 p<0.001	(3 – 100)	(1.0 – 9.7)	0.680	-0.318

a. R^2 and significance values for the line of best fit. Values for CF Equations [13,29] describe the data base as it is presented in each respective manuscript. All remaining values are derived from the data bases and analyses included in this assessment.

b. Regression slope and y-intercept values presented in Table 4 describe a log:log relationship between nestling plasma total PCB concentration (x = ng/ml, ww) and fresh/addled whole egg total PCB concentration (y = µg/g, ww).

c. Individual nestling plasma and egg sample pairings representative of exposure conditions within an active raptor nesting territory.

d. Geometric mean nestling plasma and egg sample pairings representative of exposure conditions within a regional raptor breeding area.

There were no significant differences between the slopes of regression lines for concentrations of PCB in eggs and nestling blood plasma (ANCOVA, $p>0.05$) for the three bald eagle groups. A graphical representation of the PCB and *p,p'*-DDE egg to nestling blood plasma relationships between the four PCB sample groups and three *p,p'*-DDE sample groups is provided (Figure 2.5, Figure 2.6). There were some statistically significant differences among elevations for these three groups (ANCOVA, $p<0.003$). The elevation of the Great Lakes bald eagle group was significantly different from the two Pacific Coast groups (Tukey's Test, $p<0.03$). These findings indicate that the three population samples are all estimates of the common population regression coefficient and are approximately parallel, but with differing elevations and differing values for a

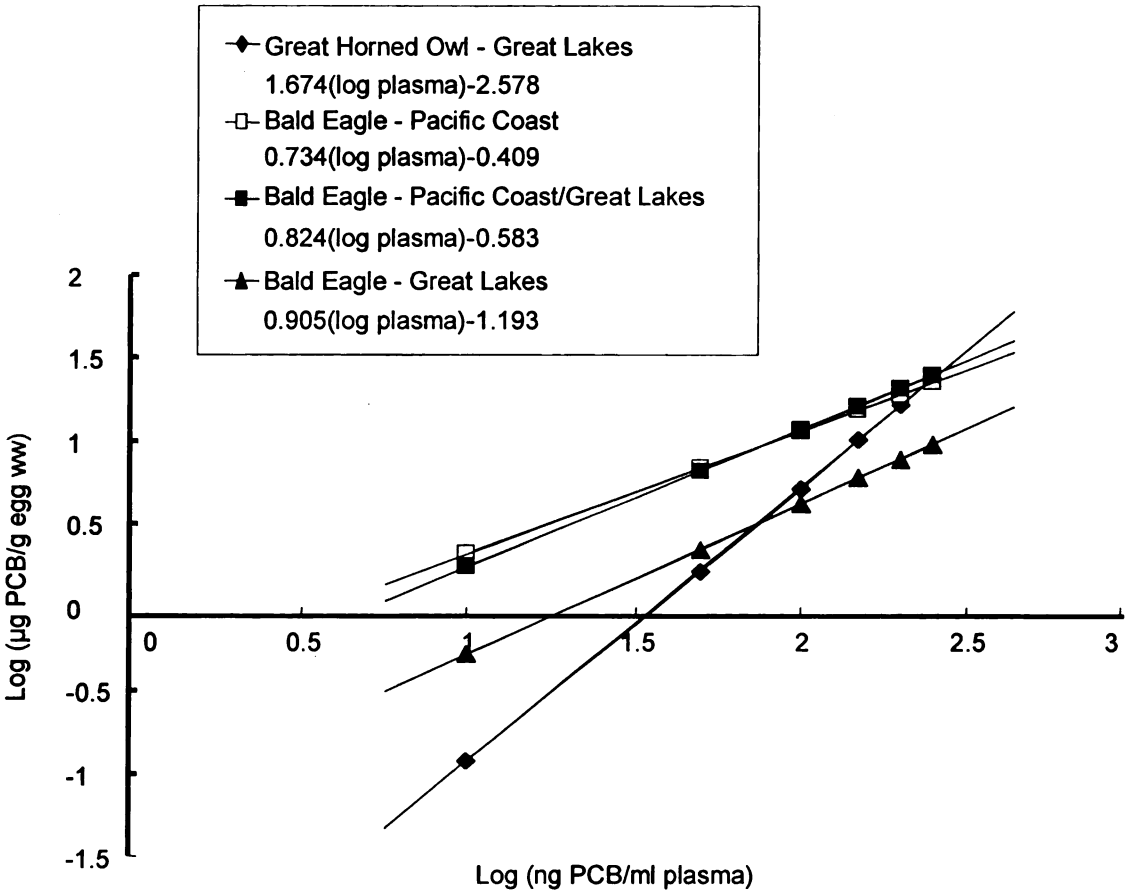

Figure 2.5. Concentration of total polychlorinated biphenyls (PCBs) in eggs of GHO and bald eagles as a function of PCBs in blood plasma of nestlings, of the same species, respectively. Regression lines are plotted and predictive equations are given in the figure legend.

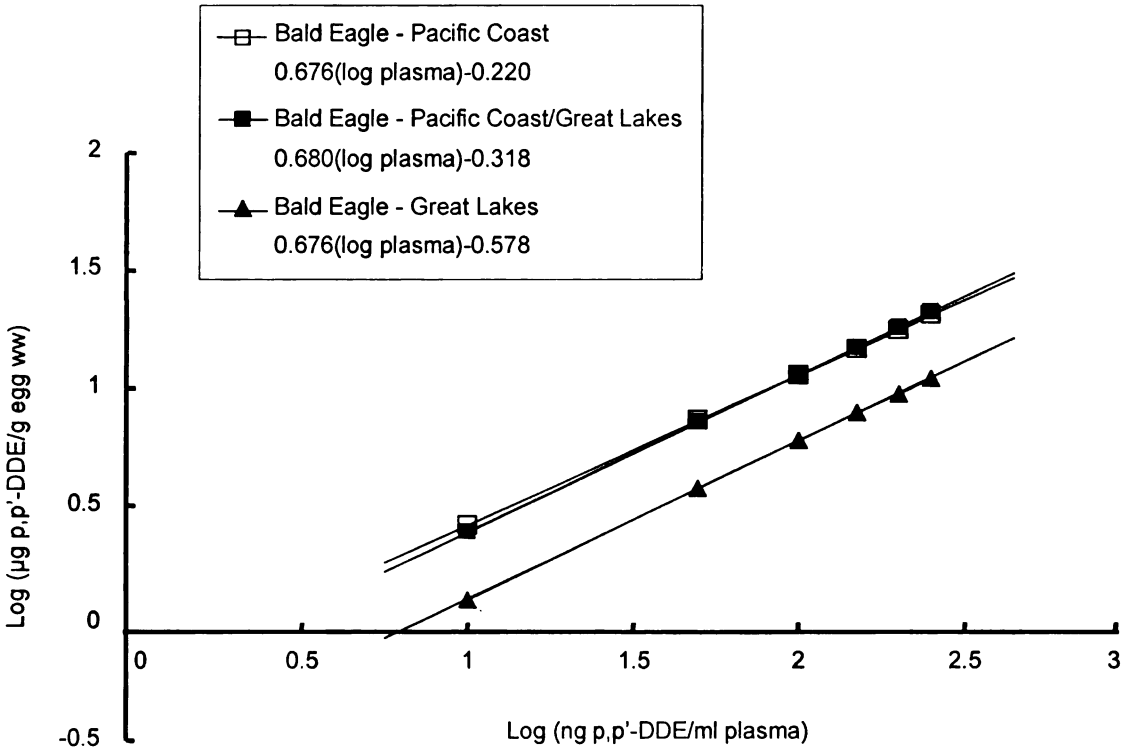

Figure 2.6. Concentration of *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) in eggs of bald eagles as a function of *p,p'*-DDE in blood plasma of nestlings, of the same species, respectively. Regression lines are plotted and predictive equations are given in the figure legend.

predicted Y (egg concentration). The slope of the relationship for the GHO from the Kalamazoo River was not different from that of the bald eagles (ANCOVA, $p>0.05$), but the elevation was significantly different (Tukey's Test, $p<0.001$). The elevation of the relationship for GHO was significantly different from that of the two Pacific Coast bald eagle groups (Tukey's Test, $p<0.03$) but not significantly different (Tukey's Test, $p>0.05$) than the Great Lakes bald eagle group. Taken together, the results of the ANCOVA and Tukey's Tests of differences in elevation for the PCB egg to plasma relationship indicate that the observed differences among the four groups is unlikely to be related to differences between species. For the *p,p'*-DDE data set, there were no statistically significant differences among either slopes or elevations of the relationship between concentrations in eggs and nestling blood plasma for the three bald eagle groups (ANCOVA, $p>0.05$). This indicates that the three sample groups could have been drawn at random as sub-populations from the same population.

Egg Predictions

To examine the predictive variability of results obtained from the various relationships, measured nestling blood plasma concentrations from the Great Lakes bald eagle data base were used in each of the four PCB conversion factor equations and three DDE conversion factor equations to predict concentrations of PCBs and DDE in eggs. The predicted PCB/DDE egg concentration was then compared to the measured egg PCB/DDE concentration comprising the plasma/egg pair from the same Great Lakes bald eagle data base. This approach allowed predictions made from each relationship to be compared to measured PCB/DDE concentrations in eggs. Differences between the predicted and

measured egg PCB/DDE concentrations were assessed by calculating the RPD between the two values. [RPD = (($|measured_{egg}-predicted_{egg}|$) ÷ ($measured_{egg} + predicted_{egg}$) /2)) * 100]. The analysis was conducted with three randomly selected sub-samples from the Great Lakes eagle data base. Using a random number generator, three sets of 10 paired plasma/egg samples were selected from the 30 PCB samples and 31 DDE samples comprising the Great Lakes bald eagle data base.

A second and more restrictive evaluation included calculating the mean predicted versus measured egg RPD for a subset of the Great Lakes eagle plasma/egg samples with a restricted range of plasma concentrations that matches or falls within the range of plasma concentrations used to establish each specific egg to plasma relationship. Predictive variability was assessed in each of the three randomly selected subsets and the single restricted subset. Results of the RPD analysis are expressed as mean RPD. (Table 2.5, Table 2.6). For the randomly selected subsets and PCB relationships, predicted egg PCB concentrations from the Great Lakes eagle data base produced the lowest range of mean RPD (73 to 78%), which would be expected since this relationship was derived from the same plasma and egg samples used in the comparison. Using the GHO conversion factor, mean PCB RPD values ranged from 74 to 97%. Mean PCB RPD values for the two Pacific Coast eagle conversion factors had the widest ranges from 73 to 97% and 70 to 100%. Predictive variability for the restricted PCB subsets show that the GHO conversion factor produced the lowest mean RPD (55%) among the four PCB conversion factors. For *p,p'*-DDE, the predicted egg concentrations from the Great Lakes eagle data base again produced the lowest range of mean RPD (52-81%) for the randomly selected subsets and the restricted sample (77%), as expected. Mean DDE RPD ranges

for the two Pacific Coast eagle conversion factors included 89 to 93% and 95 to 97%, and restricted mean RPDs were 99% and 105%.

Table 2.5. Bald eagle and great horned owl percent difference[a] (RPD) assessment for predicted versus measured egg polychlorinated biphenyl (PCB) concentrations across three randomly selected[b] cohorts (n=10) and the plasma-restricted[c] cohort drawn from the Great Lakes bald eagle data base[d].

PREDICTED VS. MEASURED PCB CONCENTRATIONS

	MEAN PREDICTED RELATIVE PERCENT DIFFERENCE (%) FROM MEASURED			
Random Sample # Plasma Range (ng PCB/ml) Egg Range (µg PCB/g ww)	Pacific Coast Bald Eagle [13]	Pac. Coast./G.L. B. Eagle [29]	Great Lakes GHO [17]	Great Lakes Bald Eagle[e] [17]
#1 (16 – 866, ng/ml) (1 – 55, µg/g)	100	97	93	77
#2 (16 – 773, ng/ml) (0.5 – 55, µg/g)	92	90	74	73
#3 (106 – 866, ng/ml) (4 – 42, µg/g)	70	73	83	78
Plasma-restricted Sample	117	97	55	74
Plasma range (ng PCB/ml)	(14 – 52, ng/ml)	(14 – 206, ng/ml)	(14 – 146, ng/ml)	(13 – 901, ng/ml)
Egg range (µg PCB/g ww)	(0.5 – 2.2, µg/g)	(0.5 – 362, µg/g)	(0.5 – 13, µg/g)	(0.5 – 67, µg/g)
(n)	(11)	(18)	(13)	(30)

Table 2.5 (Cont'd)

a.

$$RPD = \frac{|Y_P - Y_M|}{\frac{(Y_P + Y_M)}{2}}(100)$$

Y_P = predicted egg
Y_M= measured egg

b. Randomly selected sub-sample identified using random number generator to select n=10 plasma/egg paired samples from N=30 (PCB) and N=31 (DDE) possible individual paired data points in the Great Lakes bald eagle data base.
c. Plasma-restricted sub-sample includes all individual paired data points in the Great Lakes bald eagle data base lying within the range of plasma concentrations used to derive each specific PCB and p,p-DDE conversion factor data bases examined in this manuscript. Sample sizes specific to the plasma-restricted sub-sample are noted below.
d. See Table 2 for individually paired plasma and egg data.
e. Great Lakes bald eagle data base included for comparison purposes, illustrating effects of sample variability and site-specific exposure potentials on the intrinsic predictive accuracy of the line of best fit across the source data base.

Table 2.6. Bald eagle percent difference[a] (RPD) assessment for predicted versus measured egg *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) concentrations across three randomly selected cohorts[b] (n=10) and the plasma-restricted cohort[c] drawn from the Great Lakes bald eagle data base[d].

PREDICTED VS. MEASURED *p,p'*-DDE CONCENTRATIONS

	MEAN PREDICTED RELATIVE PERCENT DIFFERENCE (%) FROM MEASURED		
Random Sample # Plasma Range (ng/ml) Egg Range (µg/g)	Pacific Coast Bald Eagle [13]	Pacific Coast/G. Lakes Bald Eagle [29]	Great Lakes Bald Eagle[e] [17]
#1 (3 – 39, ng/ml) (0.1 – 15, µg/g)	95	89	81
#2 (3 – 361, ng/ml) (0.1– 15, µg/g)	99	93	52
#3 (4 – 361, ng/ml) (0.3 – 15, µg/g)	97	93	66
Plasma-restricted Sample	105	99	77
Plasma range (ng DDE/ml)	(7 – 83, ng/ml)	(3 – 83, ng/ml)	(3 – 361, ng/ml)
Egg range (µg DDE/g ww)	(0.1 – 15, µg/g)	(0.1– 15, µg/g)	(0.1– 30, µg/g)
(n)	(16)	(28)	(31)

Table 2.6 (Cont'd)

a.

$$RPD = \frac{|Y_P - Y_M|}{\frac{(Y_P + Y_M)}{2}}(100)$$

Y_P = predicted egg

Y_M= measured egg

b. Randomly selected sub-sample identified using random number generator to select of n=10 plasma/egg paired samples from N=30 (PCB) and N=31 (DDE) possible individual paired data points in the Great Lakes bald eagle data base.

c. Plasma-restricted sub-sample includes all individual paired data points in the Great Lakes bald eagle data base lying within the range of plasma concentrations used to derive each specific PCB and *p,p*-DDE conversion factor data bases examined in this manuscript. Sample sizes for the plasma-restricted sub-sample are noted below.

d. See Table 2 for individually paired plasma and egg data.

e. Great Lakes bald eagle data base included for comparison purposes, illustrating effects of sample variability and site-specific exposure potentials on the intrinsic predictive accuracy of the line of best fit across the source data base.

DISCUSSION

Because nestlings can be captured and handled more easily than adults, it is less likely to cause harm to individuals or the population [13,14]. The use of blood plasma from nestlings eliminates the potentially confounding influence of adult exposures during migrations or on their wintering grounds or in instances where resident, non-migratory species or individuals shift or greatly expand winter foraging territories [30]. Because most of the residues in the blood plasma of nestlings is accumulated from the area proximate to the nest, these results can be more easily compared with the results of diet studies to identify significant contributing sources of environmental contaminants in the food web [31]. Also, because the embryo and nestling are the most sensitive life stages for population-level effects of chlorinated hydrocarbon contaminants, field monitoring of nestling plasma combined with laboratory feeding and egg injection studies often provides the best opportunities to integrate laboratory and field studies in efforts to derive and verify field-based toxicity reference values for plasma-based endpoints [32,33].

Predictive Accuracy of the Plasma to Egg Conversion Factor

Focused studies to specifically examine the predictive accuracy of the plasma to egg relationship are not available in the literature. Nevertheless, the relationship has been used in several studies as a tool for further interpretation of adult and nestling plasma data. The earliest efforts to predict concentrations of a residue in eggs from measurements in blood plasma was for ΣDDT concentrations in adult falcons and accipiter hawks. In wild American kestrels (*Falco sparverius*) of the Pacific Northwest,

ΣDDT residues in adult female plasma closely paralleled ΣDDT residues in eggs laid by the same birds [34]. Concentrations of DDE in eggs (lipid-basis) corresponded well with concentrations of DDE in adult European sparrow hawks (*Accipiter nisus*) [35]. A significant decrease in total concentrations of DDE in the bodies of females due to translocation to eggs was also observed. In both laboratory and field studies, concentrations of DDE in blood plasma of American kestrels correlated with exposure in the diet. Concentrations of ΣDDT in adult female blood plasma in populations of three accipiters (goshawk (*Accipiter gentilis*), coopers hawk (*Accipiter cooperii*), sharp-shinned hawk (*Accipiter striatus*)) were correlated with ΣDDT concentrations in eggs [12]. Those authors also described a large decline in female kestrel plasma ΣDDT concentrations due to egg laying/transfer to eggs, similar to that observed for sparrow hawks. Studies of ΣDDT exposure were conducted during a period when researchers were investigating the egg-thinning effects of DDT and its metabolites DDD/DDE, and the maternal transfer of contaminates to eggs was of primary interest to researchers who were deciphering the mechanisms of action for this compound. Separate adult plasma (female) to egg conversion factors for ΣDDT were developed for wild, nesting kestrels (*Falconidae*) and accipiter hawks (*Accipitridae*) [12]. The parameters describing the two relationships were not statistically different for the two species. Therefore, those authors suggested use of a pooled regression to predict egg concentrations from concentrations of DDT in adult blood plasma for these two families. Use of the pooled data set in the log-log relationship for the two species resulted in a relationship that could be used to make comparisons among species. While these relationships provide useful background

information, the DDT relationships were not compared to the relationships developed in this paper because they did not meet the selection criteria in this study.

The pooled relationship developed for kestrel and accipiter hawks in one region [12] has been used to predict concentrations of ΣDDT and PCBs in eggs from concentrations in blood plasma from wintering adult eagles in Colorado and Missouri [36]. The pooled relationship was used to predict concentrations of ΣDDT in eggs from plasma and to assess the potential for DDE-caused eggshell thinning and potential impacts to reproductive success of individual eagles. The egg to adult plasma ΣDDT relationship also was used to estimate the potential hazard to reproductive success of migrating peregrine falcons exposed to DDE on their wintering grounds [37]. The same relationship was used to predict concentrations of DDE in egg from concentrations of DDE in blood plasma of adult bald eagles so that the exposure of those eagles could be compared to addled eggs collected from the same sub-region [38]. In that study predicted concentrations of DDE in eggs corresponded very well with measured values (RPDs<10%). However, concentrations of DDE in blood plasma were predicted from concentrations of DDE in whole-blood that had been adjusted for loss of DDE during storage and dilution. The relationship developed for kestrels and accipiter hawks also was used in two separate studies to predict concentrations of ΣDDT in eggs of several South African raptors, including the greater kestrel (*Falco repicoloides*) and the lanner falcon (*Falco biarmicus*), and pied kingfishers (*Ceryle rudis*). In both studies a screening-level hazard assessment was conducted by comparing the predicted concentration of ΣDDT to a toxicity reference value based on concentrations of DDE in the eggs of sensitive raptor and piscivorous species [39-42]. More recently, the USFWS

and MDEQ [26] derived toxicity reference values based on concentrations of PCBs and DDE in blood plasma of nestling bald eagles using plasma to egg relationships [29] and field-based benchmarks (concentrations in eggs that were associated with reduced productivity) [43,44].

The major obstacle to assessing the predictive accuracy of the plasma to egg relationship is the absence of an extensive data base containing individually matched plasma and egg concentrations from the same nest. The data base assembled from the USFWS addled egg monitoring [19] and the MDEQ plasma sampling [20-23] that is presented here provided a basis for this evaluation. The predictive variability RPD results indicate that applying the conversion factor between species (GHO to bald eagle) and among geographic regions (Pacific Coast to Great Lakes) yielded predicted egg concentrations that are within the natural variability observed for residue concentrations among eggs of raptors within a species and region. Within-clutch RPD values for total organochlorine pesticide concentrations in non-migratory sparrow hawks in Great Britain have been observed to be as great as 32%, while mean concentration (clutch means) between-clutch RPD values from the same female on the same territory are as great as 63% [45]. Relative percent difference values as great as 171% for PCBs (3-year interval) and 80% for DDE (4-year interval) have been seen for sparrow hawks from a sub-population of females nesting within the same geographic sub-region [46]. Studies of PCB and DDE concentrations in eggs sampled from the same population of eagles nesting in the vicinity of Green Bay exhibited between-clutch variability that ranged from 38% (one-year interval) to 125% (four-year interval) RPD for PCBs, and from 31% (one-year interval) to 133% (four-year interval) RPD for *p,p'*-DDE [43].

UTILITY AND APPLICATION

There is variability inherent in predicting concentrations of PCBs and *p,p'*-DDE in eggs using the egg to plasma relationships for GHO and bald eagle presented in this assessment. The predicted values and widely-bounded 95% prediction intervals for a relevant range of nestling blood plasma concentrations illustrate the homogeneity of slopes and heterogeneity of elevations among the four PCB and three DDE relationships (Table 2.7, Table 2.8). The predicted PCB and DDE egg concentrations for the Pacific Coast bald eagles show a consistent difference from the Great Lakes eagle and GHO predicted values. Even at their greatest, the observed divergences represent only a three-fold difference in the range of predicted egg concentrations that would be significant for an ecological risk assessment (e.g., at or above the toxicity reference value threshold concentration). This indicates that for ecological risk assessment applications, the use of a plasma to egg conversion factor would introduce minimal levels of uncertainty to calculations of risk.

Table 2.7. Estimated polychlorinated biphenyl (PCB) concentration in egg (+/- 95% PI)[a] from a relevant range of field-based nestling exposure/plasma concentrations.

"Predicted" Egg Concentrations

(μg PCB/g ww)

Total PCB Exposure

	INDIVIDUAL PAIRED SAMPLES[b]				GEOMETRIC MEAN PAIRED SAMPLES[b]			
Plasma Concentration	G. H. Owl		Bald Eagle		Bald Eagle		Bald Eagle	
(ng PCB/ml)	Great Lakes		Great Lakes		Pacific Coast		Pacific Coast/G. Lakes	
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
	1.674	-2.578	0.905	-1.193	0.734	-0.409	0.824	-0.583
10	0.12		0.52		2.11		1.74	
	(0.01 – 1.49)		(0.06 –4.57)		(0.83 – 5.39)		(0.58 – 5.25)	
50	1.66		2.22		6.89		6.56	
	(0.17 – 15.8)		(0.27 – 17.9)		(2.98 – 16.0)		(2.46 – 17.5)	
100	5.20		4.15		11.5		11.6	
	(0.51 – 52.4)		(0.52 – 33.0)		(4.28 – 30.8)		(4.17 – 32.4)	
150	10.1		5.99		15.43		16.2	
	(0.92 – 111)		(0.75 – 47.7)		(5.12 – 46.7)		(5.53 – 47.6)	
200	16.3		7.77		19.1		20.6	
	(1.36 – 193)		(0.97 – 62.2)		(5.76 – 63.4)		(6.7 – 63.2)	
250	23.5		9.51		22.4		24.7	
	(1.83 – 300)		(1.18 – 76.5)		(6.28 – 80.7)		(7.73 – 79.1)	

Table 2.7 (Cont'd)

a. Upper and lower 95% prediction interval for predicted egg "Y".
b. Regression slope and y-intercept values describe a log:log relationship for total PCB concentrations in plasma (ng PCB/ml, ww) and egg (µg PCB/g, ww).

Predicted egg concentrations (+/- 95% PIs) are for plasma values lying beyond the range of data used to derive each respective conversion factor.

Table 2.8. Estimated *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) concentrations (+/- 95% PI)[a] in egg from a relevant range of field-based nestling exposure/plasma concentrations.

	"Predicted" Egg Concentrations (μg DDE/g ww)					
***p,p'*-DDE Exposure**	INDIVIDUAL PAIRS[b]		GEOMETRIC MEAN PAIRED SAMPLES[b]			
PlasmaConcentration (ng DDE/g)	Bald Eagle Great Lakes		Bald Eagle Pacific Coast		Bald Eagle Pacific Coast/G. Lakes	
	Slope 0.676	Intercept -0.578	Slope 0.637	Intercept -0.220	Slope 0.668	Intercept -0.282
10	1.26 (0.08 – 20.0)		2.61 (1.29 – 5.29)		2.43 (1.34 – 4.41)	
50	3.72 (0.22 – 61.9)		7.28 (3.52 – 15.0)		7.13 (3.86 – 13.2)	
100	5.94 (0.34 – 105)		11.3 (5.03 – 24.4)		11.3 (5.85 – 21.9)	
150	7.81 (0.42 – 144)		14.7 (6.1 – 35.1)		14.9 (7.39 – 29.8)	
200	9.48 (0.50 – 180)		17.6 (6.95 – 44.4)		18.0 (8.70 – 37.2)	
250	11.0 (0.56 – 216)		20.3 (7.68 – 53.4)		20.9 (9.86 – 44.2)	

Table 2.8 (Cont'd)

a. Upper and lower 95% prediction interval for predicted egg "Y".
b. Regression slope and y-intercept values describe a log:log relationship for p,p'-DDE concentrations in plasma (ng DDE/ml, ww) and egg (µg DDE/g, ww).

Predicted egg concentrations (+/- 95% PIs) are for plasma values lying beyond the range of data used to derive each respective conversion factor.

CONCLUSION

Birds will continue to be used as indicators of environmental contamination due to their ubiquitous global distributions, high metabolic rates and diverse foraging habits. The advantages that bird eggs provide as a medium for assessing the bioavailability of lipophilic contaminants will undoubtedly encourage the selection of egg-based sampling programs and toxicity reference values will continue to be based on concentrations of residues in eggs. To minimize effects on populations and maximize the site-specific assessment of exposures in some cases, measurement of residues in blood plasma will be more practical.

We have demonstrated that concentrations of residues in blood plasma can be used to predict concentrations of persistent and bioaccumulative compounds in eggs by use of a blood plasma to egg conversion factor. The egg to plasma relationships derived herein from individually paired great horned owl and bald eagle samples in Great Lakes populations are not statistically dissimilar than comparable egg to plasma relationships provided in the literature for Pacific Coast bald eagles. These findings also indicate that raptors express similar relationships between nestling plasma and egg concentrations across closely related avian orders and across geographically isolated subpopulations. The plasma to egg conversion factor can be used as an accurate and reliable tool to translate nondestructive plasma-based contaminant exposure measurements to comparable egg-based concentrations. These "calculated egg-basis concentrations" can then be used with egg-based toxicity reference values derived from population-level benchmark effects (e.g., embryo mortality, developmental deformities, fledgling

productivity) to assess the health of animal communities. The plasma to egg conversion factor also offers ecological risk assessors an additional tool to aid with interpretations of dissimilar data. It is our hope that by incorporating plasma-based sampling protocols into long-term monitoring plans and site-specific hazard assessments that this method will contribute to more efficient and less disruptive studies of raptor populations in all habitats.

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Chapter 3

Risk Assessment of Great Horned Owls (*Bubo virginianus*) Exposed to Polychlorinated Biphenyls (PCBs) and DDT along the Kalamazoo River, Michigan

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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). The study examined risks from total PCBs, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), and total DDTs (sum of DDT/DDE/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/relative abundance measures for KRSS GHOs were compared with other GHO populations. Egg shell thickness was measured to assess effects of *p,p'*-DDE. PCBs in eggs ranged up to 4.7 $x10^2$ and 4.0 $x10^4$ ng PCB/g, ww from the RA and combined KRSS sites, respectively. Egg $TEQ_{WHO\text{-}Avian}$ calculated from aryl hydrocarbon receptor-active PCB congeners and World Health Organization Toxicity Equivalency Factors ranged to 8.0 and 1.9 x 10^2 pg $TEQ_{WHO\text{-}Avian}$/g, ww at the RA and combined KRSS, respectively. Egg ΣDDT ranged to 4.2 $x10^2$ and 5.0 $x10^3$ ng ΣDDT /g, ww at the RA and combined KRSS, respectively. Hazard quotients (HQs) for the upper 95% confidence limit (UCL) (geometric mean) and least observable adverse effect concentration (LOAEC) for COCs in eggs were $\leq$ 1.0 for all sites. The NOAEC (no observable adverse effect concentration) 95% UCL HQs in eggs were $\leq$ 1.0, except at the Lower KRSS (PCB HQ=3.1; $TEQ_{WHO\text{-}Avian}$ HQ=1.3). Productivity/relative abundance measures indicated no population level effects in the Upper KRSS.

