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Exposure and Multiple Lines of Evidence Assessment of Risk for PCBs Found in the Diets of Passerine Birds at the Kalamazoo River Superfund Site, Michigan

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ABSTRACT

Dietary exposures of passerine birds at the Kalamazoo River, Michigan, were examined due to the presence of polychlorinated biphenyls (PCBs) in the terrestrial and aquatic food webs. Average potential daily doses in diets were 6- to 29-fold and 16- to 35-fold greater at a contaminated location than at a reference location for PCB exposures quantified as total PCBs and 2,3,7,8–tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), respectively. Birds with diets comprised of primarily aquatic insects had greater dietary exposure than birds with diets of primarily terrestrial insects. Risk associated with dietary exposure varied with the selection of the threshold for effects including hazard quotients, which exceeded 1 in instances where the most conservative toxicity reference values were utilized. Risk based on concentrations of PCBs in the tissues indicated little risk to avian species, and co-located studies evaluating reproductive health did not suggest that observed incidences of diminished reproductive success were related to PCB exposure. Measures of risk based on comparison to toxicity reference values (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 these species especially based on TEQs. This is largely due to the absence of species-specific, dose-response relationships. Therefore, the best estimate of risk is through the application of multiple lines of evidence.

Key Words: insects, birds, bioaccumulation, dietary exposure, risk assessment, trophic level.

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INTRODUCTION

The purpose of this study was to describe the exposure pathways for passerine birds exposed to polychlorinated biphenyls (PCBs) at the Kalamazoo River and to estimate risk associated with PCBs through those pathways. A secondary goal was to compare the two established methodologies for estimating exposure and subsequent risk. Finally, the results of the risk assessments based on the two methods of estimating exposure were compared to measures of reproductive fitness at the more PCB-contaminated site and the less contaminated, upstream reference location. The first method for estimating exposure, the "top-down" approach, measured concentrations of PCBs in the eggs, nestlings, and adults of each species. The other approach, which predicts exposure based on concentrations of PCBs in dietary items, is referred to as "bottom-up" (Fairbrother 2003). The two approaches, although inherently linked, have become disjointed in the risk evaluation process. The reason for this separation is often related to constraints on funding and time, but the assessor cannot be unequivocally assured that their selection of methodology does not overlook some important interaction in determining risk, or for that matter, does not over or underestimate risk based on uncertainties due to the adjustment of models from the given data.

It has been suggested that several lines of evidence be used to evaluate risk instead of implementing only one type of methodology (Fairbrother 2003). However, it may not always be possible to apply multiple lines of evidence simultaneously. For this reason, the levels of risk estimated by the two methods were compared so that in the future, assessors will have an estimate of the similarity of the two methods. Based on this concept, a system of evaluating representative species in all trophic levels of the ecosystem was used to describe the complex dynamics present. This broad-based trophic level approach generates a bottom-to-top description of contaminant exposure and effects in the system. Detailed results of the top-down approach, including tissue concentrations and measurements of reproductive fitness, are presented elsewhere (Neigh *et al.* 2006a,b), but the degree of concordance between the top-down and bottom-up approaches is evaluated in this article. Specific measurement endpoint comparisons include (1) estimate of exposure and subsequent risk calculation for four passerine species based on dietary exposure; (2) comparisons of measured site-specific dietary exposure to dietary exposures derived from a literature based diet; (3) the contributions of terrestrial and aquatic food web–based exposures as related to generalized feeding guilds; (4) tissue-based exposure assessments; (5) species productivity; (6) and hazard quotients based on the total concentration of PCBs and 2,3,7,8–tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) of the PCB exposure mixture.

Exposures to PCBs and the potential effects of these exposures on passerine birds have been examined in several aquatic ecosystems (Custer *et al.* 1998; Bishop *et al.* 1999; McCarty and Secord 1999; Custer *et al.* 2003), but the effects on species exposed through terrestrial food webs have been less well documented. Even fewer studies have evaluated differential accumulations of PCBs by wildlife from contaminated media with the same point of origin but environmentally weathered in substantially different ways. This study was conducted to determine the differential sources of PCB dietary exposure, aquatic and terrestrial, in order to quantify risk based on exposure parameters. The demarcation between terrestrial and aquatic food webs within riverine systems is indistinct because of the interaction between the two exposure pathways. In this manuscript, the aquatic system was defined as the food web based on invertebrates with aquatic life stages exposed through in-stream sediments. Species in a food web based primarily on plants and invertebrates with essential terrestrial life stages will be referred to as the terrestrial system.

The Kalamazoo River, Michigan, was designated a Superfund site in 1990 due to PCB contamination released during the paper recycling process at multiple locations along its banks (MDEQ 2003). Clays, inks, and paper fiber stripped from recycled paper pulp were originally deposited over in-stream sediment, but the area of contamination is now partitioned between in-stream sediment, floodplain soils, and former impoundment sediments. Floodplain soils became exposed through the frequent and regular flooding of the river over its banks; the floodwaters exposing, carrying, and depositing sediments over broad areas of the 100-year floodplain. An even greater source of terrestrial PCB exposure originated from the removal of three dams from the river's watercourse, which led to the establishment of a terrestrial ecosystem when water levels were lowered and former sediment exposed. Kalamazoo River wildlife can be exposed to PCBs via both aquatic and terrestrial food chains due to this ubiquitous contamination of sediments and vegetated former lake bottom soil.

Risks posed by exposure of insectivorous and omnivorous birds to PCBs within the Kalamazoo River Superfund Site were evaluated through the use of four passerine birds. These species were selected because they represent exposure to upper-trophic level predators via different routes in the terrestrial and the aquatic food web. PCBs in the diet are known to be persistent and bioaccumulative (Kannan *et al.* 1989) and can cause reproductive impairment (Dahlgren *et al.* 1972), behavioral anomalies (Halbrook *et al.* 1998), and physiological abnormalities in offspring (Ludwig *et al.* 1993). The tree swallow (*Tachycineta bicolor*) was selected to monitor dietary exposure to passerines primarily through the aquatic food web because it had been used in other studies and found to be suitable (Ankley *et al.* 1993; Jones *et al.* 1993; Froese *et al.* 1998; Bishop *et al.* 1999; McCarty and Secord 1999; Harris and Elliott 2000; Custer *et al.* 2002), it tolerates handling (Rendell and Robertson 1990), and individuals are relatively abundant along the river. In a novel approach to risk assessment, the house wren (*Troglodytes aedon*) was selected as an indicator of terrestrial food web exposure. Its ability to colonize much of the habitat in the river basin and its abundance were important determinants for choosing the species. The American robin (*Turdus migratorius*), another terrestrial species, was chosen to represent omnivorous species and was also considered to be maximally exposed to soil contaminants because of the large proportion of earthworms, which are in direct contact with contaminated soil, in their diet. Finally, the eastern bluebird (*Sialia sialis*) was selected for study because little is known about PCB accumulation by this species, although there is a very large body of literature on its biology.

