This is to certify that the
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PASSERINE PRODUCTIVITY AND PCB EXPOSURE AT
THE KALAMAZOO RIVER SUPERFUND SITE, MICHIGAN

presented by

Arianne Marie Neigh

has been accepted towards fulfillment
of the requirements for the

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Major Professor's Signature

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Date

PASSERINE PRODUCTIVITY AND PCB EXPOSURE AT THE KALAMAZOO RIVER SUPERFUND SITE, MICHIGAN

By

Arianne Marie Neigh

A DISSERTATION

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ABSTRACT

PASSERINE PRODUCTIVITY AND PCB EXPOSURE AT THE KALAMAZOO RIVER SUPERFUND SITE, MICHIGAN

By

Arianne Marie Neigh

The Kalamazoo River basin contains soils and sediments contaminated with polychlorinated biphenyls (PCBs). In an effort to assess risk to wildlife species, studies in support of an ecological risk assessment were conducted using four passerine species. Risk to the tree swallow (*Tachycineta bicolor*), eastern bluebird (*Sialia sialis*), house wren (*Troglodytes aedon*), and American robin (*Turdus migratorius*) were quantified using three lines of evidence. These included, the bottom-up approach (dietary exposure assessment), top-down approach (tissue-based exposure assessment), and measurements of population health. Goals of the studies were to: quantify risk in the passerine receptors; evaluate various approaches to the risk assessment process; advance the scientific understanding of passerine exposures and effects from this class of compounds utilizing field-based methodologies; provide site-specific data to aid risk managers in making effective remedial decisions; and provide baseline data to evaluate remedial decisions.

Overall, the lines of evidence with the highest certainty arrived at similar conclusions based on studies consisting of 144 nest boxes spread equally between a downstream location contaminated with PCBs and an upstream reference site with background levels

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of contamination. The top-down approach, which investigated tissue concentrations of PCBs contained in eggs, nestlings, and adults of each bird species, found concentrations in tissues at the contaminated location to be significantly greater than concentrations at the reference site. Concentrations of PCBs in tissues were below the threshold at which effects would be expected, and so, little risk was associated with concentrations in tissues. The bottom-up approach quantified concentrations of PCBs in the diet of the avian species. Concentrations of PCBs in the diet at contaminated locations were 6 to 35-fold greater than at background locations. Risk associated with dietary exposure was below the threshold for effects when contaminants were quantified as total PCBs. Dietary PCB exposures converted to a measure of dioxin-like toxicity exceeded one of two dietary threshold values. However, the confidence associated with these thresholds was low and ultimately suggested the presence of risk due to dietary exposure is low. Dioxin-like toxicity approaches based on dietary exposure were highly dependent on the selection of threshold values. In this instance, little data was available to derive species-specific thresholds, which introduced uncertainty to the calculation of risk. The final line of evidence suggested little harm to population health due to PCB exposure. Based on these studies, it is unlikely that the four passerine species examined are experiencing adverse effects from PCBs present in the local environment. It should be noted that at many sites similar to this one, a single line of evidence, most often dietary exposure, is utilized to derive risk conclusions. This study highlights the necessity of utilizing a site-specific, weighted, multiple lines of evidence approach to arrive at the best estimate of risk.

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ACKNOWLEDEMENTS

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KEY TO ABBREVIATIONS

Dry weight..dw
Fort Custer State Recreation Area..FC
Kalamazoo River Area of Concern......................................KRAOC
Number..n
Polychlorinated biphenyl..PCB
Standard deviation...SD
Trowbridge Impoundment..TB
2,3,7,8 -tetrachlorodibenzo-p-dioxin...................................TCDD
2,3,7,8 -tetrachlorodibenzo-p-dioxin equivalents.................TEQ
Wet weight...ww
Part per billion..ppb

Units of measure
Kilogram...kg
Gram...g
Milligram...mg
Microgram...µg
Nanogram..ng
Picogram...pg
Days..d
Hours...h
Years...yr

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Chapter 1

Productivity of tree swallows (*Tachycineta bicolor*) exposed to PCBs at the Kalamazoo River Superfund Site

Arianne M. Neigh,[†] Matthew J. Zwiernik,[†] Monica A. MacCarroll,[†] John L. Newsted,[†‡] Alan L. Blankenship,[†‡] Paul D. Jones,[†‡] Denise P. Kay,[‡] John P. Giesy,[†§]

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ABSTRACT

A 123 km stretch of the Kalamazoo River in Michigan, USA, was designated a Superfund site in 1990 due to historical releases of effluent containing polychlorinated biphenyl (PCB) contaminated paper waste. Risk to bird species in the river ecosystem was evaluated using the tree swallow (*Tachycineta bicolor*) as a monitor for possible effects due to PCB exposure at nesting locations. There were no statistically significant differences in hatching success, fledging success, productivity, predicted brood size, predicted number of fledglings, or growth of nestlings between the Kalamazoo River Superfund Site and an upstream reference location with lesser concentrations of PCBs in the sediments and riparian soils. However, in two of the three years of the study, clutch size at the contaminated location was 3.7 ± 1.4 and 4.8 ± 0.73 eggs per nest (mean ± SD), which was significantly less than the clutch size at a reference location (5.0 ± 1.1 and 5.3 ± 1.1 eggs per nest). The results of this study indicate that tree swallows exposed to PCBs in the Kalamazoo River may have decreased clutch size, but productivity and recruitment were not statistically different between sites. Therefore, tree swallow populations in the contaminated area had overall reproductive success that was not impaired by exposure to PCBs at a level that overcame the natural variation inherent in tree swallow populations.

Keywords: Reproductive success, Polychlorinated biphenyls, Aquatic food chain, Birds

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INTRODUCTION

In 1990, 123 km of the Kalamazoo River in southwest Michigan was designated a Superfund site based on the presence of polychlorinated biphenyls (PCBs) in multiple matrices including fish, sediments, and floodplain soils. PCBs have been linked to adverse effects in numerous avian species (Giesy *et al.*, 1994). These effects include reproductive dysfunction (Dahlgren *et al.*, 1972; Britton and Huston, 1973; Lillie *et al.*, 1974; Ludwig *et al.*, 1993; Halbrook *et al.*, 1998) , embryonic deformities (Ludwig *et al.*, 1993; Summer *et al.*, 1996), and growth impairment (Lillie *et al.*, 1974; Ludwig *et al.*, 1993). As part of this multiple lines of evidence approach, we evaluated the effects of PCBs on behavior and productivity of resident tree swallow populations on the Kalamazoo River.

Passerine birds, particularly tree swallows (*Tachycineta bicolor*), have been effectively utilized as monitors of exposure to environmental contaminants. The tree swallow has been frequently used as a contaminant monitor in North American riverine and lacustrine systems, which has resulted in a large information base on accumulation of PCBs by tree swallows including bioavailability, diet, tissue concentrations, and subsequent effects on reproduction and reproductive behavior (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.*, 2003). The tree swallow has even been termed a "new model organism" for its frequency of use and favorable life history traits (Jones, 2003). In this study, the tree swallow was selected as a representative for passerine species exposed to sediment-derive

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derived PCBs through the aquatic food chain because of a number of distinct attributes including the presence of a previously established nest box population at the baseline location; an abundance of tree swallows residing throughout the river basin; and a large database of information on similar PCB exposure and productivity studies. As a PCB tolerant species, the tree swallow is a useful monitor of PCB contamination (McCarty and Secord, 1999). The tree swallow also has a small feeding range of approximately 0.1 km (McCarty and Winkler, 1999) to 5 km (Robertson *et al.*, 1992) during the nesting period, which assures that pairs nesting near the river will feed on prey that are in direct contact with PCB-contaminated sediment. Tree swallows are insectivorous, typically feeding on emergent aquatic insects just above the water surface (Quinney and Ankney, 1985; Cohen and Dymerski, 1986; McCarty, 1997). Tree swallows readily nest in boxes and are tolerant of human disturbances during nesting (Rendell and Robertson, 1990). Researchers have used tree swallows for a variety of studies without a notable occurrence of observer-induced abandonment.

Concentrations of PCBs were significantly greater in tree swallow eggs and nestlings within the Kalamazoo River Superfund Site, as compared to concentrations in birds from an upstream reference location (Neigh *et al.*, 2004). The risk of PCB-induced effects was determined by comparing site-specific measured concentrations of PCBs in bird tissues to toxicity reference values (TRVs) determined in controlled laboratory studies with surrogate species (Neigh *et al.*, 2004). However, extrapolating from laboratory experiments to tissue concentrations observed in the field has inherent uncertainties (USEPA, 1998). Thus, in conjunction with the tissue-based assessment of risk, this study monito

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monitored tree swallow reproductive and developmental health on the Kalamazoo River directly by measuring population-level responses, including fecundity, fertility, and weight gain of nestling tree swallows exposed to PCBs in their diet, from contaminated sediments and riparian soils.

MATERIALS AND METHODS

Site details

Two sites within the Kalamazoo River floodplain were selected to study nesting tree swallows, the Fort Custer State Recreation Area (FC) and the former Trowbridge impoundment (TB) (Figure 1.1). The FC reference or baseline location is situated 48 km upstream of known sources of PCBs between the villages of Augusta and Galesburg in southwest Michigan. The site, which is comprised primarily of sandy oldfield grasslands interspersed with maple-beech forest, was the most similar to the TB impoundment in habitat and proximity to the river's course. Michigan State University and the Kalamazoo Nature Center have conducted nest box studies on the site for the last 30 yr, and the tree swallow population has demonstrated normal levels of reproductive success (R. Adams, personal communication).

Figure 1

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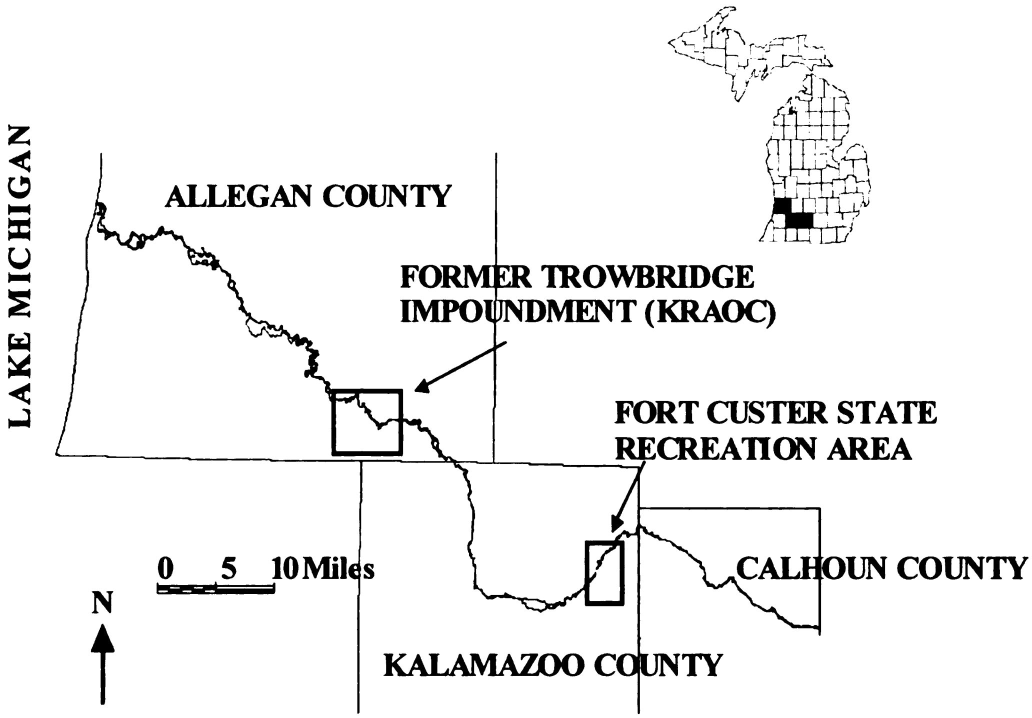

Figure 1.1. Map of the Kalamazoo River Area of Concern (KRAOC) and reference site. The inset describes the location of the two counties in Michigan where reproductive studies were conducted. A box designates the boundaries of the upstream reference location (Fort Custer State Recreation Area) and the Trowbridge Impoundment, located within the KRAOC.

Concentrations of PCBs and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs), based on World Health Organizations toxic equivalence factors for birds ($TEF_{WHO\text{-}Bird}$) (van den Berg *et al.*, 1998), in tissues of tree swallows at the FC reference location are reported elsewhere (Neigh *et al.*, 2004). The following concentration data are from the reference location and are reported on a wet weight (ww) basis. Mean concentrations of PCBs and TEQs in eggs were 810 ng PCB/g (ppb), (n = 19) and 56 pg/g, (n = 12), respectively. Mean concentrations of PCBs and TEQs in nestlings were 460 ng PCB/g, (n = 12) and 20 pg TEQ/g, (n = 12), respectively. Mean concentrations of PCBs and TEQs in adults (n = 2) were 1500 ng PCB/g and 220 pg TEQ/g, respectively.

The target study area (TB) is located upstream of the former Trowbridge Dam Impoundment near Allegan, MI. The study area was wholly within the Kalamazoo River Area of Concern (KRAOC), which is designated as a Superfund site by the US Environmental Protection Agency. The TB dam was removed to its sill in 1986, which resulted in the exposure of a large and contiguous landmass of former lake bottom, on which nest boxes were established. The former Trowbridge impoundment includes approximately 132 ha of currently exposed former sediments flanking 7 km (70 ha) of remaining impounded water. The TB study area is approximately 67 km downstream from the reference area and was considered a worst case scenario for exposure to wildlife. The former sediments have been minimally developed, so the extensive floodplain remains under natural vegetation of lowland forest, wet grassland, lowland shrub, and emergent marsh. Mean concentrations of total PCBs in eggs, nestlings, and adults were 5100 (n = 14), 3100 (n = 13), and 8700 ng PCB/g (n = 9), ww, respectively (Neigh *et al.*, 2004). The presence of non-*ortho* and mono-*ortho* PCB congeners, reported as $TEQ_{WHO\text{-}Bird}$, were found to be 760 (n = 12), 600 (n = 13), and 2200 pg TEQ/g (n = 9), ww in eggs, nestlings, and adults, respectively (Neigh *et al.*, 2004).

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Productivity and attentiveness observations

In April 2000, nest boxes were placed at both the former Trowbridge Dam Impoundment (TB) and the Fort Custer State Recreation areas (FC). At the FC site, nest boxes (n = 38) had been previously established during long-term studies, but an additional 26 boxes were added for a total of 64 nest boxes. The 2000 field season was the first year boxes were available for nesting throughout the TB area (n = 68), so occupancy rates at this site were expected to be less than the established FC site. For this reason, 2000 was designated a baseline year to establish nesting populations in the newly placed boxes at both FC and TB. The establishment and weathering of boxes in 2000 was intended to improve the correlation of data gathered from individuals nesting in the newly erected boxes and individuals utilizing the previously established nest boxes at the FC site.

Nest boxes were selectively placed in open/prairie grassland habitats to increase occupancy rates by secondary-cavity nesting passerine species. All boxes were placed within the floodplain, not more than 200 m from the river. Two types of boxes with different dimensions were placed on greased metal poles. One set of spruce-pine-fir-constructed boxes had internal dimensions of 10 x 14 x 23 cm, while cedar-constructed boxes had internal dimensions of 8 x 18 x 40 cm. Wire mesh predator guards surrounded the entrance hole and extended 6 cm from the box. Beginning in early April, boxes were checked every third day for the initiation of nest building. Once nest construction was complete, the nest was checked daily to determine date of clutch initiation, which was reported as Julian days. Within 24 h of laying, the mass and length of eggs were recorded, and each egg was given a unique identification code with a permanent marker.

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Clutch size was determined as the total number of eggs laid by a single female. Incubation was determined by touch. Warm eggs indicated incubation, while nests with no defending adults and cold eggs for 7 d were considered abandoned. Upon completion of the clutch, the nest was checked every few days until the end of the 14-d incubation period, at which time, the nest was again examined daily to determine day of hatch. Day of hatch was also confirmed by assessing physical development of the nestling. Each nestling was examined for gross morphological abnormalities. With hatch day designated as day 0, weight of each nestling was recorded on days 3, 9, and 12 as an approximation of growth. Nestling body measurements were also used to approximate growth. On day 12, chicks were weighed and the length of the entire body (tip of beak to longest retrix), tarsus (tibiotarsal joint to hind toe base), and unflattened wing chord were recorded. The sex of individual nestling tree swallows could not be determined due to the nestling's sexually dichromatic plumage, but when possible, female age was determined according to plumage characteristics described by (Hussell, 1983).

The study's sampling design, in which eggs were removed after clutch completion for residue analyses, prevented the brood size (number of nestlings at hatch) and the number of fledglings from being determined directly, so we calculated a predicted brood size and number of fledglings based on reproductive success measurements. Predicted brood size was calculated as the product of the clutch size and hatching success. The number of fledglings was calculated as the clutch size multiplied by the productivity, which is a product of hatching and fledging success. Hatching success was determined as the number of nestlings at the time of hatching per egg laid. Sampled eggs were excluded from t

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from the analyses because the viability of the eggs was unknown at sampling. Fledging success was determined as the number of fledglings divided by the number of nestlings at the time of hatch. It was assumed that all nestlings taken live would have fledged and that all nestlings at the latest nest check had fledged, unless their carcasses were found. One fresh egg or one live nestling was sampled from each nest.

Parental attentiveness of tree swallow adults was assessed during 30-min observation sessions of the nest box. The number of visits by the adults is correlated with the number of feedings (McCarty, 2002). On day 1 through 17 of the nestling period, each box was observed for a minimum of 3 sessions to determine the approximate number of times the adults fed the nestlings. Observations took place during the most active feeding periods between 08:00-12:00 and 18:00-21:00. The number of visits per nestling were averaged over all observations for a box in order to minimize the effect of nestling age and to avoid pseudoreplication. Average nestling age at both locations during nest observations were 9, 8, and 10 days for 2000, 2001, and 2002, respectively.

Statistical Analyses

Meristic data were examined by a combination of parametric and nonparametric statistical procedures, depending on the structure of the data. The normality of each sample set was assessed with the Kolmogorov-Smirnov one sample test with Lilliefor's transformation. Data sets that were determined to have normally distributed values were compared by Student's t-test, or in the case of multiple comparisons, by a one-factor analysis of variance (ANOVA). Data with non-normal distributions and/or unequal varian

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variances were analyzed by Mann-Whitney U or Kruskal-Wallis non-parametric tests for two or multiple samples. All data, regardless of normality and homogeneity of variance, was evaluated for interaction with cofactors based on an analysis of covariance (ANCOVA). Although the assumption of normality was violated in some instances, we do not believe that ANCOVA based on the ranks would have been a more reliable estimate of interaction because of the loss of discriminatory ability inherent in the ranking process. The criterion for significance used in all tests was $p < 0.05$.

Measurements from some nests were excluded from analysis after a disturbance, but measurements taken before the disturbance were included. Several nests were excluded after predation, which was determined by the presence of house wrens near the nest box or when the nest material was disturbed. Abandoned nests were included in the calculation of hatching success, fledging success, and productivity until the point where they were abandoned.

RESULTS

Reproductive success

The mean number of clutches initiated during the study was greater at the Fort Custer State Recreation Area than at the Trowbridge Impoundment. During the three-year study period, 34 clutches were initiated at the TB target site and 71 clutches were initiated at the FC reference site. At TB there were 9, 12, and 13 clutches initiated during 2000, 2001, 2002, respectively, and 20, 24, 27 clutches initiated during the same three-year period at FC

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period at FC. Female age was evaluated at each nest during 2001 and 2002 to address the influence of adult age on reproductive parameters such as clutch size and date of clutch initiation. There was a difference between years in the proportion of second year (SY) to after second year (ASY) females during 2001 (Mann-Whitney U, $p = 0.038$) but not during 2002. SY females were in their first breeding season and ASY females were in at least their second breeding season. TB had a greater number of SY birds during 2001 compared to 2002 and compared to FC during both years (Figure 1.2). Female age was not a statistically significant cofactor for any reproductive parameters (ANCOVA, $p > 0.05$).

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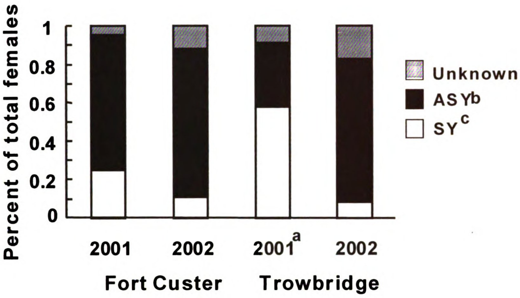

Figure 1.2. Percentage of the total population in each year represented by each female age class at the Fort Custer (FC) reference location and the Trowbridge (TB) contaminated location.

[a]Mean female age was significantly different from the Fort Custer reference population (Mann-Whitney U, $p = 0.038$).

[b]After second year (ASY) birds were in at least their second breeding season.

[c]Second year (SY) birds or subadults are in their first breeding season.

Tree swallows produce a single brood annually, but may renest when nests are predated (Kuerzi, 1941). Several incidences of renesting were probable at FC. For one nest box, a pair appeared to have raised two successful broods for two consecutive years, but this was not confirmed since the adults were not captured for banding. All nesting attempts were assumed to be independent events because adults were not banded at FC or TB.

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Clutch size differed significantly between years at each site, so years were analyzed separately. Mean clutch size at FC was significantly greater than at TB during 2000 and 2002 (Mann-Whitney U, $p < 0.05$) (Table 1.1). Date of clutch initiation was a significant covariate with clutch size in 2000 and 2001 but not 2002. Even when date of initiation was included as a co-factor, clutch size was still not significantly different between sites in 2001. All data sets violated assumptions of normality, and therefore, were evaluated by nonparametric tests. No measures of productivity, besides clutch size, were significantly different between sites in any year. The predicted number of nestlings and predicted number of fledglings were also not significantly different between sites in any year (Mann-Whitney U, $p > 0.05$). Although non-normal distribution prevented parametric tests, female age and date of clutch initiation were evaluated for covariation with reproductive parameters. They described some of the variation in the data sets, but no cofactor interacted with reproductive parameters to such a degree that the significance of the test statistic changed. The majority of nest failures at FC during 2002, and over the course of the study, were due to competition by house wrens. At TB, about 57% of the nest failures were due to abandonment and 14% to predation (Table 1.2).

Table 1.1. Reproductive productivity measurements (mean (SD)) for tree swallows at the reference site (FC) and at a PCB contaminated target site (TB) at the Kalamazoo River Area of Concern (KRAOC).

	2000				2001				2002			
	N	FC	N	TB	N	FC	N	TB	N	FC	N	TB
Hatching success[a]	20	0.77 (0.26)	9	0.68 (0.41)	23	0.77 (0.26)	10	0.83 (0.19)	17	0.71 (0.26)	13	0.64 (0.36)
Fledging success[b]	19	1.00 (0.00)	7	1.00 (0.00)	22	0.96 (0.21)	9	1.00 (0.00)	17	0.94 (0.13)	11	0.98 (0.08)
Productivity[c]	20	0.77 (0.26)	9	0.68 (0.41)	23	0.74 (0.30)	9	0.81 (0.20)	17	0.66 (0.26)	13	0.62 (0.35)
Clutch size	20	5.0 (1.1)	9	3.7 (1.4)[d]	24	5.3 (0.91)	11	5.3 (0.79)	27	5.3 (1.1)	13	4.8 (0.73)[e]
Predicted brood size	19	3.9 (1.1)	7	3.5 (1.4)	22	4.3 (1.3)	10	4.3 (1.2)	17	3.9 (1.4)	11	3.8 (1.6)
Predicted fledglings per nest	19	3.9 (1.1)	7	3.5 (1.4)	21	4.2 (1.3)	9	4.3 (1.2)	17	3.6 (1.4)	11	3.7 (1.6)

	All years combined			
	N	FC	N	TB
Hatching success[a]	60	0.75 (0.25)	32	0.71 (0.33)
Fledging success[b]	58	0.97 (0.15)	27	0.99 (0.05)
Productivity[c]	60	0.72 (0.27)	31	0.69 (0.33)
Clutch size	71	5.2 (1.0)	33	4.6 (1.1)[f]
Predicted brood size	58	4.2 (1.3)	28	3.9 (1.4)
Predicted fledglings per nest	57	4.0 (1.3)	27	3.8 (1.4)

Table 1.1 (cont'd)

[a]Percent of eggs hatched in each nest.

[b]Percent fledged per nestling hatched.

[c]Percent fledged per egg laid.

[d]Significantly different from the FC reference population (Mann-Whitney U, $p = 0.012$).

[e]Significantly different from the FC reference population (Mann-Whitney U, $p = 0.032$).

[f]Significantly different from the FC reference population (Mann-Whitney U, $p = 0.010$).

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Table 1.2. Nest fate and percent of total nests initiated at the Fort Custer (FC) reference site and the Trowbridge (TB) target site for nests in which eggs were laid.

	2000		2001		2002	
	FC	**TB**	**FC**	**TB**	**FC**	**TB**
# Successful nests[a]	**19 (95%)**	**7 (78%)**	**21 (88%)**	**9 (75%)**	**17 (63%)**	**11 (85%)**
# Abandoned[b]	**1 (5%)**	**2 (22%)**	**1 (4%)**	**0 (0%)**	**0 (0%)**	**2 (15%)**
# Predated[c]	**0 (0%)**	**0 (0%)**	**1 (4%)**	**1 (8%)**	**9 (33%)**	**0 (0%)**
# Other[d]	**0 (0%)**	**0 (0%)**	**1 (4%)**	**2 (17%)**	**1 (4%)**	**0 (0%)**
Total # initiated	**20 (100%)**	**9 (100%)**	**24 (100%)**	**12 (100%)**	**27 (100%)**	**13 (100%)**
Total # of boxes available	**64**	**68**	**64**	**68**	**64**	**68**

[a]Nests in which at least one nestling fledged.

[b]Nests containing eggs and/or nestlings but without an adult present for at least 7 d.

[c]Nests had signs of disturbed nest material or had evidence of tossed or damaged eggs attributable to house wren competition.

[d]Nests with an unknown fate or failed due to disease.

Mean egg lengths and weights are presented for each location (Table 1.3). Mean egg length and weight exhibited a statistically significant interaction (ANCOVA, weight x length $p < 0.001$), but the interaction with clutch size was not significant. There were no significant differences between years at either site, so measurements were combined for multiple years. Average egg weight was significantly greater at TB than FC (Mann-Whitney U, $p = 0.006$). Average length of the eggs was not significantly different between locations.

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Table 1.3. Mean (SD)[a] reproductive endpoints for tree swallow eggs and nestlings (12-d-old) reared at the Fort Custer State Recreation Area and at the Trowbridge Impoundment along the Kalamazoo River Area of Concern.

	Fort Custer	Trowbridge
Eggs		
N	**71**	**34**
Egg weight (g)	**1.80 (0.02)**	**1.86 (0.15)[b]**
Egg length (mm)	**19.0 (0.8)**	**19.0 (0.7)**
Nestlings		
N	**13**	**8**
Body weight (g)	**22.12 (1.47)**	**22.37 (1.87)**
Body length (mm)	**93.8 (8.6)**	**90.8 (6.0)**
Tarsal length (mm)	**13.3 (1.9)**	**13.7 (2.7)**
Wing chord (mm)	**49.9 (11.0)**	**50.9 (5.7)**

[a]Mean values were calculated based on the mean measurements per nest for all nests with eggs or nestlings present during the entire study period from 2000 to 2002.

[b]Mean was significantly different from the Fort Custer reference population (Mann-Whitney U p=0.006).

Date of clutch initiation was significantly different among years at both sites (Kruskal-Wallis, $p < 0.05$) and was significantly different between sites during 2001 and 2002

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(Mann-Whitney U, $p > 0.05$). Median clutch initiation dates were generally earlier at TB than at FC, but the range of dates over which nests were initiated was later at FC. For all years, clutches initiated more than ± 1 SD from the mean at a site were designated as early or late nesters. A total of 7 nests at TB and 13 nests at FC were beyond the cutoff dates during the three-year study period. Upon recalculation of reproductive success to exclude outlying early and late nesters, only the significance of clutch size between sites changed in 2002 ($p = 0.032$ to $p = 0.082$). Thus, differences in clutch initiation dates did not significantly affect the conclusions about other parameters.

Growth and growth curves

Growth parameters of mean weight, tarsal length, wing chord length, and body length for sampled nestlings (12 d) were not significantly different between sites when all sample years were combined (Table 1.3). Differences between years could not be evaluated due to small sample sizes. When growth parameters were evaluated for covariation, body length and wing chord variances were significantly associated, but this did not change the significance of mean growth parameters between sites.

There were few differences in average mass of nestlings between locations when years were combined. Mean nestling mass was greatest on day 11 at both sites. Nestlings at TB weighed more at day 3, although not significantly, but weights between the two sites were very similar by day 11 (Table 1.4). There were no statistically significant differences in nestling weights between sites, except on day 13 when TB nestlings were significantly heavier than FC nestlings (Student's t-test, $p = 0.044$). There were statistically significant differences between years, so sites were also compared within each study year. When sample sizes were sufficient to make comparisons, no comparisons were significant, except nestlings at TB were heavier than FC nestlings on day 5 in 2001 (Student's t-test, $p = 0.041$).

