



# Principle component analysis of PCB congener profiles in passerines and their diet.

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## ABSTRACT

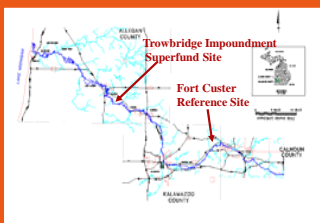
In this study, PCA was used to evaluate PCB congener profiles in tree swallow (*Tachycineta bicolor*) and house wren (*Troglodytes aedon*) adults, nestlings, eggs, and diet at a PCB contaminated site within the Kalamazoo River, MI. The importance of identifying pattern composition differences among trophic levels is a critical component in understanding metabolism, accumulation, and biomagnification of PCBs in a contaminated ecosystem. An analysis was conducted to determine whether PCB congener patterns of adults, nestlings, and eggs were associated with their life-cycle stage. The study examines the role of selective accumulation and metabolism of PCB profiles in nestlings and adults by a profile comparison of co-located dietary items. Average total PCB concentrations in adult passerines (8.0 mg/kg w.w.) were greatest for all samples, while insects had total PCB concentrations of 0.5 mg/kg w.w. Analysis of log transformed data suggests that >70% of the variation in samples across life stages can be explained by 3 factors with >40% of the variation described by hexa- and hepta-homologue groups. Patterns of PCBs in passerine species separated from each other across life stages based on the loading of lower chlorinated congeners. Aquatic emergent insects were most similar to their primary predator, the tree swallow, while there was no consistent grouping of terrestrial insects. This data suggests that patterns associated with the aquatic exposure route have less variation than patterns associated with a terrestrial exposure route.

## INTRODUCTION

Traditional risk assessments characterize risk by examining tissue concentrations in upper trophic level key receptors or by extrapolating risk to key receptors based on concentrations in the bottom trophic levels of the food chain. The current approach of risk assessments assumes that the top-down and bottom-up methodologies will arrive at the same characterization of risk. It has been established that individual congeners can exhibit different metabolism, bioaccumulation, and toxicity[1]. We are examining if species contain different profiles based on their exposure regime and if top-down and bottom-up assessments arrive at similar conclusions in describing PCB profiles.

At the Kalamazoo River Superfund Site several species were identified as species of special concern, such as the bald eagle (*Haliaeetus leucocephalus*), mink (*Mustela vison*), and American robin (*Turdus migratorius*). To address concerns about the American Robin, this study evaluates the current health of songbird populations in the KRAOC and identifies the current and future risks to songbird reproduction due to PCB exposure. We selected a novel species, the house wren (*Troglodytes aedon*), to model a terrestrial-based dietary exposure route similar to the robin's (Figure 1). The tree swallow (*Tachycineta bicolor*), a commonly used species in PCB characterization studies [2,3,4,5], was selected to model PCB exposure in songbirds with an aquatic-based diet.

Figure 2. The Kalamazoo River risk assessment areas of interest.



## METHODS

Tree swallow and house wren tissue and dietary sampling events took place during the 2000-2003 nesting period at the Fort Custer reference site and at the Trowbridge Superfund Site (Figure 2). Samples were Soxhlet extracted, cleaned, analyzed by GC-ECD, and results were compiled on a congener-specific basis. Each sample was analyzed for 98 congeners, including several co-elution groups (Table 1).

Congener profiles for each matrix at the Superfund site were analyzed by PCA to discern similarities in profile composition. Due to interferences of elution peaks by other compounds, all of the congener concentrations could not be reported for every sample. Congeners from the full data set were eliminated based on preset criteria (Figure 3). An overarching assumption made throughout the analysis states that congeners co-eluting during analysis are assumed to metabolize, bioaccumulate, and occur in the same proportion as other congeners in the same co-elution group.

Figure 3. Congener Elimination Criteria

- Congeners without variance.
- Congeners with >15% of the samples having interfering peaks.
- Congeners varying in their co-elution groups depending on the instrument used.
- Congeners with varimax factor loadings for all factors below [0.300].
- When analyzing across matrices, congeners that appear at the bottom of the factor loadings and have >15% of the a single matrix's samples with interfering peaks.