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 yr [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, MI to Lake Michigan at Saugatuck, MI. The primary contaminants of concern (COCs) are PCBs, including total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents [$TEQ_{WHO\text{-}Avian}$] 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 weight (dw) to 8.5 $x10^4$ ng PCB/g, dw with a mean concentration of approximately $1.1x10^4$ ng PCB/g, dw [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 environmental monitors due to their longevity (up to 28 yr 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 (Frank RA, 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 five-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, MI, a distance of approximately 190-river km (Figure 3.1, Table 3.1). 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, dw). 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

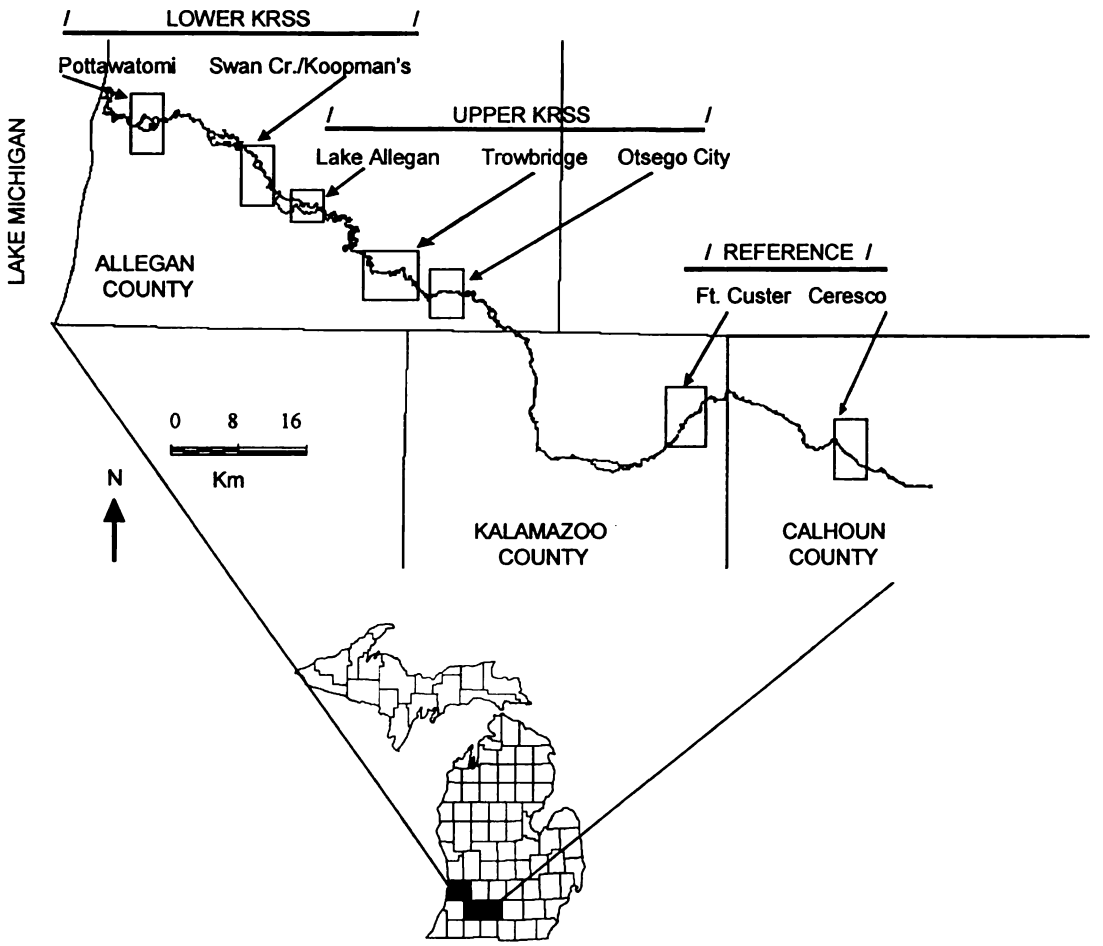


Figure 3.1. Kalamazoo River superfund site (KRSS) great horned owl (*B. virginianus*) study sites.

Table 3.1. Kalamazoo River great horned owl (*B. virginianus*) study sites, 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				
Ceresco	UH, SS	11.5	NA[e]	170
Ft. Custer	FH	5.6	NA	9
Upper KRSS[f]				
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[g]
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 concentration in 0 to 15 cm depth.
e. Not applicable.
f. Kalamazoo River Superfund Site.
g. Not sampled.

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) vs. 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 "worst-case exposure" by maximizing 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-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/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 wk 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 2 to 8 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 wk 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/syringe and sterile technique. Blood was transferred to a heparinized Vacutainer™ and labeled. Vacutainers™ containing whole blood were centrifuged at 1200 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 United States Fish and Wildlife Service (USFWS) 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 Reference area 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 co-located 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 World Health Organization (WHO) toxic equivalence factors [22]. Polychlorinated-dibenzo-*p*-dioxins (PCDDs) and polychlorinated-dibenzo-furans (PCDFs) 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/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/location/month) to determine the relative abundance and site use characteristics for juvenile, non-territorial individuals (foraging adults) and territorial nesting pairs of owls. Surveys were completed under dry, windless conditions during crepuscular hours, beginning ~ 60 min prior to sunrise or ~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/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/quality control sets composed of similar tissues were included with each group of 20 samples. Concentrations of PCBs, including di- and mono-*ortho*-substituted congeners (coplanar) were determined by gas chromatography (Perkin Elmer AutoSystem and Hewlett Packard 5890 series II) equipped with a ^{63}Ni electron capture detector (GC-ECD). Concentrations of non-*ortho*-substituted PCB congeners and ΣDDT were determined by gas chromatograph mass selective detector (GC-MS) (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 (ww) 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- and mono-*ortho*-substituted PCB congeners was conservatively estimated (minimum surface to noise ratio of 10.0) to be 1.0 ng

PCB/g, ww, 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, ww. Either TurboChrom (Perkin Elmer, Wellesley, MA, USA) 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.

Toxicity Reference Values

In this 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 co-contaminants 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, ww. 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 $x10^3$ ng/g, ww and a maximum concentration of 1.8 $x10^4$ ng/g, ww [24] (Table 3.2). Since a LOAEC was not determined in that study,

Table 3.2. 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 (*B. virginianus)* eggs. Reference number is located next to each value.

	Tissue Based TRV	Response Endpoint[a]	Reference
Total PCBs (ng/g, wet wt)			
NOAEC	7000	EST,CS,EV,FS	[24]
LOAEC	21000	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	3600	EST,FS	[29,30]
LOAEC	12000	EST,EV,FS	[31,32]

a. EST-egg shell thickness; CS-clutch size; EV-egg viability; FS-fledgling success; EI-enzyme induction.

the LOAEC was estimated by multiplying the NOAEC by an uncertainty factor of three [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, ww 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, ww, based on CYP1A induction, was also adopted from the lowest observable effect concentration determined in the same eagle study [26] (Table 3.2). 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, ww. 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, ww as a LOAEC-threshold for bald eagles. A LOAEC concentration of 1.2×10^4 ng ΣDDT /g egg, ww was selected from the study of effects in the barn owl (*Tyto alba*) [31] and is supported by recommendations for bald eagles [32] (Table 3.2).

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 3.2). 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]. HQs were calculated by dividing concentrations of each COC in egg (using both the lower and upper 95% 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 HSD (multiple comparisons) and T-test for simple pair-wise 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), non-parametric statistical methods were used, including Kruskel-Wallace 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 (X^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 6 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 3.3). 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

Table 3.3. Numbers of active great horned owl (*B. virginianus*) nests and samples collected by year (2000-2004).

	SAMPLE SITE			
	Reference		Kalamazoo River Superfund Site	
YEAR	Ceresco	Ft. Custer	Upper KRSS[a]	Lower KRSS
2000				
Active Nests	0	0	1	2
Plasma	0	0	1	0
Eggs	0	0	0	3
Sampling Scope[b]	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[b]	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[b]	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[b]	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[b]	E,NP	E,NP	E,NP	E,NP

a. Kalamazoo River Superfund Site.
b. NS-not sampled; E-egg; NP-nestling plasma; RA-relative abundance; P-productivity.

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 Reference area and the greatest concentrations occurred in eggs from the LKRSS (Table 3.4, Figure 3.2). Geometric mean egg PCB concentrations at the Reference and UKRSS sites were not significantly different from each other (Kruskel-Wallace, p=0.157), but the geometric mean concentrations at both of these sites were significantly less than concentrations at the LKRSS (Kruskel-Wallace, 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 Reference (n=3) and LKRSS (n=2) plasma samples. Geometric mean concentrations of PCBs in blood plasma at both the Reference and LKRSS sites were not significantly different from each other (Kruskel-Wallace, p=1.36), the geometric mean concentrations at both of these sites were significantly less than concentrations at the UKRSS (Kruskel-Wallace, 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

Table 3.4. Geometric mean, ww (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 (*B. virginianus)* eggs and plasma (total PCBs only) from the Kalamazoo River Superfund Site (KRSS).

	Plasma PCB		Egg PCB[a]		$TEQ_{WHO\text{-}Avian}$[b]		Relative Potency[b,c]		Lipid[b]	
STUDY SITE	ng/ml (range)	N	ng/g (range)	N	pg/g (range)	N	µg/g (range)	N	% (range)	N
Reference[d]	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[e]	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[f]	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. PCB concentrations include fresh eggs and addled eggs.
b. TEQ, relative potency, and lipid concentrations include fresh and addled eggs.
c. Relative potency = TEQ(pg/g)/PCB(µ/g).
d. Reference sample site includes samples from the Ceresco and Ft. Custer locations.
e. Upper KRSS sample site includes samples from the Trowbridge, Otsego City Dam, and Lake Allegan impoundments.
f. Lower KRSS sample site includes samples from Koopman's marsh, Swan Creek Highbanks, and Pottawatomi marsh.

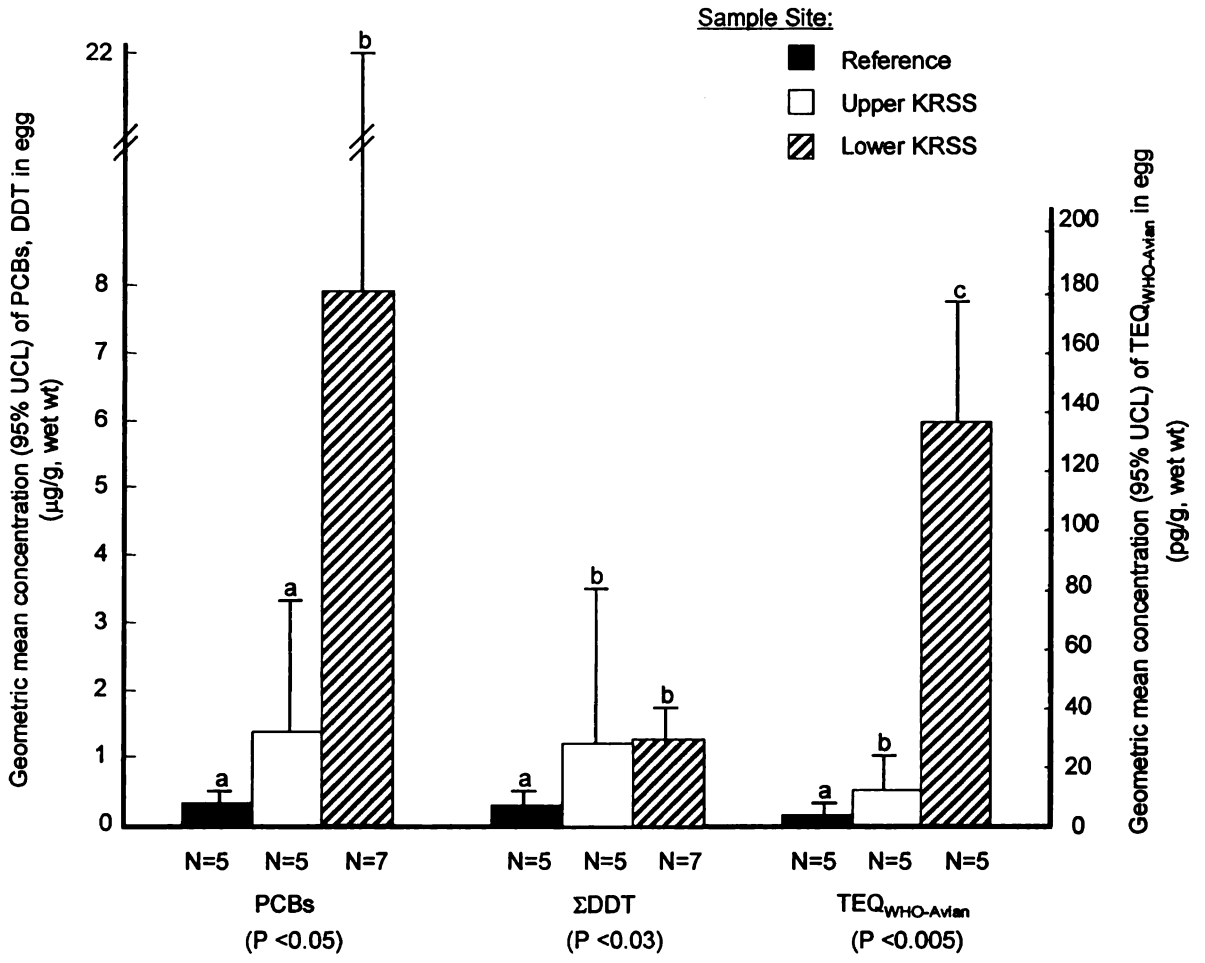


Figure 3.2. Geometric mean (ww) total polychlorinated biphenyls (PCBs), total dichlorodiphenyltrichloroethane (ΣDDT), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*B. virginianus*) eggs collected from the Kalamazoo River superfund site (KRSS), error bars show the 95%UCL.

any of the study sites where samples were collected in at least three of the five years of sampling (Kruskal-Wallace, 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 are plotted in Figure 3.3. 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, ww) and ranges are given along with corresponding values measured in eggs for each sample site (Table 3.5). Predicted and measured geometric mean concentrations of PCBs in eggs from the Reference location and UKRSS were not significantly different (Mann-Whitney U test, p=0.65 (Reference), 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}-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 Reference and both KRSS sites were significantly correlated with concentrations of total PCBs (r =0.89, p<0.001). Concentrations of $TEQ_{WHO\text{-}Avian}$

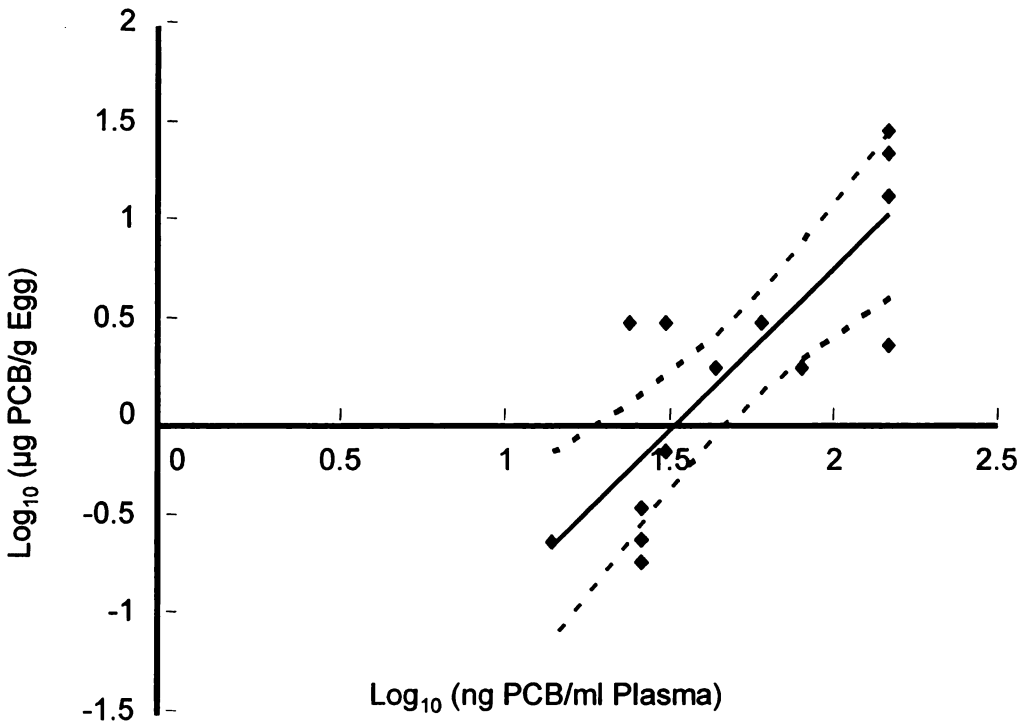


Figure 3.3. Egg to plasma polychlorinated biphenyl (PCB) relationship for Kalamazoo River superfund site (KRSS) great horned owls (*B. virginianus*), including the 95% confidence limits on the line of best fit.

Table 3.5. 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 (*B. virginianus)* from the Kalamazoo River Superfund Site (KRSS).

	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	1305 – 39,722
Geometric Mean	209	258	1462	1441	2798	7897
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 ± 1SD	32 - 532	154 - 400	644 – 2,876	350 – 3,606	0 – 12,275	340 – 29,394
NOAEC HQ (Geomean)	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(ug/g) = 1.674(log plasma,ng/ml)-2.578.

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 w/Tukey's, $p<0.005$) (Table 3.4, Figure 3.2). 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 Reference, UKRSS and LKRSS, respectively (Figure 3.4). 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 Reference 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: Reference: 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 Reference location, 61.8% at the UKRSS, and 42.1% at the LKRSS.

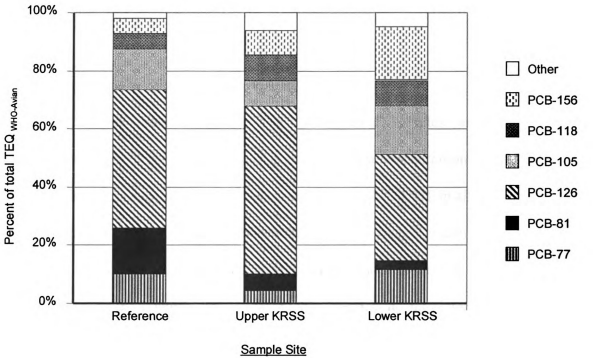


Figure 3.4. Percent contribution of polychlorinated biphenyl (PCB) coplanar and mono-*ortho*-substituted congeners to total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*B. virginianus*) egg samples at the Kalamazoo River superfund site (KRSS).

Relative Potency

The relative contributions of non-*ortho*- and mono-*ortho*-substituted congeners can be evaluated by standardizing the $TEQ_{WHO\text{-}Avian}$ 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 in evaluations of 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 Reference sites. The greatest geometric mean

concentration, (1.3 x10^1 μg/g, ww) was observed for eggs collected at LKRSS (Table 3.4).

ΣDDT concentrations/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 Reference location, and concentrations in the UKRSS and LKRSS were approximately equal. Geometric mean concentrations of ΣDDT were significantly different between the Reference site and both UKRSS and LKRSS (Kruskel-Wallace, $p<0.03$). Geometric Mean ΣDDT concentrations in eggs were not significantly different between UKRSS and LKRSS (Kruskel-Wallace, $p=0.95$) (Table 3.6, Figure 3.5). 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 w/Tukeys $p=0.22$). Egg shell thickness and Ratcliffe index measurements displayed similar trends among the Reference site and KRSS (Table 6, Figure 5). The mean Radcliff index at LKRSS was slightly less than values observed at the UKRSS and Reference 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 w/Tukey's, $p=0.27$, $p=0.44$, respectively). Additionally, eggshell thickness and Ratclffe index were not

Table 3.6. Geometric mean, wet wt (range), total dichlorodiphenyltrichloroethane (ΣDDT) concentrations, eggshell thickness and Ratcliffe Index values for great horned owl (*B. virginianus)* eggs and plasma (ΣDDT only) from the Kalamazoo River Superfund Site (KRSS).

STUDY SITE	Plasma ΣDDT[a]		Egg ΣDDT[a]		Eggshell Thickness		Ratcliffe Index	
	ng/ml	N	ng/g	N	mm	N	#	N
Reference[b]	47 (14 – 168)	3	314 (246 – 417)	5	0.376 (0.346 – 0.393)	5	1.9 (1.81 – 1.96)	5
Upper KRSS[c]	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[d]	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 DDT and metabolites DDE and DDD.
b. Reference sample sites include the Ceresco and Ft. Custer locations.
c. Upper KRSS sample sites include the Trowbridge, Otsego City Dam and Lake Allegan impoundments.
d. Lower KRSS sample sites include Koopman's marsh, Swan Creek Highbanks and Pottawatomi marsh.

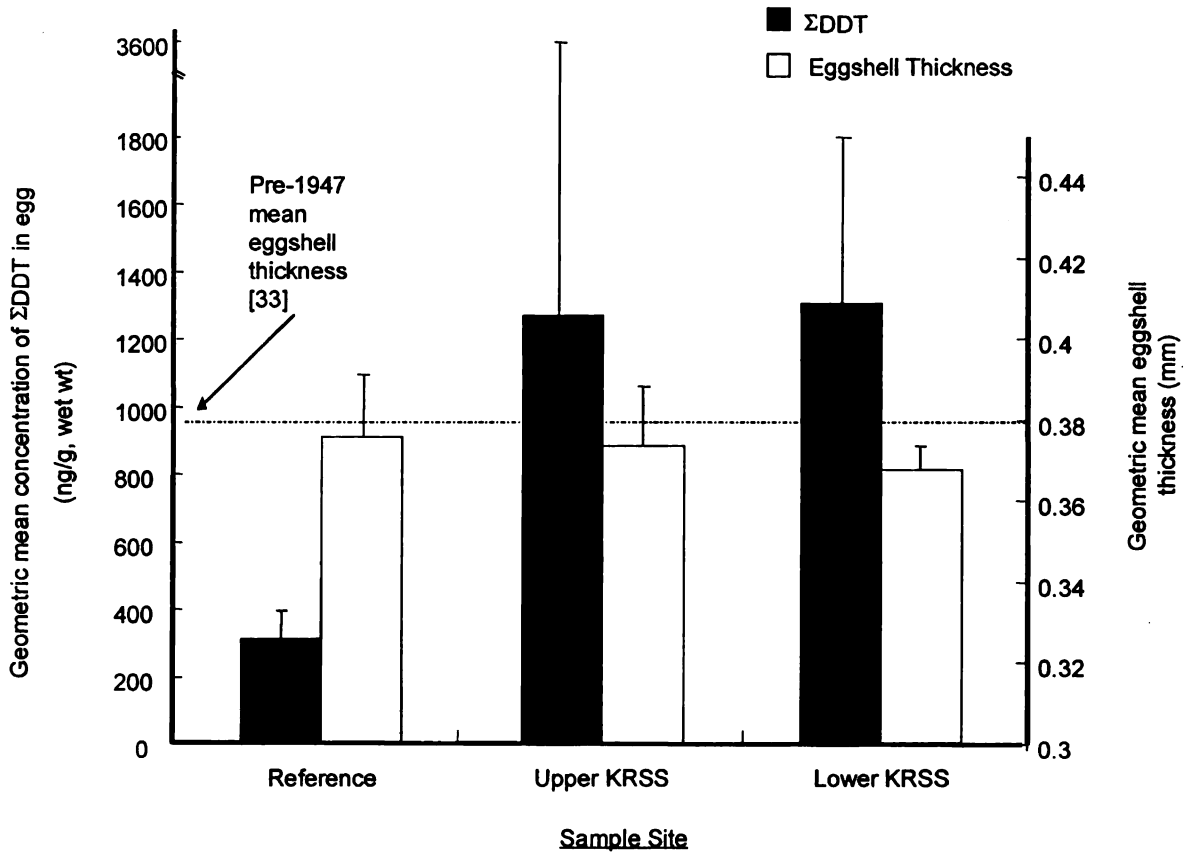


Figure 3.5. Geometric mean (ww) total dichlorodiphenyltrichloroethane (ΣDDT) concentrations in great horned owl (*B. virginianus*) eggs and eggshell thickness at the Kalamazoo River superfund site (KRSS), error bars show the 95%UCL.

significantly correlated with ΣDDT concentrations in eggs (r=0.35, p=0.17 and r=0.04, p=0.8 respectively).

Relative Abundance/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 3.7). Significant differences in the distribution and frequency of responses of individual (X^2=16.79, df=2, p=0.001; X^2=16.6, df=1, p=0.001) and the frequency of responses of paired owls (X^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 (X^2=7.57, df=1, p=0.01) at TB.

Productivity

Over the period of 2000 to 2002, there was no discernable 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 three-year study period, the arithmetic mean rate of productivity was 1.0 successful fledglings per active nest at both locations (Table 3.7). There were more active nests (6 vs. 1) and fledglings at TB (6 vs. 1) than FC. At TB there were two nests that each produced 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

Table 3.7. Relative abundance and productivity of resident great horned owls (*B. virginianus*) at Ft. Custer (Reference) 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]	N[c]=4	N=7	N=9	N=7	N=11	N=8	N=24	N=22
Adults	Mean Response Rate[d]		Mean Response Rate[d]		Mean Response Rate[d]		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[h]		Response Frequency[h]		Response Frequency[h]		Response Frequency[h]	
	n, (%)		n, (%)		n, (%)		n, (%)	
Fledgling[i]	0(0)	0(0)	1(11)	7(100)	0(0)	1(12)	1(04)	8(36)
PRODUCTIVITY[J]	N[k]=0	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

Table 3.7 (Cont'd)

a. Kalamazoo River Superfund Site.
b. Relative abundance estimates derived from hoot call/response surveys completed at dawn and dusk.
c. N=number of complete surveys.
d. Mean response rate is averaged across N 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 (n=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 (# fledglings/# 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.

the call/and 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.

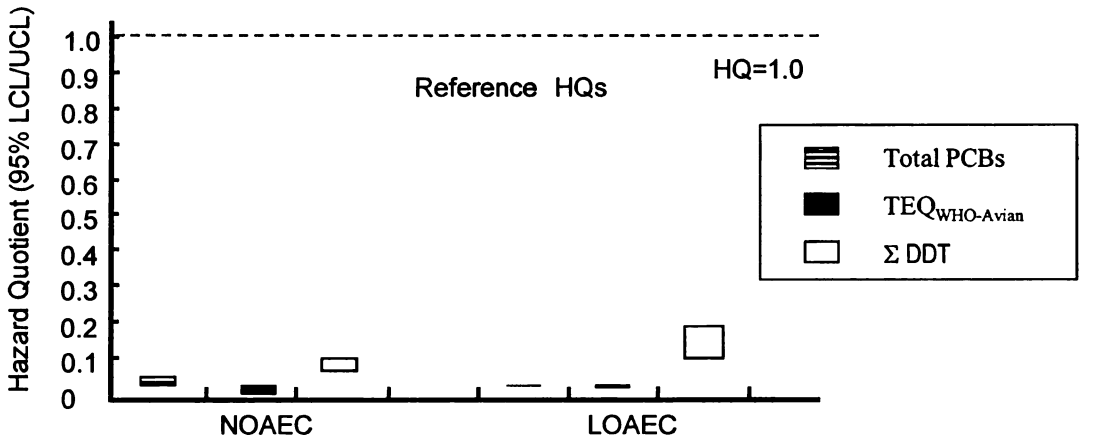
Risk Assessment

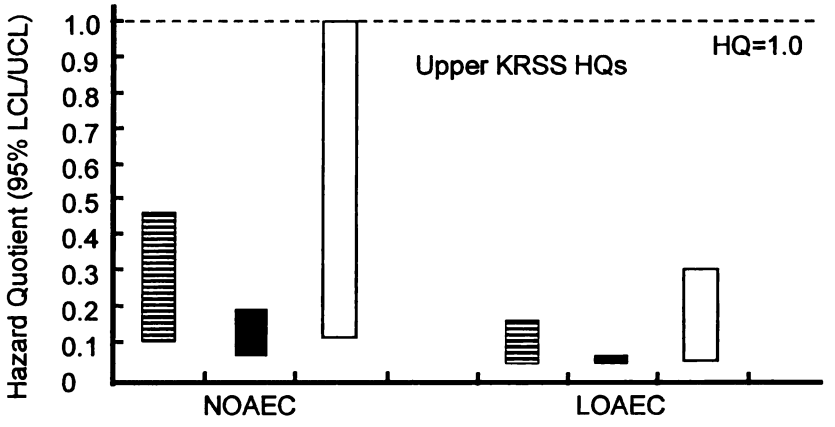
Measured 95% UCL (geometric mean) concentrations of PCBs in eggs collected from the UKRSS did not exceed the egg-based NOAEC TRV. The maximum HQ_{NOAEC} in the UKRSS was <1.0 (HQ=0.5). PCB concentrations in eggs from LKRSS included the four greatest individual PCB concentrations out of 12 eggs for the entire KRSS. In the LKRSS, the 95% UCL (geometric mean) concentration of PCBs in eggs, resulted in HQs of 1.0 and 3.1 when compared to the LOAEC and NOAEC, respectively (Figure 3.6).

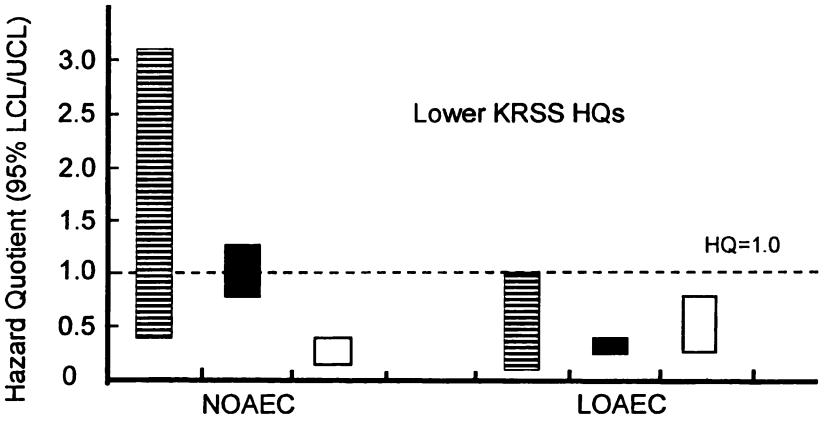
Hazard quotient values for $TEQ_{WHO\text{-}Avian}$, based on both the LOAEC and NOAEC, were <1.0 for all individual egg samples from the Reference and UKRSS locations. The greatest HQ_{NOAEC} was 0.2 for the UKRSS 95% UCL (geometric mean). Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) 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% UCL (geometric mean) concentration was compared with the LOAEC and NOAEC (Figure 3.6).