Table 1.4. Tree swallow nestling weight (g) expressed as the mean nestling weight in each nest at the upstream Fort Custer reference area and the former Trowbridge Impoundment from 2000 to 2002.

	Fort Custer		Trowbridge	
	N	**Mean (SD)**	**N**	**Mean (SD)**
Nestling day				
2	**4**	**4.71 (0.46)**	**2**	**4.11 (0.14)**
3	**34**	**5.81 (1.60)**	**17**	**6.26 (1.55)**
4	**7**	**8.45 (2.18)**	**4**	**8.63 (2.78)**
5	**4**	**8.52 (1.70)**	**3**	**10.97 (2.50)**
6	**3**	**14.28 (0.92)**	**1**	**13.72 (NA)**
7	**4**	**17.80 (3.96)**	**3**	**16.99 (3.12)**
8	**2**	**18.75 (1.84)**	**4**	**19.59 (2.63)**
9	**40**	**20.31 (1.97)**	**17**	**19.55 (1.77)**
10	**7**	**21.95 (2.64)**	**4**	**22.27 (1.69)**
11	**7**	**22.97 (0.94)**	**6**	**22.96 (0.98)**
12	**43**	**22.15 (2.34)**	**12**	**22.01 (1.06)**
13	**8**	**22.80 (1.16)**	**5**	**24.08 (0.60)[a]**

[a]Significantly different from reference location (Student's t-test, $p = 0.044$).

NA= Not Available

Over the nestling period at each location, logistic growth curves were fitted to the average nestling mass in each box across all nests. Curves based on a logistic model have been shown to be the most appropriate for describing nestling growth in tree swallows (Zach and Mayoh, 1982). Growth curves were virtually identical between sites and am

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and among years (Figure1.3). Logistic growth curves yielded R^2 values of between 0.90 and 0.93 for the tree swallow. Growth curves between sites were statistically compared based on average nestling mass gain from day 3 to day 10 over the nestling period. Nestling masses were log-transformed, and the mass gain, calculated as the mass gained per day of life, was compared between sites. There were no statistically significant differences in mass gain between sites in any year (Student's t-test, $p > 0.05$) or when all years are combined (Mann-Whitney U, $p = 0.368$).

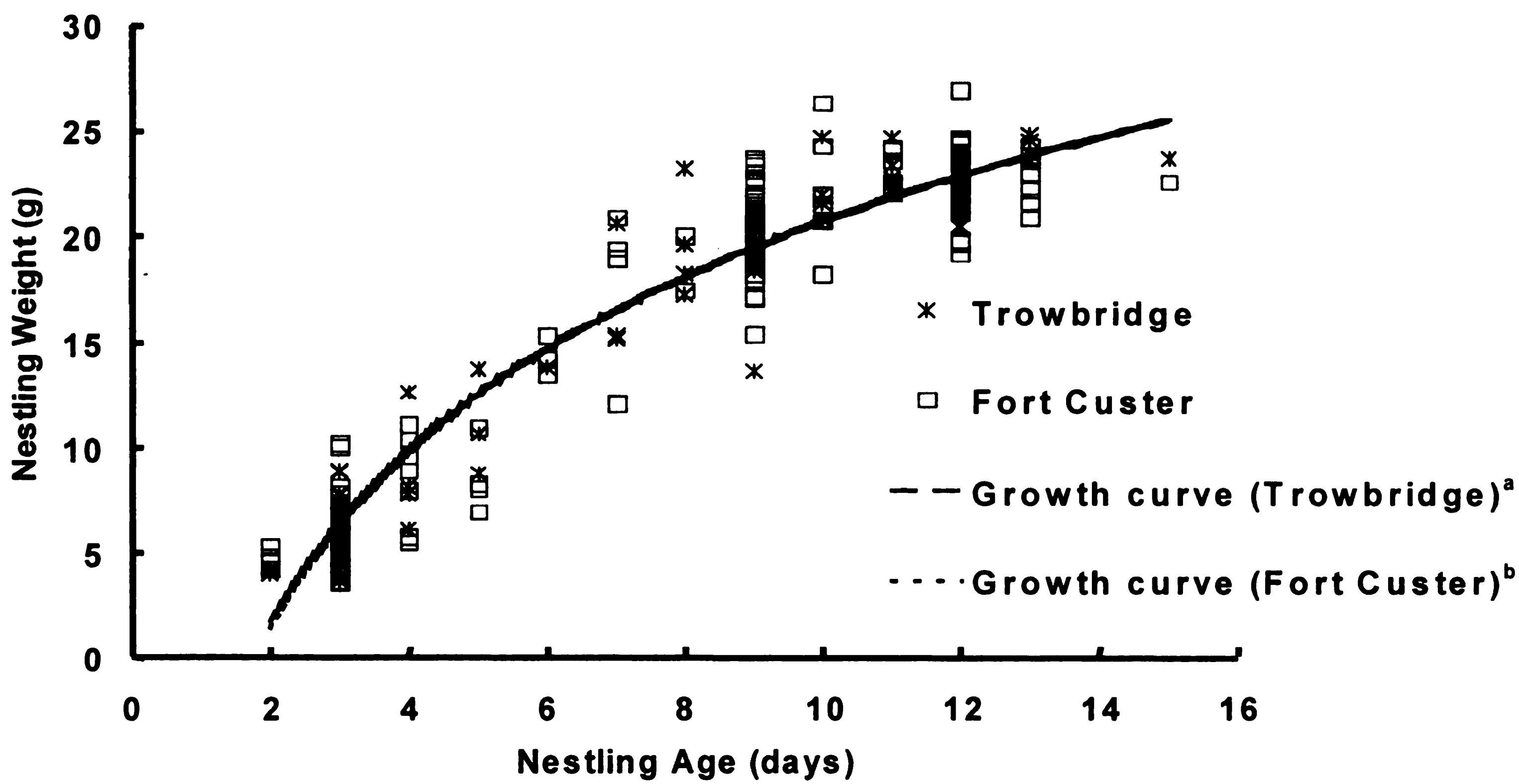

Figure 1.3. Tree swallow (TRSW) nestling growth curves for Fort Custer (FC) reference site and Trowbridge (TB) target site based on mean nestling mass for each nest box.

[a]Logistic growth curve defined by the line of best fit at TB, $y = 11.898Ln(x)-6.6376$, $r^2 = 0.903$.

[b]Logistic growth curve defined by the line of best fit at FC, $y = 11.962Ln(x)-6.9272$, $r^2 = 0.934$.

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Parental attentiveness

At the Kalamazoo River, a total of 127 h of feeding observations were made during a 3-yr period. In order to minimize the effect of brood size on parental attentiveness, the number of visits per 30-min period was normalized to the number of nestlings in each nest. Years were analyzed separately because the mean number of visits per nestling was significantly different between years at TB (ANOVA, $p = 0.002$). When the covariates time of observation, nestling age, and date of observation were factored into the model to compare among years within each site, 2002 remained significantly different from other years at TB (ANCOVA, $p = 0.023$). The mean number of visits per nestling was not significantly different between sites for either 2000 or 2001. However, the number of visits per nestling was significantly different in 2002 (Student's t-test, $p = 0.006$). TB had a mean number of visits per nestling of 0.49 ± 0.37 (± 1 SD) while the mean number of visits for FC was 1.00 ± 0.44 (± 1 SD) in 2002. Weather does not appear to have been a factor explaining this difference because the times and dates of observation were randomly dispersed between sites. No interactions with time, nestling age, or date of observation were significant in any analysis between sites.

Gross morphology and abnormalities

Upon examination of the external morphology of nestlings and contents of eggs, no external developmental abnormalities were observed at any site during any sampling year.

DISCUSSION

Reproductive success

Fewer clutches were expected at TB than FC because FC has had a nesting population established for 30 yrs with adults and offspring returning to nest annually (Kuerzi, 1941; Cohen *et al.*, 1989; Robertson *et al.*, 1992). The number of nests established by adults increased at TB as the study progressed, probably due to the return of birds raised on the site in previous years. Unfortunately, we could not determine the proportion of returning birds in the population because nestlings were not banded. The increase in the number of nesting tree swallows at FC during the course of the study may be a result of the establishment of additional nest boxes at FC during 2000. In addition to the increase in nest establishment, the number of ASY females at TB increased from 2001 to 2002. The large number of SY females during 2001 at TB may be a result of establishing a new nest box trail in 2000 (Robertson *et al.*, 1992). We have dismissed the possibility that the large juvenile population in 2001 was due to PCB-induced reduction in adult survival during 2000. In this study, reproduction, the most sensitive response to PCB exposure measured, was not different from that at the reference site. Thus, it can be concluded that exposures to PCBs were not sufficient to cause population-level effects on tree swallow adults at the KRAOC.

Age of females has been identified as an important factor in tree swallow reproduction. SY birds may have lesser reproductive output due to lack of breeding experience (Wheelwright and Schultz, 1994; Saether, 2003). These factors may contribute to smaller clutches and lesser productivity in SY birds during portions of the nesting season (De Steven, 1978; Wheelwright and Schultz, 1994; Stutchbury and Robertson, 1988). Although there was a statistically significant difference in female age between years and sites, it is unlikely that reproductive success was affected by the age of the females, since all reproductive parameters were evaluated for covariance with female age and no interaction was significant. This suggests that female age had little effect on the outcome of reproduction at sites along the Kalamazoo River.

Date of clutch initiation was also considered as a possible cofactor with reproductive success of tree swallows (Stutchbury and Robertson, 1987; Stutchbury and Robertson, 1988; Winkler *et al.*, 2002). Adults who initiate earlier tend to have larger clutches than adults who initiate later in the season, which translates into larger broods and more fledglings from early nesting adults (Stutchbury and Robertson, 1988). This relationship may not be due to direct interaction between date of initiation and clutch size, but may be due to other confounding factors (Winkler *et al.*, 2002). Nest initiation may be constrained early in the season due to low temperatures (Kuerzi, 1941; Stevenson and Bryant, 2000; Winkler *et al.*, 2002), but adults who nest early may obtain prime nesting locations before other adults arrive at the site (Stutchbury and Robertson, 1988). Early nesters may also benefit from coincidental timing of temperature-dependent insect emergence during the nestling period (Stevenson and Bryant, 2000). Timely nesting is beneficial to the growth and development of the nestlings and overall reproductive success because nesting coincides with insect prey abundance during the peak growing period of the nestlings (Quinney *et al.*, 1986). In our studies, there was no interaction between overall productivity and date of initiation, but clutch size did significantly interact with date of initiation in 2000 and 2001. The relationship between reproduction and earlier nest initiation at TB could possibly have offset some effects induced by PCBs, but effects caused by PCBs are likely to overshadow interactions with early initiation. The lack of significant interactions between reproduction and date of nest initiation also suggests that initiation date does not have a significant role in reproductive outcome at the Kalamazoo River.

Reproductive success reported as hatching success, fledging success, and overall productivity, are ecologically important parameters that if affected by exposure to PCBs could result in population-level effects. These measures of reproduction were similar between sites and within the normal range for populations as summarized by Robertson *et al.* (1992). This suggests that the KRAOC population was not exposed to concentrations of PCBs that were sufficient to cause adverse effects. The power to detect differences in the mean between locations was generally insufficient ($1-\beta < 0.20$), and the power of the analyses was generally insufficient to detect a 20% reduction in reproductive health at TB compared to FC (Table 1.5). Much of the loss of power is likely attributable to the variability associated with reproductive measurements, but insufficient sample sizes also contribute to the inability to detect differences. For some instances, the power was exceptionally low because the calculated mean at TB was greater than the mean at FC, which was the reverse of the alternative hypothesis. Although power may have been low for statistical tests comparing mean reproductive measurements between locations, the reproductive success of both the FC and TB population were generally within the normal range reported for other tree swallow populations (Robertson *et al.* 1992).

Table 1.5. Power (1-β) and sample size (N) needed to detect a 20% reduction in reproductive success at the Trowbridge Impoundment compared to the Fort Custer State Recreation Area.

	2000		2001		2002		All Years Combined	
	1-β	N	1-β	N	1-β	N	1-β	N
Hatching success	0.15	62	0.01	28	0.15	61	0.15	48
Fledging success	NA	NA	0.01	8	<0.01	5	0.01	5
Productivity	0.15	62	0.01	37	0.10	68	0.11	55
Clutch size	0.80	20	0.05	8	0.52	10	0.85	13
Predicted brood size	0.17	33	0.05	27	0.07	46	0.24	32
Predicted number of fledglings	0.17	33	0.03	28	0.03	54	0.15	36

NA= Not Available

The reproductive endpoint measured in this study that exhibited a statistically significant difference between locations in some years, was clutch size. In two out of three years, the clutch size was greater at the reference location than at TB in the KRAOC. However, clutch size at both the FC and TB locations along the Kalamazoo River were slightly less than those reported for other populations (unweighted grand mean (± SD) for 28 populations = 5.40 (0.37)) (Robertson *et al.*, 1992). Other studies where tree swallows have been exposed under field conditions to concentrations of PCBs that were similar or greater than those at the KRAOC did not exhibit effects on clutch size that could be related to exposure to PCBs (Custer *et al.*, 1998; Custer *et al.*, 2003). At some locations with elevated concentrations of PCBs, such as Hudson River, a greater incidence of supernormal clutches was observed (McCarty and Secord, 1999). This phenomenon has also been documented in gull species in contaminated areas (Conover, 1984). We did not observe more supernormal clutches at TB, but we did observe 3 clutches at FC with 7 eggs each.

During incubation, it is possible that abandonment may increase due to contamination (McCarty and Secord, 1999), but there are other factors that can cause abandonment. There were several incidences of interference by house wrens at both FC and TB and several cases of abandonment (FC n = 2, TB n = 4). Over the study period, the percentage of total nests abandoned at TB (12%) was greater than at FC (3%). Some adults experienced attacks by house wrens during the incubation stage, and therefore, some or all of the documented cases of abandonment may be attributed to this disruption or to inclement weather. Several cases of egg burial were observed at both sites. This behavior is believed to be a precursor to abandonment (Secord *et al.*, 1999), but in each case of egg burial at TB, the nest was not abandoned. Although beyond the scope of this paper, it is possible that the eggs were infertile and subsequently buried by the female to better incubate the remaining fertile eggs. Studies designed to evaluate PCB-induced infertility and resource partitioning would be needed to further address this hypothesis.

Eggs exposed to PCBs may have a slightly smaller size and mass, but the relationship is not considered to be strong (Secord and McCarty, 1999). Eggs from the KRAOC, where adults were exposed to greater concentrations of PCBs, were significantly heavier, but PCB concentration was not correlated with egg weight or hatchability. Egg weight and length from TB were within the range for unexposed populations, but egg mass at FC was slightly less than normal populations (De Steven, 1978; Wheelwright and Schultz, 1994). In western bluebirds (*Sialia mexicana*), exposure to PCBs has been associated with smaller egg volume, which was mainly associated with variation in egg width (Fair and Myers, 2002). In contrast, PCB exposure in one study was linked to larger yolk mass in American kestrels (*Falco sparverius*) (Fernie *et al.*, 2000), which may account for the significantly heavier eggs at TB.

Growth and growth curves

Growth parameters and nestlings' weights measured for both sites were within the normal range for tree swallows (Wheelwright and Schultz, 1994; Harris and Elliott, 2000). Thirteen days after hatching, tree swallows at TB were slightly heavier than those at FC, which may be associated with the heavier eggs at TB (St.Louis and Barlow, 1993). Moreover, other studies have not found a causal link between growth in tree swallows and PCB exposure (McCarty and Secord, 1999; Harris and Elliott, 2000), although it is suggested that growth measurements based on weight are suitable indicators of stress

(Zach and Mayoh, 1982). The apparently normal growth of tree swallows exposed to PCBs observed in this study and in other studies suggests that the tree swallow may be less sensitive than other species (Ludwig *et al.*, 1993).

Growth rates of nestlings were within the range expected for uncontaminated populations, although mean weights at each day were slightly greater at the Kalamazoo River than in other populations (Kuerzi, 1941; Harris and Elliott, 2000). Growth curves for tree swallows on the Kalamazoo River were based on a logistic equation as suggested by Zach and Mayoh (1982), but the logistic curve underestimated mean nestling weight for the populations at day 11 and overestimated weight at day 12. The curves fit well between days 3 and 10. It is suggested that the logistic curve overestimates maximum mass in nestlings and does not properly model weight recession, but a polynomial model fits the curves more precisely (Harris and Elliott, 2000). At 12 d of age, the logistic model underestimated nestling mass, but the data did conform to this function over the rest of the growth curve. The evaluation of growth recession was beyond the scope of the study since most nestling weights were not evaluated after 12 d. In this study, the logistic model seems to be an adequate fit over the portion of the nestling period that was evaluated. Mass gain per day, which described the slope of the growth curve at individual boxes, was also not significantly different between sites. The lack of significant differences in nestling weight at each day and the similarity of growth curves between FC and TB suggest that there were no PCB related effects on growth of tree swallow populations within the KRAOC.

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Parental Attentiveness

Some studies have shown that behavior of birds can be altered by exposure to contaminants, including PCBs (Peakall and Peakall, 1973; Mora *et al.*, 1993; Halbrook *et al.*, 1998). In doves (*Columba livia*), there was a decrease in attentiveness (Peakall and Peakall, 1973), prolonged courtship, and failure to nest when exposed to PCBs (Tori and Peterle, 1983). Likewise, Forster's terns (*Sterna forsteri*) exhibited longer incubation time, less attentiveness to nests, nest abandonment, and egg disappearance (Kubiak *et al.*, 1989). Such an effect on attentiveness of the adults, measured as the number of visits per nestling, was not observed among TB birds in 2000 or 2001, but in 2002 the number of visits was significantly less at TB compared to FC. The difference between sites can not be attributed to environmental factors such as weather or time of sampling because observations took place randomly, and there was no change in the significance of the relationship when time of observation and age of nestlings were considered as interacting cofactors. There is some evidence that feeding rates can speed up or slow down during certain periods (Kuerzi, 1941), and it is possible that observations were conducted during slow periods in 2002.

One other possible explanation for fewer feeding visits per nestling at TB during 2002 was the likely relationship between large brood size and the ability of adults to capture prey for the large brood. As brood size increased, the number of feedings per nestling decreased (Leffelaar and Robertson, 1986). The correlation between visits and the number of nestlings was examined, and there was only a slight relationship ($r^2 < 0.20$). This relationship could not account for the observed difference in visitations because brood size at TB during 2002 was smaller than in other years. Tree swallow populations are believed to respond to insect abundance (Quinney *et al.*, 1986), so one other explanation for the fewer visits at TB than FC involves the availability of aquatic insects at TB during 2002. Aquatic insects make up a larger proportion of the diet at TB (A. Neigh, unpublished data), so if there were fewer insects one might expect fewer visits to the nest due to the increased prey collection times. While the reason for fewer visits observed at TB in 2002 is unknown, the fact that such an effect was not observed in every year suggests that it was not related solely to exposure to PCBs. While it can not be ruled out that this effect might have been caused by some interaction between exposure to PCBs and nutritional or climatic factors, this seems unlikely. Regardless of the reason, the fewer number of nest visits did not result in differences in growth, survival, or overall productivity. Thus, the observation was not deemed to be ecologically relevant.

Gross morphology and abnormalities

Tree swallows are not particularly sensitive to morphological defects caused by exposure to PCBs (McCarty and Secord, 1999). This conclusion is supported by the lack of abnormalities at PCB concentrations greater than the threshold for effects in other species (Barron *et al.*, 1995). Caspian terns in Saginaw Bay exhibited a range of abnormalities, including, among others, scoliosis, clubbed feet, and gastrochisis with body concentrations of 8.0 to 18 μg PCB/g (Ludwig *et al.*, 1993), while tree swallows at the Hudson River had concentrations up to 62 μg PCB/g, ww in nestlings with no morphological abnormalities (Secord *et al.*, 1999). Kalamazoo River populations did not exhibit any morphological abnormalities at concentrations as great as 32 μg PCB/g (Neigh *et al.*, 2004).

CONCLUSION

This study is the first report of investigations into the reproductive function of tree swallow populations exposed to aquatic sources of PCB contamination through the diet at the Kalamazoo River Superfund Site. Multiple parameters focusing on PCB-induced effects, including several measurements of reproductive success, growth, and behavior, were examined in KRAOC tree swallows where tissue concentrations were significantly greater than at a reference population (Neigh *et al.*, 2004). Reproduction was not significantly different between the contaminated and reference site with the exception of clutch size. The difference in clutch size was not observed in all years, and the difference in clutch size became inconsequential as the nesting season progressed. The most predictive measures of population recruitment, productivity, and the number of fledglings were not significantly different between the two locations. Thus recruitment was the same at both sites. Growth was also similar between sites and was similar to mass gain rates for other populations. Behavior was also evaluated based on the number of adult visits to the nest during the nestling period. There was a significantly greater number of visits at FC than at TB during 2002, but the ecological significance of this difference appears to be minimal. If the number of visits was an ecologically relevant population-level effect of PCBs, it would have been expected to have led to reduced nestling growth and overall productivity. However, growth and productivity were similar between sites in 2002, which suggests that this was not an ecologically significant population-level response. B

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response. Based on three years of observation evaluating multiple parameters and comparisons to other tree swallow populations, the birds at the KRAOC did not appear to be affected by exposure to current concentrations of PCBs at the KRAOC.

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Chapter 2

Tree swallow (*Tachycineta bicolor*) exposure to polychlorinated biphenyls at the Kalamazoo River Superfund Site

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ABSTRACT

In 1990, a portion of the Kalamazoo River in Michigan, USA, was designated a Superfund site due to the presence of polychlorinated biphenyls (PCBs) in the sediment and floodplain soils. During a four-year period from 2000 to 2003, several avian species were monitored for reproductive effects and concentrations of PCBs in tissues attributed to food chain transfer from contaminated sediments. The tree swallow (*Tachycineta bicolor*) was chosen as a model receptor for contamination of passerine species. A "top-down" methodology was used to evaluate the bioaccumulation of PCBs, including non-*ortho* and mono-*ortho* congeners, in tree swallow eggs, nestlings, and adults at the Kalamazoo River Area of Concern (KRAOC) and at an upstream reference site. There was generally a 6-fold difference in tissue concentrations of total PCBs between the two sites with concentrations at the KRAOC ranging from 0.42 to 32 μg PCB/g, wet weight. Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents ($TEQs_{WHO\text{-}Avian}$) for PCBs, based on bird-specific World Health Organization Toxic Equivalence Factors, were 10 to 30-fold greater in the KRAOC than at the reference location. $TEQs_{WHO\text{-}Avian}$ ranged from 4.8 to 51 ng TEQ/g, wet weight at the KRAOC. Hazard quotients calculated from literature-derived toxicity reference values were below 1.0 at both the target and reference sites based on the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL).

Keywords: Birds, Bioaccumulation, Aquatic food chain, TEQs, PCBs

INTRODUCTION

PCBs were used in the production of carbonless copy paper and paper inks over approximately 15 yrs (USEPA, 1976). Effluent containing polychlorinated biphenyls (PCBs) was released into the Kalamazoo River during the recycling of carbonless copy paper. As a result, a 123 km portion of the Kalamazoo River in southwest Michigan was designated a Superfund site due to the presence of PCBs in fish, sediments, and floodplain soils. PCBs have been linked to adverse effects in numerous mammalian (Grant *et al.*, 1974; Aulerich *et al.*, 1977; Bursian *et al.*, 2003) and avian species (Giesy *et al.*, 1994), especially reproductive effects. Recent studies have investigated mink exposure (Millsap *et al.*, 2004) and tree swallow reproduction (Neigh *et al.*, 2004) at the Kalamazoo River Area of Concern (KRAOC), but little information was available regarding concentrations of PCBs in the tissues of passerine species. Two basic types of methodologies were employed to assess trophic transfer and ecological risk to resident avian species. The first methodology, the "bottom-up" or food web approach, predicted dietary exposure, which was compared to threshold concentrations determined in laboratory feeding studies (Fairbrother, 2003). The alternative approach, on which we report here, measures concentrations of PCBs in the tissue of the receptor and compares these to tissue-based toxicity reference values (TRVs).

Exposure to PCBs for all tissues was based on congener-specific analysis of 100 congeners and was reported as 2,3,7,8- tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents ($TEQs_{WHO\text{-}Avian}$) and total PCBs in order to assess risk to tree swallow populations on the

KRAOC, and to evaluate the consistency of the two methodologies for quantifying PCB exposure. Both methods are assumed to evaluate the toxicity of PCB mixtures, but each measure has its advantages and disadvantages. The TEQ method, adopted by the USEPA (USEPA, 1989), describes the toxic action of non-*ortho* (coplanar) and mono-*ortho* congeners, which are mediated through the aryl-hydrocarbon receptor (AhR) and additive in toxicity. Effects mediated through this pathway are expected to be critical, and therefore, overshadow the effects elicited through other pathways (Giesy and Kannan, 1998). Regulations based on this pathway are therefore considered protective of all toxic responses. However, TEQs are species and endpoint-specific, so there are uncertainties when extrapolating to wild species and other endpoints. A major weakness of the concept is the disregard for pathways that do not act through the AhR pathway but may contribute to the overall toxic response of the organism to the mixture (Giesy and Kannan, 1989). The total PCB method of evaluating toxicity assumes the toxicities of all congeners in the mixture are similar and additive, so all potential toxic contributions from individual congeners are included in the assessment. The total PCB method does not assess the toxicity of the different pathways and interactions between the pathways, so the toxic response to the total PCB concentration can only be generalized and not specifically described based on the mode of action.

The Kalamazoo River is located in southwest Michigan beginning at the confluence of the north and south branches in Albion, Michigan, and flows northwest for approximately 200 km to Lake Michigan at Saugatuck, Michigan. The main stem of the Kalamazoo River was dammed at ten locations, but three of the dams were partially dismantled in

1986, exposing approximately 205 ha of former PCB contaminated sediments, which are now floodplain soils. Surveys of in-stream surface sediment (0-10 cm) indicate concentrations range from less than 1.0 ng PCB/g, dry weight (dw) to 153 μg PCB/g, dw with a mean concentration of ~ 3 μg PCB/g, dw (BBL, Inc., 1994a; Weston, Inc., 2002). Surficial floodplain soils (0-25 cm) in the former impoundments range from less than 1.0 ng PCB/g, dw to 85 μg PCB/g, dw with mean values of ~ 11 μg PCB/g, dw (BBL, Inc., 1994b; BBL, Inc., 1994c; BBL, Inc., 1994d).

The tree swallow (*Tachycineta bicolor*) was selected as a surrogate species to determine site-specific bioavailability and trophic transfer from sediments contaminated with PCBs to upper trophic level avian receptors. This study focused on tree swallows as model receptors of PCB accumulation in avian wildlife because of the presence of a previously established nest box population at the baseline location and an abundance of tree swallows residing throughout the river basin. The tree swallow diet consists largely of emergent aquatic insects (Quinney and Ankney, 1985; Cohen and Dymerski, 1986; McCarty, 1997), which are in contact with sediment during large portions of their life cycle. The aquatic insects comprise an indirect exposure pathway of PCB contamination from the sediment to tree swallows, and therefore, tree swallows are suitable monitors of sediment contamination. Prior to egg laying, tree swallows spend two or more weeks constructing nests (Kuerzi, 1941), and thus, concentrations of PCBs in eggs, passed by maternal exposure, are related to local sources of contamination. A large database of similar studies on tree swallow exposure to PCBs and productivity exist, which suggests tree swallows are both tolerant of PCB contamination (McCarty and Secord, 1999) and are adequate monitors of aquatic sources of PCBs (Ankley *et al.*, 1993; Jones *et al.*, 1993; Bishop *et al.*, 1999).

This study evaluated the risk of exposure to PCBs in tree swallows through the aquatic food chain at a portion of the Kalamazoo River known to be contaminated with PCBs and at an upstream baseline site unaffected by point sources and having background concentrations of PCB in sediments. Previous reports based on this study elucidate no statistical difference in productivity, fledging success, hatching success, brood size, and the number of fledglings between the reference and KRAOC locations, but clutch size was significantly greater at the reference location (Neigh *et al.*, 2004). Based on these data, overall reproductive health did not appear to be impaired at ecologically relevant levels. Here, we present an alternate "top-down" approach in which site-specific concentrations of PCBs (total PCBs and 2,3,7,8-tetrachloridbenzo-dioxin equivalents ($TEQs_{WHO-Avian}$) in tree swallow adults, nestlings, and eggs are compared to literature-derived threshold values to estimate risk in aquatic food web based passerine birds.

MATERIALS AND METHODS

Site details

Two locations were selected to evaluate exposure to PCBs in tree swallows. In 2000, nest boxes were established within the KRAOC and additional boxes were added to an already existing nest box trail at the reference location. Nest boxes were within the 100-year floodplain and not more than 200 m from the river. The Fort Custer (FC) reference site was located upstream of known sources of PCB contamination between the villages of Augusta and Galesburg in southwest Michigan (Figure 2.1). The target study area was located entirely within the KRAOC and 67 km downstream of the reference location. The target area, the former Trowbridge Impoundment (TB), was formed in 1986 when the superstructure of the dam was removed, which exposed the impoundment's former lake bottom. The former Trowbridge Impoundment stretches 7 km and encompasses 132 ha of exposed former sediments, now vegetated, and 70 ha of remaining impounded water. As one of three similar impoundment areas, the TB study areas was selected as the worst case scenario for wildlife exposure because it has the greatest surface area, total PCB mass, and mean surficial concentration of PCBs in soils (~11 μg/g, dw) of any of the Kalamazoo River impoundment areas (BBL, Inc., 1994c).