## RESULTS

• Previous work has established that concentrations in tissues and dietary items of passerine species are significantly greater within the KRAOC as compared to an upstream reference site (p<0.05) (Table 2).

• PC-1 consists primarily of hexa- and hepta- PCB homologue groups. PC-2 consists of lower chlorinated PCB congeners in the tri- and tetra-homologue groups (Figure 4).

• Individual passerine species group together but are separated on PC-1 and PC-3 when analyzed across life stages.

• Passerine species separated from each other based on PC-2 for all life stages including eggs, nestlings, and adults. Tree swallow tissues contained tri- and tetra-homologue groups while house wren tissues had non-detect levels of these congeners (MDL <1 µg/kg).

• The profile of the tree swallow closely matches their main dietary items: aquatic emergent insects.

• Aquatic insect species grouped closely with tree swallow life stages but were separate from house wren based on PC-2 (Figure 5).

• Aquatic insects grouped tightly along all principle components but patterns in terrestrial insects were highly variable.

• One outlying adult tree swallow sample was dropped from the data set. The sample contained non-detect levels of lower chlorinated congeners associated with PC-2, and therefore, grouped with house wren tissue samples.

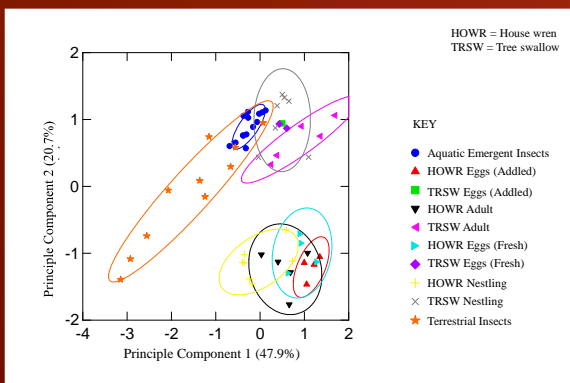


Figure 5. Principle component loading plots for all passerine tissues and dietary items.

Table 1. Congeners analyzed in the complete data set (IUPAC #). Co-planar congeners and their co-eluting congeners were analyzed but are not reported here.

Co-elution groups	Individual congeners
4 and 10	6 40 99 170 205
5 and 8	9 44 107 174 206
15 and 17	18 45 113 176 207
16 and 32	19 49 118 177 209
20, 33, and 53	22 52 119 180
37, 41, and 42	25 70 136 183
47 and 75	26 74 137 185
56 and 92	27 83 138 194
66 and 95	28 91 153 199
82 and 151	31 67 158 201

	HOUSE WREN (mg/kg)		TREE SWALLOW (mg/kg)	
	Fort Custer	Trowbridge	Fort Custer	Trowbridge
Egg (Addl)	0.156(0.155)	6.223(1.374)	0.750(0.430)	2.286(0.801)
Egg (Fresh)	0.094(0.151)	4.630(2.455)	0.629(0.391)	2.305(0.749)
Nestling	0.027(0.021)	1.361(2.439)	0.238(0.163)	3.415(2.130)
Adult	0.087(0.029)	3.109(2.210)	1.417(0.631)	7.923(11.904)

Table 2. Total PCB concentrations for passerine tissue sample at the Fort Custer reference site and the former Trowbridge Impoundment Superfund Site.

Figure 4. Congener component loadings.