Hazard quotient values for the 95% UCL (geometric mean) ΣDDT concentrations, based on both the NOAEC and LOAEC were $\leq$1.0 for all three sites with a maximum HQ_{NOAEC} value of 1.0 at the UKRSS (Figure 3.6). Geometric mean eggshell thickness values at the Reference and UKRSS were not significantly different and $\leq$1% below the

Figure 3.6. Hazard quotients (HQ) for the effects of total polychlorinated biphenyls (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), and total dichlorodiphenyltrichloroethane (ΣDDT) for great horned owl (*B. virginianus*) eggs at the Kalamazoo River superfund site (KRSS) based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentration (LOAEC). Each box encompasses the 95%CI about the geometric mean concentration.


Hazard Quotient (95% LCL/UCL)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Reference HQs
HQ=1.0
Total PCBs
TEQWHO-Avian
Σ DDT
NOAEC
LOAEC


Hazard Quotient (95% LCL/UCL)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Upper KRSS HQs
HQ=1.0
NOAEC
LOAEC


Hazard Quotient (95% LCL/UCL)
3.0
2.5
2.0
1.5
1.0
0.5
0
Lower KRSS HQs
HQ=1.0
NOAEC
LOAEC

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 Reference location than the pre-1947 values while values were 4% and 7% less at UKRSS and LKRSS, respectively.

Hazard quotient values for predicted 95% UCL (geometric mean) concentrations of total PCBs in eggs at all three sites are less than values based on measured concentrations in eggs (Table 3.5). 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.

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, this 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, Strigiforms 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 wild GHO eggs (Rosenberg BG, 1990, Master's thesis, University of Manitoba, Winnipeg, MB, Canada) [37], 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 [37] and Saskatchewan (Rosenberg BG, 1990, Master's thesis, University of Manitoba, Winnipeg, MB, Canada) found arithmetic mean PCB concentrations of 3.1 $x10^3$ ng/g, ww and 3.3 $x10^3$ ng/g, ww, in eggs, respectively. These concentrations are greater than the arithmetic mean PCB concentration observed in GHO eggs from the Upper KRSS (2.0 $x10^3$ ng/g egg, ww). 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.

PCB congener profiles

The relative concentrations of PCB congeners used to calculate $TEQ_{WHO\text{-}Avian}$ 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 $TEQ_{WHO\text{-}Avian}$. 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 $TEQ_{WHO\text{-}Avian}$.

ΣDDT/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 non-point source exposures to ΣDDT (Rosenberg, BG, 1990, Master's thesis, University of Manitoba, Winnipeg, MB, Canada) [37,42]. 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 Reference area. 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 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 (TRVs)

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. Co-contaminants 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 co-contaminants 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 co-contaminants.

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 3.1). 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 comprised of Anseriform (waterfowl) or Charadriform (gulls) prey.

In this 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% UCL (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 (Figure 3.6).

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 (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 Reference 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% UCL (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. 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 / 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/site use were monitored in the FC and TB areas of the Reference and UKRSS sites.

Mean productivity rates were similar among locations where exposures to PCBs were much different, with 1.0 successful fledglings/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 mid-western [47] GHO populations residing in varied upland habitats. Measures of site-use indicate TB populations, as reflected by territory-holding nesting pairs, were near the carrying capacity (roughly one pair per 1600 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 Strigiforms 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 2000-2004. 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 worst-case exposure, state-of-the art analytical techniques, multiple lines 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 this study concur with earlier investigations at the KRSS that indicated GHOs would effectively integrate exposures from primary environmental media through multiple trophic levels [46]. This 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 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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Chapter 4

Evaluation of Risk Assessment Methodologies for Exposure of Great Horned Owls (*Bubo virginianus*) to PCBs on the Kalamazoo River, Michigan

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ABSTRACT

Dietary exposures of great horned owls (GHO; *Bubo virginianus*) at the Kalamazoo River, Michigan, were examined due to the presence of polychlorinated biphenyls (PCBs) in the terrestrial food web. Average potential daily doses (APDD) in GHO diets were 7- to 10-fold and 3-fold greater at a contaminated location than at a reference location for site-specific exposures quantified as total PCBs and 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), respectively. Wetland and aquatic prey species with links to the aquatic food web contributed significantly to PCB exposure and calculations of APDD. Measures of risk based on comparison of modeled dietary intake (APDD) to toxicity reference values (TRVs), using hazard quotients (HQs), varied with the selection of diet composition method (mass-basis vs. numeric-basis) with mass-basis compositions yielding greater HQs at both study sites. Risk associated with dietary exposures ("bottom-up" risk assessment methodology) were below population-level effects benchmarks (HQ <1) for all dietary compositions, and were consistent with risk based on concentrations in tissues ("top-down" risk assessment methodology) and indicated PCBs at the site posed little risk to terrestrial raptor species. Co-located studies that evaluated reproductive health and relative abundance were consistent with results of the risk assessment. Measures of risk based on comparison to TRVs were consistent with direct measures of ecologically relevant endpoints of reproductive fitness, but uncertainty exists in the selection of threshold values for effects in GHO especially based on $TEQ_{WHO\text{-}Avian}$ because of the absence of species-specific,

dose-response thresholds. Results of the evaluation indicated that the best estimate of risk is through application of a multiple-lines-of-evidence approach.

Keywords: raptors, dietary exposure, bioaccumulation, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

INTRODUCTION

Great horned owls (GHO: *Bubo virginianus*) are a useful sentinel species for site-specific baseline ecological risk assessments (ERAs) at sites with large contiguous areas of contaminated environmental media. Their sensitivity to the toxic effects of some organic contaminants, such as organochlorine, organophosphate and carbamate pesticides and their relatively great exposure as apex predators makes them valuable as a surrogate species for estimating risk to raptors in the terrestrial food chain (Sheffield 1997). Great horned owls have been used successfully in site-specific estimates of risk posed by polychlorinated biphenyls (PCBs) at the Kalamazoo River Superfund Site (KRSS) in Kalamazoo and Allegan Counties, Michigan (Strause et al. 2007). This previous study utilized a "top-down" or "tissue-based" approach in which exposure was determined by measuring concentrations of PCBs in eggs and blood plasma of nestlings. The risks from exposure to PCBs were assessed in a multiple-lines-of-evidence ERA that included both comparing the measured concentrations of PCBs to toxicity reference values (TRVs) as well as concurrent measures of productivity and abundance in a "weight of evidence" to identify cause and effect linkages between the chemical stressor and any observed suboptimal population or community structure at the site (Fairbrother 2003).

A second method for assessing risk to wildlife uses a predictive approach in which the exposure is inferred by measuring concentrations of the chemicals of concern (COCs), such as PCBs, in matrices other than the receptor of interest. This predictive approach is often referred to as the "bottom-up" or "dietary-based" approach. In this method the exposure-response is inferred from food chain modeling, based on site-

specific measures of concentrations of the chemical stressors (COCs) in wildlife food items or sediments or soils. In some cases, the actual dietary items can be identified via diet studies and quantified via forage base and food item sampling programs, while in other situations default or average values are used. Data specific to dietary composition and prey item COC concentrations are combined with receptor species' ingestion rate and body weight parameters to compute an average potential daily dose (APDD). This estimated daily dose is then compared to a dietary TRV to assess potential risks at the site. This study described the site-specific dietary exposure pathways to PCBs for GHOs at the KRSS. Site-specific dietary exposures (expressed as APDD) were then compared to TRVs for adverse effects determined in controlled laboratory studies to calculate hazard quotients (HQs) based on predicted exposure through diet. Additionally, the site-specific method of estimating dietary exposures was compared to a literature-derived APDD.

Due to limitations in the time and or resources available, few assessments apply both risk assessment methods simultaneously at the same location and time. Most assessments use the predictive method. While the two approaches are inherently linked, the accuracy and precision of the two methods are seldom compared. Therefore, the overall object of this study was to evaluate the results of the predictive assessment approach with actual measurements of exposure and population level effects at the same time at the same location. The two established methodologies of risk assessment (i.e., "top-down" vs. "bottom-up") were compared to determine how similar the predictions of risk would be based on both total PCBs, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) calculated from aryl hydrocarbon receptor-active PCBs.

Meeting these objectives required completion of the following: 1) collection of GHO pellet and prey remains samples from active nest sites to identify dietary components and enumerate dietary composition; 2) collection of representative prey item samples for the categories of prey (e.g., passerine birds, mice/voles) that contributed most significantly to GHO diet; 3) determination of concentrations of PCBs and $TEQ_{WHO\text{-}Avian}$ based on congener-specific measurements; 4) calculation and comparison of HQs based on total PCBs and total $TEQ_{WHO\text{-}Avian}$ between site-specific and literature-based diets; 5) comparisons of "bottom-up" and "top-down" estimates of risk based on total PCBs and $TEQ_{WHO\text{-}Avian}$ using the HQ methodology. Additionally, information on the PCB/ $TEQ_{WHO\text{-}Avian}$ concentrations between prey categories and food web sources (e.g., terrestrial vs. aquatic) was also assessed.

METHODS AND MATERIALS

Study Sites

The KRSS includes 123 km of river extending from the city of Kalamazoo, MI to Lake Michigan at Saugatuck, MI. The primary COCs are PCBs, including total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents from non-*ortho* (coplanar) and mono-*ortho* PCB congeners. PCBs were used in the production of carbonless copy paper and paper inks for approximately 15 yr (USEPA 1976). During this period, recycling of paper, including some carbonless copy paper resulted in releases of PCBs to the Kalamazoo River. The Kalamazoo River was placed on the Superfund National Priorities List in August 1990

due to the presence of elevated PCB concentrations in fish, sediments, and floodplain soils (BBL 1993).

Two sites within the KRSS were chosen for the GHO dietary study. These included the Fort Custer State Recreation Area (FC) and the former Trowbridge Impoundment (TB) (Figure 4.1). Characterizations of these sites have been provided in earlier assessments of GHO exposure at the KRSS (Strause et al. 2007). The FC site is an upstream reference area located approximately 7 km upstream of Morrow Pond Dam (the upstream limit of the KRSS) and 40 km upstream of TB. The FC site contains floodplain habitat similar to that present at TB and represents "current" regional background exposures in the watershed. The TB site is located in the Upper KRSS downstream of the point sources in the KRSS and it is one of three former impoundments in the Upper KRSS where removal of an in-stream dam to sill level has exposed former river sediments which are now heavily vegetated floodplain soils and riparian wetland habitat. The former TB impoundment includes the greatest area of contaminated soils (132 ha) and the greatest mean PCB soil concentrations (1.5×10^4 ng/g, dw) in the river floodplain. The FC and TB sites were selected to make direct comparisons between GHO responses on a "high potential exposure" vs. background "no elevated exposure" basis.

The GHO populations studied at each site were restricted to mated-pairs occupying natural nests and artificial nesting platforms within the 100 yr floodplain. The propensity for GHOs to use artificial nesting platforms allowed for better experimental control compared to wildlife studies that rely exclusively on natural nests. Nest platforms were placed throughout the FC and TB sites, and at TB the artificial platforms were

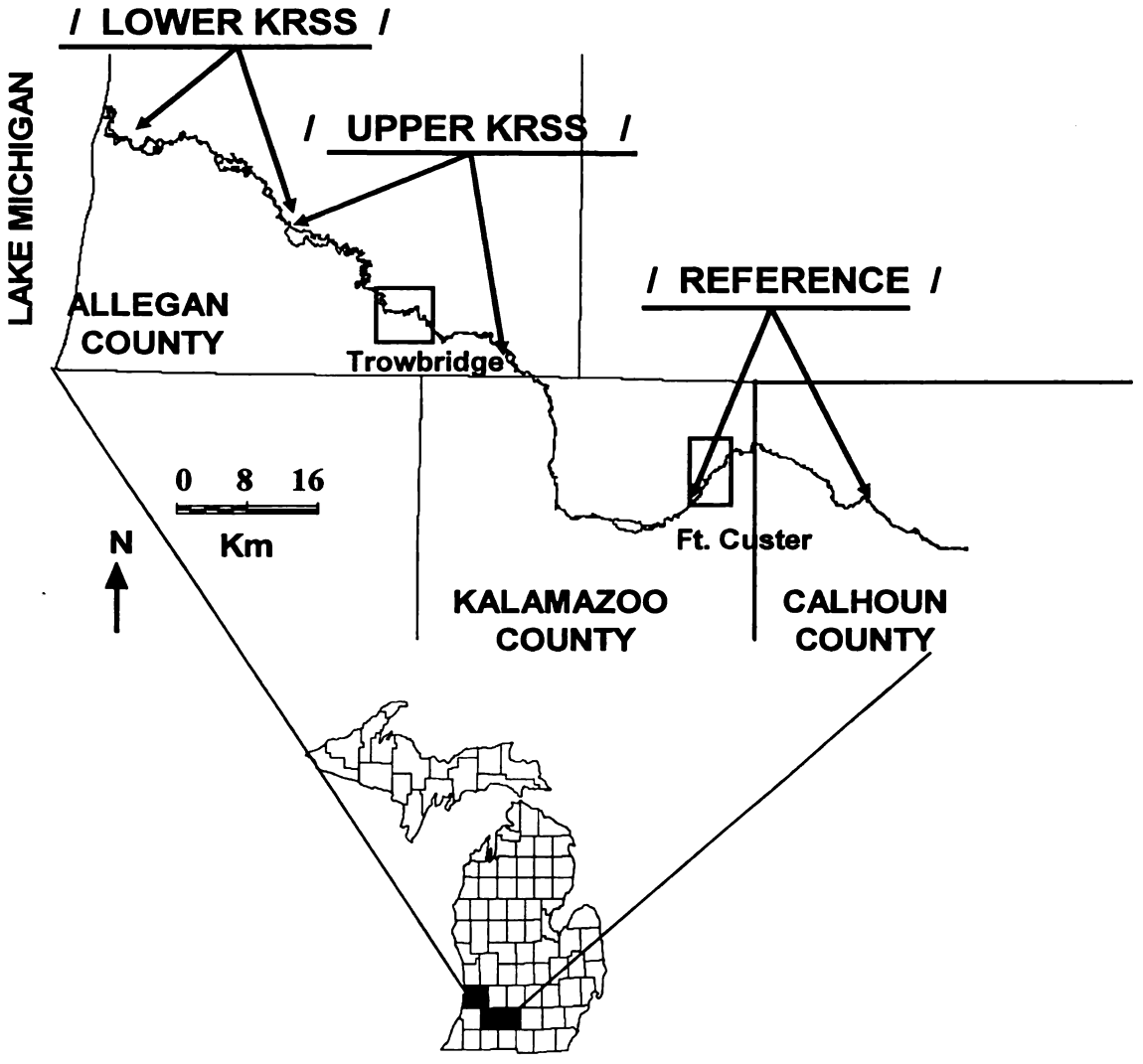


Figure 4.1. Kalamazoo River great horned owl (*B. virginianus*) study sites including the Reference sampling location (Ft. Custer), the Upper Kalamazoo River Superfund Site (Trowbridge) and Lower Kalamazoo River Superfund Site (LKRSS) sampling locations.

placed to provide for a "worst-case exposure" by maximizing GHO foraging in the most expansive areas of the contaminated floodplain.

Sample Collection

The studies of GHO exposure to PCBs at the KRSS were part of a broader study to investigate PCB congener bioaccumulation and dynamics in the terrestrial and aquatic food webs of the Kalamazoo River floodplain that included representative samples from all tropic levels in resident terrestrial and aquatic communities (Blankenship et al., 2005; Kay et al. 2005; Millsap et al. 2004; Neigh et al. 2006a, 2006b, 2006c). All sample collections were completed within the 100-yr floodplain. Representative taxa included raptors (owls and eagles), passerine birds, aquatic and terrestrial mammals, fish, terrestrial and aquatic invertebrates, plants and co-located soil and sediment samples (Blankenship et al. 2005; Kay et al. 2005). The principal components of the GHO food chain in the KRSS floodplain are likely to include terrestrial mammals and terrestrial passerine/aquatic birds, although limited numbers of aquatic invertebrates also may be eaten (Figure 4.2).

Pellets and Prey Remains

Site-specific studies of GHO diet were completed only at active nest sites. Pellets and prey remains were collected to determine the principal prey items that comprised the diet of nestlings. Diet investigations were undertaken in conjunction with other GHO study

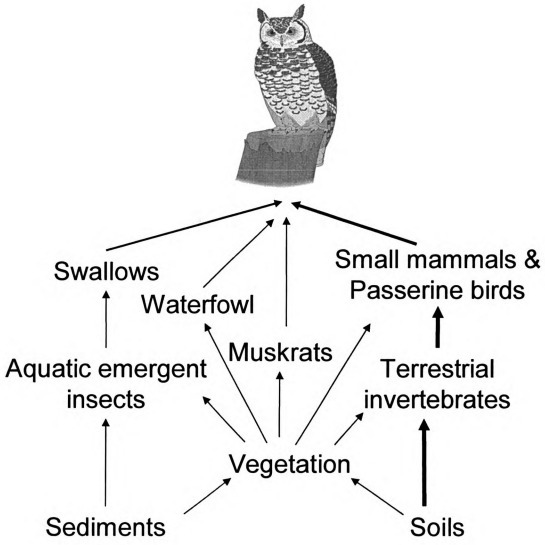


Figure 4.2. Great horned owl (*B. virginianus*) food chain and exposure pathways at the Kalamazoo River Superfund Site (KRSS).

objectives that included collection of blood plasma from nestling GHOs and monitoring of productivity. To minimize nest disturbances and to avoid bias this required that collection of pellets and prey remains were coordinated with these other investigations. Pellet and prey remains were collected from the nest, the base of the nest tree and beneath adult perch trees during the nestling blood sampling event. Additional samples were collected from the base of the nest tree and beneath feeding perches after the nestling GHOs fledged from the nest (2 to 3 wk after blood was collected), and on 10-d intervals thereafter until no more samples could be collected. A final sample was collected from the nest during a "post-fledge" nest climb to clean and maintain the artificial nesting platform. These climbs occurred between 4 and 10 wk after the young had fledged. During each collection event, pellets and prey remains were systematically and completely removed from each location to reduce the chance of overestimating the frequency of occurrence of large prey species because of their tendency to be represented in more than one pellet or prey sample (Marti 1974). Each respective sample of pellets and prey remains was assembled and packaged in a plastic jar as a composite sample representative of each collection location (e.g., nest, nest tree base, feeding/roosting perch). Samples were labeled to identify the nesting pair, dated and transported to the laboratory where they were exposed to naphthalene while drying to eliminate invertebrate scavengers. Prior to processing, pellet samples were sterilized by autoclave.

Prey Item Identification/Dietary Composition

Relative proportions of prey items in the site-specific diet were determined by examining unconsumed prey remains (bones, fur and feathers of animals too large to consume

whole) as well as the skeletal remains in regurgitated pellets (Errington 1932, 1938; Hayward et al. 1993). All identifiable remains were sorted and quantified as to the minimum number of individuals from each taxon necessary to account for the assemblage of remains present in any given composite of samples. For mammalian prey items too large for owls to swallow whole (~ 100 g) and avian prey, the remains of the same prey item were frequently present in multiple samples. When this occurred, the items from within each discrete sampling event were examined together to reconcile the frequency of occurrence of larger prey and birds. Multiple prey item identification keys were utilized for comparative identification of mammalian and avian remains including owl pellet identification keys (Carolina Biological Supply Company, Burlington, NC, USA) and the vertebrate skeletal collection from the Michigan State University (MSU) museum. Avian remains (feathers) were identified with the aid of MSU Kellogg Biological Station bird sanctuary personnel. Prey items were identified to the best practical taxonomic classification and grouped by species/family and order into seven prey categories relating to food web (aquatic vs. terrestrial) and trophic level (primary vs. secondary consumers) position. These categories included: passerine (terrestrial avian); waterfowl (aquatic avian); mice/vole (terrestrial primary consumers, small mammal); shrew (terrestrial secondary consumers, small mammal); muskrat (aquatic primary consumers, medium-size mammal); rabbit/squirrel (terrestrial primary consumers, medium-size mammal); and crayfish (detritivor/aquatic primary consumer, invertebrate).

The estimated dietary composition was based on the frequency of occurrence of all identifiable prey items and compiled on the basis of percent composition on a numeric basis (% number) and percent composition on a biomass basis (% biomass) (Wink et al.

1987). Percent biomass was calculated by multiplying each identified prey item by the mean adult weight (male + female) for the particular species or family (Dunning, 1984; Baker 1983). The small number of individual prey items that could not be positively identified to family or order was limited to unidentifiable parts of terrestrial birds and medium-size mammals. For biomass calculations, these items were assigned a mass value equal to the average mass computed for the representative species identified for that category at each respective nest site.

Prey Collections for Chemical Analyses

Prey species represented in site-specific and literature-based GHO diets were collected from the FC and TB study sites and analyzed for total PCBs. Analyses of pellet and prey remains samples collected from FC and TB identified six general categories of GHO prey. These included passerine birds, waterfowl, mice/voles, shrews, muskrats and rabbit/squirrel. A seventh category, crayfish, is represented in the literature-based diet and included in the diet analysis. Field sampling and processing methods for representative individuals from each of the seven prey categories are described below.

Passerine birds collected from the FC and TB sites included the tree swallow (*Tachycineta bicolor*), house wren (*Troglodytes aedon*), and American robin (*Turdus migratorius*). A single European starling (*Sturnus vulgaris*) also was collected at TB. All live birds were collected at the end of the nesting period. Adult wrens and swallows were captured with mist nets or a trap-door mechanism. Additionally, dead individuals found at nest boxes were salvaged for analyses (Neigh et al. 2006a, 2006b). Adult robins were collected using pellet guns [MSU Aquatic Toxicology Laboratory (ATL),

Unpublished]. The starling (carcass) was recovered beneath an active GHO nest. Birds were promptly euthanized by cervical dislocation and carcasses were placed in solvent-rinsed sample jars and frozen at –20°C. For chemical sample analysis, feathers, beaks, wings, legs, and stomach contents were removed and the whole body was homogenized in a solvent-rinsed grinder.

Waterfowl species sampled included merganser (*Mergus spp.*), mallard (*Anas platyrhynchos*), wood duck (*Aix sponsa*), and blue-winged teal (*Anas discors*). Waterfowl sampling was not included in the MSU Kalamazoo River food web investigations. Waterfowl samples used in this GHO diet exposure study were collected in August 1985 by the United States Fish and Wildlife Service (USFWS). The USFWS collected adult and immature ducks from five locations in the KRSS. Sampling locations included Morrow Pond and the Menasha and Trowbridge impoundments in the Upper KRSS, and the Allegan State Game Area and Saugatuck Lake downstream of Allegan Dam in the Lower KRSS. For chemical analysis, feathers, beaks and entrails were removed and the remaining carcass was homogenized in a solvent-rinsed grinder (MDNR 1987).

Small mammals collected included mice (*Peromyscus spp., Zapus hudsonius*), voles (*Microtus pennsylvanicus*), shrews (*Sorex cinereus, Blarina brevicauda*), red squirrels (*Tamiasciurus hudsonicus*) and chipmunks (*Tamias striatus*). All small mammals were trapped using pit-fall or Sherman live traps placed alternately within a 30 x 30 m^2 sampling grid sited in the floodplain. Two sampling grids were located at FC and four grids were set up at TB (See Figure 1 of Blankenship et al. 2005). Captured species were sacrificed by cervical dislocation and carcasses were placed in solvent-

rinsed sample jars and frozen at –20°C. Prior to chemical analysis, stomach contents were removed and the remaining whole body (including pelage) was homogenized in a solvent-rinsed grinder (Blankenship et al. 2005).

Muskrats (*Ondatra zibethicus*) were collected along the riverbank throughout FC and TB using body-gripping "conibear" traps. Samples were frozen at –20°C until processing for chemical analysis. Processing of whole-body samples included removal of the pelage, a coarse grind, and further homogenization in a commercial blender (Millsap et al. 2004).

Crayfish (*Cambarus and Orconectes spp.*) were collected along the riverbank at FC and TB by use of wire minnow traps set adjacent to the small mammal sampling grids. For chemical analyses, the whole body was homogenized in a solvent-rinsed grinder (Millsap et al. 2004).

Chemical Analysis – Extraction/Clean-up

Concentrations of PCB congeners were determined by use of U.S. Environmental Protection Agency method 3540 (SW846). The details of the soxhlet extraction and sample preparation and clean-up have been described previously (Neigh et al. 2006a). Prey items 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/Quality Control sets composed of similar tissues were included with each group of 20 samples. Concentrations of PCBs, including di- and mono-*ortho*-substituted congeners were

determined by gas chromatography (Perkin Elmer AutoSystem and Hewlett Packard 5890 series II) equipped with a ^{63}Ni electron capture detector (GC-ECD). Concentrations of non-*ortho*-substituted PCB congeners (coplanar) were determined by gas chromatograph mass selective detector (GC-MS) (Hewlett Packard 5890 series II gas chromatograph interfaced to a HP 5972 series detector). PCBs were reported on a mass wet weight (ww) 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. Di-and mono-*ortho*-subsititued PCB congeners were detected by selected ion monitoring of the two most abundant ions of the molecular cluster and the limit of quantification was conservatively estimated (minimum surface to noise ratio of 10.0) to be 1.0 ng PCB/g, ww, using an extraction mass of 20 g, a 25 pg/µl standard congener mix and 1 µl injection volume. For coplanar PCB congeners, method detection limits varied among samples but were maintained at $\leq$ 0.1 ng/g, ww for all samples using the sample-specific extraction mass and a minimum surface to noise ratio of 3.0. TurboChrom (Perkin Elmer, 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. Total PCB concentrations in waterfowl samples collected by the USFWS were quantified as Aroclor 1260 (MDNR 1987).

TEQ Computation

Concentrations of $TEQ_{WHO\text{-}Avian}$ in prey item 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 World Health Organization (WHO) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) toxic equivalency factors for avian receptors (Van den Berg et al. 1998). Polychlorinated-dibenzo-*p*-dioxins and polychlorinated-dibenzo-furans were not measured and were not included in TEQ computation. Whenever a PCB congener was not detected, a proxy value equal to one-half the limit of quantification was multiplied by the toxic equivalency factor to calculate the congener-specific TEQs. Co-eluting congeners were evaluated separately. PCB 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. Among the six GHO prey categories analyzed at the MSU-ATL (excludes waterfowl samples), the maximum combined contributions to total $TEQ_{WHO\text{-}Avian}$ of congeners 105, 156, 157 and 167 was 4.2% (passerine), 46% (mice/vole), 5% (shrew), 1.2% (muskrat), <1% (rabbit/squirrel), and <1% (crayfish), respectively.

Individual non-*ortho* and mono-*ortho* PCB congener concentrations in waterfowl samples were estimated from the quantified Aroclor 1260 total PCB concentrations by multiplying the geometric mean total PCB concentration by a congener-specific fractional composition value (Schwartz et al. 1993). The greatest observed congener-specific fractional value (percent composition basis) determined among four technical

Aroclor mixtures was selected to account for inherent differences in Aroclor batch production processes. Bioaccumulation factors of 10 and 3 were also applied to PCB congeners 126 and 169 to account for observed selective enrichment (weathering, metabolism) of these two congeners, as observed by Schwartz et al. (1993). Using this conservative format, the combined contribution of congeners 105, 156, 157 and 167 to total $TEQ_{WHO\text{-}Avian}$ in waterfowl was < 1%.

Toxicity Reference Values

In this study, TRVs were used to evaluate the potential for adverse effects due to PCBs including $TEQ_{WHO\text{-}Avian.}$ Ideally, TRVs are derived from chronic toxicity studies in which a total PCB or $TEQ_{WHO\text{-}Avian}$ dose-response relationship has been observed for ecologically relevant endpoints in the species of concern, or alternately in a wildlife species rather than a tradition laboratory species. Chronic studies should also include sensitive life stages to evaluate potential developmental and reproductive effects, and there must be minimal impact from co-contaminants on the measured effects.

Toxicity reference values used in this assessment were based on values reported in the literature for no observable adverse effect levels (NOAELs) and least observable adverse effect levels (LOAELs) for total PCBs and $TEQ_{WHO\text{-}Avian}$. The dietary PCB NOAEL for GHO was based on the controlled, laboratory study on the reproductive effects of PCBs on the screech owl (*Otus asio*) (McLane and Hughes 1980). In that study, screech owls were fed a diet that contained 3 mg PCB/kg, ww. Conversion of concentrations in the diet to a daily dose (4.1×10^2 ng PCB/g, body weight (bw)/d) was accomplished by use of the relationships presented by Sample et al. (1996). At this dose,

no effects were observed on eggshell thickness, number of eggs laid, young hatched and young fledged. The LOAEL value of 1.23 x 10^3 ng PCBs/g, bw/d was estimated by multiplying the NOAEL by an uncertainty factor of 3. No additional uncertainty factors were applied to account for potential inter-taxon variability, since the NOAEL is in the range determined for the chicken, the most sensitive bird species tested (Platonow and Reinhart 1973; Lillie et al. 1974).

No studies of the effects of $TEQ_{WHO\text{-}Avian}$ were available for deriving TRVs, and no studies were found in which there was a closely related test species to GHO. A sub-chronic laboratory study (10 wk exposure period) by Nosek et al. (1992) found that intra-peritoneal injections of 2,3,7,8 - TCDD at concentrations of 1.0 x 10^3 pg TCDD/g/wk (1.4 x 10^2 pg TCDD/g, bw/d) caused a 64% decrease in fertility and a 100% increase in embryo mortality in ring-necked pheasants (*Phasianus colchicus*). This exposure concentration was used as the dietary TEQ-based LOAEL for GHO. A NOAEL was not directly available from this study and had to be derived from the limited dose data. Because effects due to the exposure were pronounced in the test subjects, a safety factor of 10 was applied to derive a NOAEL of 1.4 x10^1 pg TEQ/g, bw/d. (Table 4.1). Limitations of the study include the use of injections of 2,3,7,8-TCDD instead of feeding 2,3,7,8-TCDD contaminated food to the test species and the evaluation of TCDD exposure and not PCB-TEQ exposure.