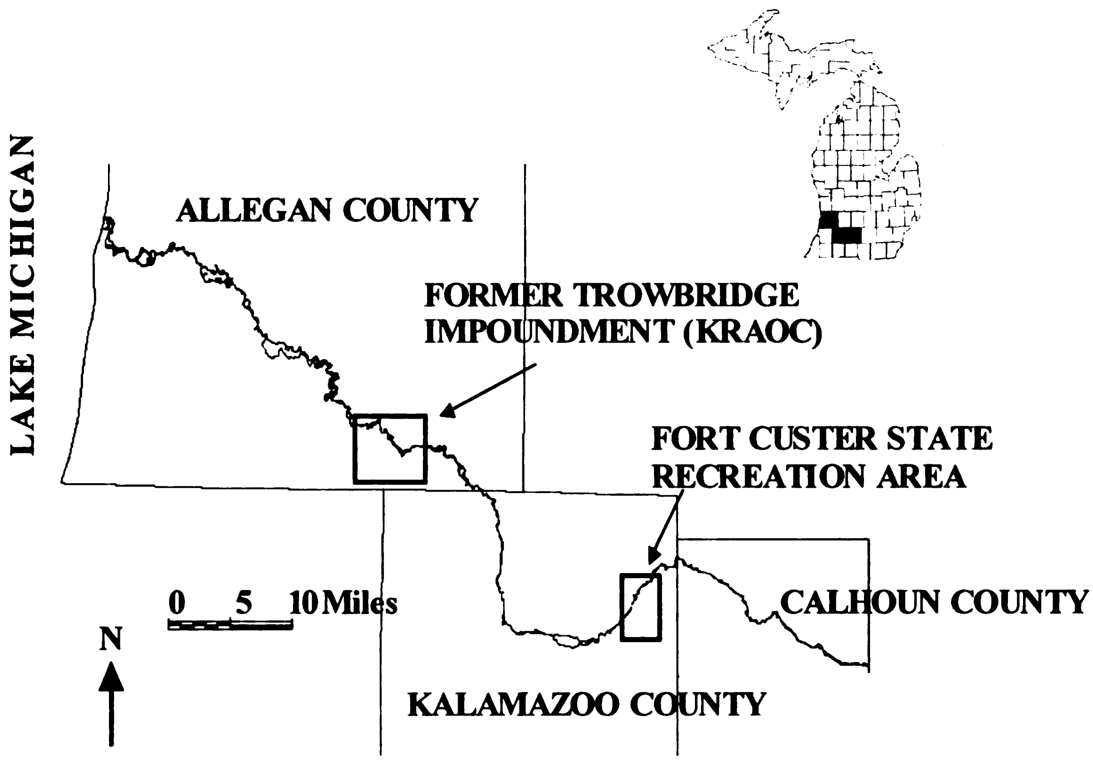

Figure 2.1. Kalamazoo River Area of Concern (KRAOC) and reference site.

Tissue sampling

Eggs, nestlings, and adults were collected during spring and summer of 2001 and 2002. No more than one fresh egg or one live nestling was taken from any box in a given year, and the type of sample was randomly predetermined for each box upon nest initiation. Dead adults, nestlings, and abandoned or addled eggs found in the nest boxes were salvaged, assessed for cause of death, and PCB concentrations determined. Fresh eggs were preferentially sampled 10 or more days after laying. Eggs were placed in a pre-cleaned Pelican Case® (Torrance, CA, USA) for transport back to the field lab where they were placed in solvent-rinsed jars and stored at 4°C. Individual nestlings were selected randomly from predetermined broods on the day of sampling. Sampling of nestlings occurred about one week prior to the expected fledge date to allow for maximum growth while minimizing the chance that nestlings would fledge when disturbed. Nestlings were quickly removed from nest boxes, transported out of audible and visual range of the nest, and euthanized by cervical dislocation. Nestling tree swallows are not sexually dichromatic, so the sex of individual nestling samples could not be determined. Chicks were then placed in solvent rinsed jars and frozen at -20°C. Adults were sampled at the end of the nestling period during 2002 and 2003. Adults were captured by mist nets erected in close proximity to the nest boxes or by a trap door mechanism. Adults were promptly euthanized by cervical dislocation, and sex, age, weight, body length, tarsal length, and wing chord were determined. Sex was determined based on the presence of a brood patch or cloacal protuberance. The carcasses were placed in solvent rinsed sample jars and frozen at -20°C.

Chemical analysis

Eggs, nestlings, and adults were processed before chemical analysis. Eggshells were removed, which left the contents of the egg, including yolk and albumen, for sample analysis. Wet weights of eggshells were measured and then dried in an oven at 80°C for 24 h to determine dry weight. Feathers, beaks, wings, legs, and stomach contents were removed from adults and nestlings, and the whole body was homogenized in a solvent rinsed grinder. Stomach contents were placed in cleaned vials and stored at -20°C.

Total concentrations of PCBs and DDT and its metabolites were determined by EPA method 3540. All concentrations were reported as wet weight unless otherwise noted. A known quantity of tissue was homogenized with anhydrous sodium sulfate (EM Science, Gibbstown, NJ, USA) using a mortar and pestle. All samples, blanks, and matrix spikes contained surrogate standards, PCB 204 (International Union of Pure and Applied Chemistry) (AccuStandard, New Haven, CT, USA) and PCB 30 (AccuStandard, New Haven, CT, USA). Extraction blanks using Na_2SO_4 were included with each set of samples and Quality Assurance/Quality Control (QA/QC) sets composed of similar tissues were included with each group of 20 samples. Samples were extracted with 400 ml of pesticide residue grade dichloromethane:hexane (3:1, v/v) for 18 h in a Soxhlet extraction apparatus (VWR Scientific, Plainfield, NJ, USA). Extracts were concentrated by rotary evaporation to a final volume of 11 ml. One ml of the hexane extract was used for lipid content determination. If deemed necessary, acid hydrolysis was performed. Briefly, 10 ml of concentrated sulfuric acid was added to the extract and shaken for at least 30 s. After separation of phases, the extract was transferred to another test tube, and 10 ml of water was added to remove acid residues from the extract. The remaining 10 ml of extract was passed through a neutral/acidic silica gel column to remove non-target analytes. Glass columns 30 cm in length and 15 mm in diameter were packed with approximately 0.5 g anhydrous sodium sulfate and alternating layers of 2 g 40% sulfuric acid (JT Baker, Phillipsburg, NJ, USA) impregnated silica gel and 2 g 100 to 200 mesh size silica gel (Aldrich, Milwaukee, WI, USA). The extract was then evaporated to a final volume of 1.0 ml under a stream of nitrogen. An aliquot of 0.5 ml was retained for total PCB and DDT analytes, while non-*ortho*-substituted (coplanar) PCB congeners were separated from the remaining aliquot, as described below.

PCBs including di- and mono-*ortho*-substituted congeners were quantified by use of a gas chromatograph (Perkin Elmer AutoSystem and Hewlett Packard 5890 series II) equipped with a ^{63}Ni electron capture detector (GC-ECD). A fused silica capillary column (Zebron ZB-5; 5% phenylpolysiloxane, 30m × 0.25 mm inner diameter) with a film thickness of 0.25 μm was used (Phenomenex, Torrance, CA, USA). The column oven temperature was programmed to change from 120°C (1 min hold) to 160°C at a rate of 10°C/min (1 min hold) and then to 250°C at a rate of 2°C/min with a final holding time of 10 min. Injector and detector temperatures were kept at 225°C and 375°C, respectively. Helium and nitrogen were used as carrier and make up gas, respectively. The standard contained 100 individual PCB congeners of known composition and content. Congeners were identified by comparing sample peak retention times to those of the known standard. In sample extracts, concentrations of each congener were determined by comparing the peak area to that of the appropriate peak in the standard mixture. The method detection limit (MDL) was estimated to be 1×10^{-3} μg PCB/g. TurboChrom (Perkin Elmer, Wellesley, MA, USA) or GC Chemstation software (Agilent Technologies, Wilmington, DE, USA) was used to integrate the peaks. A spreadsheet developed by the Michigan State University Aquatic Toxicology Laboratory was used to quantify individual congeners. Total PCB concentrations were calculated as the sum of all resolved PCB congeners.

Carbon column chromatography was used to separate non-*ortho* substituted PCB congeners (IUPAC numbers 77, 81, 126, and 169) from co-eluting congeners and interferences. Briefly, 30-cm glass columns, 15 mm in diameter, were packed with anhydrous sodium sulfate, carbon dispersed on silica gel (Wako Chemicals, Richmond, VA, USA), and anhydrous sodium sulfate. Twenty microliters of 50 μg/L radio-labeled ^{13}C coplanar PCB congeners (77, 81, 126 and 169) standard (Cambridge Isotope Laboratories, Andover, MA, USA) in isooctane were added to each extract. The first fraction, eluted with 100 ml 20% dichloromethane in hexane, was archived. The second fraction, eluted with 200 ml of toluene, contained non-*ortho* coplanar PCB congeners. The extract was then concentrated under a stream of nitrogen to a final volume of 20 μl.

Concentrations of non-*ortho* substituted PCBs and DDT metabolites were quantified by gas chromatograph mass selective detector (GC-MS) (Hewlett Packard 5890 series II gas chromatograph) equipped with a HP 5972 series detector. A fused silica capillary column (as described above) was used. Coplanar PCB congeners and DDT analytes were detected by selected ion monitoring at the two most abundant ions of the molecular cluster. Detection limit varied with each sample, but the mean detection limit for all samples was <100 pg PCB/g.

TEQ computation

Concentrations of $TEQs_{WHO\text{-}Avian}$ in bird tissues were calculated by multiplying the concentration of individual PCB congeners by their respective bird-specific World Health Organization (WHO) toxic equivalence factor (TEF) (van den Berg *et al.*, 1998). Total

TEQ concentrations were calculated as the sum of detectable non-*ortho* and mono-*ortho* PCB congeners (77, 81, 105, 118, 126, 156, 157, 167, and 169) and reported as wet weight unless otherwise noted. Polychlorinated-dibenzo-dioxins and polychlorinated-dibenzo-furans were not measured and were not included in TEQ computation. All data were log-transformed for statistical analyses. These congeners are considered to have the greatest relative potency to cause effects mediated through the AhR (Giesy and Kannan, 1998; Kannan *et al.*, 2000). A sensitivity analysis was conducted in which congeners that were not detected were assigned a proxy value equal to the detection limit or to zero (Coady *et al.*, 2004). The results of this analysis demonstrated that the calculated concentrations of $TEQ_{WHO\text{-}Avian}$ did not differ substantially with either proxy value. Therefore, a value of half the detection limit was assigned to congeners with concentrations below the method detection limit. Co-eluting congeners were evaluated separately. For example, congener 105 co-eluted with congener 132, PCB 156 frequently co-eluted with congeners 171 and 202, and congener 157 frequently co-eluted with congener 200. In order to report the most conservative $TEQ_{WHO\text{-}Avian}$, the entire concentration of the co-elution groups was assigned to the mono-*ortho* congener. Overall, congeners 105, 156, and 157 contributed little to the total concentration of the $TEQ_{WHO\text{-}Avian}$ in all samples from the Kalamazoo River, 3.7%, 1.8%, 0.4%, respectively.

Bioaccumulation calculation

Bioaccumulation factors (BAFs) were calculated by dividing the lipid normalized total PCB or $TEQ_{WHO\text{-}Avian}$ in the upper trophic level by the lipid normalized concentration in the lower trophic level. BAFs were calculated using nestlings and adults sampled live and eggs sampled fresh.

Statistical analyses

Statistical comparisons were made by use of SYSTAT (Evanston, IL, USA). Sample sets were analyzed for normal distribution by Kolmogorov-Smirnov one sample test with Lilliefors transformation and for homogeneity of variance by F test. Samples were generally log normally distributed, and therefore, all concentration data were log transformed to obtain a normal distribution. Sample sets satisfying assumptions of normality and homogeneity were compared by t-test, or in the case of multiple comparisons, by a one-factor analysis of variance (ANOVA). All other data sets were compared by Mann-Whitney U or Kruskal-Wallis non-parametric tests. The criterion for significance used in all tests was $p < 0.05$.

Two eggs were sampled from three different nests. In two of the nests, eggs were addled, and the concentration of each of the eggs was not more than one standard deviation different from the mean of the population. The mean concentration of PCBs in the populations were calculated based on the concentrations in eggs of all other nests and the average concentration of eggs in the nests in which multiple eggs were sampled. In one nest, one egg was sampled fresh and the other addled, so these values were evaluated separately when considering differences between fresh and addled eggs, but they were averaged to calculate the mean concentration of PCBs in the population.

Assessment of risk

Risk was assessed through hazard quotients (HQs) by comparing concentrations of PCB measured in eggs to tissue-based toxicity reference values (TRVs) based on the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) for total PCBs and the NOAEL for TEQs (Table 2.1). Concentrations of PCBs in eggs were considered the most sensitive metric for assessing effects induced by PCBs. Therefore, the assessment of potential risks based on TRVs derived from the exposure of eggs to PCBs was considered to be a conservative estimate of risk at all life stages (Giesy *et al.*, 1994). HQs were calculated as the tissue concentration divided by the tissue-based TRV.

Table 2.1. Literature-derived tree swallow toxicity reference values (TRVs) used to calculate hazard quotients based on the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL). Reference number is located next to each value.

	Tissue Based TRV	**Reference**
Total PCBs (μg PCB/g)		
NOAEL	**26.7**	**USEPA, 2000**
LOAEL	**63.0**	**Custer *et al.*, 2003**
Total TEQ (ng/g, ww)		
NOAEL	**13**	**USEPA, 2000**
LOAEL	**NA**	

RESULTS

Total PCB and organochlorine concentrations

Concentrations of PCBs in tree swallows were significantly different between sites. Concentrations of PCBs in adult tree swallows ranged from 0.44 to 32 μg PCB/g at TB and 1.0 and 2.0 μg PCB/g at FC. There was no statistically significant difference between sexes of the adults at TB, and the sample size was too small at FC to evaluate differences between sexes. There was no statistically significant difference in total PCB concentrations between eggs taken fresh or addled eggs, so all sample types were combined for statistical analyses. Likewise, there was no statistically significant difference in concentrations of PCBs between nestlings taken alive or salvaged carcasses, so they were also combined for analysis. In both eggs and nestlings, there was a 6-fold difference between sites in concentrations of PCBs (Table 2.2). Concentrations of PCBs in nestlings and eggs from TB were both significantly greater than those at FC (Student's t-test, $p < 0.001$). PCB concentrations of nestling tree swallows ranged from 0.95 to 7.5 μg PCB/g at TB to a significantly smaller concentration at FC ranging from 0.14 to 2.0 μg PCB/g. Egg concentrations ranged from 1.2 to 15 μg PCB/g at TB and 0.18 to 2.5 μg PCB/g at FC.

Table 2.2. Mean (± 1 SD) total polychlorinated biphenyl (PCB) concentrations (wet weight) and lipid content of tree swallow tissue samples from the Fort Custer State Recreation Area (reference location) and the former Trowbridge Impoundment within the Kalamazoo River Area of Concern (KRAOC).

	Fort Custer			Trowbridge		
	N	**% Lipid**	**PCB(µg/g)**	**N**	**% Lipid**	**PCB(µg/g)[a]**
Egg						
2001	**12**	**12 (7.1)**	**0.67 (0.40)**	**7**	**6.3 (2.3)**	**2.2 (0.74)**
2002	**7**	**8.5 (4.4)**	**1.0 (0.70)**	**7**	**8.2 (2.5)**	**8.1 (4.4)**
Total	**19**	**11 (6.4)**	**0.81 (0.54)**	**14**	**7.2 (2.5)**	**5.1 (4.3)**
Nestling						
2001	**7**	**6.4 (2.4)**	**0.36 (0.16)**	**4**	**8.4 (2.6)**	**4.3 (2.3)**
2002	**5**	**8.0 (4.5)**	**0.59 (0.80)**	**7**	**8.1 (3.2)**	**2.6 (1.1)**
2003	**NA**	**NA**	**NA**	**2**	**3.2 (0.060)**	**2.7 (0.59)**
Total	**12**	**7.1 (3.3)**	**0.46 (0.51)**	**13**	**7.4 (3.2)**	**3.1 (1.6)**
Adult						
2002	**2**	**6.8 (0.33)**	**1.5 (0.65)**	**6**	**5.6 (2.5)**	**12 (11)**
2003	**NA**	**NA**	**NA**	**3**	**8.1 (3.8)**	**2.7 (3.1)**
Total	**2**	**6.8 (0.33)**	**1.5 (0.65)**	**9**	**6.5 (3.0)**	**8.7 (9.7)**

[a]All concentrations of PCBs in eggs and nestlings at the Trowbridge Impoundment were significantly greater than at the Fort Custer reference area (Student's t-test, $p < 0.05$).

NA = Not Available

DDT and its metabolites were detected in all samples from both sites. *p',p'*- DDE occurred at the greatest concentration of the measured analytes in all samples, which contributed 98% of the sum of total DDT concentrations in all samples. The sum of all measured metabolites was used to calculate total DDT concentrations. Concentrations of total DDT at TB and FC were not significantly different (Mann-Whitney U, $p = 0.465$) due to large variances in the data, but the means were 3-fold different between sites. The mean (± 1 SD) concentration when all eggs were combined was 1.5 (1.2) μg DDT/g at TB and 0.45 (0.13) μg DDT/g at FC. Mean (± 1 SD) concentrations in addled eggs were 2.3 (0.32) μg DDT/g at TB and 0.32 (0.072) μg DDT/g at FC, and concentrations in fresh eggs were 0.95 (1.3) μg DDT/g at TB and 0.54 (0.030) μg DDT/g at FC.

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

Mean concentrations of $TEQs_{WHO\text{-}Avian}$ in eggs and nestlings at FC and TB were significantly different (Student's t-test, $p < 0.001$) (Table 2.3). Although sample sizes were insufficient to evaluate differences between groups at FC, there were no statistically significant differences between fresh and addled eggs or live and salvaged nestlings at TB. Tree swallow eggs contained mean TEQ concentrations ranging from 0.21 to 2.4 ng TEQ/g at TB and 0.021 to 0.100 ng TEQ/g at FC. Nestlings at TB also had the greatest concentration (0.26 to 1.3 ng TEQ/g) and another nestling at FC the lowest (2.6×10^{-3} to 0.047 ng TEQ/g).

Table 2.3. 2, 3, 7, 8- tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) and relative potency in tree swallow tissues sampled from the Fort Custer Reference location and the former Trowbridge Impoundment within the Kalamazoo River Area of Concern (KRAOC).

	Fort Custer			Trowbridge		
	N	TEQ (ng/g)	RELPOT (ng TEQ/g PCB)	N	TEQ (ng/g)[a]	RELPOT (ng TEQ/g PCB)
Egg						
2001	**8**	**0.061 (0.030)**	**0.10 (0.035)**	**7**	**0.30 (0.11)**	**0.15 (0.069)**
2002	**4**	**0.045 (0.016)**	**0.078 (0.033)**	**5**	**1.4 (0.75)**	**0.16 (0.027)**
Total	**12**	**0.056 (0.027)**	**0.095 (0.035)**	**12**	**0.76 (0.74)**	**0.15 (0.054)**
Nestling						
2001	**7**	**0.019 (0.016)**	**0.044 (0.027)**	**4**	**0.72 (0.40)**	**0.18 (0.094)**
2002	**5**	**0.023 (0.0085)**	**0.081 (0.058)**	**7**	**0.54 (0.16)**	**0.23 (0.066)**
2003	**NA**	**NA**	**NA**	**2**	**0.57 (0.13)**	**0.21 (0.0029)**
Total	**12**	**0.020 (0.013)**	**0.060 (0.044)**	**13**	**0.60 (0.25)**	**0.21 (0.069)**
Adult						
2002	**2**	**0.22 (0.030)**	**0.16 (0.048)**	**6**	**2.7 (1.7)**	**0.30 (0.26)**
2003	**NA**	**NA**	**NA**	**3**	**1.2 (1.9)**	**0.27 (0.23)**
Total	**2**	**0.22 (0.030)**	**0.16 (0.048)**	**9**	**2.2 (1.8)**	**0.29 (0.23)**

[a]All concentrations of PCBs in eggs and nestlings at the Trowbridge Impoundment were significantly greater than at the Fort Custer reference area (Student's t-test, $p < 0.05$).

NA = Not Available

Statistical analysis could not be completed for the comparison of adult tissues between sites due to small sample sizes at FC ($n = 2$), but at TB there was no difference between sexes. All adult samples from both locations had detectable concentrations of non-*ortho* PCBs. The average (± 1 SD) $TEQ_{WHO\text{-}Avian}$ in adult tissues at TB was 2.2 ± 1.8 ng TEQ/g and 0.22 ± 0.030 ng TEQ/g at FC. An adult from TB had the greatest $TEQ_{WHO\text{-}Avian}$ for the study (4.8 ng TEQ/g).

Five of the 8 mono-*ortho* congeners were regularly detected in samples from both TB and FC, but congeners 114, 123, and 189 were not detected in samples from either site. Samples were excluded from the analysis of $TEQ_{WHO\text{-}Avian}$ concentrations when at least one non-*ortho* congener was not quantifiable due to interferences with co-eluting congeners with the exception of PCB 169, which was not quantifiable in 65% of all samples. Congener 169 represented <1% of the total $TEQ_{WHO\text{-}Avian}$ concentrations in those samples for which it was reported. At least one of the coplanar congeners 77, 81, and 126 were not quantifiable due to co-eluting congeners or were not above the detection limit in 71% of all samples at FC. Alternatively, only 11% of samples at TB had at least one of the three regularly detected coplanar congeners with concentrations that were not quantifiable or not detectable. For the remaining samples, the congeners detected at levels greater than the detection limit and the frequency of detection at TB were in the following rank order: PCB 77 = 105 = 118 (100%) > 157 (97%) > 81 = 156 (95%) > 126 (89%) > 167 (70%) > 169 (51%). Similarly, the rank order of the frequency of detection for mono-*ortho* and non-*ortho* congeners at FC was as follows: PCB 118

(100%) > 105 (97%) > 77 = 167 (86%) > 156 = 157 (77%) > 126 (66%) > 81 (43%) > 169 (20%). Coplanar PCB congeners 81 and 126 have the greatest AhR-mediated potency relative to other congeners and were detected in all TB nestling and adult samples and in 89% of egg samples at TB. Together they comprised 8.3% ± 6.3 (mean ± 1 SD) of the total concentration of $TEQ_{WHO\text{-}Avian}$ in tissues at TB and 32.2% ± 25.6 of the total $TEQ_{WHO\text{-}Avian}$ at FC (Figure 2.2). Congener 77 occurred at the greatest concentration in all matrices and contributed the greatest proportion to the total $TEQ_{WHO\text{-}Avian}$, 89.4% at TB and 67.8% at FC. Congener 167 was not reported in 22% of samples due to interference with co-eluting congeners, but in the samples without interferences, congener 167 represented <1% of the total $TEQ_{WHO\text{-}Avian}$.

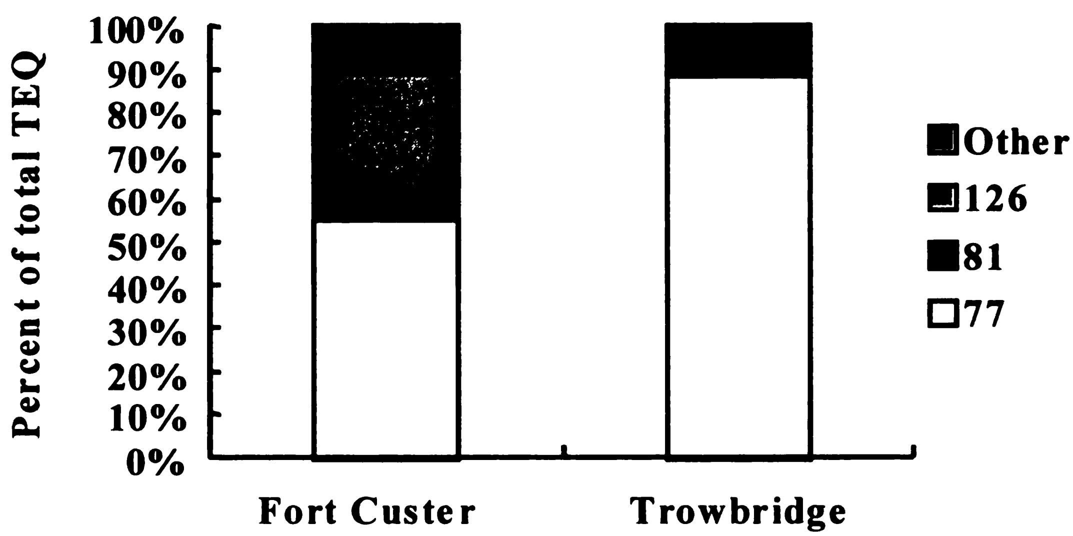

Figure 2.2. Contribution of selected congeners to total TEQ in egg, nestling, and adult samples from the Fort Custer reference site and the Trowbridge Impoundment.

Bioaccumulation

BAFs calculated from total PCBs and TEQs were less than 1.0 between egg and nestling and adult and egg but were greater than 1.0 between nestling and adult, except in 2001 when the egg to nestling BAF was greater than 1.0 at TB (Table 2.4).

Table 2.4. Biomagnification factors for tree swallows on the Kalamazoo River based on the mean of lipid-normalized total PCBs and $TEQs_{WHO\text{-}Avian}$.

	BAF (Total PCB)				**BAF (TEQ)**			
	Fort Custer		**Trowbridge**		**Fort Custer**		**Trowbridge**	
Trophic Level	**2001**	**2002**	**2001**	**2002**	**2001**	**2002**	**2001**	**2002**
Egg to nestling	**0.86**	**0.50**	**1.08**	**0.29**	**0.64**	**0.42**	**1.62**	**0.42**
Nestling to adult	**NA**	**2.90**	**NA**	**7.01**	**NA**	**11.91**	**NA**	**8.07**
Adult to egg	**NA**	**0.68**	**NA**	**0.49**	**NA**	**0.20**	**NA**	**0.29**

NA = Not available.

Accumulation rates were calculated as the difference in total mass of PCBs and TEQs between nestlings and eggs in a nest divided by the days of life (Custer and Custer, 1995). Both a nestling and an egg were sampled from 5 nests, which allowed for the calculation of accumulation rates in those nests. Accumulation rates of 4.4 and 7.7 μg PCB/d were calculated for nests at TB during 2001 and 2002, respectively, and 0.90 to 1.1 μg PCB/d at FC for 2001 and 0.053 μg PCB/d at FC for 2002. Accumulation rates of $TEQ_{WHO\text{-}Avian}$ were 0.79 and 1.2 ng TEQ/d at TB in 2001 and 2002, respectively, while accumulation rates of TEQs at FC in 2001 were 0.014 to 0.082 ng TEQ/d in 2002.

The relative contributions of non-*ortho* and mono-*ortho* congeners were evaluated by standardizing the TEQ to the total PCB concentration for each trophic level and comparing this relative potency between trophic levels via a potency ratio (Equation 1).

Equation 1:

$$\frac{(\text{Concentration of TEQs/Concentrations of Total PCBs})_{\text{trophic level 2}}}{(\text{Concentration of TEQs/Concentrations of Total PCBs})_{\text{trophic level 1}}}$$

The potency ratio was calculated via methods outlined by Froese et al. (1998) to describe changes in toxicity between trophic levels. Mean relative potencies (concentration of TEQs/concentration of total PCBs) used to calculate potency ratios are reported (Table 2.2). The egg to nestling potency ratio at TB and FC were 1.4 and 0.62, respectively. The ratios from nestling to adult and adult to egg were 1.3 and 0.52 at TB and 2.6 and 0.60 at FC, respectively.

Assessment of risk

Based on the NOAEL for both total PCBs and $TEQs_{WHO\text{-}Avian}$ (mean and 95% CI), all HQs for egg, nestling, and adult tissue concentrations were all less than 0.3 at FC. HQs based on the mean and 95% UCL for eggs, nestlings, and adults at TB were also less than 1.0 for the NOAEL and LOAEL of total PCBs and the NOAEL for TEQs (Figure 2.3). No samples from this study exceeded the LOAEL threshold, and only one adult sample from TB exceeded the NOAEL for total PCBs, yielding a HQ of 1.2. These results suggest that the tree swallow population was not at risk for effects induced by PCB exposure.