MATERIALS AND METHODS

Site Details

Two sites within the Kalamazoo River floodplain were selected for studies of passerine birds, the Fort Custer State RecreationArea (FC) and former Trowbridge

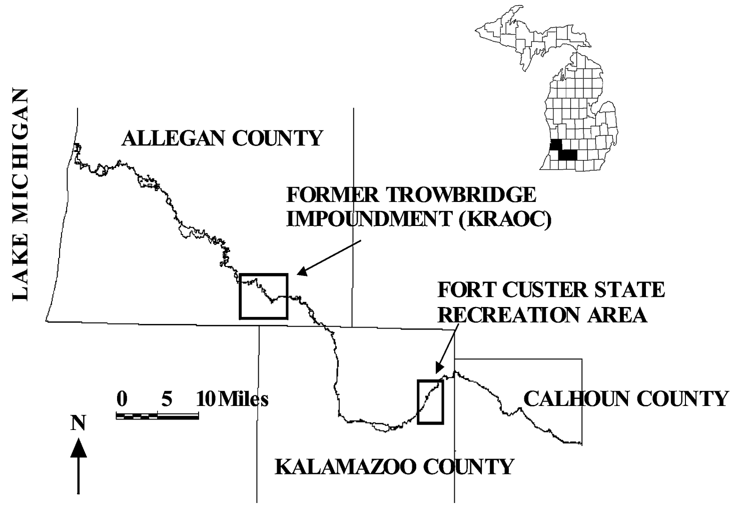

Figure 1. The PCB contaminated Trowbridge Impoundment within the Kalamazoo River Area of Concern and an upstream reference location at the Fort Custer State Recreation Area.

Impoundment (TB). FC was selected as the upstream reference area based on a number if criteria. FC is an undeveloped area of similar habitat to TB. FC is ∼7 km upstream of the initiation of the Superfund site and ∼40 km upstream of TB. FC has an established nest box trail (∼20 years) with an associated historical database concerning tree swallow tissue contaminant content and productivity. This database suggests background contaminant exposure and normal productivity. TB lies downstream of the point sources and is the most PCB contaminated location within the Kalamazoo River Area of Concern (KRAOC) (Figure 1). The Trowbridge impoundment includes 132 ha of former sediments that are now under natural vegetation. Nest boxes in both locations were limited to the 100-year floodplain.

Bolus and Tissue Collection

Bolus samples were collected from individual nestlings (day 3 to day 14) during late morning and early afternoon at TB and FC to coincide with peak feeding periods (Kuerzi 1941). However, it has been suggested that the time of day does not strongly influence the number of feeding visits by the parent, and the number of visits are comparable across the breeding season (McCarty 2002). Ligatures were placed on all nestlings in each nest according to Johnson *et al.* (1980) for 1 to 2 hours to limit the effect of food deprivation on growth. After placement of ligatures, nestlings were observed for several minutes to determine if the ligature was affecting behavior of the adults or nestlings. Ligatures were removed from nestlings that appeared to be disturbed by their presence. Each hour, the nestlings' throats and the inside of the nests were checked for boluses. The packaging of the bolus in saliva by the adult prevented the loss of small dietary items through the ligature. Once a bolus was collected from a nestling, the ligature was removed from that nestling. Bolus sampling did not exceed four separate events or 3 g of bolus material at each nest. Sampling

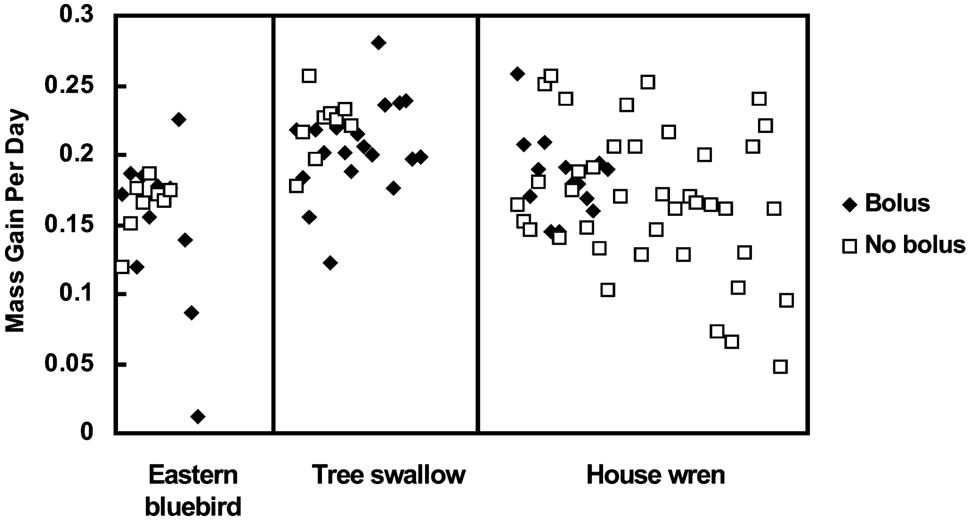

Figure 2. Comparison of mass gained per day of life for nestlings in nests from which boluses were collected (bolus) or not collected (no bolus) at the Kalamazoo River.

was discontinued when nestling tree swallows and eastern bluebirds were 14 days or house wrens were 10 days to eliminate premature fledging. This method is thought to provide an accurate representation of the nestling dietary composition (Johnson *et al.* 1980). There were no cases of abandonment at nests in which bolus sampling took place. Bolus collection did not statistically effect the growth of nestlings, and there were no discernible differences in growth of nestlings between nests from which a bolus was collected or not collected (Figure 2).

The stomachs of the birds in the study were collected to describe dietary composition in more detail. Tree swallow, eastern bluebird, and house wren nestlings were collected from randomly chosen nests, euthanized by cervical dislocation, and frozen until processing. Stomach contents were removed from nestlings and pooled based on proximity to insect sampling grids. Detailed descriptions of sampling methods are described elsewhere (Neigh *et al.* 2006a).

Average Potential Daily Dose

The amount of PCBs ingested by passerine birds was calculated using the wildlife dose equation for dietary exposures in the U.S. Environmental Protection Agency's (USEPA's) Exposure Factors Handbook (USEPA 1993). Average potential daily doses (APDD), calculated for total PCBs and TEQs based on avian-specific World Health Organization (WHO—Avian) toxic equivalency factors (van den Berg *et al.* 1998), were based on site-specific and literature-based diets for tree swallows, eastern bluebirds, house wrens, and American robins at the Kalamazoo River (Equation 1). Site-specific diets based on bolus content and literature-based diets were used to estimate dietary composition of the nestlings during the nestling period. Due to the lack of knowledge about the feeding habits of adults and nestlings after leaving the nest box, dietary dose only estimates site-specific exposure to nestlings during the nesting period.

$$APDD = \sum (C_k \times FR_k \times NIR_k) \quad (1)$$

where C_k = Concentration of PCBs (totals or $TEQs_{WHO\text{-}Avian}$, ww) in the kth prey item in the passerine diet, FR_k = Fraction of the passerine diet based on mass represented by the kth prey item, NIR_k = Normalized ingestion rate of kth prey item (g prey/g body weight/day, ww).

Concentrations of PCBs in prey items were determined for insects collected in the bolus of nestlings and during site-specific sampling of insect orders; the results of which are reported elsewhere (Blankenship *et al.* 2005; Coady *et al.* 2005). FR_k was determined based on the dietary composition of each bird species. NIR_k was derived from ingestion rates reported for the American robin (0.89 g/g/d) and marsh wren (*Cistothorus palustris*) (0.99 g/g/d) (USEPA 1993). Dietary composition was determined from bolus samples based on occurrence and were converted to composition based on mass using the order-specific weights of individual insects from each location within KRAOC.

Comparisons of risk based on dietary exposure to PCBs were based on hazard quotients (HQs). Hazard quotients were calculated as the APDD (mg PCB/kg/d or ng TEQ/kg/d) divided by the corresponding toxicity reference value (TRV).

Selection of Toxicity Reference Values

TRVs from the literature were developed for the effects of PCBs based on both total PCB concentrations and $TEQs_{WHO\text{-}Avian}$ in the diet and eggs. The selection of TRVs was based on several criteria to determine their appropriateness for use in this study. These criterion included: (1) the use of wildlife species rather than traditional laboratory species; (2) chronic exposure over sensitive life stages; (3) the evaluation of ecologically relevant endpoints; (4) minimal co-contamination; (5) multiyear studies; (6) and total PCB or $TEQ_{WHO\text{-}Avian}$ values were reported or could be calculated. There were few studies available for passerine species. Due to the uncertainty in the dose-response of passerine birds to PCB exposure, a range of TRVs matching the given criteria have been reported (Table 1).