Because co-eluting congener contributions are included in some mono-*ortho* PCB congener concentrations used in this risk assessment the PCB-based TEQs may overestimate exposure relative to 2,3,7,8-TCDD. In this instance, the use of a TRV based on 2,3,7,8-TCDD exposure is likely to yield conservative estimates of risk when applied

Table 4.1. Toxicity reference values (TRVs) used to calculate hazard quotients for total Polychlorinated Biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*B. virginianus*) diet and eggs.

	dietary-based TRVs (ng PCBs/g bw/d) ($pg\ TEQ_{WHO\text{-}Avian}$/g bw/d)		tissue-based TRVs (ng PCBs/g) ($pg\ TEQ_{WHO\text{-}Avian}$/g)	
	NOAEL[a]	LOAEL[b]	NOAEC[c]	LOAEC[d]
total PCBs	410[e]	1,230[e]	7,000[e]	21,000[e]
$TEQ_{WHO\text{-}Avian}$	14[f]	140[f]	135[g,h,i]	400[g,h,i]

a. No observable adverse effect level.
b. Lowest observable adverse effect level.
c. No observable adverse effect concentration.
d. Lowest observable adverse effect concentration.
e. McLane and Hughes (1980).
f. Nosek JA et al. (1992).
g. Elliott JE et al. (1996).
h. Elliott JE et al. (2000).
i.Woodford JE et al.(1998).

to PCB exposure. Also, tolerance to TEQ-based exposure by birds is species-specific (Woodford et al. 1998), and the TRVs derived from Nosek et al. (1992) are likely conservative because the *Galliformes* used in the study are among the more sensitive species to the effects of 2,3,7,8-TCDD (Hoffman et al. 1998). The available information indicates that raptors, such as the American kestrel (*Falco sparverius*), osprey (*Pandion haliaetus*), and bald eagle (*Haliaeetus leucocephalus*), are more tolerant than gallinaceous species to the effects of PCBs and TEQ (Elliott et al. 1997; Hoffman et al. 1998; Woodford et al. 1998; Elliott et al. 1996). Thus, for GHO a more closely related raptorial species such as American kestrels would be the ideal basis for TRVs. However,

in the few studies in which kestrels were exposed to PCBs, there was either inadequate dose-response information or incomplete assessment of ecologically relevant endpoints.

Tissue-specific TRVs (egg-basis) for total PCBs and $TEQ_{WHO\text{-}Avian}$ used in previous assessments of GHO exposure at the site (Strause et al. 2007) are included (Table 1). The egg-based TRVs are included to aid interpretation of the "bottom-up" and "top-down" methodology comparisons completed in this study.

Average Potential Daily Dose (APDD)/Risk Assessment

The amount of PCBs ingested by GHOs was calculated using the wildlife dose equation for dietary exposures (USEPA 1993). The APDDs for total PCBs and $TEQ_{WHO\text{-}Avian}$ were calculated for GHOs using the site-specific diets for GHO determined in this study, and for comparison purposes a literature-based diet for a separate population of Michigan GHOs (Craighead and Craighead 1956). All APDDs were based on diets with prey composition compiled on a biomass basis (eq 1). Average potential daily dose calculations also included the incidental ingestion of floodplain soils that could potentially be associated with GHO foraging activity.

$$APDD = \Sigma (C_k \times FR_k \times NIR_k) \qquad (1)$$

C_k = Geometric mean concentration of total PCBs or $TEQ_{WHO\text{-}Avian}$, ww in the k^{th} prey item category of GHO diet.

FR_k = Fraction of GHO diet (based on mass) represented by the k^{th} prey item category.

NIR_k = Normalized GHO ingestion rate of the k^{th} prey item (g prey/g, bw/day, ww).

Concentrations of PCBs and $TEQ_{WHO\text{-}Avian}$ in representative prey items collected from the KRSS were determined using the methods described previously and are presented in the following section. FR_k (mass-basis) was determined for the GHO subpopulation cohorts at both FC and TB, and from a previous study (literature-based diet) of GHO populations in southeast Michigan (Craighead and Craighead 1956). A conservative assumption for the value of FR_k is that GHO at the KRSS will obtain 100% of their diet requirements from the 100-yr floodplain (site use factor = 1). NIR_k (0.056 g/g bw/d) was derived from daily ingestion rates and mean body weights reported for GHO (Craighead and Craighead 1956). Additional PCB and $TEQ_{WHO\text{-}Avian}$ dietary exposure from incidental soil ingestion was calculated for TB GHOs using both the site-specific and the literature-based dietary composition and geometric mean concentrations of PCBs measured for TB soils. Incidental soil ingestion contributions to dietary exposure were not calculated for the FC GHOs because of the very low concentrations of PCBs present in FC soils. Geometric mean and upper 95% confidence level (CL) geometric mean concentrations of total PCBs in TB floodplain soils (non-detects were removed from the data set prior to computing mean and upper 95%CL values) were obtained from previous investigations at the site (BBL 1994). Concentrations of total PCBs in soils were considered to be 85% bioavailable and contain 65% moisture (estimated from Studier and Sevick 1992) to make the dry weight (dw) soil concentrations comparable to wet weight concentrations in prey. The dietary fraction of incidental soil ingestion (2%) for GHOs was based on reports in the literature (USEPA 1993). Dioxin equivalent concentrations in soils were not measured at the site and were

estimated from the Aroclor-based soil data using the methods described previously for waterfowl (Schwartz et al. 1993).

Comparisons of potential hazard estimated for dietary exposure to PCBs, were based on HQs. Hazard quotients were calculated as the APDD (ng PCB/g, bw/d or pg $TEQ_{WHO\text{-}Avian}$/g, bw/d) divided by the corresponding TRV (eq 2).

$$HQ = \frac{\text{APDD (ng PCBs/g bw/d or pg TEQs/g bw/d)}}{\text{dietary TRV}} \qquad (2)$$

Other lines of evidence from previously published studies on KRSS GHO populations were examined to minimize uncertainties in the analysis and calculation of risk from dietary exposure. These include the "top-down" risk assessment approach that quantified concentrations of PCBs present in GHO eggs and nestling plasma, and examined the effects of chlorinated hydrocarbons on egg viability through measurements of egg shell thickness and Ratcliffe index (Ratcliffe 1968; Hickey and Anderson 1968). A third and ancillary line of evidence investigated potential population-level effects of PCB exposures at the KRSS by monitoring productivity (fledgling success) and relative abundance between the contaminated floodplain habitat (TB) and the reference location (FC). By evaluating multiple lines of evidence together it was possible to provide the best available information for remedial decision-making at the site, especially when two or more lines of evidence converged on a common finding.

Statistical Analysis

Both parametric and nonparametric statistics were applied depending on which assumptions were met. Concentrations of total PCBs and $TEQ_{WHO\text{-}Avian}$ in prey populations from the site were analyzed for normality by use of the Kolmogorov-Smirnov, one-sample test with Lilliefors transformation. Concentrations of the COCs were generally log-normally distributed and therefore all data sets were log-transformed to more closely approximate the normal distribution. Data sets that were normally distributed were compared using a t-test. If the data did not exhibit a normal distribution, than a nonparametric version of the t-test (Mann-Whitney U test) was used. Associations between parameters were made with Pearson Product Correlations. Tests for normality 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 $p< 0.05$. Statistical methods for comparing COC concentrations in GHO tissues (eggs, plasma), egg shell parameters (shell thickness, Ratcliffe Index) and call/response survey measurements of GHO abundance employed in the multiple-lines-of-evidence evaluation of site-specific risk to KRSS GHO populations have been described previously [Strause et al. 2007].

RESULTS

Composition of the Diet of GHOs

A total of 285 discrete prey items were identified in 59 pellet and prey remains samples collected from a combined total of seven active nests in the FC and TB study sites from

2000 to 2002. Excepting four post-fledge prey remains samples collected between June 4 and June 28, all samples were collected prior to June 1 in each year of the study, and, as such, the data provide a characterization of the spring or nesting- season diet for KRSS GHOs. Only prey items represented by the classes *Aves* and *Mammalia* were observed. Prey from classes *Reptilia*, *Amphibia* or *Crustacae* were not observed in the diets of GHO at the KRSS.

Dietary compositions at FC and TB varied slightly when compiled on a class basis. Fort Custer GHOs consumed a slightly lesser proportion of birds and slightly greater proportion of mammals (birds; 15.5 % -numeric, 13.8%-mass: mammals; 84.5% -numeric, 86.2% -mass) compared to TB GHOs (birds; 27.5%- numeric, 24.8% -mass: mammals; 72.5% -numeric, 75.2% -mass) (Table 4.2). Similar results are produced whether one compiles the class-level data on either a numeric- or mass-basis with only a slight increase in the proportion of mammalian prey when diet is compiled on a mass-basis.

Within-class differences were observed between the FC and TB diets of GHO. Large differences were observed in the proportions of passerine/terrestrial birds represented in diets of GHOs at FC and TB (11% versus 25.8% on a numeric-basis and 5% versus 22% on a mass-basis, respectively) and in the proportion of rabbits represented in GHO diets at FC and TB (46% versus 16% on a numeric-basis and 75% versus 50% on a mass-basis, respectively). Within-class differences also are seen between diet compilations based on % number vs. % biomass. On a numeric-basis, small mammals (mice/voles) and shrews account for up to 33% and 51% of the GHO diet at FC and TB, respectively. These proportions decrease to 2.2% (FC) and 6.2% (TB) of the diet on a

Table 4.2. Great horned owl spring diet composition at the Kalamazoo River Superfund Site (KRSS)(site-specific) and from the Literature.

	Numeric Basis (percent (%) occurrence)			Mass Basis (percent (%) contribution)		
	Site-specific Diet (N = 285)		Literature-based Diet (N = 260)	Site-specific Diet (N = 285)		Literature-based Diet (N = 260)
Prey Item	Ft. Custer (FC)	Trowbridge (TB)	Washtenaw Co. MI[a]	Ft. Custer (FC)	Trowbridge (TB)	Washtenaw Co. MI[a]
Class Aves	**15.5**	**27.5**	**41.0**	**13.8**	**24.8**	**68.0**
Passerine[b]	11.0	25.8	39.0	5.0	22.0	65.5
Waterfowl[c]	4.5	1.7	2.0	8.8	2.8	2.5
Class Mammalia	**84.5**	**72.5**	**54.0**	**86.2**	**75.2**	**31.5**
Mice/Vole	31.0	49.0	41.0	2.0	6.0	2.5
Shrew	2.5	2.0	0.0	0.2	0.2	0.0
Muskrat	5.0	5.5	2.0	9.0	19.0	6.0
Rabbit[d]	46.0	16.0	11.0	75.0	50.0	23.0
Class Crustacae	**0.0**	**0.0**	**5.0**	**0.0**	**0.0**	**0.5**
Crayfish	0.0	0.0	5.0	0.0	0.0	0.5

Table 4.2 (Cont'd)

a. Craighead and Craighead (1956).
b. Passerine category includes all terrestrial birds and all unidentified bird ("unknown bird") remains.
c. Waterfowl category includes all aquatic birds.
d. Rabbit category includes squirrels and all unidentified medium-size mammal ("unknown mammal") remains.

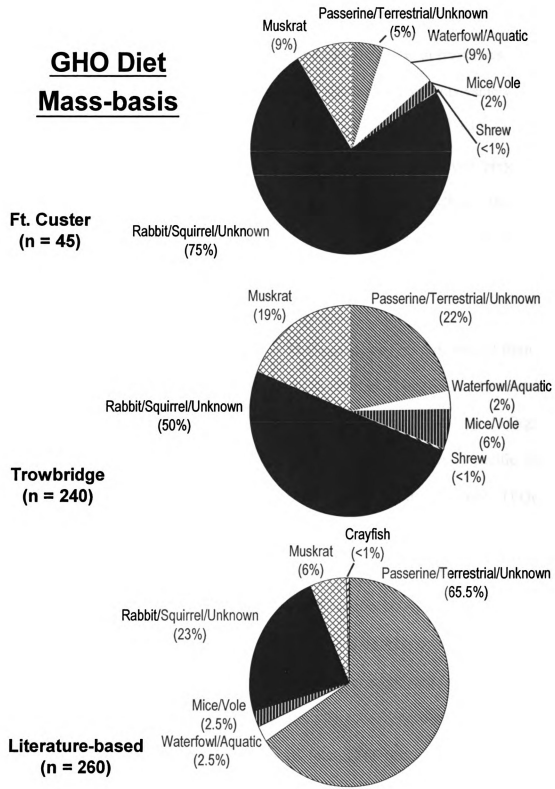
mass-basis. Likewise, the combined proportion of rabbit and muskrat prey on a numeric-basis increases from 51% (FC) and 21.5% (TB) to 84% (FC) and 69% (TB) on a mass-basis.

A diet compilation based on mass provides the most accurate characterization of the relative importance of prey to avian predators (Marti, 1987). Mass-based characterizations of KRSS GHO diet at FC and TB are compared to diet composition values for a nearby GHO subpopulation residing in southeast Michigan (Craighead and Craighead 1956) (Figure 4.3). Class-level differences in prey composition between KRSS GHOs and the literature-based (LB) values are greatest for classes *Aves* and *Mammalia* at FC (13.8% (FC) versus 68% (LB), 86.2% (FC) versus 31.5% (LB), respectively). Crayfish (class *Crustacae*) were also present in the LB diet of GHO. Within-class prey proportions for KRSS and literature-based GHO diets were similar with passerine/terrestrial birds and rabbits/muskrats comprising the great majority of bird and mammalian prey.

Total PCB and $TEQ_{WHO\text{-}Avian}$ Concentrations in Prey

Concentrations of total PCBs and lipid content of 130 discrete whole-body prey item samples were determined. Prey items collected previously from the KRSS included 17 waterfowl samples that also were used to estimate GHO exposure to PCBs via the diet. Budget limitations prevented the collection and PCB/TEQ analyses of rabbit and grey/fox squirrel samples from FC and TB. Available data for chipmunk and red squirrel were used to fill this gap in the data base. Geometric mean concentrations of total PCBs in FC prey ranged from 2 ng PCBs/g, ww in rabbits to 9.6 $x10^1$ ng PCBs/g, ww in passerines

Figure 4.3. Site-specific great horned owl (GHO; *B. virginianus*) diet composition based on a biomass contribution basis for the Ft. Custer and Trowbridge sampling locations, and a literature-based (Craighead and Craighead 1956) diet composition for GHO populations in southeast Michigan.


GHO Diet
Mass-basis
Ft. Custer
(n = 45)
Muskrat
(9%)
Passerine/Terrestrial/Unknown
(5%)
Waterfowl/Aquatic
(9%)
Mice/Vole
(2%)
Shrew
(<1%)
Rabbit/Squirrel/Unknown
(75%)
Trowbridge
(n = 240)
Muskrat
(19%)
Passerine/Terrestrial/Unknown
(22%)
Waterfowl/Aquatic
(2%)
Mice/Vole
(6%)
Shrew
(<1%)
Rabbit/Squirrel/Unknown
(50%)
Literature-based
(n = 260)
Crayfish
(<1%)
Muskrat
(6%)
Passerine/Terrestrial/Unknown
(65.5%)
Rabbit/Squirrel/Unknown
(23%)
Mice/Vole
(2.5%)
Waterfowl/Aquatic
(2.5%)

(Table 4.3). Geometric mean concentrations of total PCBs in TB prey ranged from 5.6 $x10^1$ ng PCBs/g, ww in muskrats to 1.3 $x10^3$ ng PCBs/g, ww in passerines. Total PCB concentrations in waterfowl were 8.9 $x10^2$ ng PCBs/g, ww, and this value was used for both the FC and TB sites because of the uncertainty associated with residence and exposure of these mobile and migratory species. PCB concentrations in prey items from TB were significantly greater (small mammals, muskrats, crayfish; t-test $p < 0.01$; passerines, shrews; Mann-Whitney U test $p < 0.01$) than those from the upstream reference area at FC. Waterfowl and rabbit samples (TB sample size = 1) were not tested for significant differences.

Geometric mean concentrations of $TEQ_{WHO\text{-}Avian}$ in FC prey ranged from 0.52 pg TEQ/g, ww in rabbits to 7.5 pg TEQ/g, ww in crayfish (Table 4.4). Geometric mean concentrations of $TEQ_{WHO\text{-}Avian}$ in TB prey ranged from 1.3 $x10^1$ pg TEQ/g, ww in muskrats to 7.1 $x10^1$ pg TEQ/g, ww in rabbits. Using congener-specific fractional composition values (Schwartz et al. 1993) conservatively estimated $TEQ_{WHO\text{-}Avian}$ concentrations in 17 waterfowl samples were 2.4 $x10^2$ pg TEQ/g, ww. Prey items from TB contained significantly greater concentrations of TEQ than those from FC (t-test $p < 0.02$) with the exception of muskrats and small mammals, which were not statistically different (Mann-Whitney U test $p = 0.26$ and $p = 0.22$, respectively). Waterfowl and rabbit samples (TB sample size = 1) were not tested for significant differences.

Contributions to total $TEQ_{WHO\text{-}Avian}$ from the four non-*ortho* and eight mono-*ortho* PCB congeners showed that over 90 % of total TEQ was contributed by non-*ortho* congeners 77, 81 and 126 for all prey item categories at each of the two sampling

Table 4.3. Geometric mean and upper 95% confidence level (U95% CL) concentrations of total Polychlorinated Biphenyls (PCBs) (ng PCBs/g ww) in prey items collected from two sites on the Kalamazoo River.

	Ft. Custer					Trowbridge				
	N	Range	Geometric Mean	U95% CL	Lipid (%)	N	Range	Geometric Mean	U95% CL	Lipid (%)
Passerines[a]	11	9 – 1,030	96	262	4.10	20	62 – 32,200	1,336*	3,102	4.90
Waterfowl[b]	17	130 – 28,000	889	1,751	3.80	17	130 – 28,000	889	1,751	3.80
Mice/Vole	12	2 - 180	13	27	3.59	20	30 - 548	67*	102	4.57
Shrew	16	2 – 18	8	10	3.66	17	25 – 3,150	847*	1,533	2.68
Muskrat	4	8 - 26	13	22	2.60	7	14 - 112	56*	94	2.07
Rabbit[c,d]	6	1 - 6	2	4	3.71	1	568	568	568	4.95
Crayfish	4	27 - 89	49	93	0.63	13	76 – 1,940	373*	597	1.62

a. Geometric mean total PCB concentrations in Ft. Custer house wren (5), tree swallow (2), and American robin (4) and Trowbridge starling (1), house wren (6), tree swallow (5), and American robin (8) used as representative of terrestrial passerine concentrations.

b. Waterfowl samples were collected from 5 locations on the Kalamazoo River and were not divided between upstream and downstream sampling locations because of uncertain local residence status on the river.

c. Geometric mean total PCB concentrations in Ft. Custer chipmunk (5) and red squirrel (1) used as surrogate value for rabbit concentration.

d. Total PCB concentrations in Trowbridge chipmunk (1) used as surrogate value for rabbit concentration

*Trowbridge total PCB concentrations are significantly greater than concentrations at Ft. Custer ($p \leq 0.01$).

Table 4.4. Geometric mean and upper 95% confidence level (U95% CL) concentrations of 2,3,7,8 tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{Who\text{-}Avian}$) (pg $TEQ_{WHO\text{-}Avian}$/g ww) in prey items collected from two sites on the Kalamazoo River.

	Ft. Custer					Trowbridge				
	N	Range	Geometric Mean	U95% CL	Lipid (%)	N	Range	Geometric Mean	U95% CL	Lipid (%)
Passerine[a]	11	0.47 – 238	5.3	18	4.10	19	1.1 – 4,777	56*	194	4.90
Waterfowl[b]	17	36 – 7,678	244	725	3.80	17	36 – 7,678	244	725	3.80
Mice/Vole	12	0.18 – 2.9	0.61	0.91	3.59	20	0.47 – 3.8	0.80	0.99	4.57
Shrew	16	0.45 – 49	1.28	2.18	3.66	17	4 – 249	47*	77	2.68
Muskrat	4	0.07 - 37	1.1	14	2.60	7	0.43 - 49	13	47	2.07
Rabbit[c,d]	6	0.13 – 4.0	0.52	1.51	3.71	1	71	71	71	4.95
Crayfish	4	1.5 - 58	7.5	33	0.63	13	3.1 - 374	56*	108	1.62

a. Geometric mean total $TEQ_{WHO\text{-}Avian}$ concentrations in Ft. Custer house wren (5), tree swallow (2), and American robin (4) and Trowbridge starling (1), house wren (6), tree swallow (5), and American robin (7) used as representative of terrestrial passerine concentrations.
b. Waterfowl samples were collected from 5 locations on the Kalamazoo River and were not divided between upstream and downstream sampling locations because of uncertain local residence status on the river.
c. Geometric mean total $TEQ_{WHO\text{-}Avian}$ concentrations in Ft. Custer chipmunk (5) and red squirrel (1) used as surrogate value for rabbit concentration.
d. $TEQ_{WHO\text{-}Avian}$ concentrations in Trowbridge chipmunk (1) used as surrogate value for rabbit concentration.
*Trowbridge TEQ concentrations are significantly greater than concentrations at Ft. Custer ($p \leq 0.02$).

locations excepting TB mice/voles (45% contribution) (Figure 4.4). The same three coplanar congeners were among the three greatest contributors to concentrations of total TEQ for all prey item categories at each sampling location excepting TB mice/voles and TB rabbits (chipmunk). The three greatest PCB congener contributors and their combined contribution to total TEQ for each prey item category included: PCB 77>126>81[FC passerine(95.4%), TB passerine(97%), FC and TB waterfowl(98.1%-estimated), FC and TB crayfish(99%)]; PCB 77>81>126 [TB muskrat(98.6%)]; PCB 126>77>81 [FC muskrat(99.7%), FC rabbit (chipmunk and red squirrel)(99.1%), TB shrew(93.3%)]; PCB 126>81>77 [FC shrew(99.4%), FC mice/vole(97.1%)]; PCB 126>77>118 [TB rabbit (chipmunk)(97.9%)]; PCB 105>81>126 [TB mice/vole(71.1%)].

Average Potential Daily Dose (APDD)

Average potential daily doses for GHOs were calculated based on geometric mean concentrations of both total PCBs and $TEQ_{WHO\text{-}Avian}$ of each prey item category for the numeric- and mass-based range of dietary composition at the FC and TB study sites. Calculations of both total PCB and $TEQ_{WHO\text{-}Avian}$ exposures at TB included contributions from incidental soil ingestion. Based on site-specific diet and prey item COC concentrations, GHO ingestion of total PCBs were from 7 to 10-fold greater at TB than at FC, and $TEQs_{WHO\text{-}Avian}$ were 3-fold greater at TB than FC (Table 4.5). Average potential daily doses calculated using the upper 95%CL (geometric mean) of total PCBs and $TEQ_{WHO\text{-}Avian}$ displayed a range of differences that were similar (6 to 7-fold difference and 2-fold difference, total PCBs, $TEQ_{WHO\text{-}Avian}$, respectively) to values of APDD based on the geometric mean.

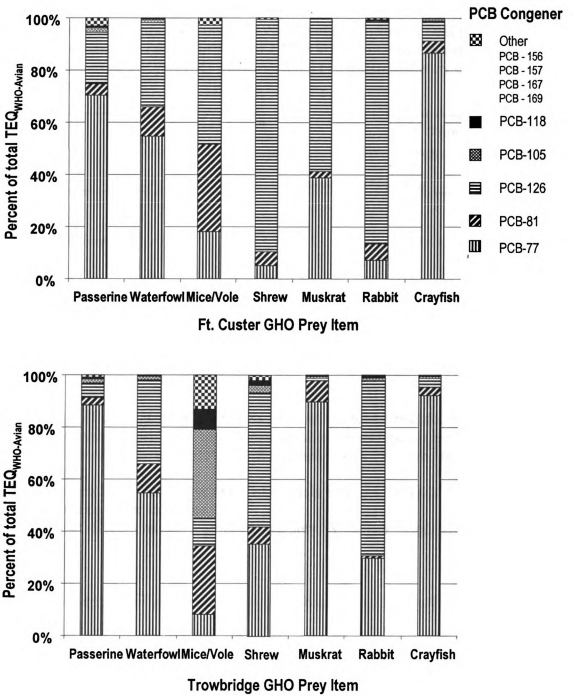


Figure 4.4. Percent contribution of polychlorinated biphenyl (PCB) coplanar and mono-*ortho*-substituted congeners to total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) in great horned owl (*B. virginianus*) prey at the Kalamazoo River.

Table 4.5. Range of Average Potential Daily Doses (APDD)[a] based on geometric mean and the upper 95% confidence level (U95% CL) of prey items for total Polychlorinated Biphenyls (PCBs) (ng PCBs/g bw/d) and 2,3,7,8 tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) (pg TEQ/g bw/d) when assuming two different dietary compositions for great horned owl (GHO) at the KRSS.

	Ft. Custer		Trowbridge[b]	
	PCBs	**TEQs**	**PCBs**	**TEQs**
		PCB Based Dietary Models		
		Site-Specific APDD[c]		
Geometric Mean	3 - 5	0.676 – 1.25	30 – 37	2.39 – 3.81
U95% CL	7 – 10	2.03 – 3.76	59 – 61	5.34 – 6.98
		Literature-Based APDD[d]		
Geometric Mean	4 – 5	0.428 – 0.549	38 – 60	2.72 – 3.97
U95% CL	9 – 12	1.34 – 1.75	80 – 127	6.81 – 10.18

a. The range of calculated APDD results from using diet estimations based on both total frequency (numeric-basis) and biomass contribution (mass-basis) (see Table 2).
b.Includes incidental ingestion of floodplain soils at the former Trowbridge impoundment.
c.Based on results of field collected GHO pellets and prey remains from active nests at each Kalamazoo River study site.
d.A study of GHO diet in Washtenaw County, Michigan (Craighead and Craighead 1956).

Comparisons of geometric mean, mass-based ranges of APDD between the site-specific and literature-based GHO dietary compositions yielded APDD values for total PCB exposures at FC that were equivalent ($\leq$ 1.5-fold difference), and TB total PCB APDD values that differed by a factor of 1.6 (literature-based > site-specific APDD) (Table 4.5). Average potential daily dose values for mean $TEQ_{WHO\text{-}Avian}$ were 1.6 to 2.3-fold greater for FC site-specific based dietary exposures and equivalent for TB based exposures. The literature-based TB APDD calculations for both total PCB and $TEQ_{WHO\text{-}}$

$_{Avian}$ included contributions from incidental soil ingestion consistent with the calculations for site-specific exposures at TB. Average potential daily doses (site-specific vs. literature-based) calculated using the upper 95%CL for total PCBs displayed a range of values similar to mean-based APDDs (FC exposures were equivalent, literature-based APDDs were 2.1-fold greater for TB exposures). Average potential daily doses calculated using the upper 95%CL concentrations of $TEQ_{WHO\text{-}Avian}$ in prey produced site-specific APDDs that were 1.5 to 2.1-fold greater than literature-based values at FC, and APDDs that were equivalent at TB.

The greatest calculated APDDs for GHOs at the KRSS originated from a mass-based dietary compilation. The 10-fold greater APDD at TB than at FC was consistent with the significant differences in total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations in prey items collected from the two sites. The APDDs based on total PCBs and $TEQs_{WHO\text{-}Avian}$ for the site-specific and literature-based diets were less than 3-fold different for both FC and TB. This indicates that the moderate differences in dietary composition observed at a class and within-class level between the two studies did not influence APDD to any great extent.

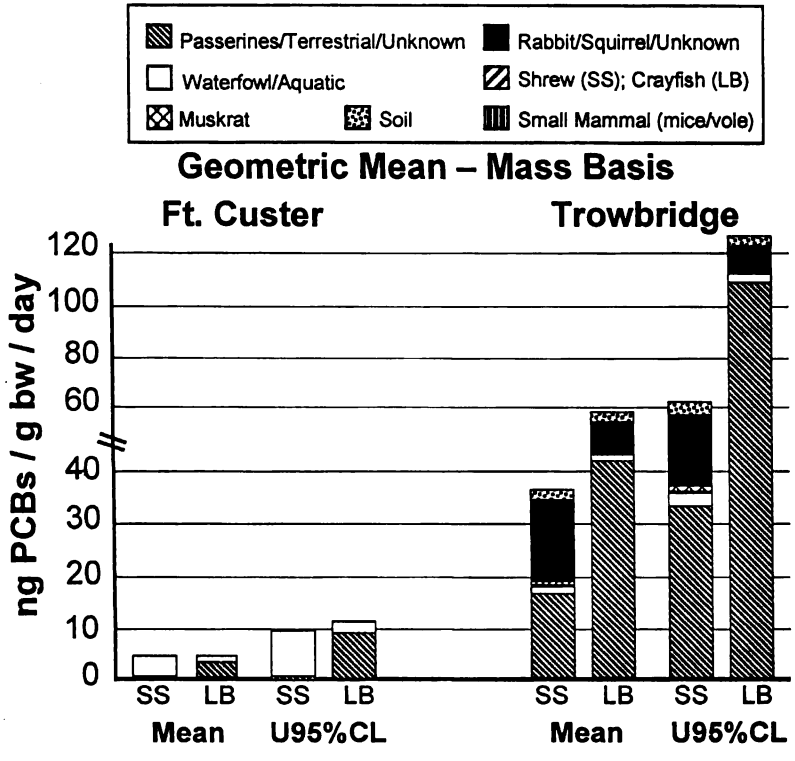
Due to greater concentrations of COCs or greater proportions in the diet, exposures are often dependent on a few types of prey. This phenomenon was observed for GHOs at the KRSS where two to four prey item categories combine to account for over 90% of the APDD at any given site and diet composition. At FC, mass-based APDDs for geometric mean total PCB concentrations in prey show that waterfowl (91% of APDD) and passerines (5%) drive exposures for the site-specific diet, and the same two prey items, albeit in an inverted ratio: waterfowl (25%) and passerines (72%), also

drive the literature-based exposure (Figure 4.5a). At TB, passerines (45%), rabbits (43%) and soil ingestion (5%) figure predominantly in $APDD_{PCBs}$ for site-specific exposures and also literature-based exposures (passerines (62%), rabbits (26%), soil ingestion (5%)). The principal prey items responsible for APDDs calculated for $TEQ_{WHO\text{-}Avian}$ include the same prey identified for $APDD_{PCBs}$ but in some cases additional prey contribute to the 90% threshold. At FC, waterfowl (97%) and rabbits (2%) drive $APDD_{TEQ}$ for site-specific exposures and literature-based exposures come from waterfowl (62%) and passerines (35%). At TB, rabbits (52%), passerines (18%), soil ingestion (16%), and waterfowl (10%) drive $APDD_{TEQ}$ for site-specific exposures, and literature-based exposures come from passerines (34%), rabbits (28%), waterfowl (16%) and soil ingestion (14%) (Figure 4.5b).