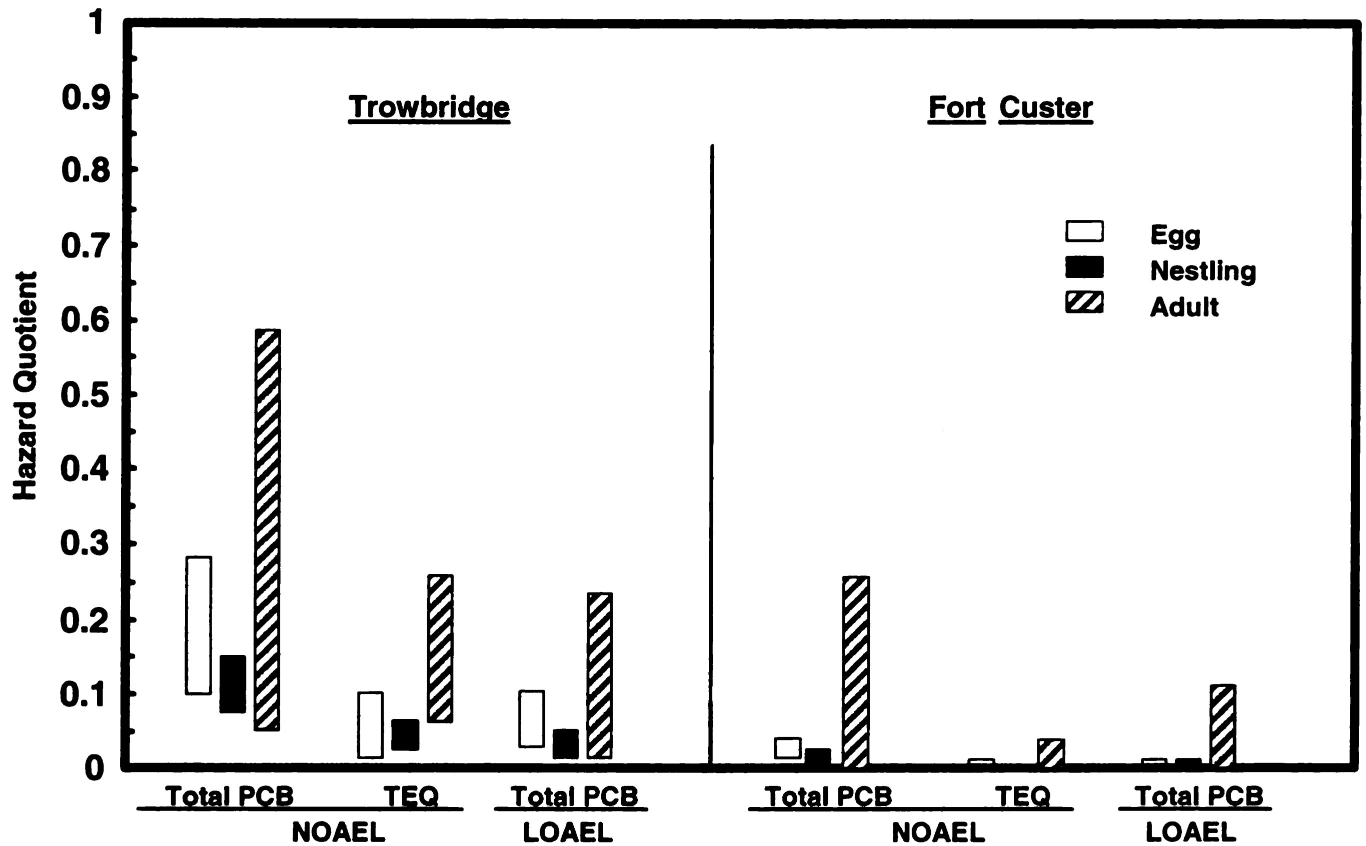

Figure 2.3. Kalamazoo River hazard quotients based on the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL). Each box encompasses the 95% CI about the mean.

DISCUSSION

Total PCB and DDT concentrations

Concentrations of PCBs in tree swallow eggs, nestlings, and adults at the FC reference site were significantly less than those at the TB site. Reference sites on the Thompson River, Fraser River, Fox River, and around the Great Lakes had PCB contaminant levels in tree swallow tissues within a 2-fold range of those found at FC with no corresponding reproductive effects (Custer *et al.*, 1998; Bishop *et al.*, 1999; Secord *et al.*, 1999; Harris and Elliott, 2000).

Concentrations of PCBs found in tree swallows from the KRAOC were generally less than at other sites in the Midwest and New York where PCBs have been identified as a chemical class of concern (Jones *et al.*, 1993; Bishop *et al.*, 1999; Secord *et al.*, 1999). Mean concentrations of PCBs in eggs and nestlings from the KRAOC were 5.1 μg PCB/g and 3.1 μg PCB/g, respectively. Tissues from tree swallows at the Fox River near Green Bay were most similar to those at the KRAOC with mean concentrations of PCBs in eggs and nestlings as great as 4.1 μg PCB/g and 3.0 μg PCB/g, respectively (Custer *et al.*, 1998). Approximately 200 km from the KRAOC, tree swallows from Saginaw Bay, MI, contained slightly lesser tissue concentrations of 0.60 to 1.4 μg PCB/g in eggs and 0.17 to 1.0 μg PCB/g in nestlings (Nichols *et al.*, 1995). An egg concentration 10-fold greater than the greatest egg concentration at the KRAOC and a nestling concentration 8-fold greater than the greatest concentration at our site were reported in tree swallows from the Great Lakes (Bishop *et al.*, 1999). To date, the greatest concentration of PCBs reported in eggs or pipping chicks were at the Housatonic River, MA (101 µg PCB/g) (Custer *et al.*, 2003), and the greatest concentration in nestlings were reported at the Hudson River (62.2 µg PCB/g) (Secord *et al.*, 1999). Concentrations of PCBs at both sites were 20-fold greater than at the KRAOC. In addition to concentrations of PCBs in eggs and nestlings, few studies besides the current study have reported tissue concentrations in adults based on more than a few incidentally salvaged samples. In a single adult female, a PCB concentration of 114 µg PCB/g was reported at one Hudson River site (Secord *et al.*, 1999). That concentration was considerably more than for adults at TB (mean = 8.7 µg PCB/g).

Sources of DDT and its metabolites at the KRAOC are unknown, but are likely related to former agricultural practices and the prevalence of orchards in downstream portions of the Kalamazoo River drainage system. Although DDT concentrations were greater at the TB site than at the FC reference site, they were not sufficient to affect the viability or hatching success of tree swallow eggs. Hatching and overall productivity were comparable between the two sites, and no unusual instances of shell breakage were noted at either of the Kalamazoo River populations studied (Neigh *et al.*, 2004). A concentration of 2.0 µg DDE/g in eggs has been suggested as a threshold for effects in raptor species, which was similar to the mean for the TB population (Elliot and Harris, 2002). Concentrations of DDE in the abandoned eggs of tree swallows near Denver, CO, were 2.8 µg DDE/g, while concentrations of DDE in attended eggs were 1.3 µg DDE/g (DeWesse *et al.*, 1985). Although concentrations of DDE may be near a threshold for effects, the lack of reproductive effects normally attributed to DDE exposure and the absence of a species specific threshold suggests that DDE did not have a significant impact on tree swallow reproduction at the Kalamazoo River.

TEQ tissue concentrations

If concentrations of TEQs and those of total PCBs were correlated it would be expected that there were few AhR-active compounds contributing to the total TEQ concentration (Jones *et al.*, 1993). Although, they were calculated with different TEQs, the calculated $TEQs_{WHO\text{-}Avian}$ for tree swallows at the KRAOC were similar to those TEQs calculated for other PCB contaminated sites in the Midwestern United States (Ankley *et al.*, 1993; Jones *et al.*, 1993; Froese *et al.*, 1998). Concentrations of $TEQs_{WHO\text{-}Avian}$ in eggs, nestlings, and adults from the KRAOC were 10 to 30-fold greater than concentrations at the reference site, compared to a 3 and 10-fold difference in concentrations of $TEQs_{WHO\text{-}Avian}$ between reference sites and contaminated sites at the Thompson and Fraser Rivers, respectively (Harris and Elliott, 2000). Concentrations of $TEQs_{WHO\text{-}Avian}$ in nestling tree swallows at the FC reference site were at least 4-fold greater than the upstream reference sites on the Thompson and Fraser Rivers and were most similar to the downstream site of the Thompson River (Harris and Elliott, 2000).

Besides the statistically significant difference in total $TEQs_{WHO\text{-}Avian}$ between sites, there was also a difference in the proportion that each congener contributed to the overall $TEQ_{WHO\text{-}Avian}$. As has been observed at other sites (Secord *et al.*, 1999; Custer *et al.*, 2002), congeners 77 and 126 contributed the greatest relative proportion of the total $TEQ_{WHO\text{-}Avian}$ concentrations at both FC and TB. However, the contribution of PCB congener 77 was significantly greater at TB in all life stages than at FC (Student's t-test, $p < 0.001$). In contrast, PCB congeners 81 and 126 contributed a significantly greater proportion to the total $TEQs_{WHO-Avian}$ at FC when evaluated together or as individual components than at TB (Student's t-test or Kruskal-Wallis, $p < 0.05$). The prevalence of PCB congener 77 at TB was likely due to the presence of this congener in the original Aroclor mixture, while the proportion of PCB congeners 81 and 126 comprising the $TEQs_{WHO-Avian}$ at FC could be attributed to the greater $TEFs_{WHO-Avian}$ of these congeners. Other mono-*ortho* congeners also contributed a greater proportion of the total $TEQ_{WHO-Avian}$ at FC than at TB, but the overwhelming presence of PCB congener 77 at TB likely contributes to this observation.

Bioaccumulation

Tissue concentrations were normalized to lipid content and compared between trophic levels. Bioaccumulation of PCBs observed in this study was similar to that observed in other studies (Ankley *et al.*, 1993; Custer *et al.*, 1998; Froese *et al.*, 1998; Custer *et al.*, 2002). The BAF from egg to nestling was less than from nestling to adult. This is due to growth dilution of the chicks and fugacity effects in the adults (Jones *et al.*, 1993). The egg to nestling BAFs would be expected to exceed 1.0 if nestlings are exposed to concentrations of PCBs in the diet that are greater than would be necessary to offset growth dilution. The BAF of 1.5 observed in tree swallow nestlings from TB suggests a dietary exposure. The contribution of site-wide contamination during the nestling stage can be assessed because the sedentary behavior of altricial tree swallow nestlings, and the relatively small feeding range of the adults ensures that dietary exposure and subsequent accumulation in chicks results from on-site dietary exposure. Therefore, the egg to nestling BAF is very informative when evaluating the bioaccumaltive potential of a toxicant at a specific site. Overall, the Kalamazoo River BAFs were slightly less than those found at the Fox River (6.18 and 3.47) (Custer *et al.*, 1998), but were comparable to those on the Hudson River (2.0 and 4.0) (Secord *et al.*, 1999).

In nestlings, the rate of accumulation of PCBs can also be evaluated based on daily accumulation rates, which account for the age of nestlings and the concentration contributed by the egg (Custer and Custer, 1995). The calculation of daily accumulation rates is useful in elucidating whether nestlings were exposed to concentrations of PCBs from the diet. Accumulation rates at TB (4.4 and 7.7 μg PCB/d) were similar to those reported for contaminated sites at the Fox River (1.34 to 6.69 μg PCB/d) (Custer *et al.*, 1998), but were less than those measured at the Housatonic River (34 to 76 μg/d) (Custer *et al.*, 2003). Accumulation rates at FC (0.053 to 1.1 μg/d) were similar to the reference site at the Housatonic River (-0.30 to 1.0 μg/d) (Custer *et al.*, 2003) and contaminated sites at the Wisconsin River (0.40 to 0.70 μg PCB/d) (Custer *et al.*, 2002). The accumulation rate in KRAOC tree swallows is less than in piscivorous birds, such as the Forster's tern (*Sterna forsteri*), which had accumulation rates of 15 μg PCB/d (Ankley *et al.*, 1993).

The greatest increase in bioaccumulation was between nestlings and adults. FC accumulation factors were generally greater than at TB, which likely resulted from the low concentrations of PCBs at the site, especially in nestlings. Concentrations near the detection limit may be difficult to quantify due to baseline noise, and so, the difference in concentrations of PCBs in the nestling and adult tissue is great. When evaluating the nestling to adult BAF, the contribution of off-site exposure and also maternal deposition in the lipids of eggs needs to be considered. At the KRAOC, most female birds were captured post-reproduction, and no difference in the concentration of male and female tree swallows was observed. Perhaps dietary contributions to the body burdens of adults had already accumulated to pre-reproductive levels when sampling was conducted, or the loss of body burden to maternal deposition was not significant in our population. Therefore, concentrations of PCBs in adults were greater than in nestlings due to on-site and off-site exposure, as well as, the lack of growth dilution.

Potency ratios were generally greater than 1.0 for both the egg to nestling and nestling to adult, which suggests that the contribution of non-*ortho* and mono-*ortho* congeners to the overall toxicity increased at higher trophic levels. A ratio of 1.0 between egg and adult was expected because there is little metabolism or growth dilution in the egg, and the egg burdens are inherited from the adult. However, at the KRAOC, potency ratios were 0.60 and 0.52. This suggests that congeners contributing the greatest concentrations to the relative toxicity did not pass into the egg. Bioaccumulating congeners in the penta-, hexa-, and hepta- homologue groups contribute the greatest amount to the total PCB concentration in adult tree swallows, while less-chlorinated congeners in the tri- and tetra-homologue groups contribute a lesser amount (Figure 2.4). The accumulation of these congeners likely led to the greater relative potency and overall toxicity of the congener mixture found in adults. A greater proportion of total PCBs in eggs and nestlings were contributed by the tri- and tetra-homologue groups, and a lesser proportion were contributed by the more chlorinated homologue groups. The greater concentration of the bioaccumulating congeners in the adults and the greater potency ratio in the adults suggests that the toxicity of PCBs in the KRAOC increases with trophic level, but that the adults contribute a metabolized, relatively less-toxic mixture to the eggs. Overall, adults appear to contain greater proportions of bioaccumulating congeners, and the mixture of congeners in adults is more toxic than in lower trophic levels. The proportionate contribution of congeners in eggs and nestlings is similar, but the toxicity of the mixture increases with trophic level.

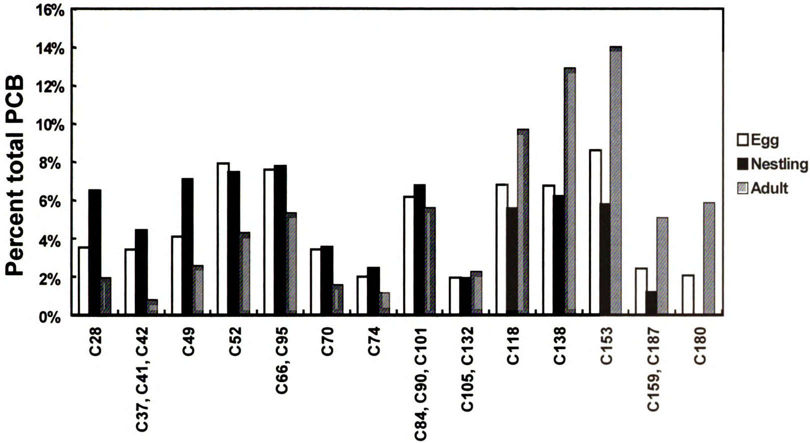

Figure 2.4. The percent contribution of selected congeners and congener co-elution groups to the concentration of total PCBs in egg, nestling, and adult tree swallows at the Trowbridge Impoundment.

Assessment of risk

Toxicity reference values (TRVs) used to calculate hazard quotients were chosen for this study based on several criteria, which included: 1) the use of wildlife species over traditional laboratory species whenever possible; 2) chronic exposure including sensitive life stages; 3) the evaluation of ecologically relevant endpoints; 4) minimal co-contamination; 5) multi-year studies; 6) and total PCB or TEQ values were reported or could be calculated. TRVs were derived from several studies for various tissue matrices (Table 2.1). Hudson River studies were included because they used the same species as in this study, and are the only studies known to observe reproductive effects in tree swallows attributable to exposure to PCBs (USEPA, 2000). Co-contaminants are also believed to be less than the threshold concentrations for effects. There were no laboratory studies using tree swallows, and therefore, this field study was deemed the most appropriate estimate of TRV values. Reproductive effects including decreased hatching, increased abandonment, lower quality nests, and abnormal plumage coloration were reported in tree swallows at PCB contaminated sites relative to an uncontaminated area. In order to be protective of wildlife species, the Hudson River location with the lowest site-wide mean concentration of PCBs in eggs was chosen as the no observed adverse effect level (NOAEL). A NOAEL value of 26.7 μg PCB/g, ww for eggs, nestlings, and adults was used. The $TEQ_{WHO\text{-}Avian}$ reported for eggs in this study was also used as the most conservative $TEQ_{WHO\text{-}Avian}$ estimate of the NOAEL (13 ng TEQ/g). The lowest observed adverse effect level (LOAEL) for total PCBs was derived from a Housatonic River study in which hatching success was impaired during two years of the study (Custer *et al.*, 2003). The least concentration of total PCBs in pipping chicks from the two years (63 μg PCB/g) was used as the LOAEL. This study was deemed appropriate because effects were observed in two consecutive years and sensitive reproductive endpoints were evaluated. A LOAEL based on $TEQs_{WHO\text{-}Avian}$ could not be derived from the literature.

Hazard quotients of less than 1.0 suggest that tissue concentrations in all stages of the tree swallow life cycle were less than the threshold for effects based on the LOAEL. In most instances, even based on the more conservative NOAEL, HQ were less than 1.0 for all tissues (Figure 2.3). It should be noted that the true effect level for individuals rests somewhere between the NOAEL and LOAEL, and that even conservatively, population effects are not expected at a HQ of 10.0. Thus, while a few individuals did have NOAEL HQ values between 1.0 and 2.5, reproductive dysfunctions would not be expected nor were any seen (Neigh *et al.*, 2004). Tree swallows on the Hudson River had concentrations of PCBs that were 10-fold greater than the concentrations observed at the KRAOC and reproductive effects were barely identifiable above the variation naturally inherent in field studies (Secord *et al.*, 1999).

Multiple lines of evidence including both exposure and effects were explored to quantify risk to passerine species with an aquatic-based diet. Both the total PCB and TEQ method of evaluating exposure concentrations resulted in similar conclusions of little to no risk to tree swallows species at the KRAOC. Even when the most conservative TRV criteria were utilized, tissue concentrations at the site suggest that only those individuals with the greatest exposure may be at risk for effects attributable to PCBs, but for the population as a whole, the current concentrations of PCBs are less than the threshold for effects

Accordingly, other studies with similar tissue concentrations did not observe reproductive effects attributable to on-site exposure to PCBs. In short, multiple lines of evidence based on three years of observation find that KRAOC tree swallow populations are not affected by PCB contamination at an ecologically relevant level (Neigh *et al.*, 2004).

CONCLUSION

Prior to this study little information was available on the extent of PCB exposure and potentially related effects on Kalamazoo River passerine birds. Passerines are not only important ecological receptors in their own right, but they are a potential prey component of higher food web receptors within the Kalamazoo River basin, including the great horned owl (*Bubo virginianus*) and bald eagle (*Haliaeetus leucocephalus*). Elevated concentrations of PCBs in TB passerine nestlings indicate environmentally available concentrations of PCBs in the vicinity of the TB nests. PCB concentrations contained in adults and eggs indicate the presence of life cycle body burdens, which are critical for evaluation of the health and sustainability of passerine populations. Here, we utilized site-specific data to perform a risk-based evaluation of passerine health and sustainability in PCB contaminated areas of the Kalamazoo River. The evaluation directly compared exposure and productivity data for the target area to that of the FC reference area of the Kalamazoo River. Concentrations of PCBs were significantly greater in tree swallows from the KRAOC than those from the upstream reference site, and they were comparable to other PCB-contaminated sites in the region. Based on a three year site specific multiple lines of evidence approach, tree swallows from the KRAOC region of the

Kalamazoo River were exposed to PCBs at concentrations that were significantly greater than similar areas without point source inputs. However, these exposures were less than those expected to cause population level effects, and no effects were observed in Kalamazoo River tree swallow populations.

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Chapter 3

Reproductive success of passerines exposed to PCBs through the terrestrial food web of the Kalamazoo River

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ABSTRACT

The eastern bluebird (*Sialia sialis*) and the house wren (*Troglodytes aedon*) were identified as ecological receptors for the identification of exposure and potential effects stemming from polychlorinated biphenyl (PCB) contamination in floodplain soils of the Kalamazoo River Superfund Site, Kalamazoo, MI. Concentrations of PCBs in tissues and diets of these two bird species were statistically greater in areas downstream of the point sources than at an upstream reference location. Here we report concurrent evaluations of hatching success, fledging success, productivity, clutch size, brood size, egg size, and growth. Productivity of eastern bluebirds (*Sialia sialis*) was significantly less at the downstream location as compared to background during this three-year study. Hathcing success, clutch size, and predicted brood size were significantly less in early clutches of house wrens (*Troglodytes aedon*) at the contaminated location relative to the upstream reference location, but fledging success was greater at the contaminated location over the entire study period. Growth was significantly different during one of the three years for each species at the contaminated location compared to the reference location, but growth was similar when the entire study period was evaluated. The presence of below threshold concentrations in the tissues and diets of the passerine birds suggest that other factors besides PCB exposure such as habitat, small sample size, and co-contaminants may be affecting reproduction.

Keywords: Polychlorinated biphenyls, Productivity, Birds, Eastern bluebird, House wren, Eggs, Hatching success

INTRODUCTION

The Kalamazoo River was designated a Superfund site in 1990 due to the presence of paper waste contaminated with polychlorinated biphenyls (PCBs) released during the carbonless copy paper recycling process (MDEQ, 2003). The Kalamazoo River Area of Concern (KRAOC) supports a diverse terrestrial ecosystem along its course, including 205 ha of formerly impounded contaminated sediment exposed when three dams were partially dismantled to their sill level. While the effects of PCB contamination on various species with aquatic-based diets is well documented (Giesy *et al.*, 1994; Kubiak *et al.*, 1989; Ludwig *et al.*, 1993; Millsap *et al.*, 2004; Yamashita *et al.*, 1993), few studies have evaluated PCB contamination in wildlife exposed through terrestrial food webs associated with riverine ecosystems. The presence of PCBs in soil and sediment at the site requires identification of effects in terrestrial and aquatic endpoints during the risk assessment process. This study focused on the effects of PCB contamination in two passerine birds with predominantly terrestrial diets, while risk to passerines dependent on the aquatic food web is presented elsewhere (Neigh *et al.*, 2004a).

The tree swallow has a well-established history as a passerine contaminant monitor in aquatic systems (McCarty and Secord, 1999), but no such favored passerine receptor exists for terrestrial systems. In terrestrial risk assessments, a number of passerine species have been utilized depending on habitat type and species applicability to site-specific monitoring criteria. These species include: the European starling (Halbrook *et al.*, 1998), American robin (*Turdus migratorius*) (Henning *et al.*, 1997; Henning *et al.*, 2002), red-winged blackbird (*Agelaius phoeniceus*) (Bishop *et al.*, 1995), western bluebird (*Sialia mexicana*) (Fair and Myers, 2002), ash-throated flycatcher (*Myiarchus cinerascens*) (Fair and Myers, 2002), American redstart (*Setophaga ruticilla*), barn swallow (*Hirundo rustica*), eastern phoebe (*Sayornis phoebe*), rose-breasted grosbeak (*Pheucticus ludovicanus*), wood thrush (*Hylocichla mustelina*), and yellow warbler (*Dendroica petechia*) (Henning *et al.*, 1997). To our knowledge eastern bluebirds (*Sialia sialis*) have been used infrequently as a reproductive monitor of exposure (Bishop *et al.*, 1995), and the house wren (*Troglodytes aedon*) is a novel monitor of terrestrial PCB exposure.

The house wren and eastern bluebird were selected as monitors of PCB exposure at the Kalamazoo River Superfund Site based on a number of criteria. Eastern bluebirds and house wrens frequently used nest boxes at the Kalamazoo River locations, so sample sizes were expected to be sufficient to demonstrate population health. It was also believed that the species' exposure would coincide with local sources of contamination in the soil due to their diet of terrestrial insects (Pinkowski, 1978), and thus, be useful surrogates to assess the potential reproductive impairment by PCBs. Here, we describe productivity and growth of house wrens and eastern bluebirds located within the Kalamazoo River Superfund Site and an upstream reference site.

MATERIALS AND METHODS

Site details

During 2000, nest boxes were established at two locations along the Kalamazoo River, Michigan, 100-yr floodplain. The Fort Custer State Recreation Area (FC) is located upstream of the sources of contamination, and therefore, was chosen to serve as a background location due to the low background levels of contamination existing at the site (Anonymous, 1997). Situated 67 km downstream, the target area was located at the former Trowbridge Impoundment within the Kalamazoo River Area of Concern (KRAOC), which was designated a Superfund site by the USEPA in 1986 (Figure 3.1). The former Trowbridge Impoundment (TB) was formed when the Trowbridge Dam was removed to the sill, which exposed some 132 ha of former depositional lake bottom sediments.

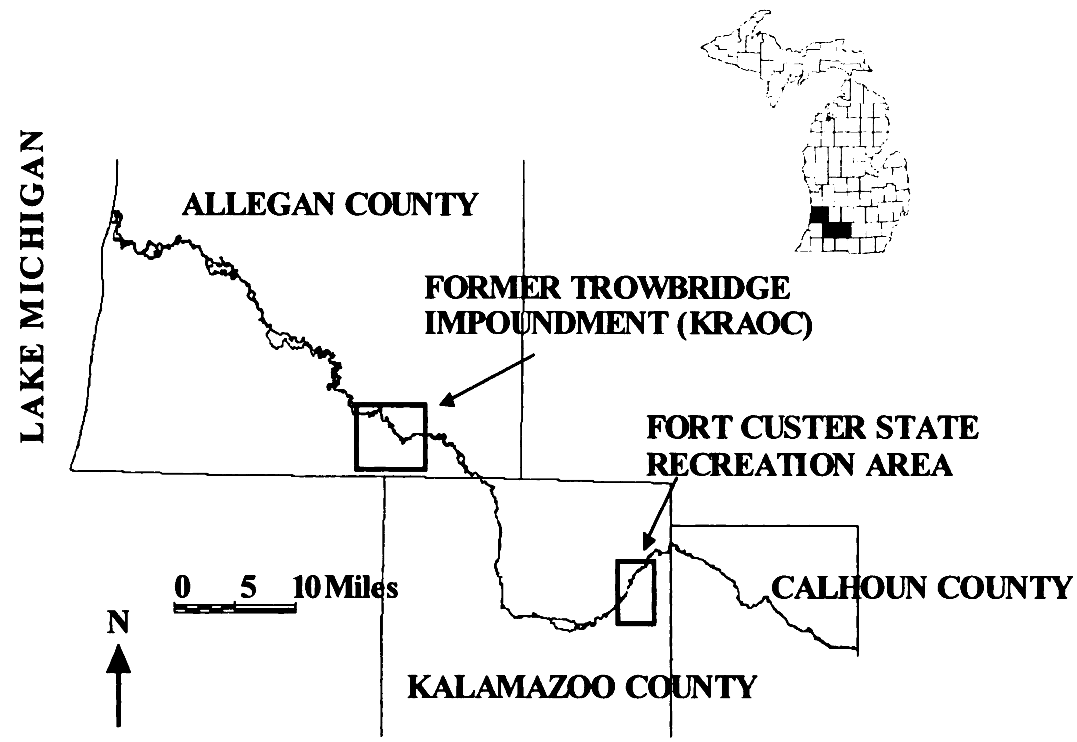

Figure 3.1. Map of the Kalamazoo River Area of Concern (KRAOC) and reference site. The inset describes the location of the two counties in Michigan where reproductive studies were conducted. A box designates the boundaries of the upstream reference location (Fort Custer State Recreation Area) and the Trowbridge impoundment, located within the KRAOC.

Previous studies quantified concentrations of total PCBs, DDT metabolites, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) (Neigh *et al.*, 2004b) based on World Health Organization toxic equivalence factors ($TEF_{WHO\text{-}Avian}$) (van den Berg *et al.*, 1998). In all tissues from both eastern bluebirds and house wrens, mean concentrations of total PCBs and TEQs were significantly greater at the KRAOC than they were at the reference location, FC (Table 3.1). Among all matrices, concentrations of total PCBs at TB were

39 to 122-fold greater than concentrations at FC, while concentrations of TEQs at TB were 5 to 47-fold greater than concentrations at FC

Table 3.1. Mean (± SD) concentrations of total PCBs and 2,3,7,8 –tetrachlorodibenzo-p-dioxin equivalents (TEQs) in the tissues of terrestrial passerine species at the Fort Custer State Recreation Area (FC) and at the Trowbridge Impoundment (TB) (Neigh et al., 2004b).

	Total PCBs				TEQs[a]			
	N	**FC**	**N**	**TB**	**N**	**FC**	**N**	**TB**
Eastern bluebird								
Egg	**14**	**0.17 (0.10)**	**7**	**8.3 (5.1)[b]**	**3**	**7.6 (8.6)**	**5**	**77 (82)[b]**
Nestling	**17**	**0.011 (0.006)**	**6**	**1.3 (1.4)[b]**	**17**	**1.3 (0.64)**	**6**	**6.3 (4.0)[b]**
House wren								
Egg	**14**	**0.12 (0.12)**	**14**	**6.3 (6.0)[b]**	**8**	**8.6 (3.9)**	**11**	**400 (450)[b]**
Nestling	**13**	**0.020 (0.020)**	**17**	**0.77 (0.64)[b]**	**13**	**1.4 (0.96)**	**17**	**63 (47)[b]**
Adult	**8**	**0.072 (0.033)**	**9**	**3.2 (2.1)[b]**	**8**	**7.1 (5.7)**	**9**	**110 (57)[b]**

[a]TEQs based on World Health Organization Toxic Equivalence Factors (van den *Berg et al.*, 1998)

[b]Mean is significantly greater than at the FC reference area (Student's t-test, $p<0.05$).