For this study as well as recent USEPA applications (USEPA 1995, 2000), a ring-necked pheasant (*Phasianus colchicus*) feeding study was selected as the most appropriate for the determination of a threshold for effects due to dietary exposure to passerine species (Dahlgren *et al.* 1972). The ring-necked pheasant study evaluates PCB toxicology during critical reproductive life stages, is of high quality, utilizes a wild species, and was found to be one of the most sensitive bird species examined. Chick survival and egg production were adversely affected in the 50 mg PCB/wk group, and hatchability was reduced by 14% in the 12.5 mg PCB/wk dose group compared to the control. The calculated daily dietary dose based on the 12.5 mg PCB/wk dose group and an adult pheasant weight of 1 kg (USEPA 1995) was 1.8 mg PCB/kg/d, and this value was considered the lowest observed adverse effect level (LOAEL). The no observed adverse effect level (NOAEL) of 0.6 mg PCB/kg/d was derived by dividing the LOAEL by a safety factor of three. A safety factor of three was determined to be acceptable because the LOAEL was established near the threshold for effects. Several species of wild passerine birds, such as the ones examined in this

Table 1. Toxicity reference values based on the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) for dietary exposure of avian species to PCBs at the Kalamazoo River.

	Reference	Dietary TRV
Total PCBs (mg PCB/kg/d)		
	Dahlgren *et al.* (1972)	LOAEL = 1.8 mg PCB/kg/d NOAEL= 0.6 mg PCB/kg/d
	Calculated from BMFs	LOAEL = 14.7 mg PCB/kg/d[a] NOAEL = 1.9 mg PCB/kg/d[b]
Total TEQ (ng TEQ/kg/d)		
	Nosek *et al.* (1992)	LOAEL = 140 ng TEQ/kg/d NOAEL = 14 ng TEQ/kg/d
	Calculated from BMFs	NOAEL = 1000 ng TEQ/kg/d[c]

[a] Calculated from TRV selected from Custer *et al.* (2003).
[b] Calculated from TRV selected from Henning *et al.* (2003).
[c] Calculated from TRV selected from USEPA (2000).

study, appear to be less sensitive to PCB exposure (Thiel *et al.* 1997; Custer *et al.* 1998; Henning *et al.* 2003) than domesticated galliformes on which dietary TRVs could be based (*e.g.*, domestic chickens, ring-necked pheasant; Dahlgren *et al.* 1972).

In addition to the laboratory study, TRVs for dietary exposure to total PCBs were calculated from site-specific biomagnification factors (BMFs) between the diet and egg at the Kalamazoo River. Decreased hatching success in pippers of tree swallows has been reported to occur at 63 mg PCB/kg, ww in eggs (Custer *et al.* 2003), but no reproductive impairment of American robins was observed at 83.6 mg PCB/kg, ww in eggs (Henning *et al.* 2003). These studies are applicable to the current study because they fulfill the criteria for TRV selection, and they are conducted on wild passerine birds. TRVs for dietary exposure were calculated using biomagnification factors (BMFs) calculated by dividing concentrations of PCBs in eggs at the Kalamazoo River by the weighted average concentration of PCBs in the diet at the Kalamazoo River. For example, the site-specific BMF from diet to egg of tree swallows is 4.3 (calculated from egg concentration of 5.1 mg PCB/kg, ww / concentration in diet of 1.2 mg PCB/kg, ww). When the biomagnification factor (4.3) was applied to the LOAEL selected for eggs (63 mg PCB/kg), a TRV of 14.7 mg PCB/kg for the diet was calculated. No LOAEL could be established for terrestrial species, so the LOAEL calculated for tree swallows was used. This same concept was applied to arrive at the most conservative estimate for all avian species based on the NOAEL established for terrestrial passerine tissues, which was 83.6 mg PCB/kg, ww in eggs (Henning *et al.* 2003). The most conservative estimate of a TRV for dietary exposure when BMFs were applied was 1.9 mg PCB/kg, ww diet.

Few studies were available to derive TRVs based on $TEQ_{WHO\text{-}Avian}$ concentrations in the diet of wildlife species, whereas even fewer studies exist for passerine birds. A laboratory study by Nosek *et al.* (1992) found that intraperitoneal injections of 2,3,7,8 –TCDD at concentrations of 1000 ng TCDD/kg/wk (140 ng TCDD/kg/d) caused a 64% decrease in fertility and a 100% increase in embryo mortality in pheasants.

The study was a subchronic exposure (10 wk exposure period), and the length of the study was greater than the length of time an adult passerine may spend on site before nesting (∼5 wk) (estimated from Adams 1979). Limitations of the study include the use of injections of TCDD instead of feeding TCDD contaminated prey to the test species and the evaluation of TCDD exposure and not PCB exposure. TEQs based on PCBs may overestimate exposure relative to TCDD (Custer *et al.* 2005), so TRVs based on TCDD exposure are likely to be conservatively estimate risk when applied to PCB exposure. 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 TCDD (Hoffman *et al.* 1996). The NOAEL (14 ng TEQ/kg/d) was calculated by applying a safety factor of 10 to the LOAEL because effects due to the exposure are pronounced in the test subjects.

TRVs for $TEQ_{WHO\text{-}Avian}$ were also derived from site-specific BMFs. The NOAEL selected for $TEQ_{WHO\text{-}Avian}$ in the tissues of birds was 13000 ng TEQ/kg (see Neigh *et al.* 2006b). This field study was based on tree swallows and fulfilled the criteria for TRV selection. Based on the greatest BMF between diet and egg for the species examined in this study (BMF = 13 for house wrens, Neigh *et al.* 2006b), the TRV for dietary exposure to TEQs was determined to be 1000 ng TEQ/kg.

Little information on the toxicity of PCBs to passerine birds was available for terrestrial diets; thus there was uncertainty associated with the selection of an appropriate TRV to compare to Kalamazoo River dietary exposure. Several species of wild passerine birds, such as the ones examined in this study, appear to be less sensitive to PCB exposure (Thiel *et al.* 1997; Custer *et al.* 1998; Henning *et al.* 2003) than domesticated galliformes on which dietary TRVs could be based (*e.g.*, domestic chickens, ring-necked pheasant; Dahlgren *et al.* 1972). Thus, the dietary HQs based on domesticated avian species are likely an overestimate of hazard potential. In order to calculate a more realistic HQ based on similar species, site-specific and species-specific BMFs were applied to TRVs chosen for tissue exposure at the Kalamazoo River (Neigh *et al.* 2006a,b). The laboratory studies on galliformes and the toxicity reference values based on Kalamazoo River BMFs are intended to give a range of hazard quotients that expresses the most conservative estimate of risk and also reports a more appropriate estimate of the true risk of passerine birds exhibiting population level effects due to current contaminant levels at the Kalamazoo River. Due to the uncertainties in selecting TRVs, this study uses an examination of dietary exposure as part of a multiple lines of evidence approach to evaluate risk.