Assessment of Hazard

Hazard quotients were calculated for each location based on the site-specific and literature-based APDDs for total PCBs and $TEQ_{WHO\text{-}Avian}$. To conservatively estimate potential hazard to resident GHOs at the KRSS and to capture the broadest reasonable

A.


Passerines/Terrestrial/Unknown
Rabbit/Squirrel/Unknown
Waterfowl/Aquatic
Shrew (SS); Crayfish (LB)
Muskrat
Soil
Small Mammal (mice/vole)
Geometric Mean – Mass Basis
Ft. Custer
Trowbridge
ng PCBs / g bw / day
120
100
80
60
40
30
20
10
0
SS LB SS LB SS LB SS LB
Mean
U95%CL
Mean
U95%CL

B.

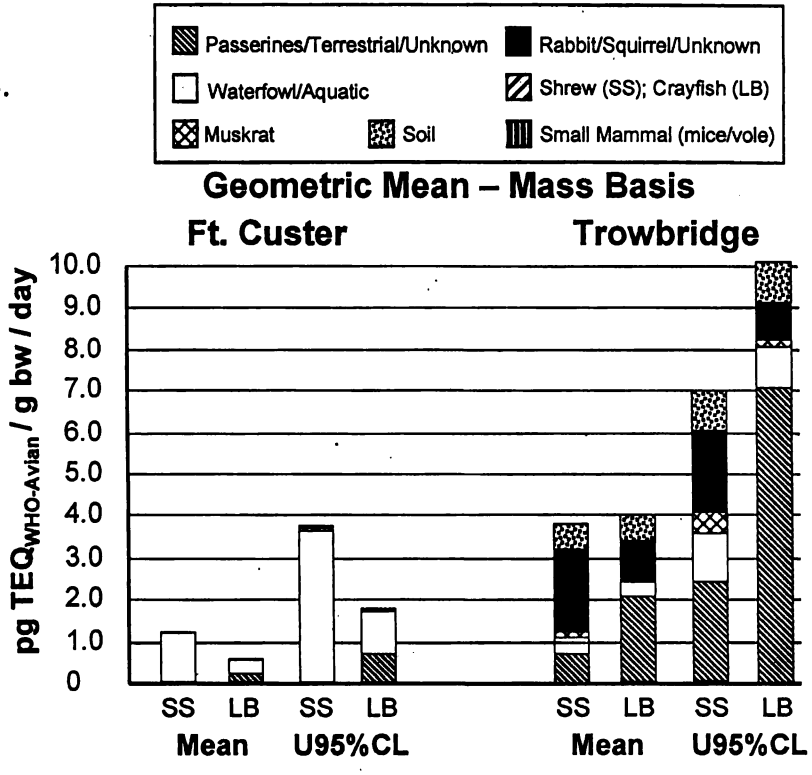

Passerines/Terrestrial/Unknown
Rabbit/Squirrel/Unknown
Waterfowl/Aquatic
Shrew (SS); Crayfish (LB)
Muskrat
Soil
Small Mammal (mice/vole)
Geometric Mean – Mass Basis
Ft. Custer
Trowbridge
pg TEQWHO-Avian / g bw / day
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
SS LB SS LB SS LB SS LB
Mean
U95%CL
Mean
U95%CL

Figure 4.5. (**A**) Percent contribution of each principal prey component and incidental soil ingestion to total polychlorinated biphenyl (PCB) average potential daily dose (APDD; ng PCBs/g bw/day, ww) for great horned owl (*B. virginianus*) based on the geometric mean and upper 95% confident level concentrations in Ft. Custer and Trowbridge prey, Trowbridge soil, and site-specific (SS) and literature-based (LB) dietary compositions (mass-basis only). (**B**) Percent contribution of each principal prey component and incidental soil ingestion to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$) average potential daily dose (APDD; pg TEQ/g bw/day, ww) for great horned owl (*B. virginianus*) based on the geometric mean and upper 95% confident level concentrations in Ft. Custer and Trowbridge prey, Trowbridge soil, and site-specific (SS) and literature-based (LB) dietary compositions (mass-basis only).

range of variability in characterizations of prey item COC concentrations and composition of the GHO diet, HQs are calculated from the range of APDD values encompassing the geometric mean and associated upper 95%CL values for each respective prey item and the dietary proportion contributed by each prey item compiled on both a numeric- and mass-basis. The range of HQs discussed for the NOAEL and LOAEL effect levels (HQ_{NOAEL}/HQ_{LOAEL}, respectively) will typically represent potential hazard associated with exposures to geometric mean concentrations for a numeric-based diet (low range) up to the upper 95%CL concentrations for a mass-based diet (high range). All HQs (total PCBs and $TEQ_{WHO\text{-}Avian}$) for site-specific and literature-based diets determined for both FC and TB geometric mean and upper 95%CL exposures were less than 1.0. The maximum FC HQ_{NOAEL} for total PCBs was 0.02 and 0.03 for the site-specific and literature-based diets, respectively (Table 4.6). The maximum TB HQ_{NOAEL} for total PCBs was 0.15 and 0.31 for the site-specific and literature-based diets, respectively. The maximum FC HQ_{NOAEL} for $TEQ_{WHO\text{-}Avian}$ ranged from 0.27 to 0.13 for the site-specific and literature-based diets, respectively; TB $HQ_{NOAEL/TEQ}$ ranged from 0.5 to 0.73 for the site-specific and literature-based diets, respectively.

Table 4.6. Hazard Quotient (HQ) values based on geometric mean and the upper 95% confidence limit (U95% CL) of average potential daily doses (APDD) of total Polychlorinated Biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), when assuming two different dietary compositions for great horned owl (GHO) at the Kalamazoo River Superfund Site (KRSS).[a]

	Ft. Custer		Trowbridge	
	HQ NOAEL	HQ LOAEL	HQ NOAEL	HQ LOAEL
		PCB Based Dietary Models		
		Site-Specific[b]		
Geometric Mean	0.01	<0.01	0.07 – 0.09[c]	0.02 – 0.03
U95% CL	0.02	0.01	0.14 – 0.15	0.05
		Literature-Based[d]		
Geometric Mean	0.01	<0.01	0.09 – 0.15	0.03 – 0.05
U95% CL	0.02 – 0.03	0.01	0.20 – 0.31	0.07 – 0.10
		$TEQ_{WHO\text{-}Avian}$ Based Dietary Models		
		Site-Specific[b]		
Geometric Mean	0.05 – 0.09	0.01	0.17 – 0.27	0.02 – 0.03
U95% CL	0.15 – 0.27	0.01 – 0.03	0.38 – 0.50	0.04 – 0.05
		Literature-Based[d]		
Geometric Mean	0.03 – 0.04	<0.01	0.19 – 0.28	0.02 – 0.03
U95% CL	0.01 – 0.13	0.01	0.49 – 0.73	0.05 – 0.07

a. Toxicity reference values used to calculate HQs are provided in Table 1.
b. Based on results of field collected GHO pellets and prey remains from active nests at each Kalamazoo River study site.
c. The range of calculated APDD results from using diet estimations based on both total frequency (numeric-basis) and biomass contribution (mass-basis) (see Table 2).
d. A study of GHO diet in Washtenaw County, Michigan (Craighead and Craighead 1956).

DISCUSSION

Dietary Composition

The three-year sampling program at active GHO nests for pellets and prey remains was an effective approach for characterizing site-specific exposures of nestling GHOs to the COCs at the KRSS, and to concurrently allow for non-intrusive monitoring of GHO productivity at each nest. Additionally, some potential biases commonly associated with pellet and prey remains sampling were also addressed in this study. Our approach capitalized on GHO preferences to use nests built by other bird species or in this instance artificial nesting platforms that were located at appropriate floodplain locations in the KRSS. In doing so, we successfully induced resident GHOs to occupy areas of the site having maximum exposure potential. Only one of the active nests sampled for pellets and prey remains was a natural nest, all remaining nesting activity included in the diet characterization study occurred at artificial nesting platforms. Additional advantages of GHO behavior incorporated into this study included their propensity to forage within relatively small areas because of their sedentary lifestyle and highly versatile prey capture ability (Marti 1974).

In this study, sampling of pellets and prey remains from beneath feeding perches and nest trees (ground collections) was augmented with collections from active nests during the blood sampling event and again after fledging was complete. Nest sites were the best locations for collecting avian prey remains, particularly feathers that could be positively classified as evidence of owl predation. Nest collections eliminated a significant source of uncertainty associated with feather remains collected on the ground

beneath or in the general vicinity of active nests and feeding perches for which solid evidence of owl predation was mostly lacking.

Feeding studies with captive great horned owls (Errington 1930; Glading et al. 1942) showed that pellets quite consistently reflected the food habits of adult and juvenile owls. Potential biases associated with pellet studies (under-representation of very small prey, prey with more easily digestible components, boneless pellets from newly hatched owlets) or prey remains collections (over-representation of larger species and avian prey) can be adequately managed through collections of both types of samples, segregation of samples among active nests, and reconciliation of all concurrently collected samples. Very small prey items (e.g., very young animals) are unlikely to carry high COC concentrations because of a very limited period of potential exposure, and even in the exception will contribute negligibly to APDD because of their low mass. Prey with easily digestible components may still be identified in prey remains collected from the nest, and the small numbers of prey that are completely absorbed by recently hatched owlets will most likely be adequately represented in subsequent pellets.

Studies of the dietary habits of North American GHO populations show the GHO is an opportunist hunter, plastic in foraging behavior, a generalist in prey selection with the broadest diet of any North American owl (Marti and Kochert 1996). Dietary preferences depend upon habitat, season, and prey vulnerability, and GHOs are capable of expressing the most diverse prey profile of all North American raptors (Voous 1998). Contributing factors to the species' broad diet include a large body size and crepuscular/nocturnal activity range. Major determinants upon prey selection by any individual include habitat, prey abundance and prey vulnerability (a measure that

combines ephemeral and interrelated determinants of prey density/prey behavioral patterns, habitat condition, and seasonality) (Houston et al. 1998).

In general, temperate North American GHO populations feed predominantly on terrestrial mammals followed by terrestrial and aquatic birds, and a minor mix of reptiles, amphibians and arthropods (Marti and Kochert 1995; Murphy 1997). Great horned owl diets vary between physiographic regions (Wink et al. 1987) and even among individual nesting territories when land use and habitat type distributions diverge within distinct physiographic units (Marti 1974). Great horned owl diets can also show significant temporal variation when pronounced changes in prey availability occur due to natural small mammal population cycles or anthropogenic modifications to habitat or prey populations (Adamcik et al. 1978; Fitch 1947). In addition, GHO diets also may vary with seasonal changes in prey vulnerability, although this source of variation tends to be minor compared to diet alterations stemming from differences in habitat and temporal prey availability (Wink et al. 1987; Fitch 1947; Errington et al. 1940). If present, significant seasonal variations in GHO diets may originate from changes in prey vulnerability caused by a combination of factors including vegetation changes, altered activity patterns of prey and GHOs, day length, GHO reproductive cycles, prey hibernation patterns, prey migration patterns, and prey reproductive lifecycle events (e.g., mating, dispersal of young).

Great horned owl foraging preferences are difficult to predict from surveys of prey populations in most North American temperate habitats and attempts to correlate GHO predation preferences with prey abundances have yielded mixed results (Murphy 1997). This is due in part to the fact that prey density apparently has little effect on prey

vulnerability in some GHO territories (Peterson 1979; Adamcik et al. 1978). GHOs tend to display a density-independent dietary relationship to prey species. Changes in prey vulnerability do not necessarily correspond with changes in numerical status. Some species remain more vulnerable to GHO predation regardless of annual fluxes in abundance/density, even in periods of low population densities (Peterson 1979; Errington 1932).

Attempts to correlate GHO predation preferences to habitat types show greater success where GHO's food habits appear to depend largely upon where the bird is situated because many birds studied seem to limit their activities to a few acres of certain favorite habitat (Errington 1932; Fitch 1947). While there is evidence to support a strong interaction between GHO foraging preferences and habitat (Rusch et al. 1972), there were still instances where some GHOs did appear to respond opportunistically to the availability of certain prey types. (Marti and Kochert 1996). However, wetlands habitats appear to be a notable exception to the habitat-prey use relationship identified for GHOs where studies of GHO use of wetland-dependent prey and proximity and extent of wetlands within nesting territories have shown almost no relationship between habitat and prey type. In these studies, GHOs sought wetland prey regardless of proximity or abundance of wetland habitats. This may stem from the fact that prey species may be more available and vulnerable due to high prey density, high prey diversity and abundance, and more favorable locations and numbers of elevated hunting perches in wetlands and wetland edge habitats (Murphy 1977; Houston 1998).

Our studies of GHO predation at the KRSS included efforts to control for spatial and temporal variability in owl diets. Great horned owl nesting platforms were

specifically located in riparian floodplain habitats that were buffered from most human disturbances and situated within 100 m of the river to provide uniformity in foraging habitat, available prey populations and habitat-dependent influences on prey vulnerability. Nest trees were selected after completing a qualitative survey of nesting habitat quality so as to provide an optimal mix of cover and foraging habitat for breeding owls. Pellets and prey remains from multiple years were collected only during active nesting and brooding periods of the annual reproductive lifecycle to provide uniformity in environmental cues on GHO behavior and seasonal influences on prey availability/vulnerability.

Site-specific and Literature-based Diets

Because the dominant factors influencing GHO diet originate from spatial differences in habitat type and temporal alterations in prey populations, a literature-based diet selected for use in the absence a site-specific value must match the physiographic region and dominant habitat types at the site. If multiple studies of equivalent quality are available, temporal considerations can also be addressed. For instance, in the 1940s, nesting habitat of GHO populations in southeastern Michigan (Superior Township, Washtenaw County) included plant and animal community assemblages that were very similar to those present at KRSS (Craighead and Craighead 1956). When dietary composition for GHOs from KRSS was compared to the Washtenaw study, differences were observed that were principally related to differing proportions of rabbits and passerine/terrestrial birds (Table 4.2). Kalamazoo River owls consumed greater proportions of rabbits, and Washtenaw County GHOs consumed greater proportions of passerines/terrestrial birds. The greater

proportion of birds in Washtenaw County GHO diets is directly attributable to a greater number of ring-necked pheasants in the diet that were at their historical peak of abundance in Michigan during the 1940's. However, their number significantly decreased by the 1980's (Luukkonen 1998) and as a result, this loss of an important dietary item was compensated for by increased GHO consumption of cottontail rabbits in the KRSS study (Springer and Kirkley 1978; Peterson 1979).

The impact of wetlands on GHO dietary composition also needs to be taken into account in that wetland habitats are well represented and in close proximity to GHO nesting territories at KRSS and the proportion of species with a direct-link (habitat-based; e.g., muskrat, waterfowl, crayfish) and indirect-link (foraging-based; e.g., insectivorous passerine birds, bats, weasels) to the aquatic food-web may have an important contribution to the overall diet. The combined diet composition for KRSS GHOs (FC + TB) shows that 8.8% (numeric-basis) and 21% (mass-basis) of GHO prey originated wholly or in-part from the aquatic food-web. In comparison, aquatic prey comprised 9% of Washtenaw Co. GHO diet on both a numeric- and mass-basis. A review of GHO diet studies available in the literature showed that aquatic prey are common in diets of GHOs residing in close proximity to wetland habitat types, and in select Western and upper Midwestern habitats, the proportion of aquatic prey (numeric-basis) in resident GHO diets can exceed 20% and 50%, respectively (Bogiatto et al. 2003; Murphy 1997).

Average Potential Daily Dose (APDD)

A conservative approach was used to calculate APDD values for GHOs such that when site-specific PCB or $TEQ_{WHO\text{-}Avian}$ concentration data were not available for a specific

component of GHO diet (e.g., rabbits, grey/fox squirrels), the shortcoming was addressed by using site-specific data for red squirrels and chipmunks to represent potential COC exposures for the group. This approach incorporated a conservative estimate of potential exposure since the omnivorous diets of squirrels and chipmunks place them in a higher trophic level compared to rabbits. The conservative nature of this substitution is evident in that the total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations used to calculate rabbit contributions to Trowbridge APDD were one to two orders of magnitude higher than concentrations expressed by both terrestrial and aquatic herbivorous counterparts to rabbits at the site (mice/vole, muskrat) (Table 4.3). A similar approach was used to address the absence of site-specific data for pheasants and other galliform prey where these species were grouped with passerine prey. Passerines at the site included insectivorous representatives (e.g., tree swallows) with forage-based links to the aquatic food-web at the site. Passerines had the highest concentration of total PCBs and second highest concentration of $TEQ_{WHO\text{-}Avian}$ among prey groups from the TB site (Table 4.3). The USFWS waterfowl database also provided an unbiased characterization of potential exposure through this aquatic pathway by including representative concentrations from both piscivorous (merganser) and omnivorous (mallard) feeding groups. Great horned owls prey indiscriminately upon waterfowl and a variety of wading birds (Rusch et al. 1972). Piscivorous waterfowl and shorebirds have been shown to accumulate total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations that are ten- to fifteen-fold greater than their closely related avian counterparts who are more herbivorous (Jones et al. 1993). Processing of small mammals and avian prey that included the removal of stomach contents (both prey types) and feathers, beaks, wings, legs (avian prey) is a common practice in exposure and

effects studies and is typically used to conservatively estimate soil to organism bioaccumulation factors. The method contributed to conservative measurements of the COCs in these samples because the substantial mass excluded from analyses (keratin, herbaceous forage) contained much lower contaminant concentrations compared to the remaining tissues (predominantly muscle and lipids) analyzed for the target organism. Finally, incidental soil ingestion was also included in the site-specific APDD calculations to account for soil that may be associated with the pelage of small mammalian prey that tunnel through vegetation or use burrows for shelter, nesting, or food storage, and also present in avian prey that consume grit and associated soil particles as a normal course of their foraging activities (Mayoh and Zach 1986).

A conservative approach also was used to estimate $TEQ_{WHO\text{-}Avian}$ from the PCB Aroclor data for waterfowl and soils in the calculation of $APDD_{TEQ}$ for GHOs. Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were estimated by selecting the greatest proportional contribution of each individual non-*ortho* and mono-*ortho* PCB congener across four technical Aroclor mixtures as the fractional composition value for each medium. The greatest potential concentrations for each congener were supplemented with additional "enrichment factor" increases to two bioaccumulative and toxic non-*ortho* congeners (PCB 126 and 169). As a result, the estimated $TEQ_{WHO\text{-}Avian}$ concentrations for waterfowl and soils have much greater toxicity than would be predicted from the original Aroclor mixtures. Waterfowl $TEQ_{WHO\text{-}Avian}$ concentrations were the highest among all prey type contributions to $APDD_{TEQ}$ by a factor of three and seven for the geometric mean and upper 95% CL concentrations, respectively (Table 4.3).

The only notable difference between estimates of APDD from a site-specific diet ($APDD_{measured}$) and literature-based diet ($APDD_{predicted}$) were higher $APDD_{predicted}$ for total PCB (geometric mean and upper 95% CL values) at TB (mass-based diet), and higher $APDD_{measured}$ for $TEQ_{WHO\text{-}Avian}$ (geometric mean and upper 95% CL) values at FC (numeric- and mass-based diets). At TB, the greater $APDD_{PCB}$ values for the literature-based diet was due to the greater proportions of pheasants and to the elevated total PCB concentrations in the passerine/terrestrial avian prey group compared to total PCB concentrations in rabbits, the predominant prey group for TB owls. The difference in predicted versus measured $APDD_{PCB}$ was not observed at FC because the large differences in the proportion of passerine/terrestrial prey (e.g., pheasant) between the site-specific and literature-based diets was mitigated by the low total PCB concentrations in the dietary items collected at FC, a larger proportion of waterfowl in the FC $APDD_{measured}$ vs. literature-based $APDD_{predicted}$, and the greater waterfowl total PCB concentrations used for the $APDD_{PCB}$ calculations (Table 4.2, Table 4.3, Figure 4.5). At FC, the greater $APDD_{TEQ}$ for the site-specific diet originated from the overriding influence of waterfowl prey. This included a larger proportion of waterfowl in the FC $APDD_{measured}$ vs. literature-based $APDD_{predicted}$, and the much greater waterfowl $TEQ_{WHO\text{-}Avian}$ concentrations used for the $APDD_{TEQ}$ calculations (Table 4.2, Table 4.4, Figure 4.5). This difference in $APDD_{TEQ}$ was not present between $APDD_{predicted}$ /$APDD_{measured}$ at TB because the much larger proportion of passerine/terrestrial prey (e.g., pheasant) in the literature-based diet was mitigated by higher mean $TEQ_{WHO\text{-}Avian}$ in rabbits vs. passerines/terrestrial avian prey coupled with additional TEQ contributed from muskrat

prey (with greater variable TEQ concentrations) to $APDD_{measured}$ that was calculated from the upper 95%CL for $TEQ_{WHO\text{-}Avian}$ in prey populations.

Overall, calculations of $APDD_{predicted}$ /$APDD_{measured}$ in this study were primarily influenced by gaps in the site-specific data for principal prey items in both the site-specific and literature-based diets. Although $APDD_{predicted}$ and $APDD_{measured}$ values were very similar across the range of prey concentration values at FC and TB, the notable differences observed in APDD can be traced to the lack of site-specific data for pheasants and rabbits, and the lack of recent, congener-specific total PCB data for waterfowl. Because all surrogate data used to address these data gaps was chosen to insure that any potential biases contributed by these data erred in a conservative "worst-case" manner, it is reasonable to assume that if site-specific data were available for these prey, the calculated $APDD_{PCB}/APDD_{TEQ}$ for both diets would have decreased and the relationships between $APDD_{measured}$ and $APDD_{predicted}$ for both total PCBs and $TEQ_{WHO\text{-}Avian}$ would have changed. This exercise also illustrates that differences between $APDD_{measured}$ and $APDD_{predicted}$ may be exacerbated at sites where the contaminant distribution between proximal aquatic and terrestrial habitats is dissimilar, and prey with links to the aquatic food-web figure predominantly in site-specific GHO diets. In these instances, the unique composition of a site-specific diet that includes aquatic prey may contribute significantly to the overall assessment of exposure, therefore posing significant risk that may be overlooked if the hazard assessment relies upon a literature-based dietary composition that fails to identify important prey items with links to aquatic exposures.

Risk Estimates Based on Total PCBs and TEQs

Hazard quotients based on $TEQ_{WHO\text{-}Avian}$ were greater than those based on total PCBs. Hazard quotients calculated from NOAEL TRVs for geometric mean and upper 95%CL concentrations of $TEQ_{WHO\text{-}Avian}$ were four- to 13-fold greater than for total PCBs at FC, and two- to three-fold greater at TB (Table 4.6). Hazard quotients based on total PCB concentrations are considered to be a more accurate estimate of potential risk because the concentration in the diet can be compared directly to values reported in the studies from which TRVs were derived. Congener-specific analyses provided for coplanar PCB congeners to be used in a calculation of TEQ. This approach eliminated the difficulties and uncertainties involved with assessing the toxicity of environmentally weathered PCB mixtures that are quantified as Aroclors, and is generally believed to correlate better with toxicity than measures of total PCBs (Blankenship and Giesy 2002). However, the scientific basis for TEQ derivation and use may contribute to bias that overestimates risk when they are applied to complex mixtures of PCBs. Concentrations of TEQs are calculated by multiplying each PCB congener by a class-specific (mammal, bird, fish) relative potency expressed as a toxic equivalency factor. Toxic equivalency factors are consensus values that were rounded up to be conservative estimates of risk (Van den Berg et al. 1998). This practice, coupled with the use of proxy values for congeners that were present at concentrations less than the method detection limit (e.g., use of one-half the method detection limit for non-detects), the summation of co-eluting congeners into a single value for some mono-*ortho* PCB congeners, and conservative estimates of congener fractional composition values for historical Aroclor PCB data are likely reasons that HQs based on $TEQ_{WHO\text{-}Avian}$ are greater than HQs estimated for total PCBs.

Additionally, a recent review of tree swallow exposure studies indicated that $TEQs_{WHO\text{-}Avian}$ calculated from field-based TCDD and PCB exposures did not elicit similar endpoints of effect and may not be toxicologically equivalent (Neigh et al. 2006c).

The Multiple-Lines-of-Evidence Approach

This study has determined that dietary exposures of resident GHO populations to total PCBs and $TEQ_{WHO\text{-}Avian}$ present in contaminated floodplain soil of the KRSS are well below the threshold for effects on reproductive success. Even when the most conservative estimates of HQ are considered in the "bottom-up" assessment of potential hazards at the site, all HQ values for calculated APDDs using a site-specific dietary composition are less than 0.5. Similarly, all HQ values for calculated APDDs using a literature-based dietary composition at the site are less than 0.75 The greater HQ value for the literature-based diet originated from overestimates of avian prey (from diet) and aquatic exposures (from COC concentrations used to calculate APDD from passerine/terrestrial avian prey) in the hazard assessment.

The "bottom up" assessment was one component of a multiple-lines-of-evidence approach that also included a tissue-based "top down" investigation of GHO exposure by investigating PCB concentrations in eggs and nestling plasma (Strause et al. 2007). Results of the tissue-based studies were consistent with the dietary findings. The observed total $PCB/TEQ_{WHO\text{-}Avian}$ concentrations in eggs resulted in HQs less than 1.0 for all exposures indicating that tissue-based exposures did not pose a significant risk to GHO populations at the Upper KRSS. Hazard quotients calculated for tissue-based and site-specific dietary exposures show strong agreement at both the Reference and Upper

KRSS study sites with less than a three-fold difference between the ranges of $HQ_{NOAEC/NOAEL}$ and $HQ_{LOAEC/LOAEL}$ (mean and upper 95%CL concentrations) for both COCs, excepting $TEQ_{WHO\text{-}Avian}$ concentrations at FC where a seven-fold range in HQs was present. (Figure 4.6).

The multiple-lines-of-evidence approach included ancillary investigations to the "top-down" assessment that focused on evaluating the relative abundance, site use and productivity of resident GHOs at the Upper KRSS relative to the upstream Reference location. (Figure 4.7). The relative abundance of territory-holding nesting pairs of GHOs in the Upper KRSS was near the carrying capacity for the available habitat area included in the study. Nest acceptance rates and nest fidelity of actively breeding Upper KRSS GHOs across all nesting seasons included in the study were consistent with previous studies of artificial nest acceptance and habitat usage by Strigiforms in Midwestern forests (Holt 1996). Mean productivity rates (fledglings/active nest) were similar among locations where exposures to PCBs were much different, and were consistent with productivity measures for healthy Midwestern GHO populations (Strause et al. 2007; Holt 196). These results agree with findings for both the "top down" and "bottom up" approaches to evaluate chemical exposures at the site, and serve to reduce the uncertainties associated with assessment endpoints and strengthen the conclusion that potential risk to GHOs from exposures to total PCB/ $TEQs_{WHO\text{-}Avian}$ in the Upper KRSS are unlikely to be sufficient to cause adverse population-level effects.