Productivity observations

Throughout the 2001 to 2003 nesting seasons, the nest boxes were monitored for occupancy by eastern bluebirds and house wrens at FC (n = 64) and TB (n = 68). Boxes were monitored daily to determine the date of clutch initiation and day of hatch. Reproductive success was determined based on nest observations. Hatching success was described as the percentage of eggs hatched in each nest. Fledging success was described as the percentage of nestlings fledged per nestling hatched, and productivity was described as the percentage of nestlings fledged per egg laid. Egg mass and length were recorded within 24 h of laying. Day of hatch was also confirmed by assessing physical development of the nestling (Pinkowski, 1975). Each nestling was examined for gross external morphological abnormalities. Weight of each nestling was recorded on days 3, 9, and 12 (hatch day = day 0) for eastern bluebirds and on days 3, 6, and 9 for house wrens. When chicks were sampled for contaminant analysis at approximately day 9 for house wrens and day 12 for eastern bluebirds, each individual was weighed and the length of the entire body (tip of beak to longest retrix), tarsus (tibiotarsal joint to hind toe base), and unflattened wing chord were recorded. Measurements were taken during 2001 and 2002 for house wrens and from 2001 to 2003 for eastern bluebirds.

Statistical Analyses

For statistical analyses, each nest box was treated as a separate experimental unit and values were reported on a per box basis. All nesting attempts were treated as separate and individual observations. Normality was assessed with Kolmogorov-Smirnov's one sample test with Lilliefors transformation, and homogeneity of variance was verified by F test. All parametric data were analyzed by one-way analysis of variance (ANOVA), and non-parametric data were analyzed by Mann-Whitney U or Kruskal-Wallis tests. The criterion for significance used in all tests was $p<0.05$.

RESULTS

Reproductive success

A total of 34 house wren and 18 eastern bluebird nests were completed at the TB study site and 71 house wren and 57 eastern bluebird nests were completed at the FC site over the study period (Table 3.2). For the entire study, 32% of eastern bluebird nests failed and 24% of house wren nests failed. The failure of the majority of eastern bluebird nests was attributed to predation, which comprised over 50% of the nest failures at both FC and TB. Abandonment accounted for 29% of the nest failures at FC and 30% at TB. Failure of house wren nests was attributed to a greater proportion of abandoned nests at TB (56% of all failures), while at FC, no nest failures were attributed to abandonment. Predation accounted for 44% of house wren nest failures at FC, but only 22% at TB.

Table 3.2. Nest fate and percent of initiated nests of eastern bluebirds and house wrens at the Fort Custer State Recreation Area (FC) reference site and at the Trowbridge (TB) contaminated site.

	2001		2002		2003	
	FC	TB	FC	TB	FC	TB
Eastern bluebird						
Successful[a]	14 (93%)	2 (40%)	15 (63%)	2 (25%)	14 (78%)	4 (80%)
Predated[b]	1 (7%)	0 (0%)	6 (25%)	4 (50%)	1 (6.5%)	1 (20%)
Abandoned[c]	0 (0%)	1 (20%)	2 (8%)	2 (25%)	2 (11%)	0 (0%)
Unknown	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other	0 (0%)	2 (40%)	1 (4%)	0 (0%)	1 (6.5%)	0 (0%)
Total	15	5	24	8	18	5
House wren						
Successful[a]	17 (89.5%)	8 (73%)	38 (73%)	17 (74%)		
Predated[b]	1 (5.25%)	0 (0%)	6 (11%)	2 (9%)		
Abandoned[c]	0 (0%)	2 (18%)	0 (0%)	3 (13%)		
Unknown	0 (0%)	1 (9%)	5 (10%)	0 (0%)		
Other	1 (5.25%)	0 (0%)	3 (6%)	1 (4%)		
Total	19	11	52	23		

[a]Successful nests fledged at least one nestlings.

[b] Predated nests included those nests with evidence of predation such as disturbed nesting material or broken eggs.

[c]Nests were considered abandoned when eggs were cold for at least 7 d and adults were not present.

Both house wrens and eastern bluebirds can produce two broods in a season. Birds were not banded at either location, and therefore, it was not possible to definitively determine when a nesting pair established subsequent nests. In all years, there were 7 and 12 suspected house wren second broods at TB and FC, respectively, and 3 and 15 suspected eastern bluebird second broods at TB and FC, respectively. Nests were still considered to be independent samples because second broods could not be verified since adults were not banded. In order to limit the effect of second nestings, reproductive parameters were also evaluated based on dates of clutch initiation, where nests from early in the season were grouped together and nests from late in the season were grouped together. Nesting attempts were categorized as early or late nests to account for potential differences in reproductive parameters between the first and second nestings. The median dates of initiation for house wrens in 2001 were May 20 and June 24 for early and late nests, respectively, and May 28 and July 1 during 2002, respectively. The median date of nest initiation for eastern bluebirds was between April 21 and April 25 for early nests and June 9 and June 22 for late nests during the study. There was little correlation (Spearman, $r^2 < 0.10$) between date of nesting and reproductive success in either house wrens or eastern bluebirds.

All measures of reproductive success, including hatching success, fledging success, and productivity were adjusted for fresh egg sampling, except clutch size, by eliminating the egg collected for residue analyses from the reproductive analyses because the subsequent potential fate of the eggs collected for residue analyses could not be predicted at the time of sampling. Nests were observed for activity until the date of fledging, so it was assumed that live sampled nestlings would have fledged successfully. Therefore, the number of nestlings sampled did not affect the outcome of statistical analyses, but the number of fresh eggs sampled could have affected the observed brood size and number of fledglings. To make comparisons of brood size and the number of fledglings, a predicted value was calculated based on other parameters. The brood size for each nest in which an egg was sampled was calculated as the clutch size multiplied by the hatching success. The predicted number of fledglings for each nest in which an egg was sampled was calculated as the clutch size multiplied by the productivity. All other interactions attributed to the collection of eggs or nestlings were considered to be negligible as compared to exposure to PCBs.

Due to small sample sizes, measurements of reproductive health in eastern bluebirds were combined among all years. When years were combined, a statistically significant difference between FC and TB in the productivity (Mann-Whitney U, $p = 0.031$) and nearly statistically significant differences in hatching success (Mann-Whitney U, $p = 0.093$) and fledging success (Mann-Whitney U, $p = 0.074$) were observed (Table 3.3). Statistically significant differences were detected in the date of clutch initiation, so to eliminate interactions between date of initiation and reproductive success, all nests were separated into one of two groups designated "early" or "late" nests. Reproductive success was not statistically different in late nests, but the clutch size (Mann-Whitney U, $p = 0.003$) and fledging success (Mann-Whitney U, $p = 0.041$) were statistically greater at FC than at TB in early nests.

Reproductive parameters for house wrens were also not statistically different between years, so all years were combined. When all years were combined, the only statistically significant difference between locations was fledging success which was greater at TB than it was at FC (Mann-Whitney U, $p = 0.030$) (Table 3.3). Although the date of clutch initiation for eastern bluebirds and house wrens was not significantly different between locations, clutches were separated into two classes, designated "early" and "late" nests, in an attempt to minimize the effect that date may have on reproduction. In early clutches, clutch size (Mann-Whitney U, $p = 0.045$), hatching success (Mann-Whitney U, $p = 0.030$), and predicted brood size (Mann-Whitney U, $p = 0.032$) were statistically greater for nests at FC than at TB.

Table 3.3. Eastern bluebird and house wren nest productivity measurements (mean ± SD) at a reference site (Fort Custer) and at a PCB contaminated target site (Trowbridge) on the Kalamazoo River for early and late clutches and all clutches combined.

	Early Clutches				Late Clutches				All Clutches			
	Fort Custer		Trowbridge		Fort Custer		Trowbridge		Fort Custer		Trowbridge	
	N	Mean (± 1 SD)	N	Mean (± 1 SD)	N	Mean (± 1 SD)	N	Mean (± 1 SD)	N	Mean (± 1 SD)	N	Mean (± 1 SD)
Eastern bluebird												
Hatching success[a]	25	0.82 (0.27)	10	0.61 (0.44)	24	0.75 (0.34)	4	0.54 (0.42)	49	0.79 (0.31)	14	0.59 (0.42)
Fledging success[b]	24	0.96 (0.20)	6	0.75[d] (0.42)	21	0.95 (0.22)	3	1.0 (0.0)	45	0.96 (0.21)	9	0.83 (0.35)
Productivity[c]	25	0.79 (0.32)	9	0.44 (0.47)	24	0.73 (0.38)	4	0.54 (0.42)	49	0.76 (0.34)	13	0.47[d] (0.44)
Clutch size	26	4.7 (0.47)	11	3.5[d] (1.4)	31	3.6 (0.93)	7	3.7 (1.7)	57	4.2 (1.0)	18	3.6 (1.5)
Predicted brood size	24	4.1 (1.2)	7	3.5 (1.2)	21	3.6 (0.93)	3	2.9 (2.0)	45	3.8 (1.1)	10	3.3 (1.4)
Predicted number of fledglings	23	4.1 (1.2)	5	3.1 (1.5)	20	3.6 (0.94)	3	2.9 (2.0)	43	3.9 (1.1)	8	3.0 (1.6)
House wren												
Hatching success[a]	32	0.83 (0.24)	15	0.58[d] (0.39)	28	0.78 (0.25)	16	0.71 (0.44)	60	0.81 (0.25)	31	0.64 (0.41)
Fledging success[b]	31	0.90 (0.26)	13	1.0 (0.0)	28	0.94 (0.21)	12	1.0 (0.0)	59	0.92 (0.24)	25	1.0[d] (0.0)
Productivity[c]	32	0.75 (0.30)	15	0.58 (0.39)	28	0.73 (0.30)	16	0.71 (0.44)	60	0.74 (0.30)	31	0.64 (0.41)
Clutch size	38	6.2 (0.93)	15	5.6[d] (1.2)	33	5.3 (1.2)	21	5.2 (1.5)	72	5.7 (1.1)	36	5.4 (1.4)
Predicted brood size	31	5.5 (1.5)	13	3.9[d] (2.2)	28	4.4 (1.6)	12	5.4 (1.3)	59	5.0 (1.6)	25	4.6 (2.0)
Predicted number of fledglings	29	5.2 (1.4)	13	3.9 (2.2)	27	4.2 (1.6)	12	5.4 (1.3)	56	4.8 (1.6)	25	4.6 (2.0)

Table 3.3 (cont'd)

[a]Hatching success is calculated as the number of eggs hatched per egg laid.

[b]Fledging success is calculated as the number of fledglings per nestling hatched.

[c]Productivity is calculated as the number of fledglings per egg laid.

[d]Mean is statistically different from Fort Custer population ($p<0.05$).

Egg measurements

No statistically significant differences in egg measurements among years were detected, so years were combined. The mean weights of eggs of neither species were statistically different between locations (Table 3.4). The mean length of house wren eggs was not statistically different between sites, but eggs were significantly longer at FC than at TB for eastern bluebird eggs (Student's t-test, $p = 0.026$). Egg parameters were evaluated for interaction with each other and with date of initiation based on ANCOVA. No interactions between any parameters existed for house wren, but a statistically significant correlation between weight and length did exist for eastern bluebirds (ANCOVA, $p = 0.008$), which changed the significance of the comparison of mean egg length ($p = 0.026$ to $p = 0.061$).

Table 3.4. Mean (± 1 SD)[a] reproductive endpoints for eastern bluebird and house wren eggs and nestlings reared at the Fort Custer State Recreation Area and at the Trowbridge Impoundment along the Kalamazoo River Area of Concern.

	Fort Custer		**Trowbridge**	
	Eastern bluebird	**House wren**	**Eastern bluebird**	**House wren**
Eggs				
N	**36**	**74**	**11**	**37**
Egg weight (g)	**3.22 (0.67)**	**1.46 (0.01)**	**3.01 (0.12)**	**1.51 (0.09)**
N	**34**	**74**	**11**	**37**
Egg length (mm)	**21.4 (1.04)**	**16.9 (0.08)**	**20.6 (0.28)[c]**	**16.8 (0.16)**
Nestlings[b]				
N	**12**	**22**	**3**	**12**
Body weight (g)	**27.79 (2.04)**	**9.43 (0.81)**	**29.17 (2.03)**	**9.32 (1.39)**
Body length (mm)	**92.3 (6.2)**	**64.9 (6.2)**	**98.1 (14.6)**	**61.5 (4.2)**
Tarsal length (mm)	**19.3 (1.8)**	**16.8 (2.7)**	**19.7 (1.4)**	**16.2 (2.4)**
Wing chord (mm)	**49.9 (8.5)**	**23.1 (4.2)**	**47.9 (4.9)**	**24.4 (4.6)**

[a] Mean values were calculated based on the mean measurements per nest for all nests.

[b] Eastern bluebird nestling measurements taken at day 12 and house wren nestling measurements at day 8 or 9.

[c] Statistically different from Fort Custer population (Student's t-test, $p = 0.026$).

Growth measurements and growth curves

Differences in growth parameters between years could not be evaluated due to small sample sizes, so all years were combined. Growth parameters between 8 and 9 day-old house wrens were similar, so ages were combined. Eastern bluebird growth measurements consisted of nestlings at day 12. Correlations between growth parameters within locations were taken into account when growth was evaluated between locations. There were no significant correlations present for eastern bluebirds, but there were several potential correlations for house wrens. The correlation between body length, wing chord length, and tarsal length drove the difference in mean body length between sites to a nearly statistically significant value (ANCOVA, $p = 0.072$), but no growth parameters for either species were statistically different between sites (Table 3.4).

Growth of nestlings was compared between sites based on curves generated from nestling mass as an indicator of stress on the species (Zach and Mayoh, 1982). A logistic growth curve was fitted to nestling masses at each site. Growth curves, based on average nestling mass for each box at each nestling day, were strikingly similar for both species at both sites when all years were combined. Growth curves of house wrens, compared between sites, overlapped over the entire nesting period (Figure 3.2). Growth curves for eastern bluebirds followed a similar trajectory, but the curve was shifted toward greater mean nestling weights at FC (Figure 3.3).

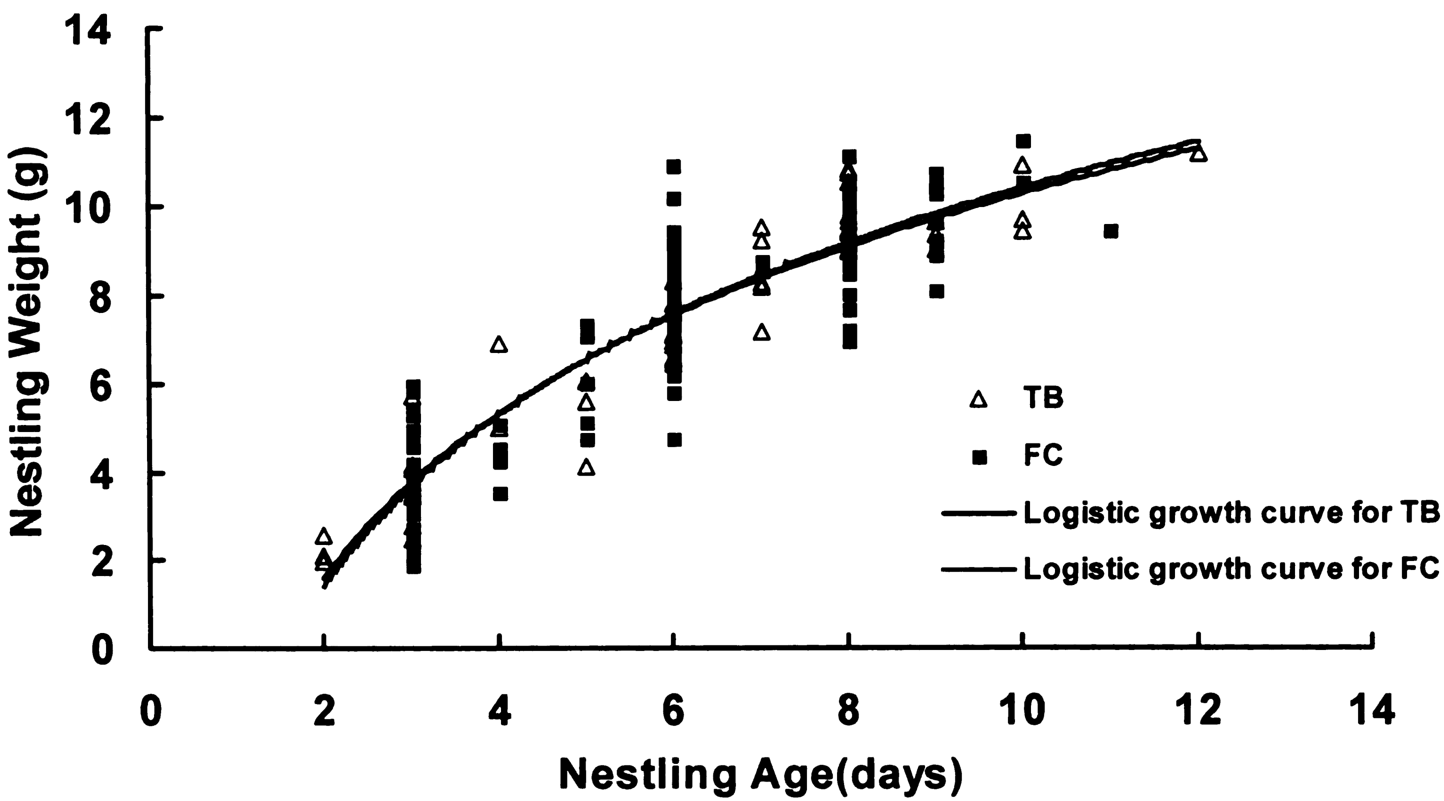

Figure 3.2. House wren nestling growth curves based on mean nestling weights for each nest box at the Trowbridge (TB) target area and the Fort Custer (FC) reference site. The equation for the line of best fit at FC is $y=5.563Ln(x)-2.4223$, $r^2=0.836$. The line of best fit for TB is $y=5.378Ln(x)-2.1186$, $r^2=0.896$.

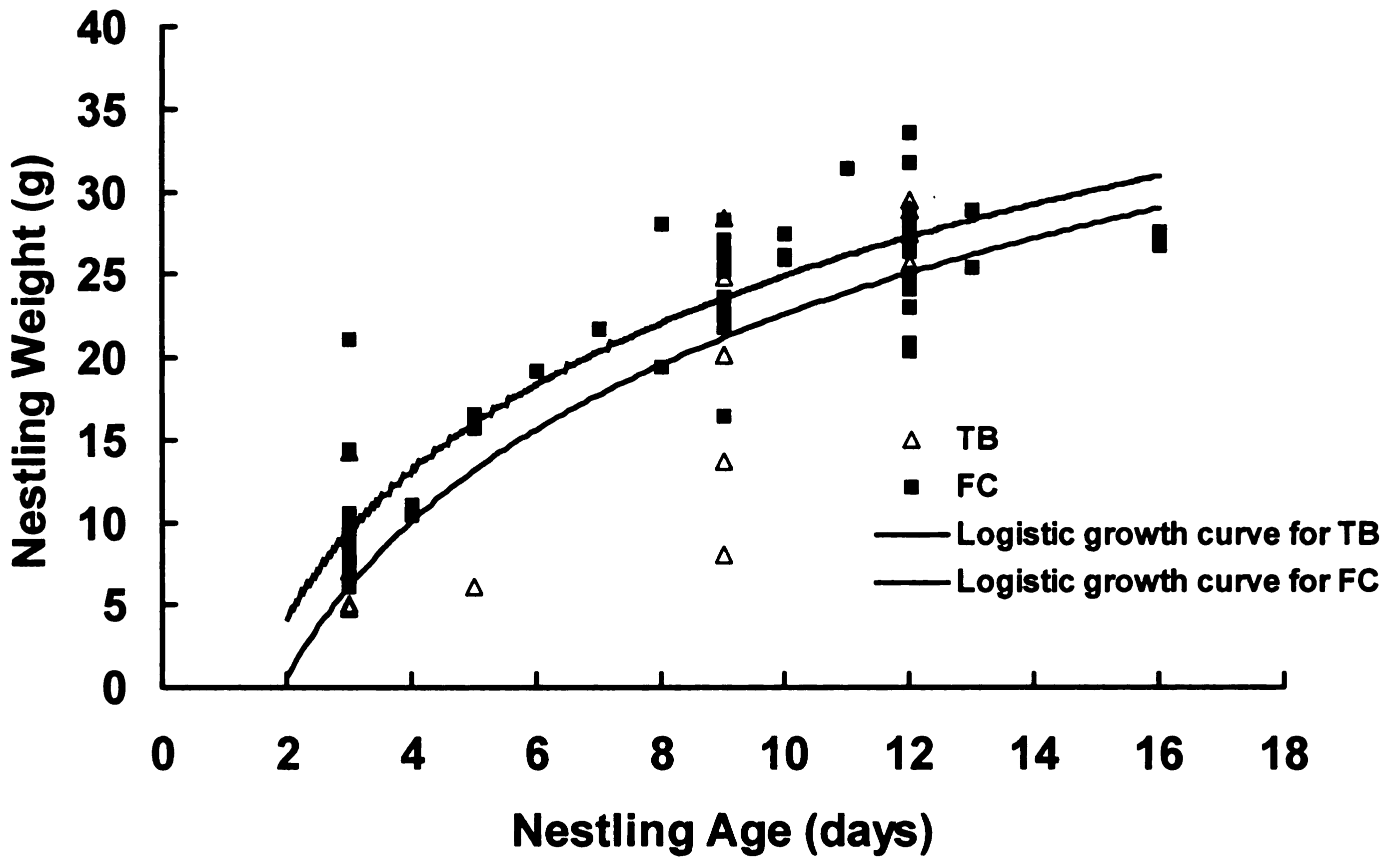

Figure 3.3. Eastern bluebird nestling growth curve based on mean nestling weights in each active nest box at the Trowbridge (TB) target site and the Fort Custer (FC) reference site. The line of best fit for the curve is described by the equation $y=13.668Ln(x)-8.8892$, $r^2=0.647$ at TB and $y=12.891Ln(x)-4.7993$, $r^2=0.86$.

Growth curves, based on average nestling mass gain over the nestling period, for the two sites were compared statistically. Nestling mass between nestling day 3 and day 10 for eastern bluebirds and day 2 and day 8 for house wrens were log-transformed and the mass gained per day of life was compared between sites. House wren mass gain per day was significantly different between years (ANOVA, $p = 0.020$), but that of eastern bluebirds was not. House wren mass gain was significantly less at TB than at FC during 2002 (Student's t-test, $p = 0.044$) but not during 2001. Eastern bluebird mass gain per day was not different between sites in 2001 or 2003, but 2002 was significantly different (Kruskal-Wallis, $p = 0.013$).

DISCUSSION

Reproductive success

Eastern bluebirds made fewer nesting attempts than house wrens at TB and FC, but confounding factors such as vegetation structure and age of the nest trail may contribute to this difference (Munro and Rounds, 1985). The bluebird box trail at FC has been established for over 30 yr, which may cause an increase the number of returning adults and nestlings to the site. An investigation of habitat suitability at both sites was conducted using a habitat suitability model for the eastern bluebird (A. Neigh, *unpublished data*). Habitat is not expected to influence house wren nesting attempts due to the large availability of the house wren's preferred edge habitat. Overall, habitat suitability for bluebirds during the early spring was determined to be 30.5% ± 32.9 (mean ± 1 SD) at FC and 42.3% ± 34.1 at TB. Due to increasing vegetation height during mid-summer, the HSI at TB decreased to 32.2% ± 25.9. Although these values are comparable, the dynamics of vegetative growth and local groundwater hydraulics during portions of the nesting season may render substantial areas of TB unusable for nesting, and therefore, less attractive to the species. The suitability for nest establishment is greater at TB, but the percentage of suitable area characterized by certain cover types is less at TB (14%) than at FC (26%), and the relationship becomes even more distinct when evaluated in mid-summer when only 3% of the total TB site is suitable for eastern bluebird nesting. The relatively high quality of the habitat at the sites limits the interaction between habitat suitability and reproductive success, but the extent of the high quality habitat is less at TB than at FC. This suggests that the lower occupancy at TB could be a result of spatial limitations, but those individuals situated on the available quality habitat would not likely be limited by habitat structure.

Abandonment rates for tree swallows have been reported to be greater at PCB contaminated sites compared to less contaminated habitats (McCarty and Secord, 1999). Little data for the bluebird or the house wren are available for abandonment rates at PCB contaminated sites, except for the tree swallow. Abandonment rates of eastern bluebirds and house wrens at the Kalamazoo River were between 0% and 25%, which was near the range (4.5% to 23.7%) reported for uncontaminated populations of bluebirds (Rustad, 1972). Abandonment rates of eastern bluebirds may be partly attributed to interactions with house wrens. House wrens are known to interfere with the nesting of other species (Finch, 1990), and unlike other studies of this type, house wren nesting was not discouraged. Abandoned nests had no physical damage to the structure of the nest, as is the case in 85% of house wren predations (Belles-Isles and Picman, 1986), so abandoned nests may have been improperly labeled as abandoned rather than associated with house wren interference. Abandonment was not observed for house wrens at FC, but some nests were abandoned at TB. Again, interactions among house wrens may have caused abandonment without damage to the nest, where in some cases, as much as 89% of unsuccessful nesting attempts have been linked to conspecific interference (Finch, 1990).

The date of clutch initiation did not affect the reproductive success of either house wrens or eastern bluebirds. Some studies have reported that second clutches were not as likely to be successful as first clutches, with only 21% of second nests producing fledglings (Pinkowski, 1979). At the Kalamazoo River, there was no reduction in nest success in the clutches classified as "late" nests, but it could not be determined which nests were in fact second nests and which nests were late first nest attempts. Other studies also did not find a correlation between Julian date and reproductive parameters (Fair and Myers, 2002).

PCBs may affect reproductive capacity by decreasing hatching success, possibly by causing infertility of eggs (McCarty and Secord, 1999). Hatching success in early clutches of housr wrens at TB was significantly less than at FC (Mann-Whitney U, $p = 0.045$), which was also observed in tree swallows from the Hudson River where hatching success was 64-79% at contaminated sites and 93% at a reference location (Secord and McCarty, 1997). Conversely, hatching success was not impaired at the Housatonic River for several species of passerines exposed to elevated levels of PCBs with 87.4% of the eggs hatching (Henning *et al.*, 1997). Of the nonviable eggs at the Hudson River, 60%

were infertile, while the remaining nonviable egg mortality was attributed to the failure of the embryo to develop and the death of the embryo (McCarty and Secord, 1999). Hatching success of both the bluebird and house wren at the Kalamazoo River is most strongly correlated with productivity (Spearman, $r^2 = 0.78$). Productivity was significantly reduced in the eastern bluebird at TB, but this value is dependent on a single female in 2001 that twice brooded unsuccessfully but did not abandon the eggs. This female alone accounted for a 40% decrease in reproductive success at TB because only a total of 5 nests were established by bluebirds during that year. If this one individual is removed from the data set, the hatching success at TB for all nests increased by 10% and the productivity increased by 9%. Productivity for all nests was no longer significantly different between locations when the nest was removed from the analyses, but all other statistical comparisons were similar to those made previously. Power to detect differences in reproductive measurements between locations was generally less than an acceptable limit for both species ($1-\beta < 0.80$) (Table 3.5). Sample sizes greater than those observed at the Kalamazoo River would have been needed to detect a 20% decrease in reproductive performance at TB compared to FC. Reproductive performance was depressed at TB compared to FC, but measured concentrations of PCBs in the tissue and diet of both species were below the threshold concentrations for effects (Neigh *et al.*, 2004b). Tissue concentrations and dietary exposure suggest little risk of PCBs eliciting reproductive effects on the birds in this study, so the observed decrease in reproductive success in these birds is likely a result of other factors present in the environment.

Table 3.5. Power (1-β) to detect a decrease in reproductive success at Trowbridge compared to Fort Custer and the sample size (N) needed at each location to detect a 20% reduction in reproductive health.

	Eastern bluebird						House wren					
	Separate Clutches				All Clutches		Separate Clutches				All Clutches	
	Early Clutches		Late Clutches				Early Clutches		Late Clutches			
	1-β	N	1-β	N	1-β	N	1-β	N	1-β	N	1-β	N
Hatching success	0.40	63	0.25	80	0.50	68	0.77	46	0.14	67	0.64	56
Fledging success	0.32	37	<0.01	9	0.26	29	<0.01	14	<0.01	8	<0.01	11
Productivity	0.65	81	0.21	92	0.70	84	0.45	67	0.07	83	0.30	74
Clutch size	0.90	15	0.07	45	0.57	28	0.50	10	0.06	20	0.37	15
Predicted brood size	0.28	26	0.14	60	0.28	33	0.77	35	<0.01	35	0.19	42
Predicted number of fledglings	0.37	35	0.15	59	0.41	39	0.64	41	<0.01	38	0.09	45

The fledging success and growth of nestlings, which may be related to nestling survival, has been associated with proximity to contamination (Fair *et al.*, 2003). Fledging success and growth over the entire study was not impaired in house wrens at TB compared to FC. In eastern bluebird nests, fledging success was less at TB in early nests but not for all clutches combined, and growth over the entire study was not different. Although growth for both species was different in one year but not over the entire study, the recruitment of the population, described as the predicted number of fledglings, was not different, so the impact of one years reduction in growth to the sustainability of the population is thought to be minimal. Some differences in fledging success and growth of nestlings were observed in the house wren and eastern bluebird, but these differences were not prevalent throughout the length of the study. Due to the inconsistency of results, nestling survival, predicted in this study as fledging success and growth, could not be linked to exposure to PCBs.