Assessment of Risk Using Multiple Lines of Evidence

In order to assist in the evaluation of risk, other lines of evidence were examined for agreement with the analysis and calculation of risk from dietary exposure (Fairbrother 2003) (Figure 3). Previously published studies on passerine birds inhabiting the riparian area of the Kalamazoo River have examined other lines of evidence to quantify site-specific risk to passerines (Neigh *et al.* 2006a,b,c,d). Two of the lines of evidence characterize exposure using the "bottom-up" approach, which is discussed here and describes dietary exposure, and using the "top-down" approach, which quantifies concentrations of PCBs present in the eggs, nestlings, and adults of the tree swallow, eastern bluebird, and house wren. A third and ancillary line of evidence investigates reproductive success of the species under investigation by

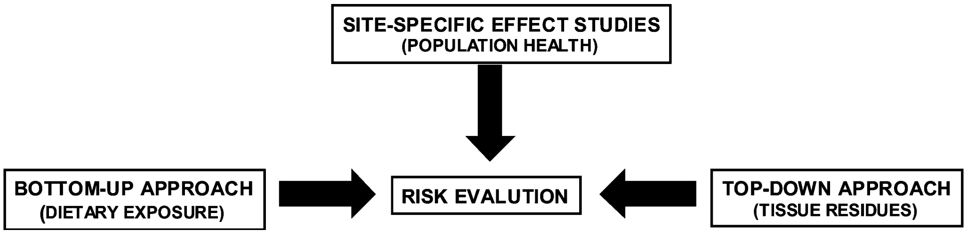

Figure 3. Multiple lines of evidence used to assess risk in Kalamazoo River passerine species.

comparing endpoints of reproductive success for birds at the contaminated location to the same endpoints of success for birds at a reference location. By evaluating these three lines of evidence together, it is possible to minimize the uncertainties associated with each approach and to make a more decisive conclusion of risk, especially when there is an agreement about risk from two or more lines of evidence.

RESULTS

Dietary Composition

The greatest number of bolus samples was collected during 2002. The most items were collected from tree swallows and the fewest from house wrens. A total of 1476 items from 64 nests were collected during the two years of diet collection in 2002 and 2003. Data for dietary composition were combined between years and between grids to increase the sample size and to provide a more representative account of passerine diets on the Kalamazoo River over the course of the study. A total of 11, 9, and 11 insect and invertebrate groups were represented in the bolus of the tree swallow, house wren, and eastern bluebird, respectively. There were a total of fourteen invertebrate groups taken by adult passerines: diptera, trichoptera, ephemeroptera, orthoptera, neuroptera, hymenoptera, araneae, odonata, hemiptera, lepidoptera, coleoptera, plecoptera, mollusca, and isopoda. For the purpose of analysis, homoptera was combined with hemiptera. Other items identified in the bolus were stones, glass, and pupae.

Aquatic insects made up the largest portion of insects in the bolus of tree swallows and were present in the bolus of all species in the following order: tree swallow (76.6%) > eastern bluebird (46.73%) > house wren (6.25%). Dipteran species represented 60.6%, 5.0%, and 8.0% of the total diet of tree swallows, house wrens, and eastern bluebirds (Figure 4). In contrast, diptera represented 79.1% and 80.0% of the aquatic species taken in the diet for tree swallows and house wrens, respectively, whereas diptera only comprised 17.1% of all aquatic species in the eastern bluebird. All dipterans were lumped into the aquatic category because the majority of dipterans captured as adults were of aquatic origin. Eastern bluebirds at TB also had a large aquatic component in the diet, but this was largely due to 1 bolus with 63 trichopterans collected from TB. When this was removed from the analysis, aquatic insects comprised 22.3% of the eastern bluebird diet.

The avian species examined differed in the occurrence of terrestrial insects in the diet relative to aquatic insects. Unlike the eastern bluebird and house wren, tree

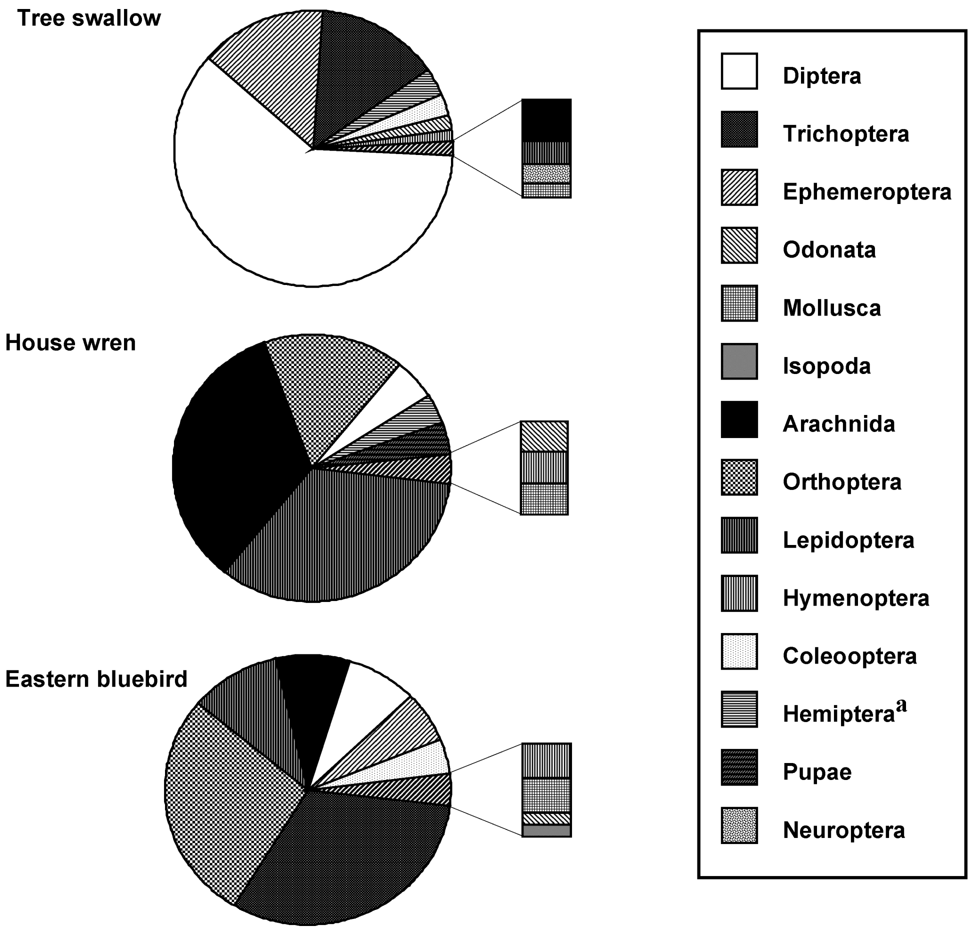

Figure 4. Dietary composition based on occurrence at the Kalamazoo River. The order hemiptera also includes the order homoptera.

swallows contained a greater proportion of aquatic insects than terrestrial insects, and only the terrestrial insect orders hemiptera and coleoptera represented more than 2% of the total diet. The order orthoptera was important in the diet of both house wrens and eastern bluebirds (house wrens = 16%; eastern bluebirds = 28%) but never occurred in tree swallow boluses. Lepidoptera were infrequently taken by tree swallow adults (<0.05% of diet), but comprised 34% and 10% of the house wren and eastern bluebird diet, respectively.

The contents of nestling stomachs were also identified. Much of the content could not be distinguished, but the indigestible portions, such as the legs, heads, and exoskeleton of orders were identifiable. The purpose of identifying stomach contents was not to quantify the diet using this method but to identify portions of the diet that were not observed during bolus sampling. Describing diet solely through this method could lead to bias because orders with indigestible portions would be easily identified, and therefore, stomach contents would appear to contain a larger proportion of orders with indigestible exoskeletons such as coleoptera (Wheelwright

Table 2. Concentration of total PCBs and TEQs$_{WHO\text{-}Avian}$ calculated for dietary exposure in avian species at the Kalamazoo River based on a site-specific diet and a literature-derived diet.