Results from this study suggest that it would be appropriate to estimate risk based on either tissue-based or dietary-based methodologies. However, if a dietary-based

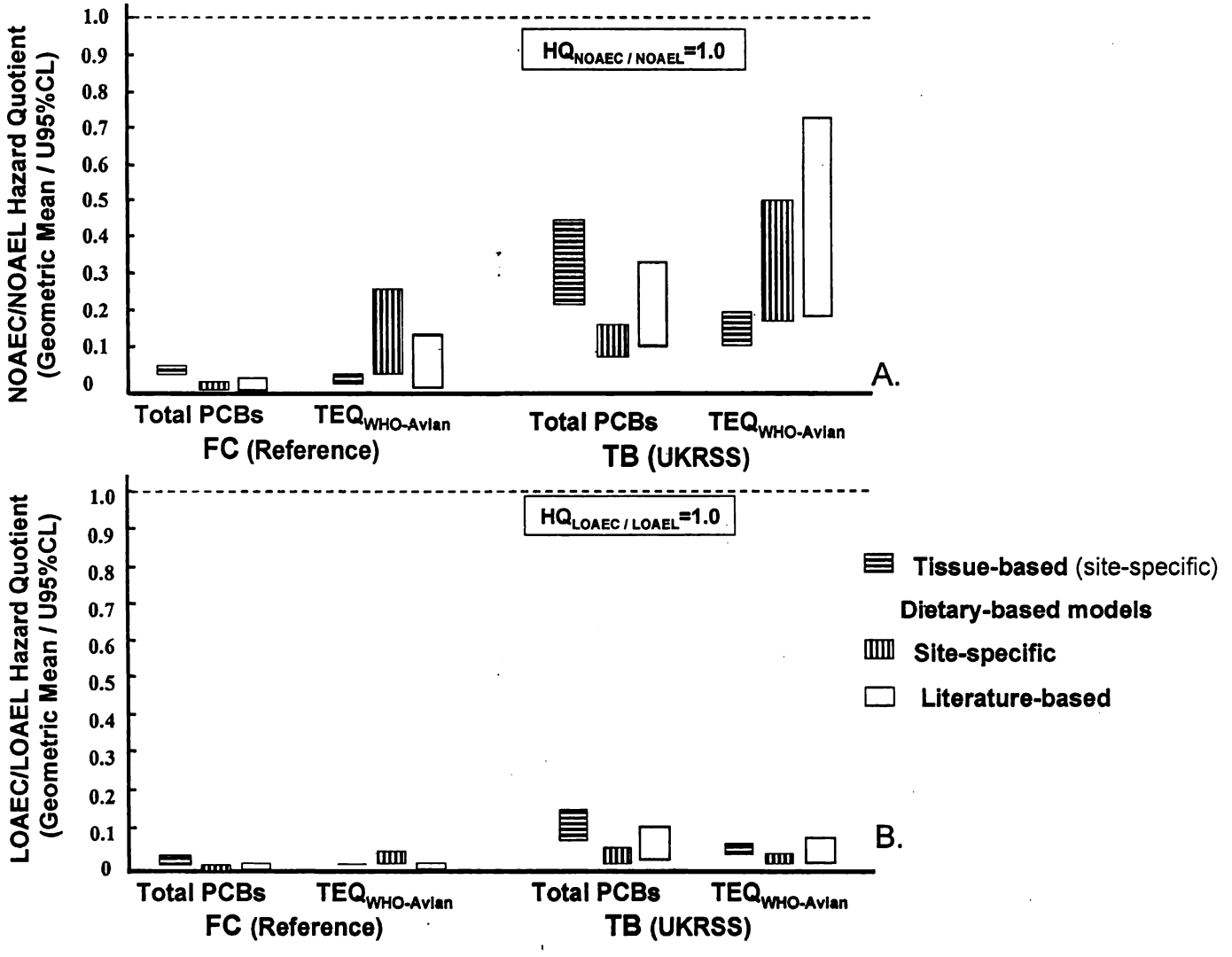

NOAEC/NOAEL Hazard Quotient
(Geometric Mean / U95%CL)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
HQNOAEC / NOAEL=1.0
A.
Total PCBs
TEQWHO-Avian
Total PCBs
TEQWHO-Avian
FC (Reference)
TB (UKRSS)
LOAEC/LOAEL Hazard Quotient
(Geometric Mean / U95%CL)
HQLOAEC / LOAEL=1.0
B.
Tissue-based (site-specific)
Dietary-based models
Site-specific
Literature-based

Figure 4.6. (**A**) Comparison of tissue-based (egg) no observable adverse effect concentration (NOAEC) and diet-based no observable adverse effect level (NOAEL) Hazard Quotients (HQs) at the Upper Kalamazoo River Superfund Site (Trowbridge) and Reference (Ft. Custer) locations calculated from NOAEC/NOAEL-based TRVs for polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$). Each box encompasses the geometric mean and upper 95% confidence level concentration. Dietary HQs also include APDD concentrations computed using frequency- and mass-based dietary compositions. (**B**) Comparison of tissue-based (egg) least observable adverse effect concentration (LOAEC) and diet-based least observable adverse effect level (LOAEL) Hazard Quotients (HQs) at the Upper Kalamazoo River Superfund Site (Trowbridge) and Reference (Ft. Custer) locations calculated from LOAEC/LOAEL-based TRVs for polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$). Each box encompasses the geometric mean and upper 95% confidence level concentration. Dietary HQs also include APDD concentrations computed using frequency- and mass-based dietary compositions.

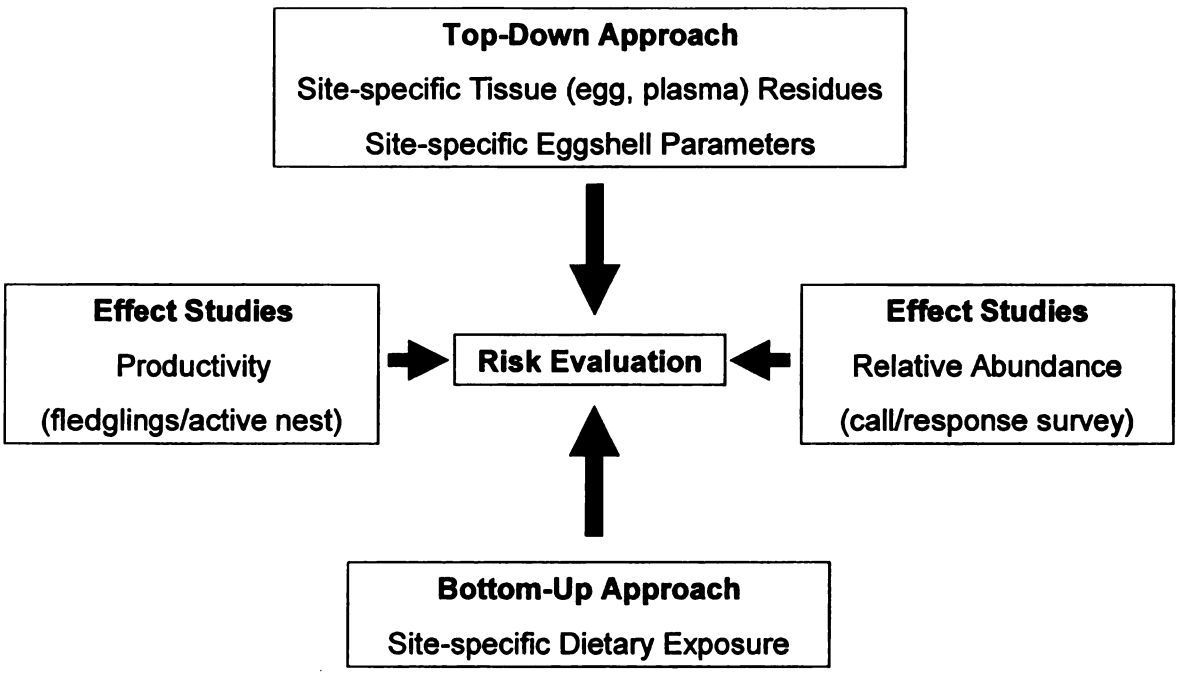


Figure 4.7. Multiple-lines-of-evidence used to assess risk to resident Kalamazoo River Superfund Site great horned owl (*B. virginianus*) populations.

approach to estimate risk to GHOs is used, studies of site-specific diet must be completed to assure that site-specific data can be collected for principal prey items representing potential exposures for both aquatic and terrestrial food webs at any site where aquatic habitats are located in close proximity to resident GHO nesting habitats. Additionally, because budget limitations will constrain the breadth of prey item sampling and analyses at most sites, it is essential that risk assessors clearly communicate all dietary assumptions applied to the data (e.g., prey groupings, gaps in the chemical database for prey comprising a low dietary proportion) and how these assumptions impact risk calculations at the site.

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Chapter 5

Risk Assessment of Bald Eagles (*Haliaeetus leucocephalus*) Exposed to Polychlorinated Biphenyls (PCBs) and DDT along the Kalamazoo River, Michigan.

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ABSTRACT

The bald eagle (*Haliaeetus leucocephalus*) was used in a multiple-lines-of-evidence study of polychlorinated biphenyls (PCBs) and *p,p'*-dichlorodiphenyltrichloroethane (DDT) exposures in the Kalamazoo River Area of Concern (KRAOC). The study examined potentials for effects of total PCBs, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQ_{WHO\text{-}Avian}$), and total DDTs (sum of DDT/DDE/DDD; ΣDDT) by measuring concentrations in nestling plasma and addled eggs in a contaminated region of the KRAOC as well as two reference locations outside of the Kalamazoo River watershed. A chemical-specific egg to plasma relationship was used to convert nestling plasma PCB and ΣDDT concentrations to an egg-basis for the hazard assessment. The assessment compared concentrations of the contaminants of concern (COCs) in eggs and calculated egg-basis samples to toxicity reference values (TRVs). Productivity observations for all three locations were also compared to benchmark values. Egg shell thickness was measured to assess effects of *p,p'*-DDE. PCBs in predicted (egg and calculated egg) egg-basis samples as great as 2.3 $x10^4$ ng PCB g^{-1}, ww at the KRAOC and as great as 9.3 $x10^3$ and 1.7 $x10^3$ ng PCB g^{-1}, ww at the Lake Michigan Tributary "Riverine" reference location and the Inland "Lacustrine" reference location, respectively. Concentrations of $TEQ_{WHO\text{-}Avian}$ calculated from aryl hydrocarbon-active PCB congeners and World Health Organization toxicity equivalency factors, in eagle eggs ranged from 1.1 $x10^3$ pg $TEQ_{WHO\text{-}Avian}$ g^{-1}, ww at the KRAOC to 1.5 $x10^3$ and 1.4 $x10^2$ pg $TEQ_{WHO\text{-}Avian}$ g^{-1} ww at the Riverine and Lacustrine locations, respectively. Concentrations of $TEQ_{WHO\text{-}Avian}$ in blood plasma of juvenile eagles ranged from 3.3 $x10^1$

pg $TEQ_{WHO\text{-}Avian}$ g^{-1}, ww at the KRAOC to 1.8 $x10^1$ and 3 pg $TEQ_{WHO\text{-}Avian}$ g^{-1}, ww at the Riverine and Lacustrine locations, respectively. Total concentrations of DDT in predicted egg-basis samples were as great as 6.3 $x10^3$ ng ΣDDT g^{-1}, ww at the KRAOC and as great as 1.1 $x10^4$ and 3.4 $x10^3$ ng ΣDDT g^{-1}, ww at the Riverine and Lacustrine locations, respectively. Hazard quotients (HQs) based on geometric mean concentrations and the least observable adverse effect concentration (LOAEC) for PCBs were 1.2, 0.5 and 0.1 at the KRAOC, Riverine and Lacustrine sites, respectively. HQs based on the NOAEC (no observable adverse effect concentration) and geometric mean concentrations of PCBs were 7.7, 3.1, and 0.6 at the KRAOC, Riverine and Lacustrine sites, respectively. The HQ values, based on the NOAEC and geometric mean concentration of ΣDDTs in predicted egg-basis samples were 1.0, 4.2 and 0.3 at the KRAOC, Riverine and Lacustrine sites, respectively. Reproductive productivity at the KRAOC site was less than that at the less contaminated reference areas. This observation, coupled with the calculated HQ vales, indicates that the contaminant exposures are likely at the threshold for adverse population-level effects for resident bald eagle populations.

Keywords: Raptors, Bioaccumulation, Aquatic food chain, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents.

INTRODUCTION

Due to the presence of elevated concentrations of polychlorinated biphenyls (PCBs) in fish, sediments and floodplain soils, in August 1990, a portion of the lower Kalamazoo River was placed on the Superfund National Priorities List (BBL, 1993). Polychlorinated biphenyls were used in the production of carbonless copy paper and paper inks for approximately 15 yr (USEPA, 1976). During this period, recycling of paper, including some carbonless copy paper, resulted in releases of PCBs to the Kalamazoo River. The Kalamazoo River Area of Concern (KRAOC) includes 123 km of river extending from the city of Kalamazoo, MI to Lake Michigan at Saugatuck, MI. The primary contaminants of concern (COCs) are PCBs, including total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents [$TEQ_{WHO\text{-}Avian}$] from non-*ortho* (coplanar) and mono-*ortho* PCB congeners. However, other persistent poly-halogenated aromatic hydrocarbons such as *p,p'*-dichlorodiphenyltrichloroethane (DDT) and its metabolites, dichloro-diphenyl-dichloro-ethylene (DDE) and dichloro-diphenyl-dichloro-ethane (DDD) (hereafter, ΣDDT) are also present.

A primary route of COC exposure to environmental receptors at the site is sediment-based and occurs through the aquatic food chain (Kay et al., 2005). The lower 35 km of the Kalamazoo River between the Calkins Dam (the first in-stream dam inland from Lake Michigan) and Kalamazoo Lake at the confluence with Lake Michigan includes greater than 2000 ha of Riparian wetland habitats (Allegan Co., 2000). These varied habitats are largely undisturbed and home to a diverse aquatic community that

includes bald eagles (*Haliaeetus leucocephalus*) and osprey (*Pandion haliaetus*) (CDM, 2003).

The bald eagle was selected as a sentinel species to estimate risk to raptors in the aquatic food chain at the KRAOC. Raptors have long been used as environmental monitors because their position at the top of the food chain increases their potential for exposure to bioaccumulative contaminants. This, combined with the fact that they are susceptible to the toxic effects of some COCs, means that raptors can be used as effective and sensitive biological monitors for contaminant exposures and assessment of environmental effects (CEQ, 1972). Raptors are often used as environmental sentinels for monitoring of contaminants or as primary or surrogate receptor species in environmental risk assessments (IJC, 1991; CDM, 2003). Because bald eagles express the biological, reproductive and ecological characteristics required for suitability as an avian biomonitoring species (Hollamby et al., 2006), they have been selected as a biological indicator of toxic effects of organochlorine compounds on piscivorous wildlife in the Great Lakes region [IJC, 1991], and as a bio-sentinel species for the State of Michigan [MDEQ, 1997].

Studies of exposure of bald eagles to persistent, bioaccumulative contaminants and subsequent reproductive productivity in Michigan and the greater Great Lakes ecosystem have found exposures to PCBs and ΣDDT to exhibit a significant negative correlation with reproductive productivity for nesting territories along the Lake Michigan shoreline compared to birds nesting further inland without access to a Great Lakes forage base (Bowerman et al., 2003). Recent investigations of environmental exposures at the

KRAOC suggested that resident bald eagle productivity had been adversely affected by exposures to PCBs originating from the site (CDM, 2003; Stratus, 2005a).

The five-year study of eagle exposures at the KRAOC was conducted in support of a baseline ecological risk assessment and used multiple lines of evidence to assess the potential effects of PCBs and ΣDDT on resident bald eagle populations (Fairbrother, 2003). The specific objectives of this study were to: measure concentrations of total PCBs, $TEQ_{WHO\text{-}Avian}$, and ΣDDT in blood plasma of bald eagle nestlings and addled eggs; 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 productivity of bald eagles at the KRAOC, relative to reference locations on uncontaminated tributaries to Lake Michigan that included exposures to the Lake Michigan food chain, and a second set of reference locations at inland sites representative of uncontaminated "background" conditions in Michigan's lower peninsula.

MATERIALS AND METHODS

Study sites

Bald eagle study sites within the KRAOC included all of the active breeding territories in the river environs during a five year period from 2000 to 2004. These included two territories in the Allegan State Game Area, including the Swan Creek High Banks Wildlife Refuge and Ottawa Marsh. A third territory is located at Pottawatomi Marsh, downstream of the city of New Richmond. Observations completed during this study

indicated that two mated pairs of eagles choose their nesting sites alternately between these three territories.

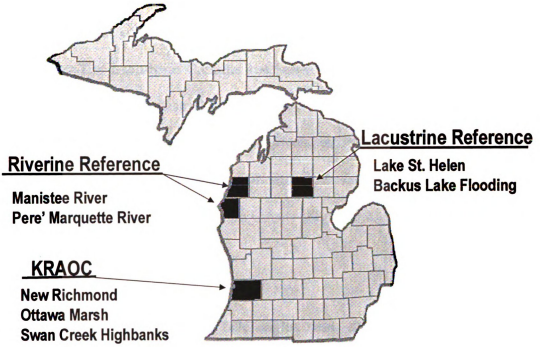
There are no known PCB sources in the lower 35 km of the Kalamazoo River downstream of the Calkins Dam. All KRAOC bald eagle breeding territories were located downstream of the known point sources of PCBs to the river which originally included paper mills and residuals management operations in the river floodplain between the cities of Kalamazoo and Otsego. Studies of PCB flux in the river have also identified three formerly impounded areas at Plainwell, Otsego and Trowbridge, where large masses of PCBs in former river sediments are now exposed as floodplain soils, and function as active sources of PCBs to the river. Additionally, Calkins dam is an active hydroelectric generating facility that impounds Lake Allegan, a 1,550 acre impoundment with approximately 2.0 $x10^4$ kg of PCBs contained in lake-bottom sediments (Stratus, 2005a). Calkins dam is approximately 3 km upstream of the nearest eagle nesting site at Swan Creek High Banks and approximately 25 km upstream of Pottawatomi Marsh, the furthest downstream eagle nesting site.

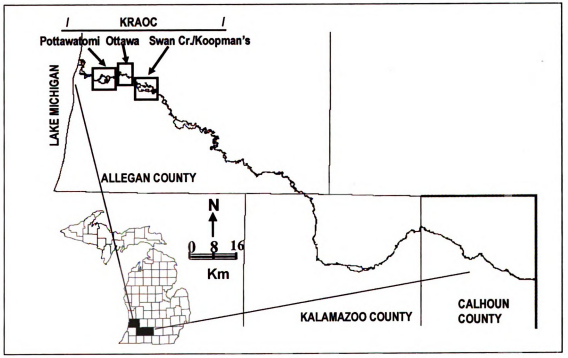
Below Calkins dam and in the immediate vicinity of the KRAOC bald eagle study sites, maximum detected PCB concentrations for in-river sediments range from 30 ng PCBs g^{-1}, dry weight (dw) to 5.9 $x10^3$ ng PCBs g^{-1}, dw and mean surface floodplain soil concentrations in Koopman, Ottawa and Pottawatomi marshes do not exceed 7.7 $x10^2$ ng PCBs g^{-1}, dw and have a maximum concentration of 2.8 $x10^3$ ng PCBs g^{-1}, dw. Upstream of Calkins dam, concentrations of PCBs in Lake Allegan surface sediments (0 to 15 cm) range from 30 ng PCB g^{-1}, dw to 6.4 $x10^4$ ng PCB g^{-1}, dw with a mean concentration of 4.0 $x10^3$ ng PCB g^{-1}, dw. Further upstream in the former Trowbridge impoundment,

mean surface floodplain soil (0-25 cm) concentrations are 1.5 x 10^4 ng PCB g^{-1}, dw (Stratus, 2005a; CDM, 2003; BBL, 1994b; 2000).

There were no eagle breeding territories present in the upper, less contaminated reaches of the Kalamazoo River drainage to use as reference sites for this study, so reference breeding territories were selected in watersheds separate from the Kalamazoo River. An important consideration of the eagle exposure assessment was the potentially confounding influence that utilization of a Lake Michigan forage base contaminated with PCBs and ΣDDT by KRAOC eagles may have on assessments of PCB exposures originating from the Kalamazoo River. To address this potential bias in the hazard assessment, two distinct reference locations were utilized in the bald eagle study. One reference location is a Lake Michigan "Riverine Control" site. Located approximately 160 km to the north of the KRAOC, the Lake Michigan Riverine Control consists of two breeding territories each of which has a pair of eagles nesting on a "clean" tributary to Lake Michigan with no evidence of upstream industrial influences or contamination (Best, 2002) (Figure 5.1). These sites are at the Manistee State Game Area along the Manistee River (Manistee County) and the Pere' Marquette River (Mason County). The Riverine Control sites are similar to the KRAOC eagle sites since they both are located on the Lake Michigan side of the most downstream dam, and allow foraging eagles access to anadromous Great Lakes fish. Additionally the territories are all situated greater than 5 km inland from Lake Michigan in marsh habitats with similar prey species. Taken together, these nesting areas were useful in establishing general background concentrations of PCBs and ΣDDT that are attributable to exposures to Lake Michigan fish and/or piscivorous prey that feed on Lake Michigan fish.

Figure 5.1. Kalamazoo River bald eagle (*H. leucocephalus*) study sites in Michigan including the KRAOC in Allegan County, the Riverine Reference sites in Manistee and Mason Counties and the Lacustrine Reference sites in Roscommon County.


Lacustrine Reference
Lake St. Helen
Backus Lake Flooding
Riverine Reference
Manistee River
Pere' Marquette River
KRAOC
New Richmond
Ottawa Marsh
Swan Creek Highbanks


KRAOC
Pottawatomi
Ottawa
Swan Cr./Koopman's
LAKE MICHIGAN
ALLEGAN COUNTY
N
0 8 16
Km
KALAMAZOO COUNTY
CALHOUN COUNTY

A second reference location included two breeding territories approximately 225 km to the northeast of the KRAOC that served as a Lower Peninsula "Lacustrine Control" site. The Lacustrine Controls each consist of a pair of eagles on a "clean" inland lake isolated from industrial influence and Great Lakes anadromous fish and thus useful in establishing general background levels of PCBs and ΣDDT in relatively undisturbed habitats. Lacustrine Control sites included territories at Backus Lake flooding and Lake St. Helen, Roscommon County (Figure 5.1).

Field sampling

Blood was collected from nestling bald eagles were collected when nestlings were approximately 6 to 9 wk of age. Nestlings were lowered to the ground and a whole blood sample of ~10 ml was withdrawn from the brachial vein with a 22 gauge hypodermic needle/syringe and sterile technique (Mauro, 1987). Blood was transferred to a heparinized Vacutainer™ and labeled and put on ice in an insulated cooler. Vacutainers™ containing whole blood were centrifuged at 1200 rpm for 10 min within 10 hr of field sampling. Plasma (supernatant) was transferred to a new Vacutainer™ appropriately labeled and shipped to the East Lansing field office (ELFO) of the United States Fish and Wildlife Service (USFWS) and stored upright at -20 °C. Sample splits were provided to the Michigan State University Aquatic Toxicology Laboratory (MSU-ATL) where analyses for total PCBs and ΣDDT were completed. Nestlings were banded with USFWS leg bands and total body weight, bill depth, and length of the culman, foot pad , and eighth primary feather measured following standard techniques (Bartolotti, 1984) [data not presented] after which the birds were returned to the nest unharmed.

Addled eggs and egg shells were collected as available when the nestling were banded and at a second post-fledge nest climb. Addled eggs were labeled, and transported back to the USFWS-ELFO 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. Sample splits of egg homogenate were provided to the MSU-ATL where analyses for total PCBs and ΣDDT were completed. All concentrations of residues in eggs were corrected for moisture loss (Stickel et al., 1973). Eggshells from whole eggs were rinsed, air-dried, and eggshell thickness measured (to the nearest 0.01 mm) at 2 to 8 places by use of a Starrett Model 1010M micrometer (L.S. Starrett Co., Athol, MA, USA). Eggshell fragments collected from nests were similarly processed and measured for thickness as the recovered quantity of shells permitted. Because whole-egg volumes were not available for eggshell fragment samples and because only three addled egg samples were recovered during the study, Ratcliffe index values were not used to evaluated the shell thinning effects of ΣDDT.

Measures of productivity (i.e., total number of fledged young per occupied nest) were made by direct observation/visual confirmation of fledgling success in each active breeding territory (Postupalsky, 1974). In instances where visual confirmation could not be obtained, the successful banding of a pre-fledge nestling (age 7 to 10 wks) was logged as a successful fledge (USFWS, 1983).

Predicted egg concentrations

Concentrations of total PCBs and ΣDDT in nestling plasma were used to calculate predicted concentrations in eggs using an egg to blood plasma relationship, or conversion factor, derived for Great Lakes bald eagle populations (Strause et al., 2007a). The "predicted egg-basis" concentrations were combined with available addled egg concentration (combined egg basis) data in assessments of hazard at the KRAOC and reference sites. The combined egg-basis data sets were used with egg-based toxicity reference values (TRVs) derived from population-level benchmark effects to assess the health of eagle populations in each of the three sub-regions, including the KRAOC, Lake Michigan Riverine Control site (Riverine site), and Lower Peninsula Lacustrine Control site (Lacustrine site).

TEQ computation

Concentrations of $TEQ_{WHO\text{-}Avian}$ in eagle 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 World Health Organization (WHO) toxic equivalence factors (Van den Berg et al., 1998). Polychlorinated-dibenzo-p-dioxins and polychlorinated-dibenzo-furans were not measured and were not included in the 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 co-eluting congeners 105, 156, 157 and 167 ranged from 0.7% to 8.6%, 0% to 7.4%, 0% to 2.2%, and 0% to 1.1%, respectively; and among all egg and plasma samples in the study, the sum contributions of these four congeners exceeded 10% of total $TEQ_{WHO\text{-}Avian}$ in only 2 (egg) samples.

Chemical analysis – extraction/cleanup

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 (Neigh et al., 2006). 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/quality control sets composed of similar tissues were included with each group of 20 samples. Concentrations of PCBs, including di- and mono-ortho-substituted congeners were determined by gas chromatography (Perkin Elmer AutoSystem and Hewlett Packard 5890 series II) equipped with a ^{63}Ni electron capture detector (GC-ECD). Concentrations of non-ortho-substituted coplanar PCB congeners and ΣDDT were determined by gas chromatograph mass selective detector (GC-MS) (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 (ww) 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- and mono-ortho-substituted PCB congeners was conservatively estimated (minimum surface to noise ratio of 10.0) to be 1.0 ng PCB g^{-1}, ww, 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 MDL for all samples at < 0.1 ng g^{-1}, ww. Either TurboChrom (Perkin Elmer, Wellesley, MA, USA) 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.

Toxicity reference values

In this study, tissue-based 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 (e.g., population-level) assessment endpoints in the species of concern, or a closely related species (e.g., other raptor species). Chronic studies must

include sensitive life stages to evaluate potential developmental and reproductive effects, and there must be minimal impact from co-contaminants 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 eagles.

Productivity of bald eagles is not easily evaluated in the laboratory and it is difficult to develop unambiguous dose-response relationships for PCBs in bald eagle eggs. Field studies of eagle productivity may be influenced by sample bias (from preponderance of addled egg data) and the confounding effects of exposures to mixtures of environmental contaminants, however the work of Wiemeyer et al., (1993) and Elliott and Harris (2000) provide conservative benchmarks that describe the potential effects of PCBs upon eagle productivity. For this study, TRVs based on the NOAEC and LOAEC were determined to be 3 $x10^3$ and 2.0 $x10^4$ ng PCB g^{-1} egg, ww (Table 5.1). PCB concentrations ranging from 3 $x10^3$ to 5.6 $x10^3$ ng PCBs g^{-1} egg, ww and from 1.3 to 2.3 $x10^4$ ng PCBs g^{-1} egg, ww were associated with a 10% and a 50% decrease in eagle productivity, respectively (Wiemeyer, 1993). A 1999 evaluation of bald eagle nesting success in the vicinity of Green Bay Wisconsin concluded that a threshold of 2.0 $x10^4$ng PCBs g^{-1} egg, ww was associated with significant decreases in the probability of eagle nesting success (Stratus, 1999). In a comprehensive review of chlorinated hydrocarbon effects on bald eagle populations, Elliott and Harris (2002) supported the NOAEC/LOAEC TRVs described above.

Table 5.1. Toxicity reference values (TRVs) for total PCB, TEQ, and total DDT in bald eagle (*H. leucocephalus)* eggs. Reference number is located next to each value.

	Tissue-Based TRV	Response Endpoint[a]
Total PCBs (ng g^{-1}, wet wt)		
NOAEC	3000[b]	FS,P
LOAEC	20000[c,d]	FS,P
Total TEQ (pg g^{-1}, wet wt)		
NOAEC	135[e]	EI,EV
LOAEC	400[e]	EI
Total DDT (ng g^{-1}, wet wt)		
NOAEC	3600[b,d]	EST,FS,P
LOAEC	12000[d,f]	EST,FS,P

a. FS-fledgling success; P-productivity; EI-enzyme induction; EV-egg viability; EST-egg shell thickness.
b. Wiemeyer et al., 1993
c. Stratus Consulting, 1999.
d. Elliott and Harris, 2002.
e. Elliott JE et al., 1996.
f. Nisbet and Risebrough, 1994.

A tissue-based NOAEC for $TEQ_{WHO\text{-}Avian}$ in bald eagle eggs was estimated to be greater than 1.4 $x10^2$ pg $TEQ_{WHO\text{-}Avian}$ g^{-1} egg, ww from the no observable effect concentration observed in bald eagle chicks (presented on an egg-basis) (Elliott et al., 1996). A LOAEC concentration of 4.0 $x10^2$ pg $TEQ_{WHO\text{-}Avian}$ g^{-1} egg, ww, based on CYP1A induction, was also adopted from the lowest observable effect concentration determined in the same study (Elliott et al., 1996) (Table 5.1). It should be noted that no adverse effects on developmental or any other ecologically relevant endpoints were observed at these concentrations. The Elliott et al. study (1996) and two studies on the osprey that yielded very similar toxicity benchmark values for $TEQ_{WHO\text{-}Avian}$ g^{-1} egg (Elliott et al., 2000; Woodford et al., 1998), are the only three dose response studies of acceptable value for deriving avian raptor TEQ TRV values for tissue exposures. Comparable effects studies that use avian plasma as the exposure/dose response medium are not available.

A TRV based on the NOAEC for ΣDDT in bald eagle eggs was estimated to be 3.6 $x10^3$ ng ΣDDT g^{-1} egg, ww and the LOAEC was estimated to be 1.2 $x10^4$ ng ΣDDT g^{-1} egg, ww (Table 5.1). The selection of the NOAEC value as a conservative estimate of the TRV is supported by the analyses of effects presented by Wiemeyer et al., (1993) where this concentration was identified as the "benchmark" p,p-DDE concentration for normal bald eagle productivity (1.0 fledge/active territory). The LOAEC concentration of 1.2 $x10^4$ ng ΣDDT g^{-1} egg, ww was selected from the study of effects of *p,p*-DDE exposure on productivity in western bald eagle populations (Nisbet and Risebrough, 1994). These selections are also identified by Elliott and Harris (2002) who suggested

adoption of TRV values within the range of 3.6 to 12 $x10^3$ ng *p,p*-DDE g^{-1} egg, ww as threshold effects levels for bald eagle productivity.

Hazard assessment

Potential hazards were assessed using hazard quotients (HQs) that were calculated by dividing concentrations of PCBs, and ΣDDT measured in a predicted egg-basis sample (e.g., direct egg measurements and predicted "egg-basis" concentrations derived from nestling plasma) by the tissue-based (egg) NOAEC and LOAEC TRVs identified for these COCs (Table 5.1). Hazard quotients for total $TEQ_{WHO\text{-}Avian}$ were calculated in the same manner for measured egg concentrations only.

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 (Giesy et al., 1994a). Hazard quotients were calculated by dividing concentrations of each COC in egg (using both the lower and upper 95% CI of the geometric mean) by the egg-based TRV. The shell thinning effects of *p,p'*-DDE were evaluated by comparing current measurements of eggshell thickness to pre-1947 benchmark values reported for bald eagles (Anderson and Hickey, 1972).

