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Site Specific PCB-Correlated Interspecies Differences in Organ Somatic Indices

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Abstract. We correlated site specific differences in the organ somatic indices of nestlings of five passerine species (tree swallow, red-winged blackbird, house wren, Carolina chickadee, and eastern bluebird) with the degree of polychlorinated biphenyls (PCB) exposure *in ovo* and post-hatching. The birds were exposed to PCBs at or downstream of four PCB-contaminated sites. Of the organs evaluated for this paper, brain, bursa, heart, kidneys, lungs, pancreas, spleen, stomach, and thyroid varied significantly ($p < 0.05$) or marginally significantly ($0.05 < p < 0.11$) between sites for at least one species. Differences were noted in the direction of the mean SI change (increased or decreased with increasing contaminant exposure by site) between different species for brain, lung, pancreas, spleen and thyroids. Our results indicated that no single passerine species fully represented the response characteristics observed in these species, and no single difference in organ SI should be used in ecotoxicological evaluations. Further, it is critical to include congener analysis in any evaluations since some endpoints in some species correlate well with total TEQs, and some with total PCBs and not TEQs. Some samples containing biologically significant levels of dioxin-like congeners would have been "non-detects" for total PCBs using common analytical methods.

Keywords: TEQ; pancreas; kidney; brain; thyroid; somatic index

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental toxicants that are the basis for about 29% of nationwide fish advisories issued in 2003, and is the contaminant responsible for the second largest number of fish advisories (EPA, 2004). As developmental toxicants, PCBs and related organochlorines are known to interact with and cause functional and morphological changes in most organs including, but not limited to, the nervous system (Henshel, 1998; McCarty and Secord, 1999a; DeWitt and Henshel, 2000), immune system (Fox and Grasman, 1999), heart (Henshel et al., 1993; Walker and Catron, 2000; Millsap et al., 2004; Yeager et al., 2004), liver (Anderson et al., 2003) and kidneys (Couture et al., 1990; Andreason et al., 2002) as a result of exposure during the embryonic and post-embryonic developmental periods. Changes in development and functioning of these organs, which control such diverse critical functions as behavior, protection against biological stressors, oxygen availability, metabolism and excretion can seriously impact survival and quality of life (Gilbertson et al., 1991).

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Standard protocols for assessing ecological risk and evaluation of the environmental impact of a source of contamination (such as PCBs) call for determination of the potential adverse effects of the contaminants on local fauna. However, both laboratory and field assessments have tended to focus on indicator organisms, specific species that have been well studied and are then used to model the potential impacts on species that are less well studied. Among the non-piscivorous avian species, for example, the most recent indicator species of choice has been the tree swallow (McCarty and Secord, 1999b). The implicit assumption underlying the practice of using indicator species is that all species of a similar kind (such as passerines, or even [more broadly] non-piscivorous birds) will respond similarly to a given contaminant. The main issue to be determined, under this assumption, is the relative sensitivity of each species for the chosen evaluation endpoints (e.g. a change in body weight or organ somatic index). This assumption of similar responsiveness needs to be evaluated using multiple types of endpoints across several species within the same study.

Our study compared the relative effects of *in ovo* and post-hatching exposures to PCBs in five passerine species: eastern bluebird (BB; *Sialia sialia*); Carolina chickadee (CC; *Parus carolinensis*); house wren (HW; *Troglodytes aedon*); red-winged blackbird (RW; *Agelaius phoeniceus*); and tree swallow (TS; *Tachycineta bicolor*). The birds were exposed to PCBs while being reared in nests (RW) or nest boxes (BB, CC, HW, TS) at or downstream from four PCB-contaminated sites, three of which are listed National Priorities List (NPL) sites. We compared the organ somatic indices of the birds by site and with the site-averaged concentrations of total PCBs and TCDD-toxic equivalents [TEQs] measured in a sibling nestling.