	Total PCB (mg/kg/d)		TEQ (ng/kg/d)	
	Fort Custer	Trowbridge	Fort Custer	Trowbridge
Eastern bluebird (*Sialia sialis*)				
Kalamazoo Diet	0.017	0.51	2.7	70
Pinkowski (1978)	0.015	0.39	2.3	70
Tree swallow (*Tachycineta bicolor*)				
Kalamazoo Diet	0.12	1.2	5.4	190
Johnson and Lombardo (2000)	0.11	0.33	5.6	50
McCarty and Winkler (1999)	0.070	0.49	1.9	95
House wren (*Troglodytes aedon*)				
Kalamazoo Diet	0.022	0.13	2.5	31
Kale (1965)	0.052	0.50	2.1	89
American robin (*Turdus migratorius*)				
Howell (1940)	0.030	0.41	0.97	52

1986). Sampling of stomach contents from 2001 and 2002 revealed that stones and mollusks were frequently found in the stomachs of nestlings, especially at FC, but large pieces of grit were not observed as frequently in nestlings from TB. Of the nestlings examined at FC (n = 50), 64% contained at least one stone and 10% contained at least one mollusk shell, while at TB (n = 11), 36% of the nestlings examined contained at least one stone and 18% contained a mollusk shell. Seeds of the garlic mustard plant (*Alliaria petiolata*) were also found in the stomachs of eastern bluebird and house wren nestlings from FC ($n = 3$).

Average Potential Daily Dose (APDD)

APDDs for passerine birds were calculated for both total PCB and TEQ$_{WHO\text{-}Avian}$ concentrations of each insect order for a diet based on dietary composition at the Kalamazoo River. Based on the site-specific diet, dietary ingestion of total PCBs and TEQs$_{WHO\text{-}Avian}$ were greatest for the tree swallow and least for house wren at TB (Table 2). Calculations of APDD for total PCBs were 10-, 29-, and 6-fold greater at TB than at FC, and TEQs$_{WHO\text{-}Avian}$ were 35-, 26-, and 16-fold greater at TB than FC in tree swallows, eastern bluebirds, and house wrens.

Soil ingestion based on literature values was also factored into the calculation of the APDD for total PCBs, but TEQ$_{WHO\text{-}Avian}$ concentrations in soils were not measured at the site and could not be factored into the APDD. 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) concentrations comparable to wet weight concentrations in prey. The mass percent of grit in the diet consumed by the bird species were calculated based on the average mass of grit in the stomach of nestlings over the nestling period (tree swallow = 17.2 mg, dw; house wren = 6.2 mg, dw) (Mayoh and Zach 1986). The APDD calculated with soil was not greatly different from the APDD calculated with insects alone, but APDD calculated with soil was greater in all cases. The APDD value for house wrens at TB was the most different with a 4% increase in concentration when soil was added to the calculation of APDD. All other APDD values did not change or increased by only 1–2% when soil was included.

The APDD was also calculated based on dietary composition found in the literature and compared to site-specific calculations (Table 2). Soil ingestion was factored into the calculation of APDD in both the literature diet and the site-specific diet for total PCBs but not for $TEQs_{WHO\text{-}Avian}$. The APDDs based on total PCBs and $TEQs_{WHO\text{-}Avian}$ for the site-specific diet were no more than 4-fold greater than in the literature diet, which suggests that the Kalamazoo River diet was similar to literature diets. The tree swallow diet was the most different from the literature diet in APDD, and the house wren was the only species in which the literature-derived diet yielded APDDs greater than those calculated for the Kalamazoo River. The site-specific dietary composition of the American robin was not quantified in this study, but an APDD was calculated using PCB concentrations of site-specific dietary items combined with a literature-based dietary composition. The APDD of total PCBs and $TEQs_{WHO\text{-}Avian}$ for the American robin were 0.41 mg PCB/kg, ww and 52 ng TEQ/kg, ww, respectively. This suggests that the exposure of the American robin is less than that of the tree swallow and eastern bluebird but more than in the house wren. It should be noted that the APDD calculation for the American robin does not include $TEQs_{WHO\text{-}Avian}$ ingested during the consumption of plant. This is because plant PCB concentrations are extremely low resulting in less than 4% of the total dietary PCB exposure. Similarly, American robin $TEQs_{WHO\text{-}Avian}$ exposure derived from plant consumption should be of an equally low proportion. A subset of plant samples analyzed for coplaner PCBs resulted in concentrations at or below limits of detection. However it should be noted that the American robin diet utilized contains 29% plants (Howell 1940), thus the APDD for $TEQs_{WHO\text{-}Avian}$ in the American robin diet may be slightly underestimated.

Assessment of Risk

Hazard quotients (HQs) were calculated for each location based on the literature-derived APDD and the site-specific APDD. Two different estimates of the NOAEL and LOAEL were calculated from TRVs derived from a laboratory study and from field studies. All HQs at FC for total PCBs were less than 1.0 based on both the NOAELs and the LOAELs. Therefore, only HQs at TB are discussed. Mean values of HQs based the NOAELs and LOAELs for total PCBs were less than 1.0 for all species, except for the tree swallow site-specific diet, which had a HQ of 2.0 based on the most conservative NOAEL (Figure 5). Species were also evaluated based on the upper 95% confidence limit (U95 CL) in order to describe a range of HQs that could be expected for a population. HQ values for all species at the U95 CL ranged from 4.8 for house wrens based on the most conservative NOAEL to 0.17 for house wrens based on the less conservative NOAEL. The HQs for all species based on the U95 CL for the LOAEL ranged from 1.6 for house wrens to 0.02 for house wrens based on the less conservative LOAEL.

HQs based on $TEQ_{WHO\text{-}Avian}$ concentrations followed similar patterns as HQs based on total concentrations of PCBs. HQs for FC were less than 1.0 for all comparisons,

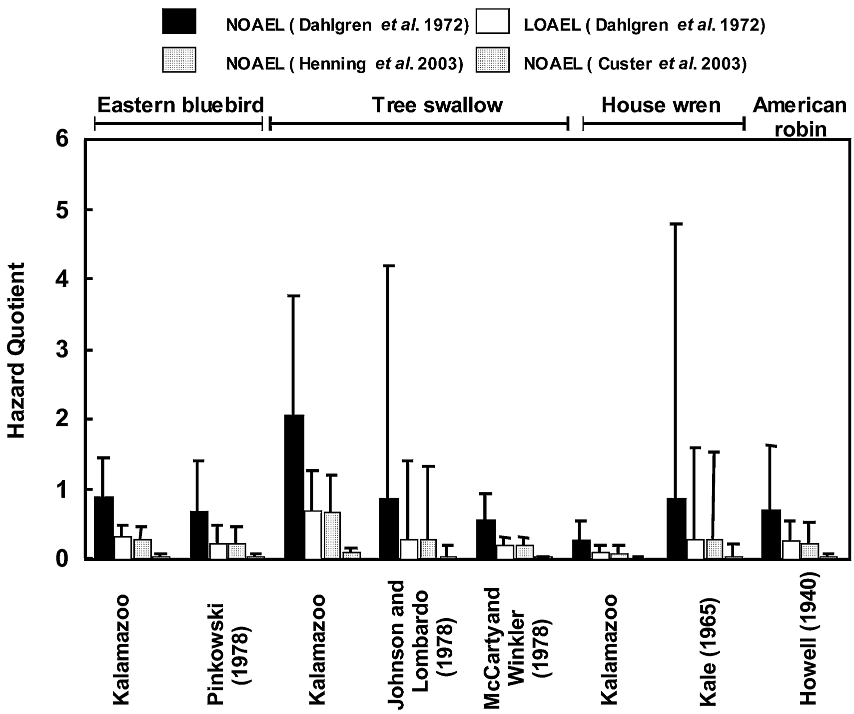

Figure 5. Passerine bird dietary hazard quotients for the Trowbridge Impoundment (Kalamazoo) based on the total PCB no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL). Dietary hazard quotients were calculated based on the Kalamazoo site-specific diet and literature-derived diets. Error bars represent the upper 95% confidence limit. HQs range from approximately 0 to 5.

so only values for TB are discussed. HQ values were greatest for tree swallows, and HQs for only the tree swallow based on LOAEL exceed 1.0 (Figure 6). HQ values based on the most conservative laboratory NOAEL (Nosek *et al.* 1992) exceeded 1.0 in all species, and in tree swallows, the HQ value exceeded 10.0. The mean HQ did not exceed 0.20 based on a less conservative NOAEL calculated from field studies on the tree swallow (calculated from USEPA 2000). Based on the U95 CL of the NOAEL derived from Nosek *et al.* (1992), HQs were as great as 70 in the tree swallow, but the greatest HQ calculated from USEPA (2000) based on the U95 CL was 0.99 for tree swallows.