No data are available regarding reproductive measurements in the eastern bluebird or house wren at other PCB contaminated sites to compare. All measures of reproductive success for eastern bluebirds at the Kalamazoo River, except productivity at TB, were similar to uncontaminated populations (Pinkowski, 1979). There is little information on the reproductive success of unmanipulated house wren populations, but one population had similar productivity to the Kalamazoo River house wren populations (Finch, 1989). Other studies with greater tissue concentrations of PCBs in terrestrial species did not observe reproductive effects (Bishop *et al.*, 1995, Henning *et al.*, 2002). The observations made in our study, coupled with those made by others at both PCB-contaminated and uncontaminated locations indicate that it was unlikely that the concentrations of PCBs measured in birds at TB were causing any population-level effects on survival of nestlings.

Egg measurements

It has been suggested that eggs containing PCBs may exhibit differential dimensions (Fair and Myers, 2002) or mass compared to uncontaminated populations (Fernie *et al.*, 2000). PCBs may contribute to the smaller volume of eggs in ash-throated flycatchers at contaminated sites (Fair and Myers, 2002), but this difference was attributed to interaction with the width parameter in the calculation of egg volume (Pinkowski, 1975). Egg length but not width was evaluated in eggs at the Kalamazoo River, so differences in egg length may not be predictive of PCB exposure. As for measurements of egg weight, the yolk of eggs at PCB contaminated sites was found to be greater in mass than at uncontaminated sites, with greater mass associated with greater lipid content (Fernie *et al.*, 2000). When comparing fresh eggs, the lipid content in house wren eggs from TB was not significantly greater than that of eggs from FC. Samples sizes of fresh eggs of eastern bluebirds were insufficient for analysis. Natural variation in unexposed populations of eastern bluebirds has been described as an egg size gradient ranging from observably smaller eggs (mean length x mean width, 19.0 x 15.3 mm) to comparatively larger eggs (22.4 x 17.7 mm). The natural variation inherent in egg size limits the discernment of PCB-induced effects on egg size in this population, but it does not appear that exposure to PCBs had any significant effects on the sizes of eggs.

Growth measurements

Growth curves generated for each species were similar between sites. Logistic curves gave the best overall fit to the growth data of the Kalamazoo River, which is similar to the results for other locations (Zach and Mayoh, 1982). There are no published growth curves for house wren nestlings, but the relative similarity of curves for birds at TB and FC, over the length of the study, suggests that growth was not affected by exposure to PCBs. Growth curves for eastern bluebirds at the KRAOC were compared to an uncontaminated population (Pinkowski, 1975), and the logistic curve at TB was similar to the uncontaminated populations until day 8. From day 8 to day 10, the rate of weight increase in Kalamazoo populations decreased relative to the uncontaminated site, but weights coincided again by day 12. TB nestling growth during 2001 had the greatest mean nestling mass at each day, but the least during 2002. There is no apparent reason for the variation among years, but the inconsistent results among years may be associated with small sample sizes at the site. Nestlings at TB had a curve similar to FC, but the mass of nestlings at each day was less. When mass gain for each box over the nestling period of all years was examined, there was no statistically significant difference between TB and FC. The results of a study conducted at another location suggests that availability of insects accounts for 18-51% of the variation, while unexplained variation (error) accounted for a similar proportion (Quinney *et al.*, 1986). Potential variations in error at the Kalamazoo River locations may be associated with variation of individuals, egg quality, or measurement error. Based on visual examination of growth curves and statistical analysis of nestling growth expressed as mass gain per day, no consistent differences in nestling growth were identified when evaluating nests over the entire study period. Although some significant differences between sites existed when years are evaluated separately, the differences were only found in one of the three years of the study. Thus, it can be concluded that the exposure to PCBs at TB did not affect the rates of growth of either bluebirds or house wrens.

CONCLUSION

Measures of growth and overall productivity were used to evaluate whether adverse population-level effects were occurring in two terrestrial avian species exposed to PCBs at the Kalamazoo River Superfund Site. While the values of some measures of reproductive success of house wrens were significantly less at TB than at FC in some years, overall fledging success, which is a predictor of population-level effects, was greater at TB than at FC. Also during one year, growth of each species was less at TB than FC, but when evaluated over the entire study period, growth of both species was not significantly different between TB and FC. Other studies also examined reproductive effects in terrestrial species due to PCB exposure and reported no observed effects at concentrations greater than those contained in birds at the Kalamazoo River (Bishop *et al.*, 1995, Henning *et al.*, 2002). Based on the results of the study on which we report here and the results of other studies investigating the effects of PCBs on terrestrial passerine birds, it can be concluded that it was unlikely that exposure to PCBs at the Kalamazoo River Superfund site was having adverse effects.

Co-located studies were conducted in conjunction with the present study in order to quantify the concentration of PCBs in the tissues and diets of Kalamazoo birds and to compare various approaches to estimating risk (Neigh *et al.*, 2004b; Neigh *et al.*, 2004c). Concentrations of PCBs and DDE were significantly greater in tissues of the eastern bluebird (*Sialia sialis*) and house wren (*Troglodytes aedon*) at TB, which was the more contaminated location, than birds at FC, which was the upstream reference area (Neigh *et al.* 2004b). However, concentrations of PCBs in eggs and nestlings did not exceed a threshold above which effects were expected. Concurrent studies also investigated dietary exposure and determined that total PCBs in the diet of terrestrial species were less than the threshold concentration to cause adverse effects on reproduction (Neigh *et al.*, 2004c). Concentrations of TEQs in the diet were in a range that, based on the most conservative threshold determined from laboratory studies with other species, could cause some adverse effects. However, it was determined that the threshold was not exceeded when toxicity reference values based on a more appropriate field-derived threshold were applied in the risk assessment. The assessment of risk for concentrations in tissues and dietary exposure depend heavily on the threshold of effects, and since there are no studies specifically dealing with the species examined here, there are uncertainties associated with the choice of a threshold concentration, especially with dietary studies.

Even based on an approach to evaluate risk that includes three lines of evidence (tissue concentrations, dietary exposure, and reproduction), it was difficult to definitively determine if PCBs are eliciting effects on birds at the Kalamazoo River. The results of the studies presented here suggest some reproductive impairment of eastern bluebirds and house wrens at some locations of the Kalamazoo River, but other factors besides PCBs, such as co-contaminants, habitat suitability, and prey availability, are likely causes for the effects. Reproductive effects at the Kalamazoo River can not be decisively linked to exposure to PCBs, and with the exception of the potentially conservative estimate of risk based on exposure to non-*ortho* and mono-*ortho*-substituted PCBs, the tissue and dietary lines of evidence arrive at similar conclusions of no unacceptable risk. Based on three lines of evidence, the authors do not believe that terrestrial passerine species are exposed to concentrations of PCBs in contaminated areas of the Kalamazoo River that may contribute to the present or future risk of reproductive dysfunction.

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Chapter 4

Accumulation of polychlorinated biphenyls (PCBs) from floodplain soils by passerine birds

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ABSTRACT

Accumulation of polychlorinated biphenyls (PCBs) from floodplain soils and associated invertebrate prey by two passerine birds were evaluated at two locations at the Kalamazoo River Superfund Site in Michigan. Eggs, nestlings, and adults of the eastern bluebird (*Sialia sialis*) and house wren (*Troglodytes aedon*) were collected at a PCB contaminated site and a reference location on the Kalamazoo River, Michigan. In eastern bluebird eggs and nestlings at the more contaminated location, mean concentrations (wet weight) of total PCBs were 8.3 and 1.3 mg PCB/kg, respectively, and concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) (wet weight) were 77 and 6.3 ng TEQ/kg, respectively. Eggs, nestlings, and adults of house wrens from the contaminated location contained 6.3, 0.77, and 3.2 mg PCB/kg, respectively, and 400, 63, and 110 ng TEQ/kg, respectively. Concentrations of total PCBs and TEQs in tissues at the more contaminated location were significantly greater than concentrations in tissues at the reference site for all tissue types of both species. Concentrations of PCBs in tissues of both species were approximately 40-fold greater at the more contaminated location compared to the upstream reference location. Concentrations of TEQs in eastern bluebird tissues were 4 to 10-fold greater at the more contaminated location, while concentrations of TEQs in house wrens were 40-fold greater at the more contaminated location. Exposure of the two species studied were different, which suggests that terrestrial-based insectivorous passerine species, foraging in the same area, may have differential exposure to PCBs depending on specific foraging techniques and insect orders targeted. Despite the greater accumulation of PCBs at the more contaminated location, the risk of exposure

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to PCBs did not exceed the threshold for adverse effects at either location. Hazard quotients (HQs) based on both total concentrations of PCBs and TEQs at both the reference and more contaminated locations were both less than 1.0 for all samples.

Keywords: Eggs, Bioaccumulation, Birds, Eastern bluebird, House wren, American robin, Multiple lines of evidence, Twin embryos

INTRODUCTION

The Kalamazoo River in southwestern Michigan was contaminated with PCBs when carbonless copy paper was inadvertently mixed into the paper recycling process. In 1986, three dams were removed to their sill, which drained approximately 132 ha of formerly impounded sediment to create a large and contiguous landmass of lowland forests and marsh. Previous studies of the Kalamazoo River quantified concentrations of PCBs in multiple matrices including: soil, sediment, plants, mink, and tree swallows (Camp, Dresser, and McKee 2002; Millsap *et al.* 2004; Neigh *et al.* 2004a). Surveys of in-stream surface sediment (0-10 cm) indicate that concentrations of PCBs range from less than 0.001 mg PCB/kg, dry weight (dw) to 153 mg PCB/kg, dw with a mean PCB concentration of ~ 3.0 mg PCB/kg, dw (BBL, Inc. 1994a, Weston, Inc. 2002). Passerine birds with terrestrial diets were identified as receptors of concern due to the contamination in the formerly impounded floodplain soils, but little was known about the trophic transfer and bioavailability of organochlorines to upper food web insectivores. Preliminary sampling quantified concentrations of PCBs in surfical floodplain soils (0-25 cm) of former impoundment to be less than 0.001 mg PCB/kg, dw to as great as 85 mg PCB/kg, dw with mean values of ~ 11 mg PCB/kg, dw (BBL, Inc. 1994b, BBL, Inc. 1994c, BBL, Inc. 1994d).

The house wren and eastern bluebird were selected as upper trophic level monitors of exposure to PCBs derived from contaminated soil at the Kalamazoo River Superfund Site. The risk of potential effects on passerine health, due to exposure to PCBs existing in Kalamazoo River floodplain soils, was estimated by comparing concentrations of PCBs to established toxicity reference values (TRVs) for eggs, nestlings, and adults of the house wren and eastern bluebird (Fairbrother 2003). The American robin was identified as a receptor of concern during a baseline ecological risk assessment (Camp, Dresser, and McKee 2002) due to modeled contamination levels in its omnivorous diet. Likewise, eastern bluebirds, also in the Family Turdidae, feed on invertebrates in close contact with contaminated soil during portions of their life cycles (Pinkowski 1978). Thus, it was hypothesized that concentrations of PCBs in bluebird tissue would be analogous to concentrations in American robin tissues. The house wren is primarily an insectivorous species and was used here to determine risk to avian species with wholly insectivorous diets. It was expected, based on a 2000 baseline study at the reference site and at the contaminated site, that a sufficient number of nests that were easily identified would be available for both the bluebird and the house wren (Neigh *et al.* 2004b).

This paper applies "top-down" methodology that is based on measured concentrations of PCBs in eggs, nestlings and adults to establish exposure to avian species of the Kalamazoo River. Exposure was quantified by congener-specific analysis of approximately 100 PCB congeners, including non-*ortho* (coplanar) and mono-*ortho* congeners, and was reported as total PCBs. In addition, 2,3,7,8 -tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) were calculated for PCBs based on relative potencies for birds (TCDD-$_{\text{WHO-avian}}$ (van den Berg et al., 1998). Concentrations of total PCBs were intended to quantify potential exposure based on all measurable congeners in the environment, while concentrations of non-*ortho* and mono-*ortho* congeners, reported as avian-specific World Health Organization (WHO-Avian) TEQs, evaluated exposure based on the additive toxicity of the PCB congeners known to interact with the Aromatic hydrocarbon receptor (AhR). It has been postulated that the most sensitive measure of toxic effects of PCBs is through the AhR-mediated pathway and that due to weathering of the total PCB mixture, the TEQ approach is a more accurate method to estimate exposure and potential toxic effects (Giesy and Kannan 1998).

Multiple lines of evidence were employed to characterize risk of exposure to PCBs in terrestrial passerine populations. Concentrations of PCBs were significantly greater in the diet of birds at the more contaminated location than in birds from the upstream reference location (Neigh *et al.* 2004c). Concentrations of total PCBs were deemed to be less than a threshold where effects would be expected, but concentrations of $TEQs_{WHO-Avian}$ may exceed a level of effect. Co-located studies of reproductive performance of passerine species were also conducted during the same time period and applied to the overall evaluation of risk as an ancillary line of evidence (Neigh *et al.* 2004b, Neigh *et al.* 2004d). These studies of reproductive performance over a two-year period indicated that house wren fledging success was significantly greater at Trowbridge (TB) compared to Fort Custer (FC). Eastern bluebirds had significantly decreased productivity at the more contaminated location relative to the reference location during the three-year study, but 30% of the decrease in productivity between locations can be linked to a single female. Although reproductive abnormalities were seen in both species, the cause of the depressed reproductive success was unlikely to be due to exposure to PCBs, but more likely due to differences in habitat suitability, co-contaminants, or prey availability. The final line of evidence, examined here, is the measured concentration of PCBs in tissues, which were then compared to TRVs. The primary objectives of the present study were to 1) determine if concentrations of total PCBs and $TEQs_{WHO\text{-}Avian}$ in passerine tissues were different between locations; 2) to determine if all avian species examined in this study were equally exposed; and 3) to determine whether the concentrations of PCBs in the tissues of passerine birds exceeded concentrations that, based on TRVs, would be expected to result in population-level adverse effects.

MATERIALS AND METHODS

Site details

Eggs, nestlings, and adults were collected from nest boxes at two locations on the Kalamazoo River from 2001 to 2003 (Figure 4.1). The upstream reference location at the Fort Custer State Recreation Area (FC) was minimally contaminated by PCBs (Neigh *et al.* 2004a). The more contaminated location, the former Trowbridge Impoundment (TB), was located 67 km downstream from the reference location. Further physical description of the study sites and nest box locations were described elsewhere (Neigh *et al.* 2004b).

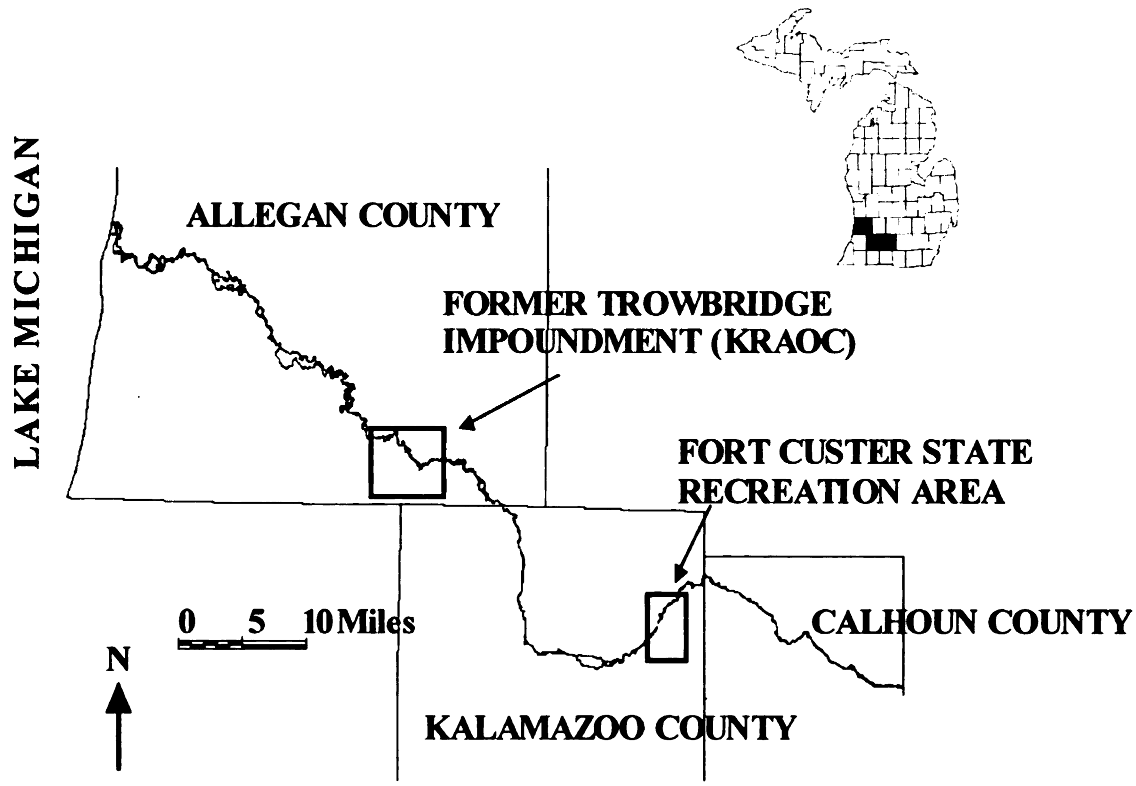

Figure 4.1. Map of the Kalamazoo River Area of Concern (KRAOC) and reference site. The inset describes the location of the two counties in Michigan where studies were conducted. A box designates the boundaries of the upstream reference location (Fort Custer State Recreation Area) and the Trowbridge Impoundment, located within the KRAOC.

Tissue sampling

Samples of eggs, nestlings, and adults were collected during spring and summer of 2001, 2002, and 2003. One predetermined sample was collected from each nest box for determination of PCB and TEQ concentrations. In addition, abandoned and addled eggs, dead adults, and dead nestlings were salvaged for additional measurements of concentrations of PCBs and TEQs Fresh eggs were sampled 7 to10 d post-laying, and nestlings were sampled 6 to 8 d prior to fledging. Adult house wrens were sampled during 2002 and 2003 by mist net.

Chemical analysis

Eggs were prepared by removing the eggshell, so the yolk and albumen remained for chemical analysis. Nestlings and adults were processed prior to chemical analysis by removing feathers, beaks, wings, legs, and stomach contents, and then, homogenizing the whole body in a solvent-rinsed grinder.

Concentrations of PCB congeners and *o,p'*- and *p,p'*-isomers of DDT, DDE, DDD were determined by previously described methods (Nakata *et al.* 1998, Neigh *et al.* 2004a). Total concentrations of PCBs were quantified by Perkin Elmer AutoSystem and Hewlett Packard 5890 series II gas chromatographs equipped with ^{63}Ni electron capture detectors. The method detection limit (MDL) was estimated to be 1 x 10^{-3} mg PCB/kg, wet weight (ww). Total PCB concentrations were calculated as the sum of all resolved PCB congeners and co-eluting congeners. Non-*ortho*-substituted PCB congeners (IUPAC numbers 77, 81, 126, and 169) were separated from co-eluting congeners and interferences by carbon column chromatography. Extracts containing the non-*ortho*-substituted (coplanar) congeners were analyzed by gas chromatograph mass selective detector (Hewlett Packard 5890 series II gas chromatograph) equipped with an HP 5972 series detector. Detection limits varied among samples, but the mean detection limit for all samples was <100 ng/kg, ww.

TEQ computation

TEQs in bird tissues were calculated by multiplying individual concentrations of AhR-active PCB congeners by their respective bird-specific World Health Organization (WHO-Avian) toxic equivalence factors (TEF) (van den Berg *et al.* 1998). Total $TEQ_{WHO\text{-}Avian}$ concentrations were calculated as the sum of detectable individual non-*ortho* and mono-*ortho* PCB congeners (77, 81, 105, 118, 126, 156, 157, 167, and 169). When at least one of the coplanar congeners could not be quantified due to interfering compounds, with the exception of congener 169, the sample was removed from statistical analyses. Congener 169 was not regularly detected in the samples. For congeners that occurred at concentrations that were less than the limit of quantification (LOQ), a proxy value of half the LOQ was assigned. Some mono-*ortho* congeners co-eluted with other congeners, so in order to report the most conservative estimate, the total concentration of the co-elution group was considered to belong to its respective mono-*ortho* congener.

Statistical Analyses

For statistical analyses, each nest box was treated as a separate experimental unit, and values were reported on a per box basis. All nesting attempts were treated as separate and individual observations. Normality was assessed with Kolmogorov-Smirnov's one sample test with Lilliefors transformation, and homogeneity of variance was verified by F test. All concentration data were log transformed before analyses. All parametric data were analyzed by one-way analysis of variance (ANOVA), and non-parametric data were analyzed by Mann-Whitney U or Kruskal-Wallis tests. The criterion for significance used in all tests was $p<0.05$.

Bioaccumulation and accumulation rates

Bioaccumulation factors (BAFs) and accumulation ratios were calculated for total PCBs and $TEQs_{WHO\text{-}Avian}$ as the lipid-normalized concentration in the upper trophic level divided by the lipid-normalized concentration in the lower trophic level. Accumulation was calculated based on live sampled adults and nestlings and fresh sampled eggs.

Accumulation rates were calculated to determine the mass of PCBs gained by the nestlings over the nestling period. The mass of PCBs in the egg was subtracted from the total mass in the nestlings. The accumulation rate was calculated as the difference in total mass of PCBs and $TEQs_{WHO\text{-}Avian}$ between nestlings and eggs divided by the nestlings' days of life (Custer and Custer 1995).

Selection of toxicity reference values (TRVs)

TRVs were derived from the results of studies reported in the literature. Studies were chosen for terrestrial passerine species based on several criteria. The studies judged to be the most suitable for TRV calculation used wildlife species chronically exposed over sensitive life stages; evaluated endpoints ecologically relevant to PCB exposure; had minimal co-contamination; and were multi-year studies.

Few studies are available for the species used in the current study except for an examination of American robin exposure and effects at the Housatonic River, New York, which found no correlation between reproduction and PCB concentrations (Henning *et al.* 2002). American robin eggs at the Housatonic site contained a mean concentration of 83.6 mg PCB/kg, ww. From this field study it can be concluded that the threshold for effects of PCBs on robins is greater than 83.6 mg PCB/kg, ww. Studies of European starlings (*Sturnus vulgaris*) were also considered for derivation of an appropriate TRV. European starlings exhibited a decrease in parental attentiveness and increased mortality at two PCB sites compared to a reference site when Aroclor 1254 concentrations (mean ± 1 SD) were 13.6 ± 2.9 mg PCB/kg, ww in eggs, 5.9 ± 0.8 mg PCB/kg, ww in nestlings, and 15.9 ± 5.3 mg PCB/kg, ww in adults (Halbrook *et al.* 1998). The study of starlings did not report reduced hatching success, and similar embryo mortality was observed at another study site contaminated with metals but with relatively low concentrations of PCBs. It has been suggested that adult incubation anomalies or co-contamination by dioxin may be leading to embryo mortality, so these egg concentrations are an unacceptable basis for a TRV for the Kalamazoo River (Halbrook *et al.* 1998). Effects found in adult starlings also may be affected by the fitness of nestlings, which may reduce feeding calls, and therefore, affect parental care. The starling study fulfilled several of criteria by taking place over several years; measuring ecologically relevant endpoints; and measuring concentrations at critical life stages in a terrestrial wild avian species. The confounding factors in the study by Halbrook *et al.* (1998) and the fact that Henning *et al.* (2002) did not observe abnormalities in reproduction at much greater concentrations suggests that Henning *et al.* (2002) may be a more appropriate choice for the TRV based on the no observed adverse effect level (NOAEL). Selected TRVs are reported (Table 4.1).

Table 4.1. Toxicity reference values based on the no observed adverse effect level (NOAEL) for tissues of avian species at the Kalamazoo River.

	Tissue-Based TRV	Reference
Total PCBs (mg PCB/kg)	**83.6**	**(Henning *et al.* 2002)**
Total TEQ (ng TEQ/kg)	**13000**	**(USEPA 2000)**

The TRV selected as a threshold for effects based on concentrations of $TEQs_{WHO-Avian}$ was that suggested by USEPA (2000). The study investigated concentrations of $TEQs_{WHO-Avian}$ in tree swallows at the Hudson River, New York, over a 2 yr period; was conducted on a wild passerine birds; evaluated relevant ecological endpoints during critical life stages; and co-contamination was considered to be inconsequential. Tree swallows exhibited abnormal plumage, decreased hatching success, and increased abandonment during one year, but these effects were not observed in the following year. The inconsistency of effects over both years suggests that values from this study should not be used as a LOAEL, but they are appropriate for a NOAEL. The greatest concentration of $TEQs_{WHO-Avian}$ in the year without effects was 13 μg TEQ/kg, ww. No LOAEL could be established for $TEQs_{WHO-Avian}$.

RESULTS

Gross morphology and abnormalities

There were no gross physiological abnormalities observed in nestlings or adults of either species, but some egg abnormalities were present in house wrens. One house wren egg from FC during 2001 contained two well-developed embryos at about 10 d of incubation. The egg was believed to be addled when sampled from the location. To our knowledge, this is the first report of twin embryos in a passerine bird, although, twin chicks have been reported in the emu (*Dromaius novaehollandiae*) (Bassett *et al.* 1999). In addition to the twin egg anomaly, the eggshells at five TB nests were irregular. One nest had irregularly shaped eggs, best described as jellybean shaped. The adults eventually abandoned the nest after a short incubation period. In four other nests, the shells had a bumpy irregular texture and seemed to be thinner than normal eggs, although, eggshell thickness was not measured directly. Also, as soon as one day after laying, egg contents were noticeably desiccated as determined by a marked weight loss, which supports the observed decrease in eggshell thickness in some nests.

Total PCB concentrations in tissue

PCB concentrations in eggs, nestlings, and adults from TB were significantly greater than those at the reference location (FC) (Student's t-test or Kruskal-Wallis, $p<0.001$) for both eastern bluebirds and house wrens (Table 4.2). For all tissues analyzed, concentrations of PCBs were 39 to 118-fold greater at TB than at FC. The trend in concentration differences between sites was the same when concentrations were lipid-normalized. In general, eastern bluebirds at TB had the greatest concentrations of PCBs in eggs and nestlings (Table 2), but the greatest tissue concentration in the study was measured in a house wren egg from TB in 2002 (36 mg PCB/kg, ww).

Table 4.2. Mean concentrations of total PCBs (± 1 SD) and lipid content (± 1 SD) of eastern bluebirds and house wrens at the Fort Custer reference area and the Trowbridge Impoundment at the Kalamazoo River Area of Concern.

	Fort Custer			Trowbridge		
	N	% Lipid	PCB (mg/kg)	N	% Lipid	PCB (mg/kg)
Eastern bluebird						
Egg						
2001	2	6.0 (2.4)	0.25 (0.077)	2	8.2 (3.1)	11 (2.9)
2002	7	8.4 (2.7)	0.13 (0.076)	3	15 (15)	11 (3.9)[a]
2003	5	8.7 (3.1)	0.19 (0.13)	2	5.9 (0.40)	1.8 (0.32)[a]
Total	14	8.2 (2.8)	0.17 (0.10)	7	11 (10)	8.3 (5.1)[a]
Nestling						
2001	6	5.7 (0.84)	0.011 (0.003)	1	4.8 (NA)	0.41 (NA)
2002	5	5.0 (0.42)	0.010 (0.004)	3	4.6 (1.2)	1.4 (2.1)[a]
2003	6	4.0 (1.2)	0.013 (0.009)	2	5.0 (1.3)	1.8 (0.83)[a]
Total	17	4.9 (1.1)	0.011 (0.006)	6	4.8 (0.96)	1.3 (1.4)[a]
House wren						
Egg						
2001	8	19 (17)	0.14 (0.15)	6	9.3 (5.4)	4.5 (2.0)[a]
2002	6	7.1 (4.0)	0.10 (0.053)	8	11 (6.2)	7.6 (7.7)[a]
Total	14	14 (14)	0.12 (0.12)	14	10 (5.7)	6.3 (6.0)[a]
Nestling						
2001	7	4.5 (1.8)	0.029 (0.024)	5	5.1 (1.4)	0.85 (0.79)[a]
2002	6	6.3 (2.6)	0.010 (0.005)	11	6.9 (2.1)	0.71 (0.62)[a]
2003	NA	NA	NA	1	2.4 (NA)	1.1(NA)
Total	13	5.3 (2.3)	0.020 (0.020)	17	6.1 (2.2)	0.77 (0.64)[a]
Adult						
2002	5	5.5 (1.1)	0.087 (0.029)	6	5.2 (1.3)	3.6(2.3)[a]
2003	3	5.3 (0.86)	0.045 (0.022)	3	5.6 (2.1)	2.5 (1.6)[a]
Total	8	5.4 (0.93)	0.072 (0.033)	9	5.4 (1.5)	3.2 (2.1)[a]

[a]Mean concentration were significantly greater than the FC reference site ($p < 0.05$).