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 $TEQ_{WHO\text{-}Avian}$ in plasma and shell thickness) were analyzed using parametric methods, including one-factor analysis of variance (ANOVA) with Tukey's HSD (multiple comparisons) and T-test for simple pair-wise comparisons. When parameters did not satisfy either or both assumptions of normality and homogeneity (log-transformed values for PCBs and ΣDDT in plasma and "egg-basis" data sets) non-parametric statistical methods were used, including Kruskel-Wallace ANOVA and Median Test (multiple comparisons) and the Mann-Whitney U test. Associations between parameters were made with Pearson Product Correlations. Tests for normality, homogeneity of variance and treatment effects (spatial trends) were completed using the Statistica (Version 6.1) statistical package (Statsoft, Tulsa, OK.). The criterion for significance used in all tests was $p<0.05$.

In instances where multiple egg samples from the same clutch were obtained within any single sampling year, the multiple samples were averaged together to yield a single data point prior to statistical testing (experimental unit = nest). This practice reflects the integrative nature of adult exposures expressed through egg contaminant levels. Alternatively, when multiple nestlings were sampled from the same nest, analytical results for each nestling plasma sample were converted to an individual "egg-basis" concentration (plasma to egg conversion factor) for statistical testing (experimental unit = nestling). This approach recognized that nesting exposures are not integrated through mechanisms of adult foraging behavior, contaminant metabolism and tissue

distribution, and that exposure of nestlings through diet reflects the varied age, prey consumption rates and metabolic development unique to each individual nestling. This approach is also consistent with efforts to develop regional population-level assessment endpoints that examine reproductive success (i.e., productivity) using relationships developed between the number of fledged young/occupied nest (regional statistic) and the concentrations of organochlorine contaminants in plasma of individual eagle nestlings (Bowerman et al., 2003).

RESULTS

Sampling success

Between 2000 and 2004, total PCB and ΣDDT concentrations were measured in a total of 2 egg and 19 nestling blood plasma samples that were collected from 31 active bald eagle nests. An additional egg sample obtained from the USFSW-ELFO egg sample archive also was analyzed for PCBs and ΣDDT. Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were calculated for 3 egg and 14 nestling blood plasma samples. Coplanar congener results for five plasma samples were removed from the data set because the analyses did not meet the strict quality assurance/quality control standards in place for this study. Eggshell thickness measurements were completed for three intact eggs and 12 eggshell fragment samples. Productivity measures (number of successful fledglings/active nesting territory) were based on observations of 30 active nests (one nest outcome undeterminable) that produced a total of 23 fledglings.

Total PCB concentrations

Geometric mean PCB concentrations in bald eagle nestling plasma samples were the greatest observed in the blood of any KRAOC birds, and the least PCB concentrations were measured in plasma samples from the Lacustrine Control site (Table 5.2). Identical spatial trends are displayed for PCBs concentrations in the single egg samples analyzed for each of the three study areas and for the combined egg and converted-plasma geometric mean predicted "egg-basis" concentrations (Figure 5.2). The formula describing the egg-to-plasma relationship (conversion factor equation) for PCBs in Great Lakes bald eagles is expressed on a log-basis as "log PCB_{egg} (ug g^{-1}) = 0.905[log PCB_{plasma} (ng ml^{-1})] -1.193" (R^2=0.623, p<0.001) (Strause et al., 2007). Geometric mean predicted "egg-basis" total PCB concentrations at the KRAOC and Riverine Control site differed by a factor of 2.5, but the margin was not statistically significant (Kruskel-Wallace, p>0.5). Geometric mean predicted "egg-basis" concentrations at the KRAOC and Riverine Control were each significantly greater than concentrations at the Lacustrine Control (Kruskel-Wallace, p<.0.001 KRAOC; p<0.02 Riverine).

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

Dioxin equivalent concentrations ($TEQ_{WHO\text{-}Avian}$) were calculated for both egg and plasma samples. Because congener specific conversion factors are not available to calculate an predicted "egg-basis" concentration from plasma, $TEQ_{WHO\text{-}Avian}$ concentrations in eagle tissues are presented separately for egg and plasma samples. Concentrations of $TEQ_{WHO\text{-}Avian}$ in bald eagle nestling plasma analyzed across all three sampling locations were significantly correlated with concentrations of total PCBs in plasma (r =0.868. p<0.05).

Table 5.2. Geometric mean, ww (range), total PCBs in bald eagle (*H. leucocephalus)* egg and plasma samples from the Kalamazoo River Area of Concern (KRAOC), Riverine and Lacustrine sites.

Study Site	Total PCBs (egg) ng PCBs g^{-1} egg	N	Total PCBs (plasma) ng PCBs ml^{-1} plasma (range)	N	Combined Total PCBs[a] (egg-basis) ng PCBs g^{-1} egg (range)	N
KRAOC[b]	44302	1	524 (339 – 773)	3	23055 (12498 – 44302)	4
Reference						
Riverine[c]	23943	1	212 (152 – 291)	7	9348 (6047 – 23943)	8
Lacustrine[d]	5516	1[e]	38 (8.8 – 114)	9	1735 (459 – 4661)	9[f]

a. PCB concentrations in nestling plasma samples converted to an "egg basis" using the Great Lakes bald eagle conversion factor: ($\log_{10}$ µg PCB_{egg} g^{-1}, ww) = (0.905[$\log_{10}$ ng PCB_{plasma} ml^{-1}]-1.193). PCB concentrations include both addled eggs and converted nestling plasma samples.
b. KRAOC sample site includes samples from the Ottawa Marsh and New Richmond bald eagle breeding areas.
c. Riverine sample site includes samples from the Manistee River SGA, Manistee River Airport and Pere' Marquette River bald eagle breeding areas.
d. Lacustrine sample site includes samples from the Lake St. Helen East and Backus Lake Flooding bald eagle breeding areas.
e. Lacustrine egg sample from a Lake St. Helen East addled egg collected in 1994.
f. Egg sample from 1994 is not included in combined egg-basis geometric mean concentration for the Lacustrine reference location.

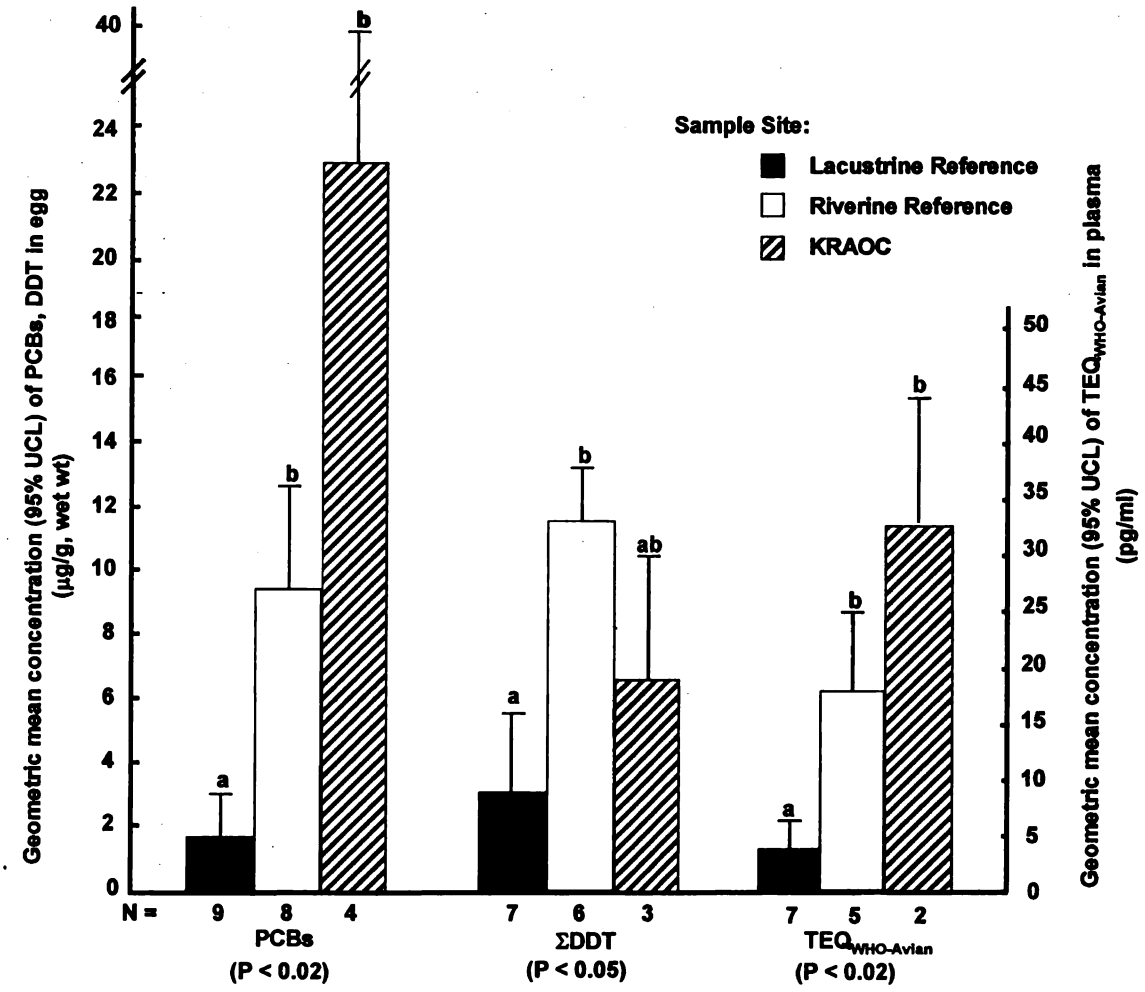


Figure 5.2. Geometric mean (ww) total polychlorinated biphenyls (PCBs) and ΣDDT concentrations in bald eagle (*H. leucocephalus)* eggs (combined addled egg and predicted egg-basis from nestling plasma) and $TEQ_{WHO\text{-}Avian}$ in nestling plasma from the Lacustrine and Riverine Reference sites and the KRAOC, error bars show the 95%UCL.

Concentrations of $TEQ_{WHO\text{-}Avian}$ in plasma displayed spatial trends that were identical to trends for total PCBs in plasma. KRAOC birds had the greatest concentrations of $TEQ_{WHO\text{-}Avian}$ and the least concentrations were in Lacustrine Control birds (Table 5.3). Statistical testing of spatial trends for $TEQ_{WHO\text{-}Avian}$ concentrations in plasma matched results for total PCBs in predicted "egg-basis" samples described above. Geometric mean concentrations at the KRAOC and Riverine Control site differed by a factor of 1.8, but the margin was not statistically significant (ANOVA w/Tukey's, p=0.75), and mean concentrations at the KRAOC and Riverine Control were each significantly greater than concentrations at the Lacustrine Control (ANOVA w/Tukey's, p<0.02 KRAOC and Riverine). The distribution of $TEQ_{WHO\text{-}Avian}$ concentrations in the three addled egg samples available for analyses differed from plasma $TEQ_{WHO\text{-}Avian}$ concentrations in that the Riverine Control egg (Pere' Marquette) had a greater $TEQ_{WHO\text{-}Avian}$ concentration than the KRAOC egg (Ottawa Marsh) (Table 5.4). Egg $TEQ_{WHO\text{-}Avian}$ concentrations at both sites were an order of magnitude greater than the concentration of the Lacustrine Control egg (Lake St. Helen). All four non-*ortho*-substituted PCBs or coplanar PCBs (IUPAC 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 and plasma samples from the KRAOC and Riverine study sites and the egg sample from the Lacustrine site. Lacustrine plasma samples displayed very low concentrations of both coplanar and mono-*ortho*-substituted PCB congeners. All coplanar PCB congeners were below the method detection limit in two of seven Lacustrine plasma samples. Coplanar PCB 77 was detected in five of seven plasma samples and in three of these samples it was the only non-*ortho*-substituted congener quantified above the method detection limit.

Table 5.3. Geometric mean, ww (range), total PCBs, $TEQ_{WHO\text{-}Avian}$ and relative potency concentrations in bald eagle (*H. leucocephalus)* plasma samples from the Kalamazoo River Area of Concern (KRAOC), Riverine, and Lacustrine sites.

Study Site	Total PCBs (plasma) μg PCBs ml^{-1} plasma (range)	N	$TEQ_{WHO\text{-}Avian}$ (plasma) pg TEQ ml^{-1} plasma (range)	N	Relative Potency[a] (plasma-basis) μg g^{-1} (range)	N
KRAOC[b]	0.43 (0.34 – 0.55)	2	33 (28 – 38)	2	75.5 (69.1 – 82.6)	2
Reference						
Riverine[c]	9.21 (0.15 – 0.25)	5	18 (13 – 32)	5	87.4 (62.2 – 127)	5
Lacustrine[d]	0.04 (0.01 – 0.11)	7	3 (0.3 – 10)	7	70.7 (34.1 – 189)	7

a. Relative potency = (pg $TEQ_{WHO\text{-}Avian}$ ml^{-1} plasma) / (μg total PCBs ml^{-1} plasma).
b. KRAOC sample site includes samples from the Pottawatomi Marsh bald eagle breeding area.
c. Riverine sample site includes samples from the Manistee River SGA, Manistee River Airport and Pere' Marquette River bald eagle breeding areas.
d. Lacustrine sample site includes samples from the Lake St. Helen East and Backus Lake Flooding bald eagle breeding areas.

Table 5.4. Geometric mean, ww (range), total PCBs, $TEQ_{WHO\text{-}Avian}$ and relative potency concentrations in bald eagle (*H. leucocephalus)* egg samples from the Kalamazoo River Area of Concern (KRAOC), Riverine, and Lacustrine sites.

Study Site	Total PCBs (eggs) $\mu g\ PCBs\ g^{-1}\ egg$	N	$TEQ_{WHO\text{-}Avian}$ (egg) $pg\ TEQ\ g^{-1}\ egg$	N	Relative Potency[a] (egg-basis) $\mu g\ g^{-1}$	N
KRAOC[b]	44.3	1	1139	1	25.7	1
Reference						
Riverine[c]	23.9	1	1474	1	61.6	1
Lacustrine[d]	5.52	1	135	1	24.5	1

a. Relative potency = (pg $TEQ_{WHO\text{-}Avian}\ g^{-1}$ egg) / (µg total PCBs g^{-1} egg).
b. KRAOC sample site includes one addled egg sample from the Ottawa Marsh bald eagle breeding area.
c. Riverine sample site includes one addled egg sample from the Manistee River Airport breeding area.
d. Lacustrine sample site includes one addled egg sample collected in 1994 from the Lake St. Helen East bald eagle breeding area.

Two remaining plasma samples included PCB 77 and 126 present at levels above the method detection limit. Just four of the eight mono-*ortho*-substituted PCB congeners (PCBs 105,118,156,167) were regularly detected in Lacustrine plasma samples, PCB 157 was not present. Mono-*ortho*-substituted congeners 114, 123, and 189 also were not detected in any of the plasma samples from the three study sites.

The relative proportion of $TEQ_{WHO\text{-}Avian}$ contributed by the three coplanar PCBs having the greatest $TEF_{WHO\text{-}Avian}$ values relative to other congeners was seen to vary between egg and plasma samples. For egg samples, PCB congener 126 contributed the greatest proportion toward total $TEQ_{WHO\text{-}Avian}$, ranging from 45.7% at the Lacustrine site to 69.9% at the Riverine site (Figure 5.3). In plasma samples, PCB congener 77 contributed the greatest proportion toward total $TEQ_{WHO\text{-}Avian}$, ranging from 47% at the Riverine site to 80.9% at the KRAOC. Together, the relative proportion of $TEQ_{WHO\text{-}Avian}$ contributed by the four coplanar PCBs did not vary greatly between the three sampling sites or between egg and plasma samples, plasma concentrations had slightly greater proportional values, ranging from 90% (Riverine) to 95.2% (KRAOC). Coplanar PCBs accounted for 85.4% (Lacustrine) to 90% (Riverine) of total $TEQ_{WHO\text{-}Avian}$ in egg samples (Figure 5.3).

Relative potency

The relative contributions of non-*ortho*- and mono-*ortho*-substituted congeners can be evaluated by standardizing the $TEQ_{WHO\text{-}Avian}$ to the total PCB

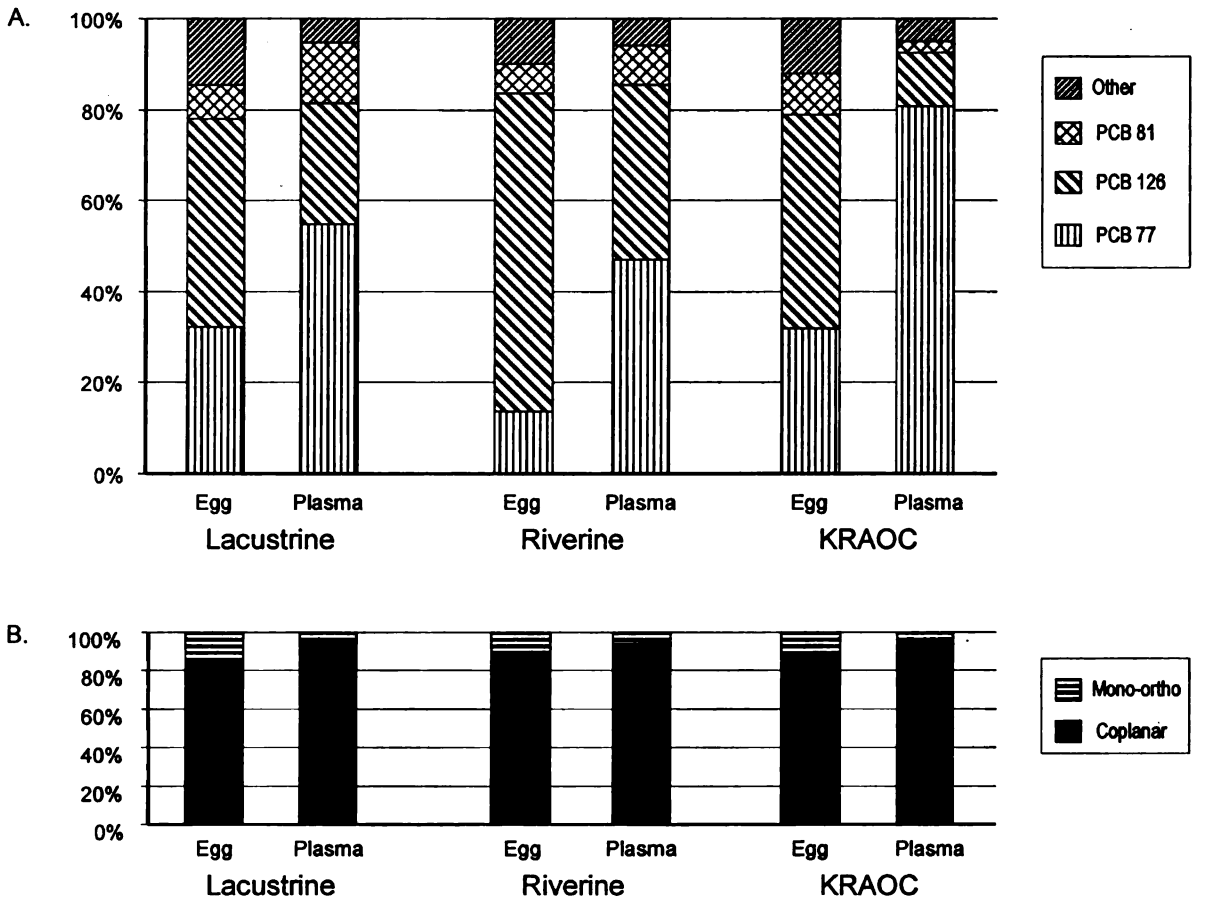


Figure 5.3. Percent contribution of polychlorinated biphenyl (PCB) coplanar and mono-*ortho*-substituted congeners to total $TEQ_{WHO\text{-}Avian}$ in bald eagle egg and plasma samples at the Lacustrine, Riverine and Kalamazoo River Area of Concern (KRAOC) sites. Contribution of individual coplanar congeners to total TEQ and total coplanar verses total mono-*ortho*-substituted coplanar contribution to TEQ.

concentration to obtain a relative potency value (Froese et al., 1998). Relative potency values can be used to assess the degree of weathering and in evaluations of 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 bald eagle plasma are similar among the KRAOC and reference sites. The greatest geometric mean concentration, (8.74×10^{1} μg g^{-1}, ww) was observed for plasma samples collected at the Riverine site (Table 5.3). Relative potency values for the three addled egg samples available for the study sites were lower than plasma at each site, with the maximum concentration in eggs (6.16×10^{1} μg g^{-1}, ww) also observed at the Riverine site (Table 5.4).

ΣDDT concentrations/Eggshell measurements

Total DDT was detected in all egg and plasma samples analyzed. p,p'-dichloro-diphenyl-dichloro-ethylene occurred at the greatest concentration of the measured DDT analytes and contributed from 92 to 99% and 85 to 100% of the ΣDDT in all egg and plasma samples, respectively. Total DDT concentrations in eggs were greater from the Riverine site compared to samples from the KRAOC and Lacustrine site (Figure 5.2). The formula describing the egg-to-plasma relationship (conversion factor equation) for p,p'-DDE in Great Lakes bald eagles is expressed on a log-basis as "log *p,p'*-DDE_{egg} (ug g^{-1}) = 0.676[log *p,p'*-DDE_{plasma} (ng ml^{-1})] -0.578" (R^2=0.324, p<0.001) (Strause et al, 2007a). Geometric mean predicted "egg-basis" total ΣDDT concentrations at the Riverine site and the KRAOC differed by a factor of two, but the margin was not statistically significantly (Kruskel-Wallace, p>0.5). Geometric mean predicted "egg-basis" concentrations at the

Riverine site were significantly greater than concentrations at the Lacustrine site (Kruskel-Wallace, $p<0.05$). Predicted egg-basis ΣDDT concentrations in KRAOC birds were not significantly greater than the Lacustrine site (Kruskel-Wallace, $p>0.5$) (Figure 5.2). Egg shell thickness measurements from addled eggs and shell fragments recovered from active nests displayed a significant negative correlation with ΣDDT concentrations in the combined egg-basis data set ($r= -0.65$, $p<0.05$), and spatial trends for geometric mean egg shell thickness measurements (mm) were inverse to trends observed for geometric mean predicted "egg-basis" ΣDDT concentrations. Shell thickness was significantly less at the Riverine site compared to the Lacustrine site (ANOVA w/Tukeys, $p<0.01$), and shell thickness at the KRAOC was not significantly less then thickness at the Riverine Site, or significantly greater than thickness at the Lacustrine site (ANOVA w/Tukeys, $p=0.17$, Riverine; $p=0.37$, Lacustrine) (Figure 5.4).

Productivity

From 2000 to 2004, a large difference in productivity (fledglings/active nest) was observed between the KRAOC and the two reference sites. During the five reproductive seasons, there were 10 nesting attempts (active nests) in the KRAOC, and 11 and 10 nesting attempts at the Riverine and Lacustrine sites, respectively. The three KRAOC territories included in the study had greater frequencies of inactive breeding territories (5 inactive territories/15 possible) and failed nesting attempts (7 failures/10 possible) compared to the Riverine (4 inactive territories/15 possible; 4 failures/10 possible + one unknown outcome) and Lacustrine sites (0 inactive territories/10 possible; 3 failures/10

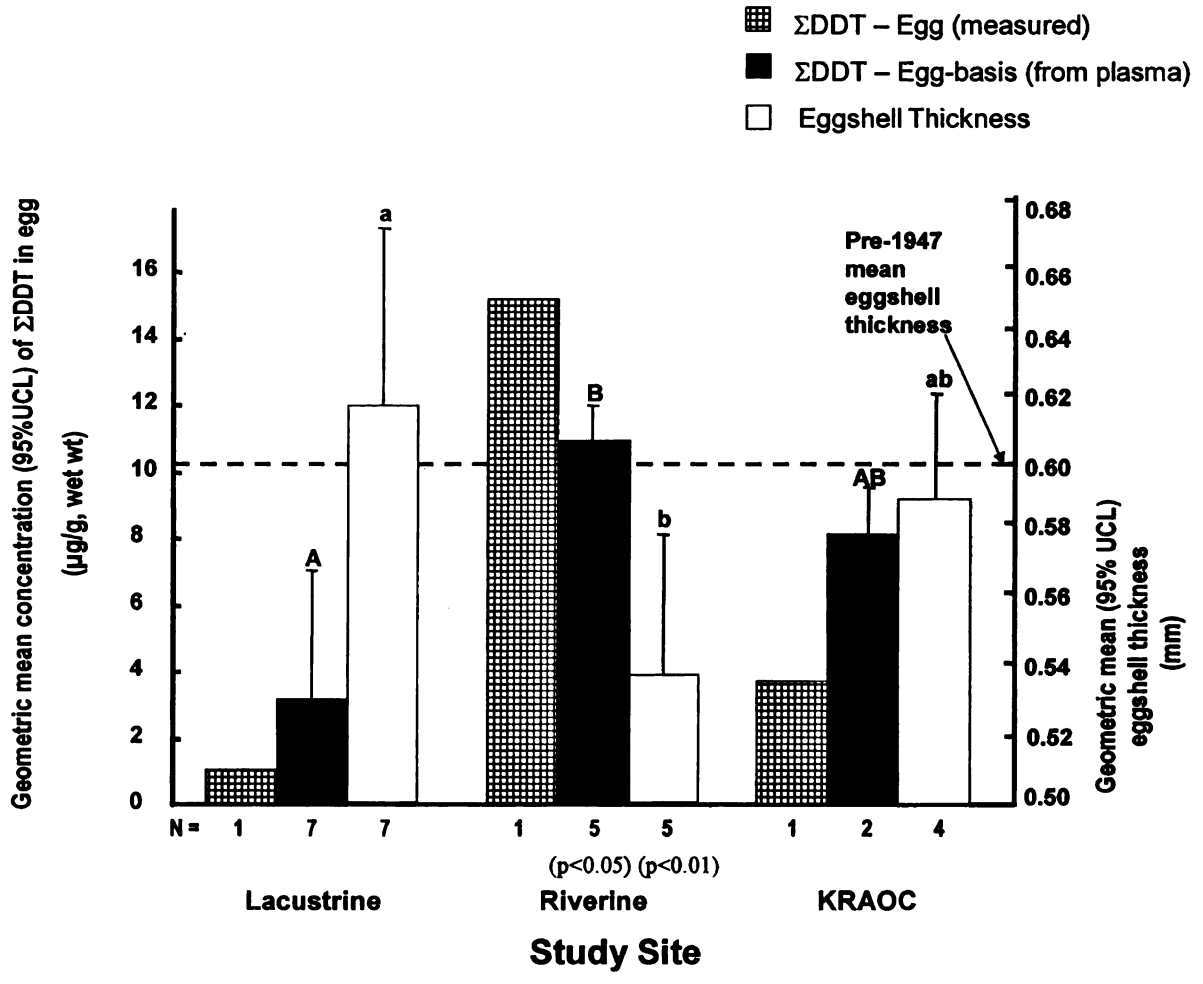


Figure 5.4. Geometric mean (ww) ΣDDT concentrations in bald eagle (*H. leucocephalus)* eggs and the predicted egg-basis (from plasma) sample compared to egg shell thickness at the Lacustrine, Riverine and Kalamazoo River Area of Concern (KRAOC) sites, error bars show the 95%UCL.

possible). Four fledglings were produced at the KRAOC for an annual productivity rate of 0.4 fledglings per active nest. At the Riverine and Lacustrine sites, successful fledglings totaled 9 birds and 10 birds for annual productivity rates of 0.9 and 1.0, respectively (Table 5.5).

Risk assessment

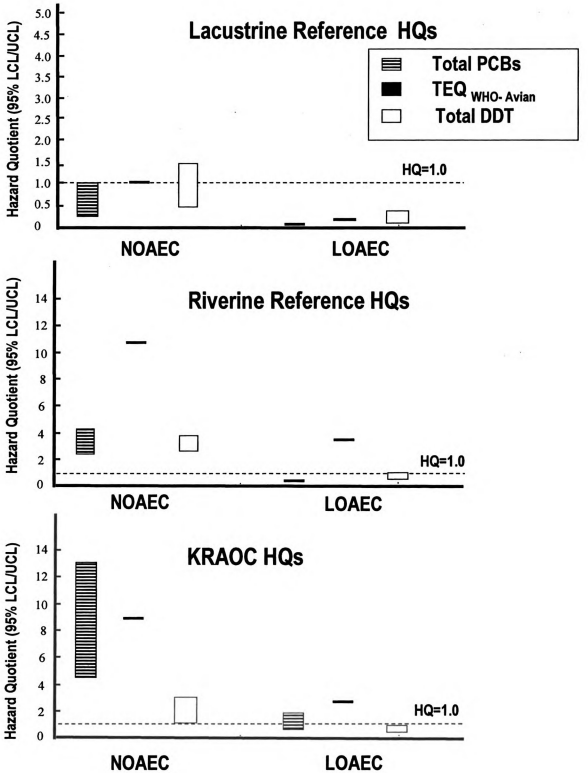
Hazard quotient (HQ) values based on the geometric mean PCB concentration for the KRAOC combined egg-basis data set ranged from 1.2 for the LOAEC TRV to 7.7 for the NOAEC TRV. On an individual sample basis, two of four (50%) KRAOC egg-basis samples exceeded the LOAEC TRV and four of four (100%) samples exceeded the NOAEC TRV. Hazard quotients for the 95% confidence interval (95%CI) on the geometric mean concentration ranged from 0.7 – 1.9 (LOAEC) and 4.6 – 13.0 (NOAEC) (Figure 5.5). At the Riverine site, HQ values for the geometric mean PCB concentration in eggs ranged from 0.5 (HQ_{LOAEC}) to 3.1 (HQ_{NOAEC}), with one of eight (12.5%) and eight of eight (100%) samples exceeding the LOAEC and NOAEC TRVs, respectively. Hazard quotients for the 95%CI on the geometric mean concentration were <1.0 for the LOAEC and ranged from 2.3 – 4.2 for the NOAEC. At the Lacustrine site, HQ values for the geometric mean PCB concentration in eggs ranged from 0.1 (HQ_{LOAEC}) to 0.6 (HQ_{NOAEC}), with zero and three of nine (33%) samples exceeding the LOAEC and NOAEC TRVs, respectively. Hazard quotients for the 95%CI on the geometric mean concentration were $\leq$1.0 for both the LOAEC and NOAEC.