DISCUSSION

Dietary Composition

The bolus sampling strategy at the Kalamazoo River seemed to accurately predict the general dietary feeding guilds of the species that were being evaluated, but it also identified some unique feeding characteristics of each species. Tree swallows were

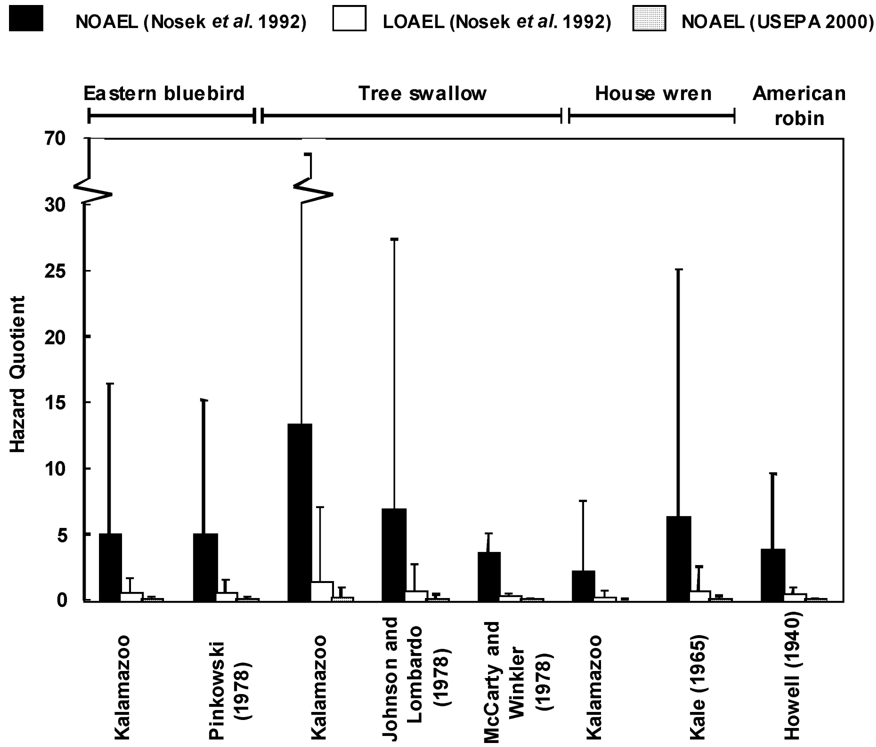

Figure 6. Passerine bird dietary hazard quotients for the Trowbridge Impoundment based on the $TEQ_{WHO\text{-}Avian}$ no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) based on toxicity reference values calculated from the literature. Dietary hazard quotients were calculated based on the Kalamazoo site-specific diet and literature-derived diets. Error bars represent the upper 95% confidence limit. HQs range from approximately 0 to 70.

expected to feed primarily near the water's surface on emergent aquatic insects (Quinney and Ankney 1985; McCarty 1997). The tree swallows at the Kalamazoo River were observed feeding in riparian areas and at the water's surface, and the diet was comprised of a majority of aquatic insects (76.6%). Dipteran species comprise a large portion of the diet in tree swallows (60.6%), which is similar to a tree swallow population in New York (57%) (McCarty and Winkler 1999) and Michigan (58%) (Johnson and Lombardo 2000). Eastern bluebirds fed primarily on insects from terrestrial origins, as was expected (Pinkowski 1978), but there were some deviations from the diet not predicted by the literature. Trichoptera was found in the diet of both tree swallow and eastern bluebird populations at the Kalamazoo River, but was not represented in any of the literature-derived diets (Figure 7). This likely resulted from the close proximity of Kalamazoo populations to an aquatic ecosystem. There is also a notable absence of earthworms in bolus contents of eastern bluebirds inhabiting riparian areas of the Kalamazoo River, which is maybe a result of unsuitable soil conditions for earthworms at study locations. Previously, little was known about