DDT metabolites were detected in all egg samples from a random subset analyzed for DDT concentrations. Concentrations of *p,p'*-DDE comprised 95% to 98% of the total sum of all DDT metabolites in eastern bluebirds and house wrens. Total DDT is defined as the sum of all isomers measured. Concentrations in eastern bluebirds were 7-fold greater than in house wrens at FC and were 3-fold greater than house wrens at TB. Concentrations of total DDT (mean ± 1 SD) contained in eastern bluebird eggs were 2.1 ± 1.1 mg DDT/kg, ww at TB and 1.5 ± 1.8 mg DDT/kg, ww at FC, but differences between sites were not statistically significant. House wren eggs at TB contained 0.66 ± 0.87 mg DDT/kg, ww and eggs at FC contained 0.20 ± 0.21 mg DDT/kg, ww, but again, differences between locations were not statistically significant. The only statistically significant difference was for concentrations of *p,p'* -DDD in eggs of house wrens between sites (Student's t-test, p=0.021), but sample sizes were small (n=10). Addled eggs of eastern bluebirds at FC contained 2.6 times greater concentrations of lipid-normalized total DDT than fresh eggs, but addled eggs of eastern bluebirds at TB had lipid-normalized total DDT concentrations 82% that of fresh eggs from TB. Addled eggs of house wrens had lipid-normalized concentrations 7.4 and 5.7 times greater than fresh eggs at FC and TB, respectively. Samples sizes were insufficient to perform statistical tests comparing addled and fresh eggs.

TCDD Equivalents ($TEQs_{WHO\text{-}Avian}$)

Concentrations of 2,3,7,8 –TCDD equivalents ($TEQs_{WHO\text{-}Avian}$) were significantly greater at TB than at FC for all tissue matrices in both species (Table 4.3). Concentrations of $TEQs_{WHO\text{-}Avian}$ in the tissues of eastern bluebirds were 5 to 10-fold greater at TB than at

FC, while concentrations of $TEQs_{WHO\text{-}Avian}$ in the tissues of house wrens were 15 to 47-fold greater at TB compared to FC. An addled egg of a house wren contained the greatest $TEQ_{WHO\text{-}Avian}$ concentration for all tissues in the study (2300 ng TEQ/kg, ww).

Table 4.3. Mean 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) (± 1 SD) and relative potency (± 1 SD) in eastern bluebird and house wren tissues sampled from the Fort Custer Reference location and the former Trowbridge Impoundment.

	Fort Custer			Trowbridge		
	N	TEQ (ng/kg)	RELPOT (ng TEQ/kg PCB)	N	TEQ (ng/kg)	RELPOT (ng TEQ/kg PCB)
Eastern bluebird						
Egg						
2001	2	10 (10)	37 (29)	2	70 (4.8)	7.4 (0.63)
2002	NA	NA	NA	1	220 (NA)	25 (NA)
2003	1	2.2 (NA)	47 (NA)	2	15 (10)	8.5 (3.3)
Total	3	7.6 (8.6)	40 (21)	5	77 (82)[a]	11 (8.0)
Nestling						
2001	6	1.5 (1.1)	130 (99.0)	1	3.2 (NA)	7.8 (NA)
2002	5	1.3 (0.34)	150 (71)	3	7.3 (4.9)[a]	25 (18)[a]
2003	6	1.2 (0.20)	120 (45)	2	6.3 (4.4)[a]	3.3 (0.91)
Total	17	1.3 (0.64)	130 (71)	6	6.3 (4.0)[a]	15 (16)
House wren						
Egg						
2001	4	8.0 (4.7)	300 (280)	5	160 (54)[a]	40 (16)[a]
2002	4	9.2 (3.6)	150 (130)	6	600 (540)[a]	62 (20)[a]
Total	8	8.6 (3.9)	220 (220)	11	400 (450)[a]	52 (21)[a]
Nestling						
2001	7	1.7 (1.2)	74 (53)	5	73 (63)[a]	94 (35)[a]
2002	6	1.1 (0.43)	130 (66)	11	61 (43)[a]	97 (42)[a]
2003	NA	NA	NA	1	41 (NA)	38 (NA)
Total	13	1.4 (0.96)	100 (64)	17	63 (47)[a]	93 (40)[a]
Adult						
2002	5	5.2 (2.6)	66 (43)	6	120 (40)[a]	43 (30)
2003	3	10 (8.7)	260 (180)	3	91 (91)[a]	47 (38)
Total	8	7.1 (5.7)	140 (140)	9	110 (57)[a]	45 (30)

Table 4.3 (cont'd)

[a]Mean concentration were significantly greater than the FC reference site ($p < 0.05$).

The relative contributions of non-*ortho* and mono-*ortho* congeners to total $TEQs_{WHO\text{-}Avian}$ were evaluated at each site. PCB 169 was not detected in 67% to 69% of all samples for eastern bluebirds and house wrens, and in the samples in which it was detected, it represented less than 1% of the total concentration of the $TEQ_{WHO\text{-}Avian}$. House wren tissues from TB were the only matrix in which PCB congeners 77, 81, and 126 were all detected in the majority of the samples. PCB 77, 81, and 126 comprised the greatest proportion of the total $TEQ_{WHO\text{-}Avian}$ for all species (Figure 4.2). In house wrens at FC, PCB 126 comprised the greatest portion of the total $TEQ_{WHO\text{-}Avian}$, but at TB, PCB 77 made the greatest contribution to the concentration of the $TEQ_{WHO\text{-}Avian}$. Contributions of every congener to the total $TEQ_{WHO\text{-}Avian}$ were significantly different between FC and TB for house wrens. In eastern bluebirds, both PCB 81 and PCB 126 comprised significantly different proportions of the $TEQ_{WHO\text{-}Avian}$ between sites, but PCB 77 was similar between locations. PCB 118 was frequently detected at both locations in all samples for each species.

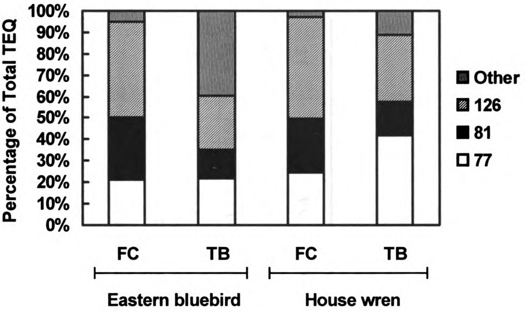

Figure 4.2. Contribution of individual non-*ortho* and mono-*ortho* congeners to total 2,3,7,8 TCDD equivalents (TEQs) in the tissues of passerine species at the Fort Custer State Recreation Area (FC) and the Trowbridge Impoundment (TB).

Bioaccumulation

Ratios of concentrations between life stages of avian species for lipid-normalized total PCBs and $TEQs_{WHO\text{-}Avian}$ were less than 1.0 for house wren eggs to nestlings, greater than 1.0 for house wren nestlings to adults, and near 1.0 for house wren adults to eggs at FC and TB. Eastern bluebird egg to nestling ratios (<1.0) were less than those in house wrens (Table 4.4). Ratios of bioaccumulation between egg and nestling life stages for eastern bluebirds and house wrens were greater at FC than for TB when comparing $TEQs_{WHO\text{-}Avian}$.

Table 4.4. Ratios of accumulation between life stages of the eastern bluebird and house wren based on the mean lipid-normalized total PCBs and $TEQs_{WHO\text{-}Avian}$ eggs and live sampled nestlings at the Fort Custer State Recreation Area (FC) and the Trowbridge Impoundment (TB).

	Accumulation ratio (Total PCB)				Accumulation ratio ($TEQ_{WHO\text{-}Avian}$)			
	Eastern bluebird		House wren		Eastern bluebird		House wren	
	FC	TB	FC	TB	FC	TB	FC	TB
Egg to nestling	0.11	0.20	0.79	0.22	0.31	0.0094	0.50	0.34
Nestling to adult	NA	NA	3.2	4.4	NA	NA	4.3	1.9
Adult to egg	NA	NA	0.40	1.0	NA	NA	0.47	1.6

NA = Not available.

Daily accumulation rates were calculated based on total PCBs and $TEQs_{WHO\text{-}Avian}$. Total PCB daily accumulation rates for house wren nests were 0.0068 µg PCB/d, ww at FC during 2001 and were 0.32 and 0.40 µg PCB/d, ww at TB during 2001 and 2002, respectively. Daily accumulation rates based on $TEQs_{WHO\text{-}Avian}$ were 1.9 pg TEQ/d, ww at FC during 2001 and 26 and 16 pg TEQ/d, ww at TB during 2001 and 2002, respectively. Accumulation rates for eastern bluebirds at FC were 0.0053 and 0.054 µg PCB/d, ww for 2002 and 2003, respectively. Total $TEQ_{WHO\text{-}Avian}$ accumulation rates for eastern bluebirds were 2.1 and 12 pg TEQ/d, ww at FC during 2002 and 2003, respectively.

Relative potency (ng TEQs/kg PCB) of PCB mixtures in samples were calculated by dividing the concentration of $TEQs_{WHO\text{-}Avian}$ by the concentration of total PCBs for each sample (Table 4.3). The resulting relative potency is an indicator of the overall effect of environmental weathering and differential bioaccumulation on the toxicity of the PCB mixture. Relative potencies were generally greater and more variable at FC than at TB for both species because concentrations of PCBs were generally low and concentrations of $TEQs_{WHO\text{-}Avian}$ were driven by the detection limit of the analytical method. Ratios of relative potencies among different sample types reflect changes in the potency of the PCB mixture. At TB, the potency ratio from egg to nestling was 1.3 and 1.8 for eastern bluebird and house wren, respectively, and the potency ratio at TB for the same life stages of these species was 3.3 and 0.45. Ratios for nestling to adult were 0.48 at TB and 1.4 at FC for house wrens.

DISCUSSION

Gross morphology and abnormalities

A proportion of house wren nests contained eggs with shells that appeared to be thinned (4% of all nests, 12% of nests at TB), although the thickness of the shells was not measured. Eggshell thinning is a well-known result of DDT exposure (Ratcliffe 1967, Hickey and Anderson 1968, Lincer 1975, Peakall 1993), but most studies observed no correlation between PCB exposure and eggshell thickness (Dahlgren and Linder 1972, Weseloh *et al.* 1989, Secord and McCarty 1997, Fernie *et al.* 2000). Although, at one PCB contaminated site, eastern bluebird eggshells had a thinner eggshell thickness index than at a reference location (Fair and Myers 2002). Total DDT residue levels were 1.3 mg DDT/kg in the attended eggs of another passerine bird, the tree swallow, and 2.8 mg DDT/kg in unattended eggs, but these concentrations were not associated with decreases in eggshell thickness between attended and unattended eggs or between the study population and historical eggshell measurements prior to 1915 (DeWesse *et al.* 1985). A concentration of 2.0 mg DDE/kg in eggs has been suggested as a threshold for effects in bald eagles (*Haliaeetus leucocephalus*) (Elliot and Harris 2002).

Although little is known about the differences in species sensitivity between other birds and the eastern bluebirds or house wrens, concentrations of DDE and DDT in the eggs of eastern bluebirds were near the threshold for effect of other species, but the eggs of house wrens had concentrations of total DDT 3-fold less than the threshold. Thinning was not observed in the eggs of eastern bluebirds, but thinning was observed in the eggs of house wrens from TB. Unless the eggshells of house wrens are much more sensitive to thinning caused by DDT than those of tree swallows, raptors or eastern bluebirds, the house wrens does not appear to be at risk for DDT-induced eggshell thinning. However, studies do find a magnitude of difference in the sensitivity of some terrestrial passerine species (Eeva and Lehikoinen 1995, Blus 1996), so it is possible that house wrens are more sensitive to the effects of DDT exposure than eastern bluebirds. No decisive conclusion as to the cause of the abnormal eggs can be garnered from the study due to the lack of data on concentrations of total DDT in abnormally formed eggs, the lack of measurement of eggshell thickness, and the existence of co-contaminants in the area.

In addition, eggs at TB with thinned shells appeared to be desiccated. A similar desiccation of eggs with thinned shells was associated with mortality of the embryo (Eeva and Lehikoinen 1995). At the PCB contaminated Hudson River, the failure of eggs to hatch was attributed mainly to lack of fertility instead of embryo mortality (McCarty and Secord 1999). The reason for failure of individual eggs at the Kalamazoo River sites was not specifically examined, but eggs with apparently thin shells did not hatch successfully in most cases. The threshold for effects of DDT on reproduction of passerine birds is likely near 8 or 9 mg DDE/kg (see DeWesse *et al.* 1985). No eggs of either species at the Kalamazoo River contained concentrations of DDE greater than the threshold, and mean concentrations were 4-fold less than the threshold. DDT is a co-contaminant at many locations, including the Kalamazoo River Area of Concern (KRAOC), which makes it difficult to attribute reproductive abnormalities to the presence of one contaminant versus another. There was DDT detected at both

Kalamazoo River locations, but the presence of DDT is not expected to be causing reproductive impairment in the species examined.

Total PCBs

Limited data are available for passerine birds exposed to PCBs through terrestrial diets to which the results of this study could be compared. Tissue concentrations were similar between species at FC and other species at other relatively uncontaminated sites in the region (Bishop *et al.* 1995, Henning *et al.* 2002). Total concentrations of PCBs in house wren and eastern bluebird eggs and nestlings at TB were less than concentrations contained in American robin eggs and nestlings at the Housatonic River (Henning *et al.* 2002), but concentrations of PCBs at TB were greater than concentrations in red-winged blackbird (*Agelaius phoeniceus*) eggs at locations in the Great Lakes-St. Lawrence River Basin with the exception of one site (Bishop *et al.* 1995). Although few comparisons to other studies can be made for these specific species, the fact remains that concentrations of total PCBs were significantly greater at TB than at FC in all matrices examined. Concentrations in the tissues corresponded to greater concentrations of total PCBs in the soil at TB compared to FC (MDEQ 2003), which suggests that concentrations in the tissues of the species examined reflect local contamination in the soil.

Tissue concentrations, expressed on a wet weight basis, were generally greater in eastern bluebirds than house wrens. When concentrations in all matrices were lipid normalized, concentrations in house wrens and eastern bluebirds were more similar, but eastern bluebirds still contained greater mean total concentrations of PCBs. Differences in concentrations of PCBs between house wrens and eastern bluebirds were likely indicative of on-site dietary exposure. For eggs and nestlings at TB, eastern bluebirds contained total PCB concentrations 1.7-fold greater than house wrens. When site-specific average potential daily doses were calculated for TB, exposure in the diet of eastern bluebirds was found to be 3.7-fold greater than that of house wrens (Neigh *et al.* 2004c). At FC, this study measured greater daily accumulation rates for the nestlings of house wrens compared to eastern bluebirds, which suggests that they obtain greater concentrations from the diet at FC. Indeed, dietary exposure for house wrens was greater than in eastern bluebirds at FC. Further study is needed, but general trends in concentration tissue gradients, described as daily accumulation rates, appear to reflect daily dietary exposure.

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

One measure of the toxicity of PCBs is based on toxic equivalency factors, which compares the toxicity of individual PCB congeners to 2,3,7,8 -TCDD (Giesy and Kannan 1998). The greatest $TEQ_{WHO\text{-}Avian}$ values were consistently measured in adults and eggs of house wrens from TB. This was opposite of what was measured for concentrations of total PCBs for which eastern bluebirds contained consistently greater concentrations in their tissues than house wrens. Mono-*ortho* and non-*ortho* (coplanar) congeners, which comprise $TEQ_{WHO\text{-}Avian}$ concentrations, apparently accumulate between life stages to a greater degree in house wrens than in eastern bluebirds. House wrens had concentrations of $TEQs_{WHO\text{-}Avian}$ in eggs 5.7 times greater than in eastern bluebird eggs, and house wren nestlings contained concentrations of $TEQs_{WHO\text{-}Avian}$ 10 times greater than in eastern bluebird nestlings. Bioaccumulation factors from the diet to tissues (calculated from

Neigh *et al.* 2004c) also suggest that accumulation of both $TEQs_{WHO\text{-}Avian}$ and total PCBs were greater in house wrens than in eastern bluebirds, especially at TB (Table 4.5). Unlike the relationship between total PCB concentrations and dietary exposure, $TEQs_{WHO\text{-}Avian}$ for dietary exposures do not correspond with tissue concentrations or accumulation rates. Eastern bluebirds were found to have greater accumulation rates and dietary exposure to $TEQs_{WHO\text{-}Avian}$ compared to house wrens at both TB and FC (Neigh *et al.* 2004c).

Table 4.5. Bioaccumulation factors for wet weight concentrations of total PCBs and 2,3,7,8 –tetrachlorodibenzo-*p*-dioxin (TEQ) in the diet (average potential daily dose) (Neigh *et al.* 2004c) to bird tissues at the Fort Custer State Recreation Area (FC) and the Trowbridge Impoundment (TB).

	Bioaccumulation factors (Total PCB)				**Bioaccumulation factors (TEQ)**			
	Eastern bluebird		**House wren**		**Eastern bluebird**		**House wren**	
	FC	**TB**	**FC**	**TB**	**FC**	**TB**	**FC**	**TB**
Diet to egg	**10**	**16**	**6.4**	**45**	**2.8**	**1.1**	**8.9**	**13**
Diet to nestling	**0.64**	**2.5**	**0.91**	**5.5**	**0.48**	**0.09**	**0.56**	**2.0**
Diet to adult	**NA**	**NA**	**3.3**	**23**	**NA**	**NA**	**2.8**	**3.5**

NA = Not available

The congener contributing the greatest proportion of the $TEQ_{WHO\text{-}Avian}$ was different at the two sites. Congeners 81 and 126 contributed more to the total TEQs at FC than at TB for both species. However, these congeners were detected in less than 30% of the samples from FC, so the detection limit of each congener contributed greatly to the analyses, being that half the detection limit was used to calculate contributions in the remaining samples. Congener 77 was the greatest contributor in most matrices at TB. The contribution of congener 77 to total $TEQs_{WHO\text{-}Avian}$ increased from eggs to nestlings of house wrens, but it decreased from eggs to nestlings in eastern bluebirds. Congener 126 followed an opposite trend by contributing more to the total $TEQ_{WHO\text{-}Avian}$ between eggs and nestlings in eastern bluebirds and contributing less between eggs and nestlings in house wrens. Adult concentrations did not follow any discernible trend. Adults are likely exposed to variety of contamination sources over a life, while patterns in nestlings more closely reflect local sources of contamination (Bishop *et al.* 1995, Secord *et al.* 1999).

Bioaccumulation

Ratios of accumulation were similar for terrestrial species and tree swallows from the same location (Neigh *et al.* 2004a). Ratios ranging from 2 to 5 between nestlings and adults suggest that birds are accumulating PCBs from their diet, but ratios less than 1.0 between eggs and nestlings suggest that the concentration or the rate of consumption is not great enough in nestlings to overcome growth dilution. Red-winged blackbirds also followed a similar trend with eggs having greater concentrations than nestlings (Bishop *et al.* 1995). The greater accumulation ratio at FC compared to TB between eggs and nestlings for eastern bluebird and house wren may be a result of variation around the limits of detection for chemical analysis due to the small sample mass of many eggs.

Daily accumulation rates were calculated to describe the mass of PCBs accumulated by the nestlings through the diet. House wrens at TB had accumulation rates over 10-fold less than the accumulation rates for tree swallows at the same location (Neigh *et al.* 2004a) and at another location (Custer *et al.* 2003). Likewise, dietary exposure was estimated to be 6 to 8-fold greater for concentrations of PCBs in the diet of the tree swallow compared to the house wren (Neigh *et al.* 2004c). House wren and eastern bluebirds also had accumulation rates less than in black-crowned night heron (*Nycticorax nycticorax*) and Forster's tern (*Sterna forsteri*) (Ankely *et al.* 1993, Custer and Custer 1995).

Relative potency and potency ratios calculated for birds were intended to describe the increase or decrease in toxicity of the PCB mixture at the study locations. Potency ratios greater than 1.0 were observed for most comparisons which suggests that the mixture of congeners in the food chain become more potent at increasingly greater trophic levels through the bioaccumulation of AhR-active congeners (Trowbridge and Swackhamer 2002). Relative potencies at FC were influenced by several samples with low concentrations of PCBs or concentrations of $TEQs_{WHO\text{-}Avian}$ driven by the detection limit of the analytical method. These scenarios led to greater relative potencies in FC samples compared to TB, but the ratio of the relative potencies between trophic levels at each location (potency ratio) was similar. Ubiquitous congeners such as PCB 118 and PCB

156 appear to bioaccumulate regardless of local sources of contamination, which contributes to the overall concentrations of $TEQs_{WHO\text{-}Avian}$ at the reference location. The presence of these ubiquitous congeners in the diet adds to the increased potency of the internal mixture as nestlings and adults feed. PCB 126 with a TEF of 0.1 (van den Berg *et al.* 1998), the greatest TEF for PCBs in avian species, contributes the greatest relative percentage to the total $TEQ_{WHO\text{-}Avian}$ concentrations in samples from FC. The contribution of this congener also increases with trophic level, which suggests that PCB 126 is not easily metabolized and bioaccumulates through the food web. These factors lead to the increase in relative potency between trophic levels at FC. At TB, PCB 77 and PCB 126 contribute large portions to the total $TEQ_{WHO\text{-}Avian}$ due to the presence of these congeners in the original mixture released at the site (Camp, Dresser, and McKee 2002).

Assessment of risk based on multiple lines of evidence

Multiple lines of evidence were utilized to assess the risk of PCB exposure to terrestrial birds inhabiting the former impoundments at the Kalamazoo River Superfund site. Exposure assessments of concentrations of PCBs in eggs, nestlings, and adults are described here. A number of reproductive parameters and dietary exposures, calculated as daily dietary dose, were also assessed for both eastern bluebirds and house wrens (Neigh et al. 2004c, Neigh *et al.* 2004d). Potential adverse effects based on exposure data were determined by calculating hazard quotients (HQs) derived from literature based TRVs. All HQs at FC for total PCB and $TEQs_{WHO\text{-}Avian}$ were less than 0.002 for all tissues of house wrens and eastern bluebirds. Similarly, HQs at TB based on the mean and U95 CL were less than 0.16 for all species and tissue types (Figure 4.3). In addition,

no individual tissue concentration of PCBs exceeded any of the threshold values for effects.

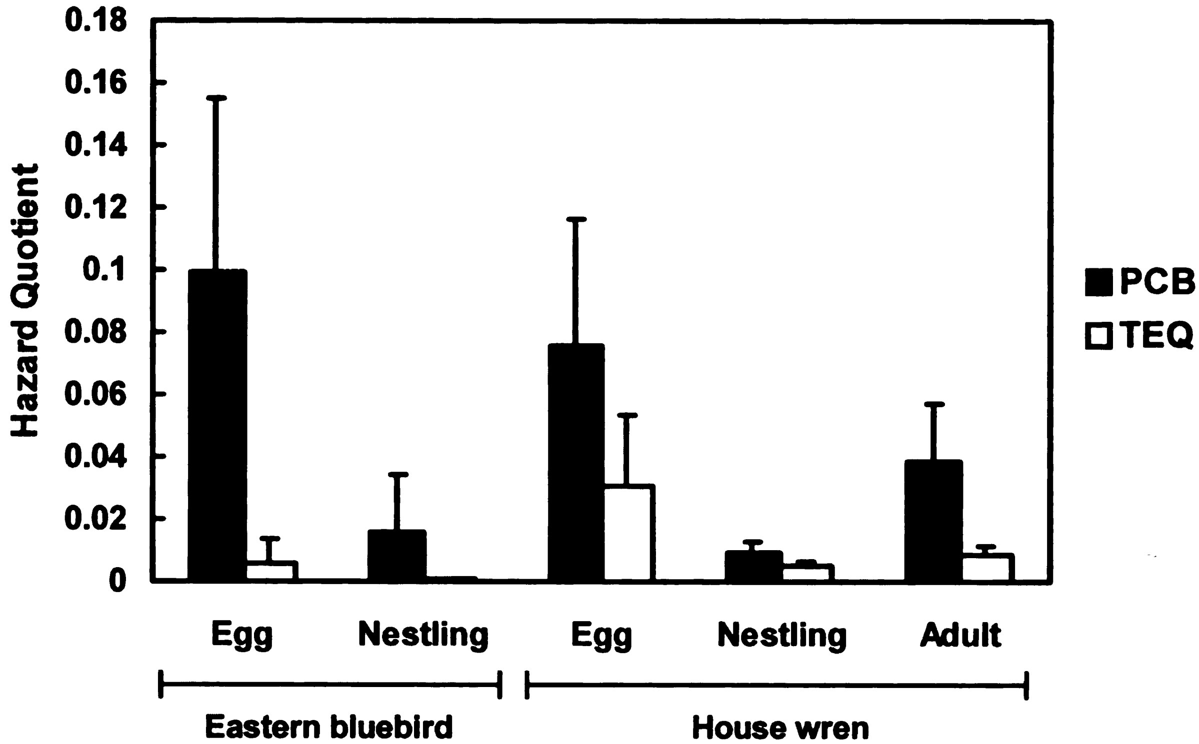

Figure 4.3. Hazard quotients (HQ) for concentrations of total PCBs and 2,3,7,8 TCDD equivalents (TEQs) based on the no observed adverse effect level (NOAEL) for house wrens and eastern bluebirds at the Trowbridge Impoundment. Error bars indicate the upper 95% confidence limit.

To assist in the evaluation of risk, dietary exposures, described in detail elsewhere (Neigh *et al.* 2004c), were examined concurrently as another way of estimating exposure and subsequent risk potential (Fairbrother 2003). Since there was little toxicity data available for terrestrial passerine birds, dietary exposure studies reported risk based on a range of HQ values. Concurrent studies suggest little risk to terrestrial passerines exposed to concentration of total PCBs in the diet (Neigh *et al.* 2004c). Given this, the only exposure calculations resulting in HQs greater than 1.0 were $TEQs_{WHO\text{-}Avian}$ in the diet of eastern bluebirds and house wrens in the exposed area (TB) based on the most conservative TRVs. HQ values, based on more appropriate TRVs calculated from field measurements of PCBs in wild passerine birds (USEPA 2000), were less than the threshold for effects based on the mean dietary exposure (Neigh *et al.* 2004c). It is expected that actual risk lies somewhere within the range of calculated HQs, but that it likely lies closer to the hazard based on the field measurements of wild passerine birds. The presence of PCBs in tissues at concentrations that were less than a threshold for effects, suggests that the HQ estimated based on dietary exposure of wild passerine birds is likely a better estimate of risk.

The final line of evidence involving reproductive health suggested some reproductive impairment that was likely attributable to other factors (Neigh *et al.* 2004d). Depressed reproduction in house wrens and eastern bluebirds was observed in some, but not nesting attempts, and in some, but not all parameters evaluated. Sample sizes were small, especially for eastern bluebirds, and a 40% decrease in reproductive success at TB during 2001 can be attributed to a single female. Conversely fledging success, which is the most important measure of reproductive success, was significantly greater in the PCB contaminated area for house wrens. Similar field studies found that there was no significant effect on reproductive performance of passerine birds at or concentrations found in birds of the Kalamazoo River or even greater concentrations (Secord and McCarty 1997; Custer *et al.* 2003). Likewise, studies of reproductive success conducted at the Kalamazoo River did not find statistically significant decreases in reproductive health of tree swallows at the contaminated TB location relative to the FC reference location (Neigh *et al.* 2004b). Another study examined dioxin exposure to eastern bluebird eggs, and no effects were observed at toxic equivalent concentrations greater than those contained in bluebird eggs at the Kalamazoo River (Thiel *et al.* 1997). Without observing consistency across all nesting attempts and indices and because of the contribution of a few adults to the reproductive measurements at TB, it can not be concluded that the observed responses were due to exposure to PCBs, but rather were due to other exogenous factors such as co-contaminants, habitat suitability, and prey availability.

The results of the lines of evidence for exposure and effects in this study suggest that concentrations of PCBs to which house wrens and eastern bluebirds were exposed are less than the threshold for effects. Few studies have directly investigated the sensitivity of these species to PCBs, so there are uncertainties as to the applicability of the species used to derive the TRVs relative to the susceptibility to the effects of PCBs in the terrestrial species in this study. Based on the lines of evidence, there does not appear to be substantial risk of adverse effects due to exposure of terrestrial passerine birds to PCBs originating the floodplain soils of the Kalamazoo River.

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Chapter 5

Multiple lines of evidence assessment of PCBs in the diets of passerine birds at the Kalamazoo River Superfund Site, Michigan.