Table 5.5. Kalamazoo river study - bald eagle (*H. leucocephalus*) productivity within the KRAOC, Lacustrine and Riverine reference locations.

Study Location	Annual Nest Site/Fledgling Success[a]					
	2000	2001	2002	2003	2004	Total Productivity[b]
KRAOC						**0.4**
Swan Creek Highbanks (AN-02)[c]	d/F	b/F	e/F	b/F	f/F	
Ottawa Marsh (AN-03)	c/1	c/F	c/F	I	I	
Pottawatomi Marsh (AN-04)	I	I	I	b/1	b/2	
Riverine Reference						**0.9**
Manistee River SGA (MN-05)	d/2	d/2	I	f/F	f/U	
Manistee River Airport (MN-11)	I	I	a/2	a/F	a/1	
Pere' Marquette River (MS-04)	a/1	a/1	a/F	I	d/F	
Lacustrine Reference						**1.0**
Backus Lake (RO-04)	b/1	b/F	b/2	b/F	b/2	
Lake Saint Helen E. (R0-01)	j/2	l/F	m/2	j/F	j/1	

a. United States Fish and Wildlife Service (USFWS) bald eagle breeding area nest site descriptors/number of successful fledglings per nest: F=nest failure, no successful fledglings; I=nest site inactive (annual activity not included in calculations of total productivity); U=fledgling success unknown (annual activity not included in calculations of total productivity).
b. Total productivity=total number of fledglings (all years, all occupied nests)/total number of occupied nests, all years)(Postupalsky, 1974).
c. USFWS bald eagle breeding area designator.

Figure 5.5. Hazard quotients (HQ) for the effects of total polychlorinated biphenyls (PCBs) and ΣDDT (combined addled egg and predicted egg-basis sample) and $TEQ_{WHO\text{-}Avian}$ (egg samples only) for bald eagles *(H. leucocephalus)* based on the no observable adverse effect concentration (NOAEC) and the lowest observable adverse effect concentrations (LOAEC). Each box encompasses the 95% CI about the geometric mean concentration.


Lacustrine Reference HQs
Total PCBs
TEQ WHO- Avian
Total DDT
Hazard Quotient (95% LCL/UCL)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
HQ=1.0
NOAEC
LOAEC
Riverine Reference HQs
Hazard Quotient (95% LCL/UCL)
14
12
10
8
6
4
2
0
HQ=1.0
NOAEC
LOAEC
KRAOC HQs
Hazard Quotient (95% LCL/UCL)
14
12
10
8
6
4
2
0
HQ=1.0
NOAEC
LOAEC

Hazard quotient values for $TEQ_{WHO\text{-}Avian}$ concentrations in eagle tissues were calculated for measured egg exposures only (sample size = 1 at each study site), because coplanar and mono-*ortho*-substituted congener-specific conversion factors are not available to calculate an egg-basis concentration from plasma measurements, and as noted earlier, there are no TRVs available for plasma $TEQ_{WHO\text{-}Avian}$ exposures/effects. At the KRAOC, HQ values for the Ottawa Marsh addled egg ranged from 2.9 (HQ_{LOAEC}) to 8.4 (HQ_{NOAEC}). The Riverine site egg from Pere' Marquette had HQ values ranging from 3.7 (HQ_{LOAEC}) to 10.9 (HQ_{NOAEC}), and the Lacustrine site egg (Lake St. Helen–collected in 1994) had HQ values ranging from 0.3 (HQ_{LOAEC}) to 1.0 (HQ_{NOAEC}).

Hazard quotient values based on the geometric mean ΣDDT concentrations for the KRAOC combined egg-basis data set ranged from 0.5 for the LOAEC TRV to 1.8 for the NOAEC TRV. On an individual sample basis, zero samples exceeded the LOAEC TRV and three of three (100%) samples exceeded the NOAEC TRV. Hazard quotients for the 95%CI on the geometric mean concentration were <1.0 for the LOAEC and ranged from 1.1 – 2.9 for the NOAEC (Figure 5.5). At the Riverine site, HQ values for the geometric mean ΣDDT concentration in eggs ranged from 1.0 (HQ_{LOAEC}) to 3.2 (HQ_{NOAEC}), with three of six (50%) and six of six (100%) samples exceeding the LOAEC and NOAEC TRVs, respectively. Hazard quotients for the 95%CI on the geometric mean concentration ranged from 0.8 – 1.1 (LOAEC) and 2.8 – 3.7 (NOAEC). At the Lacustrine site, HQ values for the geometric mean ΣDDT concentration in eggs ranged from 0.3 (HQ_{LOAEC}) to 0.9 (HQ_{NOAEC}), with one of seven (14%) and three of seven (43%) samples exceeding the LOAEC and NOAEC TRVs, respectively. Hazard quotients for

the 95%CI on the geometric mean concentration were <1.0 for the LOAEC and ranged from 0.5 – 1.8 for the NOAEC.

Geometric mean egg shell thickness at the KRAOC site was 4% less than the pre-1947 benchmark for bald eagles and was not significantly different than mean thickness measurements recorded for the Lacustrine (ANOVA w/Tukeys, p=0.37) and Riverine (ANOVA w/Tukeys, p=0.17) sites. Mean egg shell thickness at the Riverine site was 11.8% less than the pre-1947 benchmark and was significantly less (ANOVA w/Tukeys, p<0.01) than mean thickness measurements at the Lacustrine site (+1.3% pre-1947 benchmark) (Table 5.6).

Table 5.6. Geometric mean, wet wt (range) of ΣDDT concentrations in eggs, egg-basis samples, eggshell thickness and percent (%) departure from pre-1946 eggshell thickness values for bald eagle (*H.leucocephalus)* eggs from the Kalamazoo River Area of Concern (KRAOC) and Riverine, Lacustrine sites.

Study Site	ΣDDT[a] Egg ng ΣDDT g^{-1} egg	N	ΣDDT[b] Egg-basis ng ΣDDT g^{-1} egg	N	Eggshell Thickness[c] mm	N	Percent Departure From Pre-1947 Thickness[d] %
KRAOC[e]	3751	1	6310	3	0.584	4	-4.3
			(3751 – 8809)		(0.516 – 0.619)		(-15.4 – +1.5)
Riverine[f]	15155	1	11415	6	0.538	5	-11.8
			(9559 – 15155)		(0.502 – 0.575)		(-17.7 – -5.7)
Lacustrine[g]	1098[h]	1	3368	7	0.618	7	+1.3
			(1167 – 12630)		(0.566 – 0.673)		(-7.2 – +10.3)

Table 5.6 (Cont.d)

a. ΣDDT concentrations include DDT and metabolites DDE and DDD.
b. Combined egg-basis sample, includes measured egg and predicted egg (from plasma) concentrations.
c. Combined shell thickness measurements for addled egg and egg shell fragment samples.
d. Using a pre-1947 baseline egg thickness of 0.610mm (Anderson and Hickey, 1972).
e. KRAOC sample sites include the Ottawa Marsh, Swan Creek and New Richmond bald eagle breeding areas.
f. Riverine sample sites include the Manistee River SGA, Manistee River Airport and Pere' Marquette River bald eagle breeding areas.
g. Lacustrine sample sites include the Lake St. Helen East and Backus Lake Flooding bald eagle breeding areas.
h. Total DDT concentration from a Lake St. Helen East addled egg collected in 1994.

DISCUSSION

Organochlorine exposures in Great Lakes and KRAOC bald eagles

Great Lakes exposures

Studies of bald eagle productivity and eagle exposures to environmental contaminants in the Great Lakes ecosystem during the 1970s linked observations of reduced productivity with elevated concentrations of organochlorine contaminants in eagle eggs (Wiemeyer et al., 1984). On the basis of productivity benchmarks that identified the average number of successful fledglings produced for each active nest as a measure of population health, investigators frequently observed sub-populations of resident Great Lakes eagles with fledgling production rates below 0.7 fledglings per active nest, a rate insufficient for population maintenance. Fledgling productivity rates of 0.7 to 1.0 fledgling/nest, and >1.0 fledgling/nest are associated with stable and healthy eagle populations, respectively (Sprunt et al., 1973; Wiemeyer et al., 1984). Concurrent environmental investigations of contaminant exposure in bald eagles recorded elevated concentrations of *p,p*-DDE (up to 3.0 x10^4 ng g^{-1}, ww) and total PCB Aroclors (as great as 9.8 x 10^4 ng g^{-1}, ww) in eggs from active eagle nests in Michigan, Wisconsin and Ohio, with decreases in egg shell thickness of 16% compared to the pre-1947 benchmark (Wiemeyer et al., 1984). These observations provided for a weight-of-evidence consensus that environmental exposure from DDT use (e.g., manifested as *p,p'*-DDE-induced reproductive failure) and perhaps PCBs were the primary causes of impaired reproduction in Great Lakes eagle populations. Restrictions on the manufacture and use of DDT mitigated the most severe effects of *p,p'*-DDE exposures on eagle reproductive success, and as *p,p'*-DDE

concentrations in eggs declined during the 1980s and early 1990s, most regional eagle populations in the Great Lakes basin recovered to produce fledglings at rates that provided for population growth (e.g., >1.0 fledgling/active nest) (Bowerman et al., 1998). Eagle monitoring activities over the last two decades verified that the Great Lakes population is growing, but productivity monitoring and environmental sampling indicated that eagles occupying territories along some Great Lakes shorelines (including Lake Michigan) were still exhibiting depressed productivity caused by exposures to *p,p'*-DDE and PCBs. (Bowerman et al., 2003). For Lake Michigan in particular, current and uncontrolled sources of PCBs are the suggested cause of localized impacts to resident environmental communities (Stratus, 1999; 2005a), and studies of the aquatic forage base indicate that aquatic biota continue to accumulate and translocate sufficient mass of both PCBs and ΣDDT to create hazards to top consumers in the aquatic food chain including bald eagles that occupy territories along the Lake Michigan shoreline and further inland along tributaries accessible to Great Lakes anadromous fishes (USEPA, 2004; Giesy et al., 1994b).

KRAOC exposures

Concentrations of total PCBs and ΣDDT/*p,p'*-DDE in eagle eggs and nestling plasma samples at the KRAOC and the Lake Michigan Riverine site fall within the range of concentrations observed in eggs and plasma collected from these same locations by previous researchers during the 1990s (Bowerman, 1993; Best, 2002). KRAOC study contaminant concentrations in eggs and plasma also are comparable to observed PCB and ΣDDT tissue burdens in eagle eggs and plasma collected from locations on the Pacific

Coast of North America with known sources of PCBs and DDT that originated from paper making and product manufacturing activities (Elliott and Harris, 2002). An assessment of contaminant trends in the Great Lakes basin showed no trends in changes in the concentrations of *p,p'*-DDE or total PCBs in un-hatched eagle eggs through the mid-1990s (Bowerman et al., 1998), but more recent nestling plasma data indicated that between 1993 and 1999-2002, total PCB concentrations in plasma samples collected from inland nests have decreased by an order of magnitude and at Lake Michigan nests PCB concentrations have decreased by over 50% (Roe et al, 2004b).

Total PCB and ΣDDT trend monitoring data for the three collection sites included in the KRAOC study are available for addled eggs (Best, 2002), nestling blood plasma, and productivity (Michigan Wildlife Trend Monitoring annual monitoring reports - Roe et al., 2004a, 2004b, 2003; Summer et al., 2002). Additional productivity data for the Kalamazoo River eagle territories is available in Stratus (2005a). For comparisons to the KRAOC bald eagle data base, the Great Lakes egg and plasma trend monitoring data were grouped to provide for comparisons of like-exposures. To minimize spatial and temporal sources of variability from the historical data bases the data were grouped to generate descriptive statistics for samples from the Kalamazoo River, Lake Michigan lakeshore samples from the Manistee and Pere' Marquette Rivers only (PM/MR Riverine sites), and inland Lower Peninsula samples from Roscommon County lacustrine habitats. Only plasma samples from 1999 - 2002 and eggs collected from 1996 - 2000 were included in the comparison data set. The KRAOC results were examined to determine if the contaminant concentrations at the three study sites were consistent with the historical trend monitoring data for site-specific exposures and spatial contaminant distributions.

The historical trend data includes five samples (two eggs, three plasma) from the Kalamazoo river, eight samples (one egg, seven plasma) from the PM/MR Riverine sites, and 18 samples (one egg, 17 plasma) from Roscommon County Lacustrine sites. For comparison purposes, all samples were treated in the same manner as described previously for KRAOC study samples. Multiple egg samples from the same clutch were combined for an average concentration and individual plasma samples were converted to an egg-basis using a plasma-to-egg conversion factor. An additional adjustment of the Michigan Wildlife Trend Monitoring PCB plasma concentration data was required to convert the sum value for the 20 quantified PCB congeners to a total PCB equivalent (all resolvable PCB congeners). This conversion was completed using the relationship: Total PCBs = 4.57(sum 20 PCB congeners, ng g^{-1} ww) + 0.98 developed for the Wildlife Trend Monitoring data set by Stratus (2005).

Spatial distributions of total PCBs in the KRAOC study data are identical to the trends displayed by the combined egg-basis historical trend monitoring data, but differences are found in results of the statistical testing. For the trend monitoring samples, geometric mean PCB concentrations in egg-basis samples were the greatest in Kalamazoo river birds (2.5 $x10^4$ ng PCB g^{-1}, ww), and the least PCB concentrations were measured in data for the Roscommon County sites (9.8 $x10^2$ ng PCB g^{-1}, ww). Geometric mean total PCB concentrations at the Kalamazoo river and PM/MR Riverine sites (1.1 $x10^4$ ng PCB g^{-1}, ww) differed by a factor of 2.3 (compared to 2.5 for KRAOC study samples), and the difference was statistically significant (Kruskel-Wallace, $p<0.5$). Geometric mean concentrations at the Kalamazoo river and PM/MR Riverine sites were each significantly greater than concentrations at the Roscommon County sites (Kruskel-

Wallace, $p<0.001$). Direct comparisons between the two data sets show that the trend monitoring and KRAOC study PCB data are not significantly different at any of the three locations (Kruskel-Wallace, $p=1.0$ KRAOC/Kalamazoo river comparison; $p=0.34$ Riverine sites; $p=0.17$ Lacustrine sites, respectively).

Historical ΣDDT concentrations in the KRAOC sample displayed spatial distributions that were inconsistent with the trend monitoring data. The trend monitoring data set showed slightly greater geometric mean egg-basis concentrations of ΣDDT in samples from the Kalamazoo river (3.7×10^3 ng ΣDDT g^{-1}, ww) compared to the PM/MR Riverine sites (2.7×10^3 ng ΣDDT g^{-1}, ww) and much lesser concentrations were measured at the Roscommon County sites (7.7×10^2 ng ΣDDT g^{-1}, ww). Statistical testing of spatial trends show that geometric mean "egg-basis" total ΣDDT concentrations at the Kalamazoo river and PM/MR Riverine sites were not statistically significant (Kruskel-Wallace, $p=0.88$), but both the Kalamazoo river and PM/MR Riverine site concentrations were significantly greater than concentrations at the Roscommon County sites (Kruskel-Wallace, $p<0.005$). Direct comparisons between the two data sets show that the historical trend monitoring geometric mean ΣDDT concentrations are 42% less at the Kalamazoo River site (not statistically significant, Kruskel-Wallace, $p=0.3$) and 76% less at both the Riverine and Roscommon County sties (statistically significant, Kruskel-Wallace, $p<0.005$).

Productivity data for the years 1999 thru 2002 included monitoring results for "Anadromous" and "Inland Lower Peninsula" eagle populations. Anadromous eagle populations included all eagle territories on Michigan tributaries to the Great Lakes with access for anadromous fish up to the first dam on the River. Tributaries to lakes

Michigan, Superior, Huron, and Erie are included in this group. Productivity data for the Kalamazoo River eagle territories includes nesting success for the years 1990 through 2003. Productivity rates from the Michigan Wildlife Trend Monitoring database show four-year average Inland and Anadromous productivity rates of 1.0 and 0.8 fledglings/active nest, respectively. These values are consistent with the productivity rates for the KRAOC study Lacustrine (1.0 fledge/nest) and Riverine (0.9 fledge/nest) reference sites. The comparable four-year (1999-2002) productivity rate for Kalamazoo Bald eagles computed using only the 1999 data from Stratus (2005a) and observations made during this study (2000-2002) is 0.4 fledgling/active nest, a value identical to the five-year (2000-2004) productivity rates identified in this study. Because productivity rates are typically examined on a five-year basis (Wiemeyer et al., 1984) a rate could be calculated for the five-years immediately preceding the KRAOC study (1995-1999). These data show four fledglings were produced after 10 nesting attempts, or 0.4 fledglings/active nest, and indicate no net change in productivity for this population over the last ten years.

PCB congener profiles/$TEQ_{WHO\text{-}Avian}$

The relative proportions of coplanar PCB congeners present between egg and plasma samples collected for the KRAOC study are generally consistent with proportions observed in these tissues of resident eagles throughout North America. Similarities include the predominance of PCB126 as the maximum detected coplanar congener in egg samples, and the predominance of PCB77 as the maximum detected coplanar congener in plasma samples (Elliott and Harris, 2002). This is consistent with observations that

PCB77 and 81 are more susceptible to metabolism than PCB126 and 169 (Smith et al., 1990) and thus less likely to be available in stored lipids that might be translocated to yolk lipids during oogenesis. Relative contributions between PCB77 and PCB126 to total $TEQ_{WHO\text{-}Avian}$ in egg samples were consistent for KRAOC and Pacific Coast (Canada) data sets, but KRAOC samples differed from the data set compiled by Elliott and Harris (2002) regarding the relative contributions from PCB77 and PCB126 to total $TEQ_{WHO\text{-}Avian}$ in plasma. PCB77 predominates as the greatest relative contributor to total TEQ for plasma samples from each of the three KRAOC study sites in contrast to trends for the 10 plasma sample groups from the Pacific Coast of Canada which show PCB126 as the greatest contributor to total TEQ for the PCB group.

Comparable $TEQ_{WHO\text{-}Avian}$ concentrations for eagle egg or plasma samples from the Great Lakes basin are available for only a single Lake Huron addled egg collected in 1986 (Schwartz, et al. 1993). This egg contained a deformed embryo and had a total PCB concentration of 1.0×10^5 μg PCB g^{-1}, ww and a $TEQ_{WHO\text{-}Avian}$ concentration of 9.8×10^3 pg $TEQ_{WHO\text{-}Avian}$ g^{-1}, ww , nearly 7-fold greater than the maximum KRAOC concentration of $TEQ_{WHO\text{-}Avian}$ in eggs (1.5×10^3 pg $TEQ_{WHO\text{-}Avian}$ g^{-1}, ww, Riverine site - Pere' Marquette River). The available data for PCB-based $TEQ_{WHO\text{-}Avian}$ concentrations in eagle tissues show that Great Lakes eagles carry the greatest PCB $TEQ_{WHO\text{-}Avian}$ burdens of all North American eagle populations studied to date. Eagle egg samples from the KRAOC contained PCB contributions to $TEQ_{WHO\text{-}Avian}$ that were 2- to 5-fold higher than comparable PCB contributions to $TEQ_{WHO\text{-}Avian}$ in eagle eggs from New Jersey tributaries to the Delaware Bay (Clark et al., 1998), the Columbia River (Buck et al., 2005) and the Pacific Coast of Canada (Elliott et al. 1996). Similarly, nestling plasma

samples from the KRAOC contained PCB contributions to $TEQ_{WHO\text{-}Avian}$ that were 10-fold higher than comparable PCB contributions to $TEQ_{WHO\text{-}Avian}$ in nestling plasma from the Pacific coast of Canada (Gill and Elliott, 2002; Elliott and Norstrom, 1998).

Calculated $TEQ_{WHO\text{-}Avian}$ concentrations in eggs and plasma were of limited value to this examination of exposures and potential risks to bald eagles and were used sparingly to reinforce conclusions drawn from the total PCB, ΣDDT, and productivity data. Major contributing factors to this condition include the difficulty of collecting an adequate number of egg samples from a protected avian species, the lack of congener-specific conversion factors for converting plasma sample TEQ concentrations to an egg-basis, and the absence of a suitable plasma-based $TEQ_{WHO\text{-}Avian}$ TRV.

Hazard assessment

Results of the focused KRAOC eagle investigations are consistent with previous Great Lakes basin-wide monitoring efforts for the bald eagle undertaken by the USFWS and the focused wildlife contaminant trend monitoring by the Michigan Department of Environmental Quality (MDEQ). All three of these monitoring efforts have produced contaminant data and productivity measurements that can be interpreted as strong evidence that Kalamazoo River eagle populations are experiencing impaired reproductive success and on a broader scale, indicate an ecosystem with decreased fitness. A close examination of the KRAOC eagle data shows productivity rates (0.4 fledglings/active nest) below the minimum level (0.7 fledglings/active nest) associated with a stable population (Sprunt et al., 1973). The KRAOC data show total PCB HQ_{LOAEC} ranges for the 95%CI on the geometric mean egg-basis concentration were from 0.7 to 1.9, and the

95%CI HQ_{NOAEC} ranged from 4.6 to 13. The geometric mean HQ_{LOAEC} and HQ_{NOAEC} values were 1.2 and 7.7, respectively. The HQ_{NOAEC} value for $TEQ_{WHO\text{-}Avian}$ concentrations in the single egg sample from the KRAOC was 8.4. The hazard assessment for PCBs indicated that the degree of exposure to PCBs was at or above the threshold for effects on reproduction in bald eagles. This assessment is supported by the ΣDDT data for the KRAOC and Riverine sites.

The greatest ΣDDT concentrations observed for this study were observed at the Riverine site. Together, the Riverine site combined egg basis sample set displayed individual ΣDDT concentrations that exceeded the egg-based TRV for the NOAEC in 100% of the samples. The geometric mean HQ_{LOAEC} and HQ_{NOAEC} values were 1.0 and 3.2, respectively and the 95%CI HQ_{NOAEC} ranged from 2.8 to 3.7. Geometric mean egg shell thickness from two addled egg shells and 3 egg shell fragments showed a decrease of over 11% compared to the pre-1947 benchmark. Two of five egg shell samples (40%) showed shell thinning in excess of the -15% value generally expressed as a conservative benchmark of reproductive failure in raptors (Newton, 1979), and both nests failed the year these samples were collected. Despite these elevated ΣDDT exposures, productivity rates at the Riverine site (0.9 fledglings/active nest) were above maintenance levels and close to values indicative of a healthy population. Exposures to ΣDDT for KRAOC birds were roughly half the levels observed at the Riverine site, and compared to the much greater PCB exposures, effects related to ΣDDT can be expected to be negligible.

Risk Assessment Uncertainties

Riverine exposures to ΣDDT provided a clear illustration of the fact that the true effect level for individuals' lies somewhere between the NOAEC and LOAEC, and that population effects are seldom observed when HQ values exceed the 1.0 threshold by small margins. Even conservatively, population effects have not been expected at HQs of 10.0 (Neigh et al., 2006). The uncertainties associated with HQ estimates between 1.0 and 10.0 supported using a multiple lines of assessment and weight of evidence approach to assess population health. Taken together, contaminants data in tissues and direct observation of productivity provided a compelling argument for population level effects at the KRAOC study site.

As part of the weight-of-evidence approach, additional factors that have been observed to influence productivity outcomes at individual eagle nest sites should be considered in the ecological risk assessment. Correlations between contaminant exposure and lower nest productivity can be confounded by other correlated factors, anthropogenic or natural, that an investigator fails to recognize when a study is completed (Karasov and Meyer, 2000). One of these factors is human disturbance to the nest site during critical time periods of eagle courtship and egg incubation (USFWS, 1983), or later in the reproductive cycle, when disturbances to foraging adults can adversely impact foraging success and ability to provide sufficient food to nestling birds. Human activities that have been shown to greatly disturb eagles include residential development and associated daily activities, hiking, and boating (fishing). Studies of disturbances to nesting and foraging eagles indicated that individual eagles vary in their tolerance to human encroachment. Some birds become habituated to human activities and are extremely

tolerant of incursions into their territories, and others are quickly disturbed to the extent they leave the nest or abandon all activity in the zone of disturbance during all or part of the day (e.g., foraging activity). Studies of foot traffic in proximity to actively nesting eagles have indicated that birds can be flushed from the nest from mean distances of over 400 m (Fraser et al., 1985). Studies of boating activity under similar conditions of eagle activity showed that the distance (from the nest) and duration of a boating activity are factors that cause eagles to be flushed from the nest, and the authors recommended buffer zones that included critical minimum distances of 100 m to pass and 400 m to stop (Grubb et al., 2002).

The lower reach of the Kalamazoo River where eagles are currently nesting is a popular destination for sportspersons and outdoor recreationists. Overland foot access is available to critical buffer areas (e.g., $\leq$ 400 m) surrounding most of the actively maintained nests in each of the three eagle territories in the KRAOC, via residential property, public hiking trails, or through illegal trespass in restricted areas. There are presently few institutional controls available to restrict overland access to nest sites in these areas. A recent survey of recreational fishing activity in the lower river downstream of Calkins Dam estimated that there were a total of 19,416 to 20,193 fishing days by Kalamazoo River anglers in 2001 (Stratus, 2005b). These figures did not include winter fishing, and most of the fishing activity focused on salmon, trout, walleye and bass. Trout fishing is concentrated in the early spring (March/April) when substantial runs of steelhead trout make their way into the river from Lake Michigan and run to the Calkins dam. Walleye also make spawning runs up the river and the popular opening day/week of the walleye season is in late April. Both of these periods of high fishing

activity coincide with the critical period for eagle courtship and egg incubation in the KRAOC which typically extends from mid-February through early May. Trout and walleye can be fished with efficiency from the shore and from a boat. There are public launch facilities in the immediate vicinity of each of the three established eagle territories in the KRAOC, at Calkins Dam (2 km upstream of Swan Creek), at the M-89 bridge (adjacent to Swan Creek and 7 km upstream of Ottawa Marsh), and at the New Richmond and 63rd Street ramps (5 km upstream and 2 km downstream, respectively of Pottawatomi Marsh). Most of the actively maintained eagle nests are fairly isolated from the potential effects of boat traffic on the river, however the cumulative effects of heavy fishing pressure during the early spring critical period have not been investigated with regard to potential adverse effects on reproductive success at the KRAOC.

Prey delivery rates and nestling energy intake are a principal determinant for successful fledgling production. Previous studies of eagle productivity and contaminant exposure in the Great Lakes basin also have identified the overriding influence of prey availability and the necessity to also consider energy intake by nestlings to avoid erroneous assignment of causation for observations of depressed productivity (Dykstra et al., 1998). Delivery rates are influenced by prey availability, human disturbances to foraging activities, and perhaps contaminant exposures. Nest observations to record prey delivery rates and adult attentiveness can provide an indication of how energy provision to nestlings affects productivity. Nest attentiveness data are available for nests included in the KRAOC eagle study, and these data are discussed in a companion assessment of potential risks through dietary exposure for KRAOC eagles (Strause et al., 2007b).

Predation also can adversely affect nest productivity. Nestling eagles are subject to predation by great horned owls and nestlings that fall from the nest and land unharmed at the base of the nest tree can succumb to a variety of mammalian predators. Aside from constant surveillance of the nest site (e.g., continuous video surveillance) accurate estimates of predation impacts to eagle productivity are unavailable. Predation may certainly have played a role in depressing eagle productivity at the KRAOC. Great horned owls nest in close proximity to active eagle nests and at the KRAOC site have been observed to nest in existing eagle nests. The potential for owl predation on eagle nestlings was present throughout the duration of the study. At the 2001 Swan Creek nest, the predated remains of a single 7 to 9 wk old nestling were found at the base of the nest tree during mobilization to access the nest and band said nestling. Although all indicators suggest that rough weather caused the nestling to fall from the nest, there is no way to know if the young bird was severely injured or expired from the fall, or was preyed upon while healthy and active at the base of the nest tree. Nestling falls from the nest are a fairly common event, and as long as the nestling is visible, adults will continue to tend to grounded birds until they reach the fledgling stage.

CONCLUSIONS

The "top-down assessment of potential hazards to resident bald eagles at the KRAOC, and the Riverine and Lacustrine reference sites has employed an unbiased and conservative investigation of "worst-case" exposures to environmental contaminants. Sampling of nestling plasma is recognized as the most reliable medium for sensitive

measurements of localized contamination at the point of collection, much more so than are concentrations of residues in eggs or adult plasma samples (Donaldson et al., 1999; Olsson et al., 2000; Bowerman et al., 2000). Investigative methods included state-of-the-art analytical techniques with multiple levels of quality assurance/quality control safeguards for identification and quantification of PCB congeners and ΣDDT analytes, multiple lines to estimate exposure to PCBs, and assessment of potentially confounding chemical stressors. Use of a Great Lakes derived plasma to egg conversion factor with conservative consensus benchmarks of toxicity furthers a technique for evaluating hazard using plasma data, and has broad recognition in the literature (Stratus, 2005; Elliott and Harris, 2002). Additionally, all field sampling activities were completed with the assistance of USFWS oversight personnel and split samples of all tissue samples were provided to USFWS scientists to encourage reproducibility and confidence in the analytical data.

The KRAOC eagle study demonstrated the utility that bald eagles provide as a 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 productivity. Additional opportunities for focused monitoring using bald eagles are possible. In certain instances eagles will select artificial nesting platforms that are properly constructed and sited (Grubb, 1995). With eagle populations expanding in most North American habitats the competition for optimal nesting sites will increase. Lesser availability of optimal nesting sites and increased tolerance of proximal human activity are two contributory trends that

may further facilitate the use of eagles as biological monitors of long term ecosystem health at areas of concern in the Great Lakes and throughout North America.

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