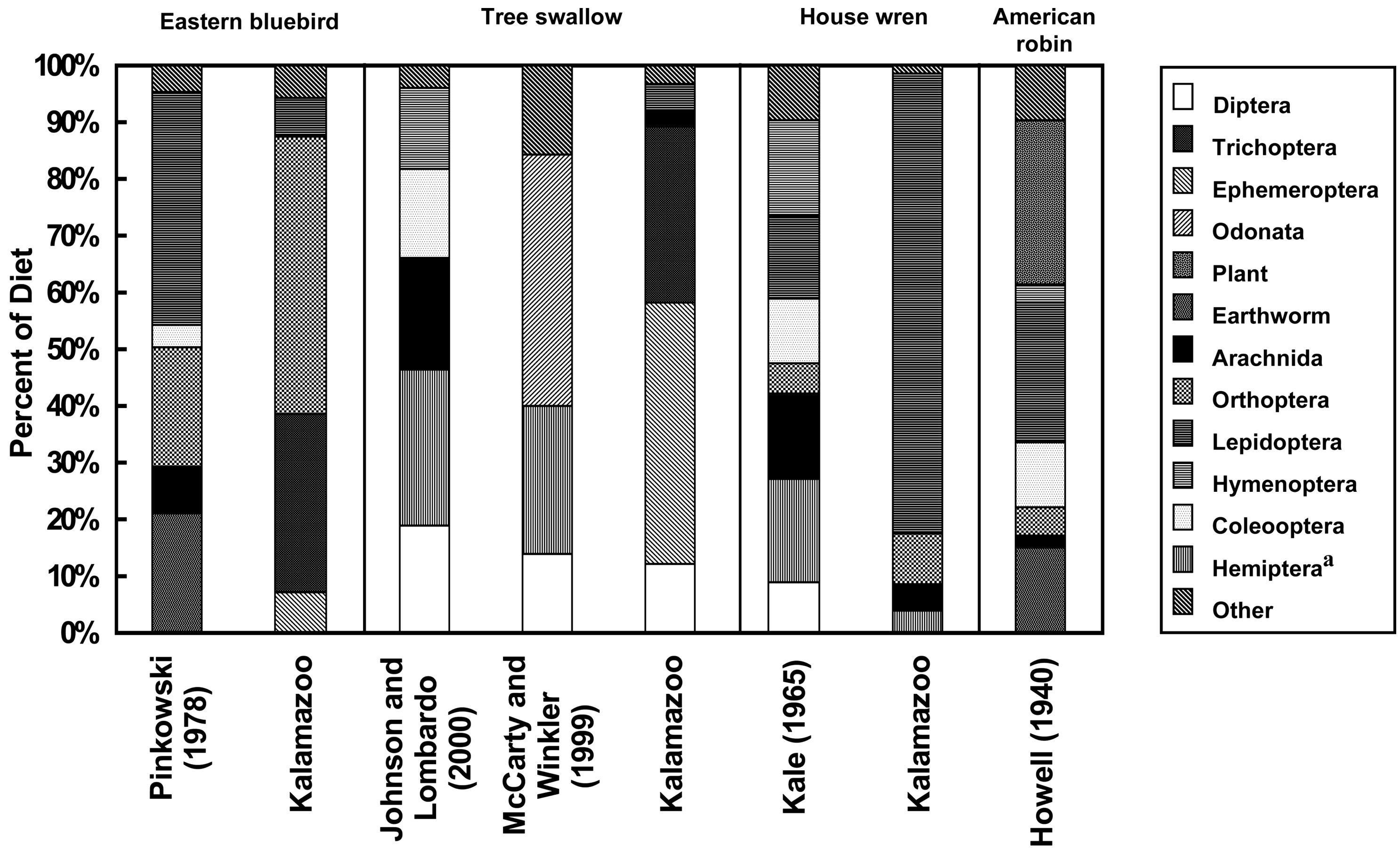

Figure 7. Comparison of literature-derived dietary composition (wet weight) to the site-specific diet (wet weight) for eastern bluebirds, tree swallows, house wrens, and American robins at the Trowbridge Impoundment on the Kalamazoo River. The order hemiptera also includes the order homoptera.

specifics of the house wren diet, but they also fell within the terrestrial feeding guild as predicted.

The differences between the diet of the Kalamazoo River and other studies suggest that although diets of each species may be predictably composed primarily of aquatic or terrestrial prey items, there may be important site-specific interactions and opportunistic feeding events that alter the general diet of a population. In the stomachs of nestlings, there were stones, mollusk shells, bits of glass, and the seeds of the garlic mustard plant, and likewise, metal shavings and plastic have been reported in the stomach of tree swallows (Mayoh and Zach 1986). It is believed that all these items were opportunistically accumulated as sources of grit. Ephemeroptera and trichoptera were found in some boluses of eastern bluebirds at TB, which possibly shows that this species will feed opportunistically. Diets may differ regionally or temporally depending on the amount of opportunistically selected prey. Although these changes did not result in large differences in exposure predictions at our site it is foreseeable that sites where the contaminant dispersion among proximal aquatic and terrestrial habitats are dissimilar, the unique composition of a site-specific diet may contribute significant concentrations to the overall assessment of exposure, and therefore, pose significant risk that may be overlooked when applying literature-based dietary composition to the assessment of risk.

Habitat dynamics may be a potential cause or even a tool to predict site-specific differences in the diet of passerine birds. Habitat is a critical factor in the determination of the diet of key receptors and the eventual determination of risk to those receptors. For example, Blancher and McNicol (1991) examined tree swallow diet in relation to wetland acidity. In wetlands with a high pH, tree swallows predominately preyed on mollusks and ephemeroptera, but in wetlands with low pH, they preyed on a greater percentage of diptera. The adult tree swallows also fed nestlings fewer aquatic orders relative to terrestrial orders in areas of wetland acidity, which was possibly linked to the inability of low calcium aquatic prey to satisfy the calcium requirements of nestlings (St. Louis and Barlow 1993). In a case such as this, the exposure of tree swallows through terrestrial pathways may be much more important than in a typical area. When diets are predicted instead of measured, interactions between habitat and prey availability must be identified. Only by measuring site-specific dietary composition or prey availability can these interactions be taken into account.

Average Potential Daily Dose

The intake of grit, or soil ingestion, was factored into calculations of the APDD as a conservative approach to calculating risk. Concentrations present in the soil are greater than concentrations in insects (soil = 4.3 mg PCB/kg, ww; insect = 0.55 mg PCB/kg, ww), so the contribution of soil to overall exposure could be substantial depending on the amount of soil ingested and the bioavailability of the PCBs through absorption in the gut. The majority of the exposure to PCBs through grit ingestion is not expected to be from grit itself, which is composed of stones, mollusk shells, and sand (Mayoh and Zach 1986), but from soil associated with the surface of the grit. Tree swallows feed aerially, so the only soil present in the diet would exist as incidental particles on prey or would be consumed in conjunction with grit particles.

In addition to grit ingestion, eastern bluebird, house wrens, and American robins may ingest some additional soil particles from the ground as they are feeding, but American robins likely have the greatest soil ingestion of all species due to the ingestion of earthworms with soil in the gut and soil adhering to the surface of the earthworms. This exposure pathway could potentially yield extensive exposure in species consuming large amounts of soil, so soil intake should be carefully considered when evaluating exposure in various species.

The APDD based on a theoretical diet derived from the literature was an underestimate of the site-specific diet in most species. The underestimation can be attributed to the proportion of aquatic insects in the diet at the Kalamazoo River, relative to that of the literature diet. The aquatic insects contain some of the greatest concentrations of PCBs for insects of the Kalamazoo River (Kay *et al.* 2005), which results in the greater concentrations of PCBs in the site-specific diet. Only for the house wren did the literature diet contain greater concentrations than in the diet of the Kalamazoo River. Lepidoptera and orthoptera contained lesser concentrations of total PCBs and $TEQs_{WHO\text{-}Avian}$ than all insect orders (Blankenship *et al.* 2005). Because the site-specific diet for house wrens was comprised of a much greater proportion of lepidoptera and orthoptera (90% by mass) than did the literature diet (20% by mass), the APDD calculated from the site-specific diet was also less than the literature diet. The differences in the proportion of insects may be a result of the fact that a literature diet could not be located for the house wren, so a published diet for the marsh wren was used (Kale 1965). The differences between the diets does not greatly effect the estimate of risk based on the mean concentration in the diet, but based on the U95 CL, the most conservative HQ is 0.53 from the site-specific diet and 4.8 from the literature diet. Depending on the weight given to this line of evidence during the risk evaluation process, a very different estimate of risk may be reached, so it is critical to establish a diet specific to the species.

Relationship Between Risk Estimates Based on Total PCBs and TEQs

Hazard quotients based on $TEQs_{WHO\text{-}Avian}$ were greater than those based on total concentrations of PCBs. HQs based on the total PCBs is thought to be a more accurate estimate of possible risk because the concentration can be compared directly to values reported in the studies from which TRVs were derived. There are difficulties and uncertainties with assessing the toxicity of environmentally weathered PCB mixtures that are quantified as Aroclors. Congener-specific analyses, including coplanar PCB congeners combined with a calculation of TEQs, are generally thought to correlate better with toxicity than measures of total PCBs (Giesy and Kannan 1998; Blankenship and Giesy 2002). However, recent work by Custer *et al.* (2005) calls into question whether toxic equivalency factors (TEFs) developed for PCBs are appropriate to predict effects in some bird species. One reason for the possible overestimate of risk posed by complex mixtures of PCBs is that concentrations of TEQs are calculated by multiplying each aromatic hydrocarbon receptor (AhR)–active PCB congener by a relative potency expressed as a TEF. TEF values are consensus values that were rounded up to be conservative estimates of risk (van den Berg *et al.* 1998). Thus, they tend to overestimate the risk. This coupled with the use of proxy values for congeners that were present at concentrations less than the method detection limit were the most likely reasons that HQs estimated based on $TEQs_{WHO\text{-}Avian}$ were much greater than those estimated based on total PCBs. For example, based on tree swallow studies on the Woonasquatucket River, an LC50 based on TEQs was estimated to be 1700 pg TEQ/g, ww (primarily due to TCDD) (Custer *et al.* 2005). However, if one compares this LC50 to concentrations of TEQs (calculated from PCBs) between 1,730 and 12,700 pg TEQ/g, ww in tree swallow eggs from the Hudson River, one would expect considerable population-level effects due to mortality. However, there were minimal effects on subtle endpoints at TEQ concentrations (based on PCBs) in tree swallow eggs from the Hudson River (McCarty and Secord 1999). In other words, a concentration of TEQ was not toxicologically equivalent to the same concentration expressed as calculated TEQs (based only on PCBs).