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ABSTRACT

Dietary exposures of passerine birds to polychlorinated biphenyls (PCBs) through the terrestrial and aquatic food webs were examined at the Kalamazoo River, Michigan. The tree swallow (*Tachycineta bicolor*) represented those species with primarily insectivorous aquatic diets, while the house wren (*Troglodytes aedon*), eastern bluebird (*Sialia sialis*), and American robin (*Turdus migratorius*) were chosen to represent those with primarily terrestrial diets. Average potential daily doses (APDDs) in site-specific diets were 6 to 29-fold and 16 to 35-fold greater at Trowbridge than at Fort Custer for total PCBs and 2,3,7,8 –tetrachlorodibenzo-p-dioxin (TCDD) equivalents (TEQs), respectively. This study evaluated the risks of exposures of these birds to PCBs using both the “top-down” (measured concentrations in tissues of birds) and “bottom-up” (predicted from concentrations in the diet) approaches and compared the predicted effects with measured reproductive fitness. Based on the best estimates of the threshold for effects in these species, both the “top-down” approach based on concentrations of PCBs in the tissue and the “bottom-up” approach based on dietary exposure suggest little risk of passerine birds exhibiting effects expressed as total concentrations of PCBs or TEQs. Two of the three species exhibited some reduction in reproductive success during some years at the contaminated location relative to a reference location in some years. However, the effects were not consistent from year to year, and when three years of pertinent measures of reproductive success were considered, there were no statistically significant differences in population-level reproductive outcomes, such as fledging success. Only through the application of multiple lines of evidence can the best estimate of risk be

quantified. Both the "top-down" and "bottom-up" methods to estimate exposure based on field-derived thresholds for effects, suggested little risk of PCBs to passerine birds at the Kalamazoo River locations. These observations based on measures of exposure with comparisons to TRVs developed from both laboratory and field studies were consistent with direct measure of ecologically relevant endpoints of reproductive fitness.

Keywords: Birds, Reproduction, Bioaccumulation, Dietary exposure, House wren, Eastern bluebird, American robin, Risk assessment, Trophic level

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 can not 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 both 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.* 2004a, Neigh *et al.* 2004b), but the degree of concordance between the top-down and bottom-up approaches is evaluated in this paper. The first priorities of the study were to 1) estimate risk to four passerine species based on dietary exposure; 2) to compare measures of exposure based on a site-specific diet to exposure based on a literature-derived diet; and 3) to estimate the contribution of food web based exposure related to generalized feeding guilds. Secondarily, this study examined the effectiveness of approaching a risk assessment based on the examination of all trophic levels instead of choosing an evaluation based solely on the top-down or bottom-up methodologies. Furthermore, the results of the assessments based on the total concentration of PCBs or the TEQ were compared and contrasted.

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 striped 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 Recreation Area (FC) and former Trowbridge Impoundment (TB). FC is an upstream reference area of known PCB sources, and TB is the most PCB contaminated location within the Kalamazoo River Area of Concern (KRAOC) (Figure 5.1). The Trowbridge impoundment includes 132 ha of former sediments that are now under natural vegetation. Nest boxes were established within the 100-y floodplain in both the Trowbridge Dam Impoundment and the Fort Custer State Recreation Area reference location.

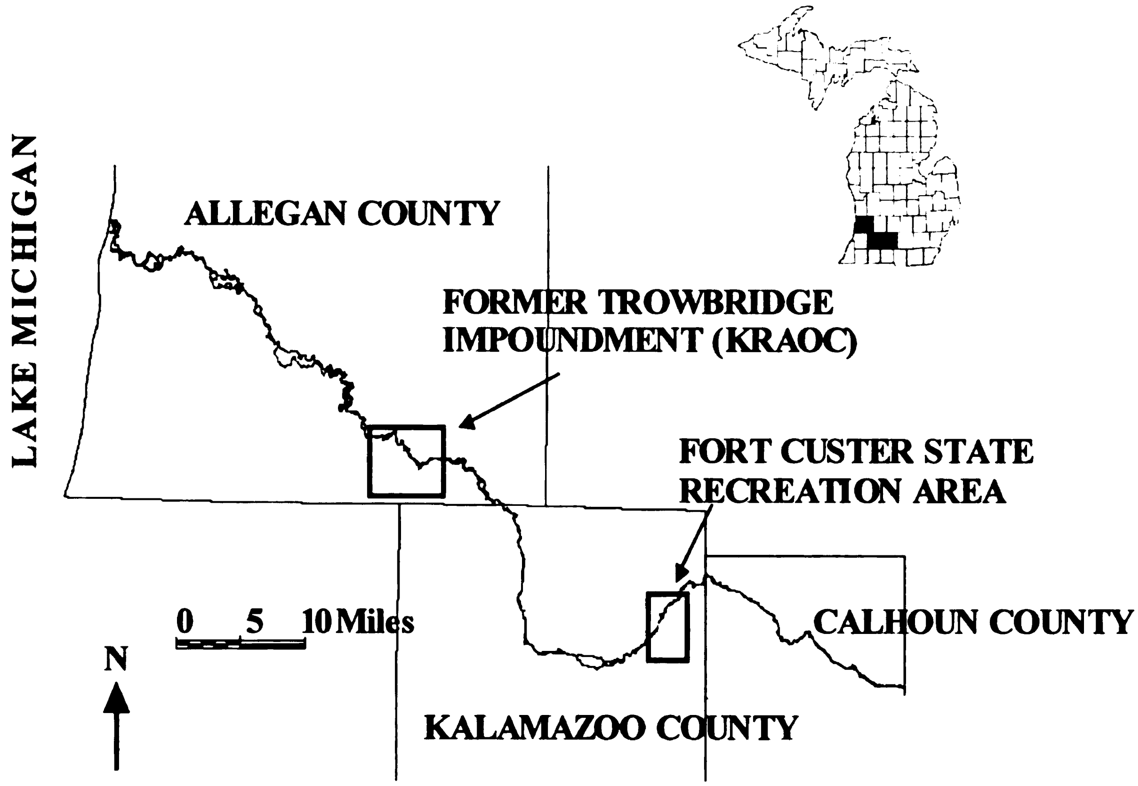

Figure 5.1. The PCB contaminated Trowbridge Impoundment within the Kalamazoo River Area of Concern and an upstream reference location at the Fort Custer State Recreation Area.

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 h 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 nesltings. 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 3g of bolus material at each nest. Sampling was discontinued when nestling tree swallows and eastern bluebirds were 14 d or house wrens were 10 d 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 5.2).

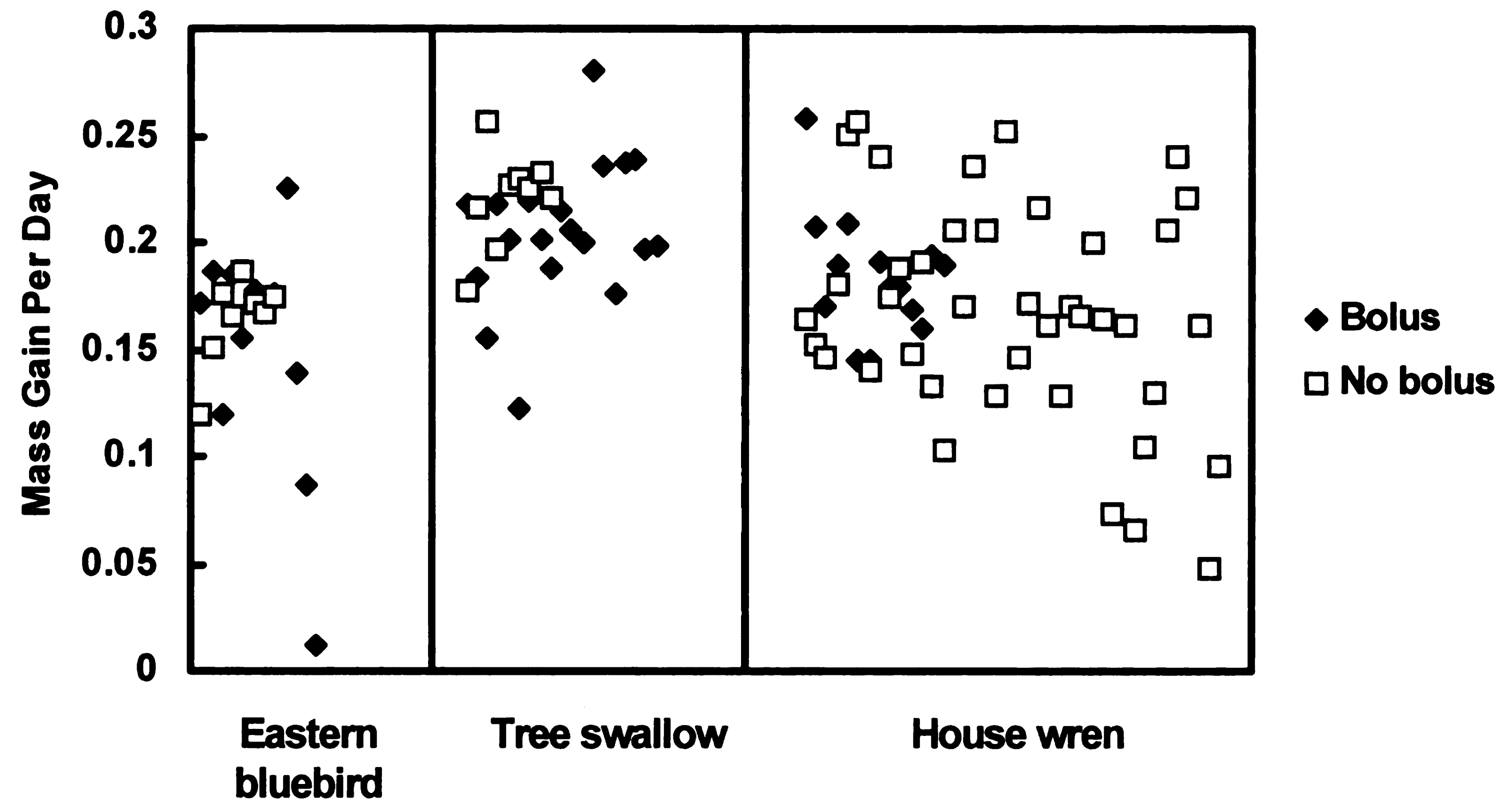

Figure 5.2. Comparison of mass gained per day of life for nestlings in nests from which boluses were collected (bolus) or not collected (no bolus) at the Kalamazoo River.

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.* 2004a).

Average potential daily dose

The amount of PCBs ingested by passerine birds was calculated using the wildlife dose equation for dietary exposures in the USEPA Exposure Factors Handbook (USEPA 1993). Average potential daily doses (APDD), calculated for total PCBs and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents (TEQs) based on World Health Organization (WHO) 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.

$$APDD = \sum (C_k \times FR_k \times NIR_k) \qquad \text{(Equation 1)}$$

C_k = Concentration of PCBs (totals or $TEQs_{WHO\text{-}Avian}$, ww) in the k^{th} prey item in the passerine diet.

FR_k = Fraction of the passerine diet based on mass represented by the k^{th} prey item.

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

Concentrations of PCBs in prey items were determined in 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.* 2004; Coady *et al.* 2004). 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 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) multi-year 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 5.1).

Table 5.1. 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.* (2002).

[c] Calculated from TRV selected from USEPA (2000).

A study in which ring-necked pheasants (*Phasianus colchicus*) were dosed with Aroclor 1254 was selected as an appropriate study from which to determine a threshold for effects due to dietary exposure to PCBs during critical reproductive life stages (Dahlgren *et al.* 1972). 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. Galliformes are among the most sensitive avian species (Hoffman *et al.* 1996), so TRVs based on Dahlgren *et al.* (1972) are expected to be a conservative estimate of the threshold for effects in passerine species.

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.* 2002). 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.* 2002). 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, while 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 food to the test species and the evaluation of TCDD exposure and not PCB exposure. It has been suggested that TEQs based on PCBs may overestimate exposure relative to TCDD (Custer *et al.* 2004), 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 also expected to be 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 as 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.* 2004b). 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.* 2004b), the TRV for dietary exposure to TEQs was determined to be 1000 ng TEQ/kg.

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 including: 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 5.3). In contrast, diptera represented 79.1% and 80.0% of the aquatic species taken in the diet for tree swallows and house wrens, respectively, while 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 one bolus with 63 trichopterans collected from TB. When this was removed from the analysis, aquatic insects comprised 22.3% of the eastern bluebird diet.

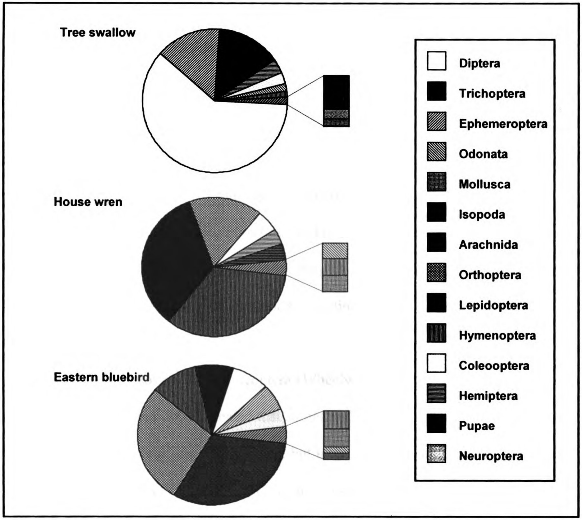

Figure 5.3. Dietary composition based on occurrence at the Kalamazoo River.

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 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 was used 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 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 on 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 several eastern bluebird and house wren nestlings from FC (n = 3).

Average potential daily dose

Average potential daily dietary doses (APDDs) for passerine birds were calculated for both total PCB and $TEQ_{WHO\text{-}Avian}$ concentrations from 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 5.2). 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, 16-fold greater at TB than FC in tree swallows, eastern bluebirds, and house wrens.

Table 5.2. 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

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 5.2). 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. A site-specific diet of the American robin was not

quantified in this study, but an APDD was calculated using a literature-derived diet. The literature-derived ingestion of total PCBs and $TEQs_{WHO-Avian}$ 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 this study did not measure concentrations of $TEQs_{WHO-Avian}$ in plants. Since the American robin diet contains 29% plants (Howell 1940), the APDD for $TEQs_{WHO-Avian}$ in the American robin diet may be 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 below. 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 5.4). 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.

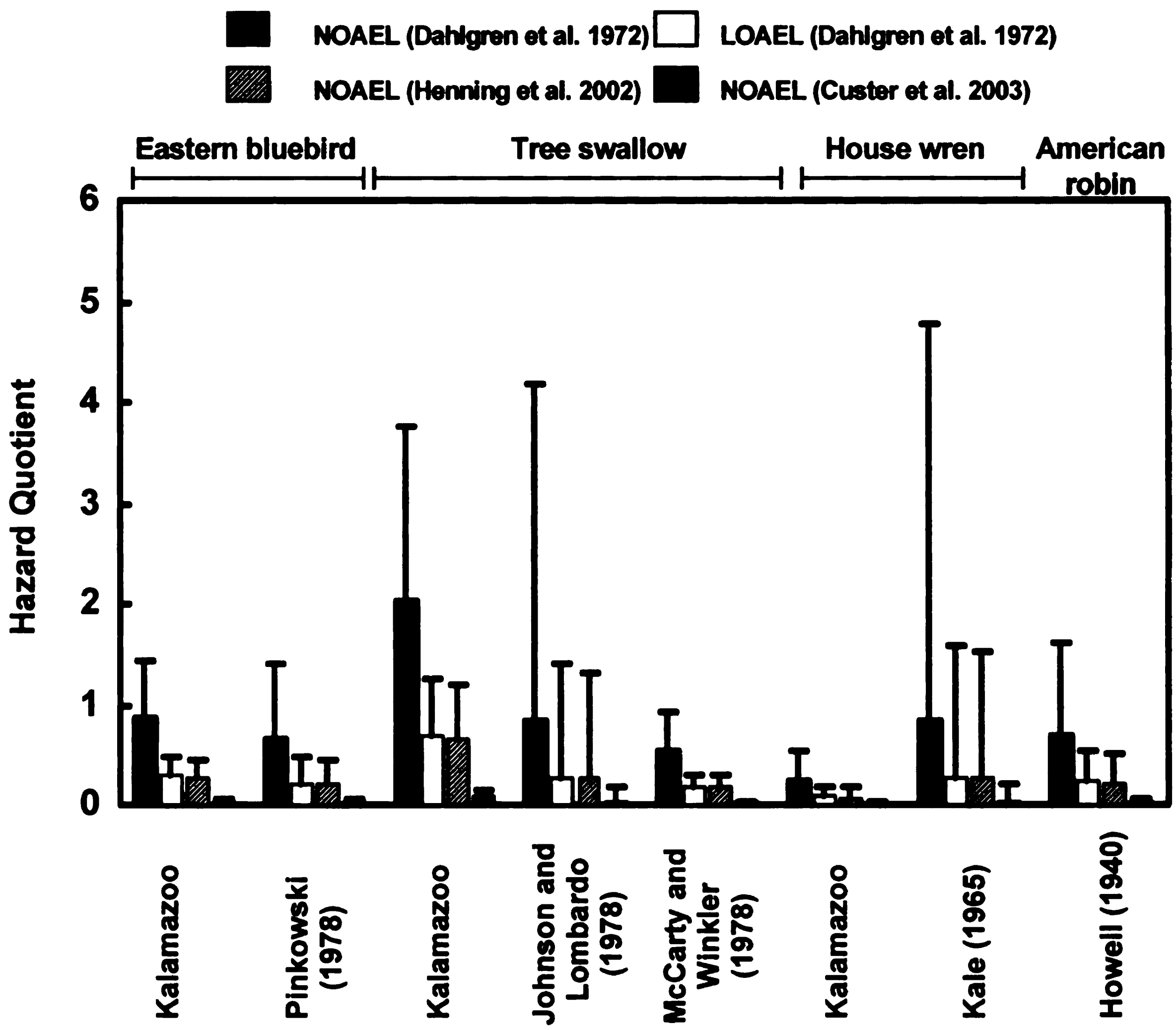

Figure 5.4. Passerine bird dietary hazard quotients for the Trowbridge Impoundment (Kalamazoo) based on the total PCB no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL). Dietary hazard quotients were calculated based on the Kalamazoo site-specific diet and literature-derived diets. Error bars represent the upper 95% confidence limit.

HQs 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, so only values for TB are discussed. HQ values were greatest for tree swallows, and HQs of only the tree swallow based on LOAEL exceed 1.0 (Figure 5.5). 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.

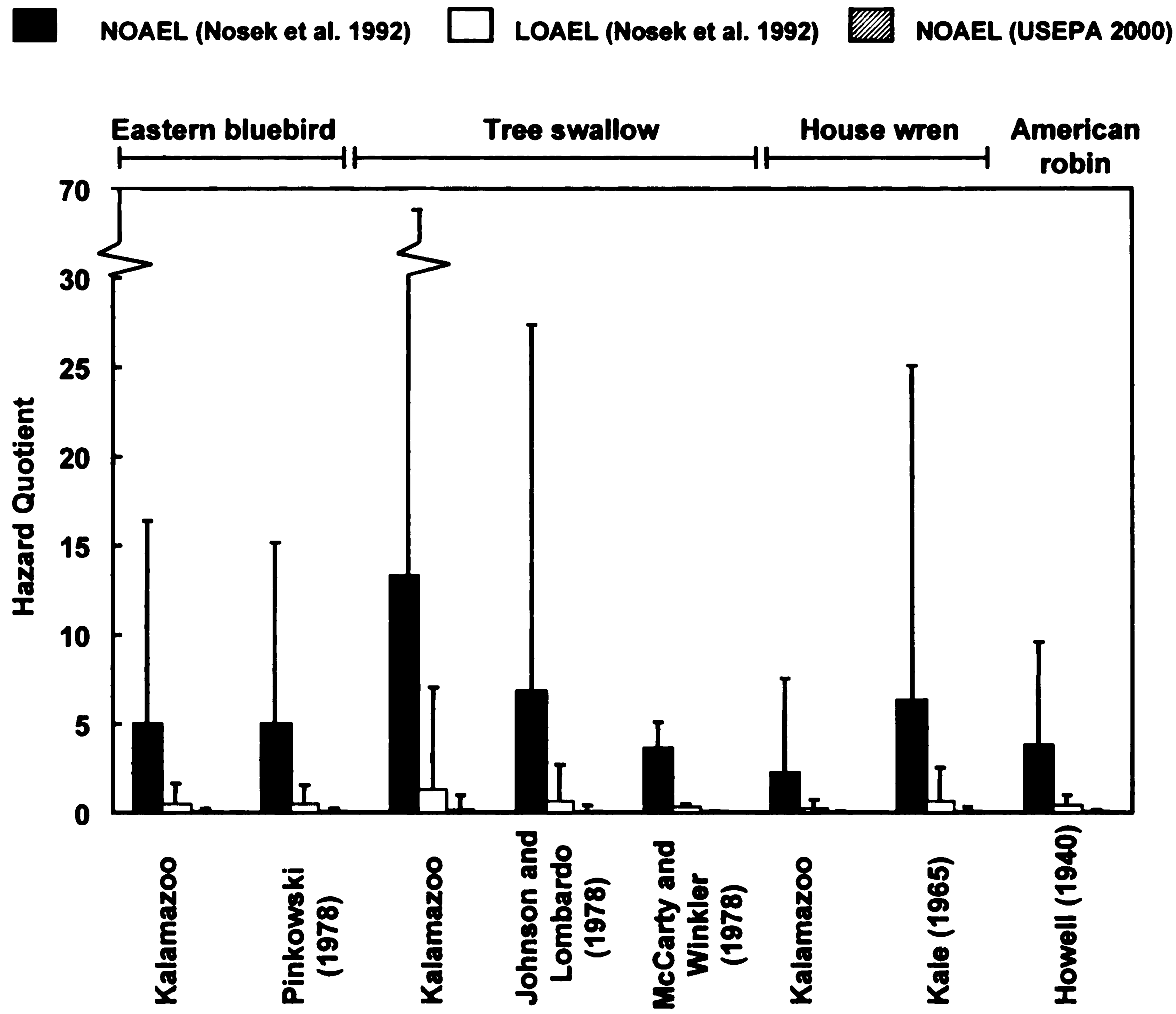

Figure 5.5. Passerine bird dietary hazard quotients for the Trowbridge Impoundment based on the $TEQ_{WHO\text{-}Avian}$ no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) based on toxicity reference values calculated from the literature. Dietary hazard quotients were calculated based on the Kalamazoo site-specific diet and literature-derived diets. Error bars represent the upper 95% confidence limit.

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 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%), 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 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 5.6). 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 a likely result of unsuitable soil conditions for earthworms at study locations. Previously, little was known about specifics of the house wren diet, but they also fell within the terrestrial feeding guild as predicted.

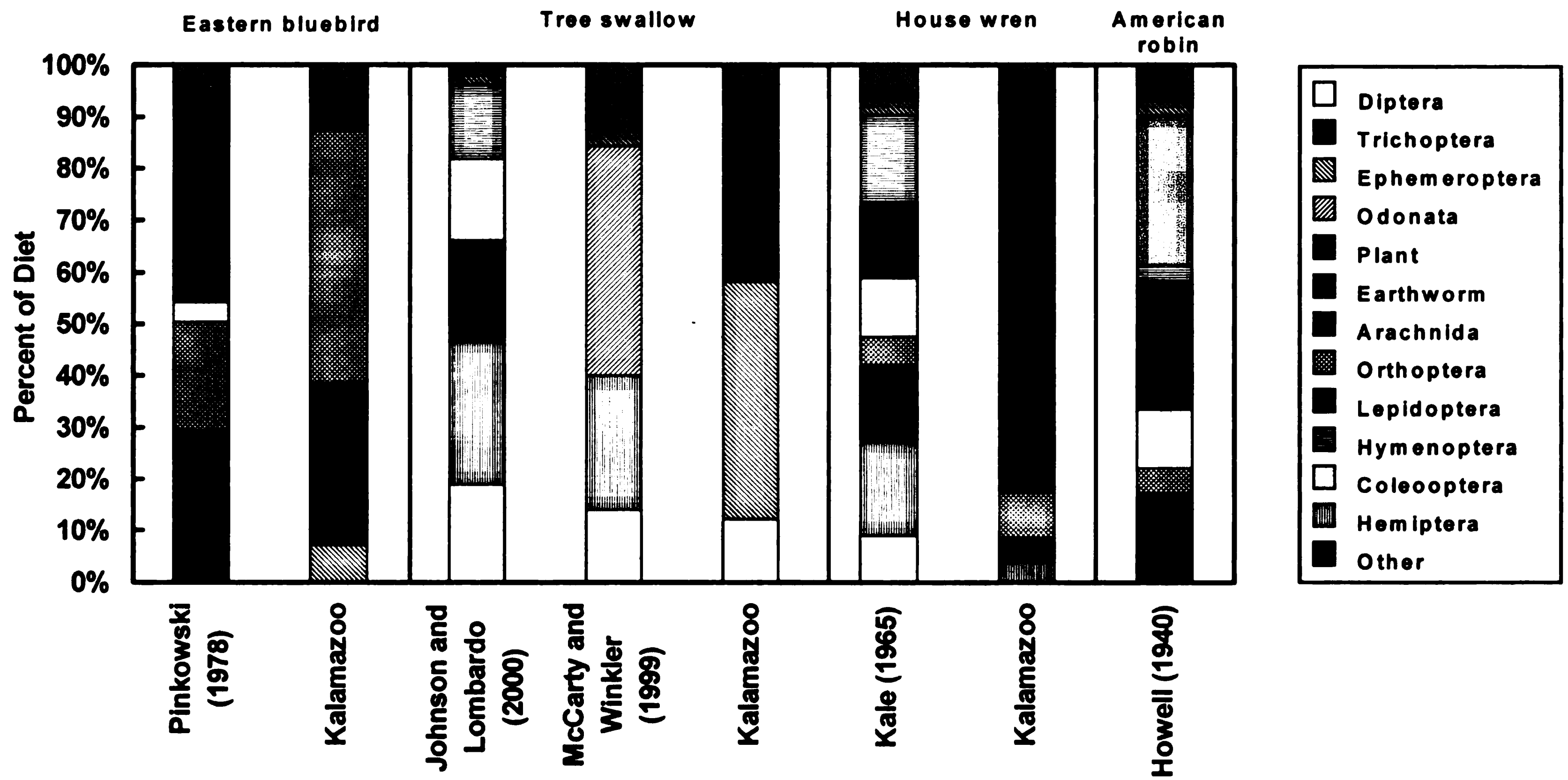

Figure 5.6. Comparison of literature-derived dietary composition (wet weight) to the site-specific diet (wet weight) for eastern bluebirds, tree swallows, house wrens, and American robins at the Trowbridge Impoundment on the Kalamazoo River.

The 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 sampling 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. These changes become important in the calculation of exposure to contaminants at a site because the unique composition of a site-specific diet may contribute significant concentrations to the overall assessment of exposure, and therefore, pose significant risk that would not be recognized by 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 in the soil could be relatively great compared to concentrations in insects (soil = 4.3 mg PCB/kg, ww; insect = 0.55 mg PCB/kg, ww), so potential 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. The amount of soil associated with the grit surface is likely to be minimal in these species, especially when compared to domestic ground feeding species such as the chicken (*Gallus domesticus*) or pheasant. 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. 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 (Coady *et al.* 2004), 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.* 2004). Since 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 since 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, is 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. (Custer *et al.* 2004) 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 toxic equivalency factor (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.* 2004). 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

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. Wild passerine birds, such as the ones examined in this study, appear to be less sensitive to PCB exposure than domesticated galliformes on which dietary TRV could be based (Hoffman *et al.* 1996). 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 TRV values chosen for tissue exposure at the Kalamazoo River (Neigh *et al.* 2004a, Neigh *et al.* 2004b). 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 value we feel is a more appropriate estimate of the true risk of passerine birds exhibiting population level effects due to current contaminant levels at the Kalamazoo River. Based on the most conservative HQs, there appears to be risk to passerine species exposed through the diet due to dietary concentrations being 13 times 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. Additional lines of evidence should be evaluated in order to arrive at the best estimate of risk, especially given the uncertainty in selecting appropriate TRVs. This information coupled with the discussion above on the use of HQs based on TEQs indicate that the more appropriate estimates of risk are those based on total PCB concentrations.

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 5.7). 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.* 2004a; Neigh *et al.* 2004b; Neigh *et al.* 2004c, Neigh *et al.* 2004d). Studies using the top-down approach to evaluate egg, nestling, and adult tissue concentrations in the Kalamazoo River tree swallows, eastern bluebirds, house wrens, and American robins suggest that there is little risk to passerines based on tissue concentrations at the site. Other field studies found that there were no pronounced effects on the reproductive fitness of passerine birds at the concentrations of PCBs measured in birds of the Kalamazoo River (Secord and McCarty 1997; Custer *et al.* 2003).

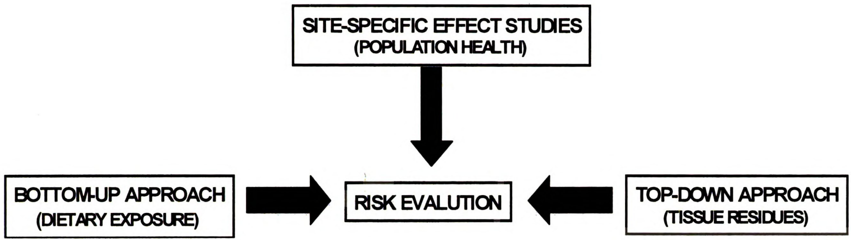

Figure 5.7. Multiple lines of evidence used to assess risk in Kalamazoo River passerine species.

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.* 2004d). 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 the observed reproductive effects were likely caused by PCBs. 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, 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, while 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.* 2002), 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 above those present at the Kalamazoo River. Although it can not 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

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 exposure 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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