Variance explained by principle component factor 1 and corresponding congeners

Component loadings	1	2	3
C158	0.945	-0.168	-0.120
C174	0.943	-0.262	-0.101
C090A0A101	0.942	-0.125	0.151
C15AB932	0.939	-0.156	-0.059
C097	0.937	-0.293	0.036
C176	0.935	-0.175	-0.069
C064B95	0.921	-0.123	-0.046
C121A129A178	0.920	-0.151	0.044
C130	0.915	-0.144	-0.020
C137	0.910	-0.253	0.045
C139	0.908	-0.208	0.035
C118	0.907	-0.139	0.000
C107	0.902	-0.380	0.091
C56AB92	0.898	-0.189	-0.004
C181	0.890	-0.032	-0.038
C01	0.887	-0.232	0.028
C010	0.877	-0.246	-0.130
C139AB197	0.873	-0.292	0.095
C137AB194	0.872	-0.241	-0.184
C74	0.871	-0.278	0.030
C20	0.865	-0.065	0.084
C197AB200	0.860	-0.143	0.132
C40	0.857	-0.074	-0.093
C12AB151	0.856	-0.248	-0.006
C70	0.852	-0.338	0.030
C184	0.849	-0.234	0.035
C185	0.848	0.234	0.134
C02	0.846	0.424	0.130
C195AB208	0.842	-0.249	0.070
C199	0.850	-0.053	0.027
C82	0.831	0.633	0.149
C18	0.817	0.853	-0.076
C12AB17	0.815	0.845	0.021
C12AB17	0.810	0.839	-0.030
C12AB32	0.806	0.835	-0.112
C83	0.804	0.823	0.034
C44	0.828	0.810	-0.042
C132	0.812	0.770	0.116
C20A2A57	0.196	0.807	0.137
C40	0.189	0.74	0.054
C19	-0.002	0.646	0.219
C7	-0.001	0.625	0.237
C31	-0.124	0.549	0.009
C12AB167	-0.075	0.601	-0.214
C15AA17A20	-0.124	0.504	-0.479
C18ABD	0.199	0.387	0.374
C18	0.466	-0.018	0.201
C205	0.484	-0.304	0.026

Variance explained by principle component factor 2 and corresponding congeners

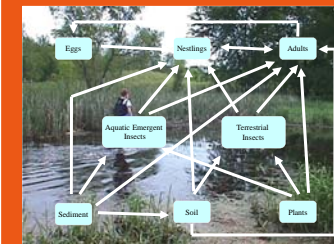


Figure 1. Kalamazoo River passerine food chain and sample matrices.

## DISCUSSION

• Based on differences between passerine species and the agreement of the aquatic insects with their major predator, the aquatic-based exposure pathway varies less than the terrestrial pathway in its congener profiles. One explanation of this phenomenon may be the homogeneity of PCB sources in aquatic systems. Sediment and river water are the primary sources of exposure to aquatic emergent insects. Over time, juvenile stages of aquatic insects equilibrate with the sediment and river water resulting in the homogenous profiles in aquatic system[6].

• Based on the results of this study, risk assessors should be cautious when choosing to use either a bottom-up or top-down methodology for PCB contaminated sites because the variable profiles between different exposure pathways may lead to a mischaracterization of risk. The sampling scheme should include both aquatic and terrestrial based diets to accurately quantify risk to all receptors.

• Exposure routes for terrestrial insects likely have greater variability because terrestrial insects may be obtaining PCB body burdens through atmospheric deposition, ingestion of plant matter, or exposure to soil and water. Due to this variability, narrow sampling schemes may mischaracterize exposure when using lower trophic levels as a basis for bottom-up assessments. When devising sampling plans for areas with PCB contaminated soils risk assessors should maintain a broad sampling scheme over several trophic levels to properly characterize risk in the entire system instead of using the traditional bottom-up and top-down methodologies.

• Passerine species exhibit different congener patterns depending on their food web. Sampling schemes for risk assessments should take these different patterns into consideration as there may be an alternate conclusion based on the selective accumulation and associated toxicity for some congeners in key receptors.

## CONCLUSIONS

Key receptors in upper-trophic levels will reflect the difference in PCB profiles of terrestrial and aquatic systems. The study also shows that there is a greater variability in lower trophic levels of terrestrial systems, although the variability is less in the upper-trophic levels of the same system. Risk assessors must adjust sampling schemes to identify differing patterns in species exposure from diet.

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