Assessment of Risk Using Multiple Lines of Evidence

The study presented here was part of a larger study to examine PCB exposure and associated risk in aquatic and terrestrial ecosystems. In particular, risk associated with dietary exposure to total PCBs and TEQs, or the "bottom-up" approach, determined that based on the most conservative HQs, there appears to be risk to passerine species exposed through the diet due to dietary concentrations of TEQs being greater than the threshold for effects. These values suggest that effects may occur due to exposure to non-*ortho* and mono-*ortho* PCBs at the Kalamazoo River (USEPA 1998). When the more realistic, field-based TRVs are applied, the HQs are near to or less than 1.0, which suggests little risk of the population exhibiting reproductive effects. The lack of knowledge about sensitivity of passerine birds compared to other well-studied species suggests that for understudied passerines in particular, HQs based on total PCBs may be a more appropriate estimate of risk than those based on TEQ concentrations. As suggest by the multiple lines of evidence approach (Fairbrother 2003), additional lines of evidence besides dietary exposure should be evaluated in order to arrive at the best estimate of risk, especially given the uncertainty in selecting appropriate TRVs.

In addition to the "bottom-up" approach, a secondary line of evidence used the "top-down" approach to evaluate egg, nestling, and adult tissue concentrations in tree swallows, eastern bluebirds, house wrens, and American robins from the Kalamazoo River. This line of evidence suggested little risk to passerines based on tissue concentrations of total PCBs and TEQs at the site. Similar field studies completed at other sites found no effects on the reproductive fitness of passerine birds at concentrations of PCBs as great or greater than those measured in birds of the Kalamazoo River (Secord and McCarty 1997; Custer *et al.* 2003).

A third line of evidence was used to investigate population health of passerine species in contaminated portions of the Kalamazoo River compared to an upstream reference location. Studies of reproductive performance did not find statistically significant decreases in reproductive fitness of tree swallows at the more contaminated TB location relative to the FC reference location. However, there were some measures of reproductive success in the eastern bluebird (productivity) and house wren (clutch size, brood size, and hatching success) that were statistically less at TB than FC, but these differences were not found consistently across all of the reproductive measures or throughout the entire nesting season (Neigh *et al.* 2006d). Also, samples sizes were small for eastern bluebirds, and so, a 10% decrease in reproductive success at the contaminated location during the study can be linked to a single female who made two unsuccessful reproductive attempts during one year.

Guidelines based on criteria established for describing the chemical causation for effects in ecoepidemiological studies were applied to this study to determine whether PCBs likely caused the observed reproductive effects. They include temporality, strength of association, consistency, and biological plausibility (Hill 1965). PCB contamination remained constant throughout the study but reproductive success varied over the course of the field season, among years, and among species, likely due to normal population variability, so the first criterion requiring consistent trends over the course of exposure (temporality) was not satisfied. Also, strength of association between PCB exposure and reproductive effects could not be identified. Some individuals with comparatively great concentrations of PCB reproduced successfully, whereas other individuals with background levels of contaminants failed to reproduce. Consistency between results of available studies is also needed in order to assign causality to PCBs for reproductive dysfunction in passerine birds. Few studies have investigated dosing in a laboratory setting in these species, but there are several studies available describing PCB concentrations in the field. Other field studies of passerine species did not find significant reproductive effects when concentrations of PCBs in tissues were similar or greater than in the birds at the Kalamazoo River (McCarty and Secord 1999; Harris and Elliot 2000; Henning *et al.* 2003), which suggests that PCBs are not the primary causative agent of effects. It is biologically plausible based on laboratory and field studies that PCBs deposited in the sediments can bioaccumulate up the food chain and elicit effects on the reproduction of upper trophic level species (Giesy *et al.* 1994). PCBs seem unlikely to be the primary cause of the observed reproductive effects based on the criteria described due to the lack of temporality, strength of association, and consistent findings of no effects at concentrations similar or greater than those present at the Kalamazoo River. Although it cannot be denied that PCBs cause reproductive impairments at certain concentrations, the below-threshold concentrations present in the tissues and diet at the Kalamazoo River suggest that other factors such as co-contamination by DDT and its metabolites, habitat quality, inclement weather, or prey abundance may be affecting reproduction.

There are several potential reasons for different conclusions between the lines of evidence. The evaluation of risk from tissue and dietary concentrations depends heavily on the selection of the TRV. Little data for these species exist based on concentrations of total PCBs, but even less data exist on the dose-response of $TEQs_{WHO\text{-}Avian}$ in the tissue or diet and their relation to reproductive effects. Upon selection of a TRV, uncertainty factors are often applied to compensate for unknown differences between species, exposure time, or exposure route (Chapman *et al.* 1998). The application of these factors is often an inexact science and can introduce a negative bias into the proper calculation of TRVs, which would then result in an overestimate of risk. In particular, in the case of PCBs, the use of total PCB concentrations as a measure or exposure, instead of TEQs seems to more accurately represent the actual risk based on field measures of effects. A strength of this study is that it used measured concentrations of PCBs to quantify exposure in the diet and tissues instead of predicting exposure based on concentrations in other matrices and then applying biomagnification factors to predict other trophic level or life stage concentrations. As for the population health line of evidence, observations of reproductive performance may also be affected by environmental stressors, which may be confounded with exposure to PCBs.

CONCLUSION

The main purpose of this study was to quantify exposure pathways for four passerine birds and determine risk associated with exposure. As expected, the diet of the tree swallow consisted of primarily aquatic insects, whereas the diets of the eastern bluebird and house wren consisted of primarily terrestrial insects. Aquatic insects contained greater concentrations of total PCB and TEQs than terrestrial insects, and therefore, dietary exposure to PCBs was calculated to be the greatest in tree swallows. Risk varied with the TRV selected, but risk for total PCBs was generally below the threshold for effects. Risk associated with TEQs was below the threshold for effects when the most appropriate TRV was applied, but when the most conservative TRV was selected, all species were above the threshold for effects. Risk based on dietary exposure to passerine species is uncertain due to the lack of sound laboratory derived effects data in the literature for passerine species. There is a serious need to fill these data gaps in the literature, especially given the frequent use of bird species in risk assessment. Another primary goal of the study was to test the assumption that “top-down” and “bottom-up” assessments arrive at similar estimates of risk. The study also was an exercise in the application of a multiple lines of evidence approach to risk evaluation. Many factors can play a role in the calculation of risk, and by selecting only one method, there is a possibility of missing an important interaction and improperly characterizing a site. Three lines of evidence based on reproductive health, tissue concentrations, and dietary exposure arrived at differing conclusions of risk. Overall, the various lines of evidence suggest that the inconsistent differences in reproductive performance observed for some species of passerine birds at the more contaminated site were caused by factors other than exposure to PCBs. In addition, the top-down approach (concentrations measured in the tissues of the birds) suggested little risk to any species, but the bottom-up approach (exposure predicted based on the diet) arrives at different conclusions of risk depending on the TRV selected and whether they were based on concentrations of total PCBs or TEQs. When exposures were predicted through application of biomagnification factors, uncertainty factors, or if the appropriateness of the TRV is in question, the actual dynamics at the site can become even more difficult to characterize. As suggested by Fairbrother (2003), the application of several lines of evidence in multiple matrices seems to be the best approach to gather the most complete and appropriate information on which to gauge important risk decisions. By considering all of the lines of evidence simultaneously, it was concluded that the current concentrations of PCBs in the aquatic and terrestrial food chains were not causing population-level adverse effects on the populations of passerine birds studied here.

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