Methods

Sites

Six sites were evaluated in this study: five contaminated sites and one relatively uncontaminated reference site. The PCBs at each of the contaminated sites originated from two separate industrial sources. All environmental analyses indicated that PCBs are the primary contaminant of concern at all of these sites. Of the five contaminated sites, the two least contaminated sites (Richland Creek [RC] and Conard's Branch [CB]) feed into the West Fork of the White River in connected streams, which receive effluent from a single superfund site. The other three contaminated sites (Winston-Thomas [WT], Illinois Central Springs [ICS] and Pleasant Run [PR]) feed into the East Fork of the White River and have distinctly different sources of PCB contamination. The reference site (Goose Pond [GP]) is on the upper end of the Salt Creek watershed, which also feeds into the East Fork of the White River upstream of any PCB contamination. However, birds migrate through the contaminated area enroute to the nesting area at Goose Pond. Because house wrens were not present at Goose Pond, Conard's Branch (a moderately contaminated site) was used as the "reference site" for the house wren data.

Field collections

Nests at each site were monitored daily throughout the nesting period. Egg laying and hatching dates were recorded. All nestling birds from each nest were collected prior to fledging (approximately day 7 for red-winged blackbirds, days 10–11 for house wrens, and approximately day 14 for bluebirds, chickadees, and tree swallows) and transported to the laboratory. At the laboratory, one sibling chick from each nest was sacrificed by decapitation and the whole body was placed in a chemically clean glass jar for later chemical analysis. The remaining chick(s) were sacrificed and the livers and brains were collected immediately. Livers were weighed and half was quick-frozen and archived for biochemical analysis (−80 °C in glycerin), while half was archived in 10% neutral buffered formalin (NBF) at 4 °C for future histological study. Brains were removed from the brain case and rapidly placed in NBF. Brains and the rest of each body were immersion fixed in NBF and stored at 4 °C for at least 2 weeks prior to analysis. Later, the fixed birds were rinsed (to remove excess NBF), dissected, and the organs were weighed using a semi-ultra balance (Mettler AE240; +0.000005 g accuracy).

Chemical analysis

All samples were analyzed through the US Fish and Wildlife Service's Patuxent Analytical Control Facility (PACF) with the analyses actually done at PACF or by the Geochemical & Environmental Research Group (GERG) at Texas A&M University, College Station, TX under contract to PACF. GERG analyzed total PCBs and PCB congener numbers 1, 7, 8, 15, 16/32, 18, 22, 24, 25, 26, 28, 29, 31, 33, 39, 40, 41/64, 44, 45, 46, 47/48, 48, 49, 52, 53, 60/56, 63, 66, 67, 69, 70, 72, 74, 77, 81, 82, 83, 84, 85, 87, 92, 95/80, 97, 99 101, 105, 107/108/144, 110, 114, 118/108/149, 119, 126, 128, 129, 130, 135, 136, 138, 141, 146, 149, 151, 153, 156/171/202, 158, 166, 167, 169, 170, 171/202, 172, 174, 175, 177, 178, 180, 183, 185, 187/182/159, 189, 191, 193, 194, 195, 196, 197, 199, 200, 201, 205, 206, 207, and 209 as designated by the International Union of Pure and Applied Chemists. Congeners that co-eluted during chemical analysis and could not be distinguished from one another are grouped together by slashes.

Data analysis and statistics

This paper focuses on the site specific analyses of the adrenals (left, right and total), bursa, brain, heart, kidneys (left, right and total), liver, lungs (total), pancreas, spleen, stomach (minus stomach contents), and thyroids (left, right and total). Organ somatic indices were determined by calculating each organ weight as a percentage of body weight in order to correct for variations in body size. Because some nestlings had relatively large stomach contents, body weights used for this normalization were pre-corrected for stomach content weight.

All data were analyzed both graphically, to observe trends, and statistically, to quantitatively analyze between site means. All statistical analyses were run using the SAS system (SAS Institute Inc, Cary, NC). The organ weights and organ somatic indices were compared by species and study site using the SAS PROC GLM (General Linear Model) analysis of variance (ANOVA), a procedure that fits general linear models using least squares analysis, and is especially suitable for unbalanced data. Multivariate ANOVA (within PROC GLM) was used for the multifactoral analysis of the confounding factors. For house wrens, we had to use one of the moderately contaminated sites as our reference site because no house wrens built nests in the nest boxes at the study reference site (Goose Pond) during the 2 years of the study.

Organ somatic indices were correlated against total PCB concentrations and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-based toxic equivalents (TEQs) using linear regression analysis (PROC REG) in SAS. To obtain optimal regression equations, contaminant concentrations were also evaluated using log, squared, and square root transforms of the original data. TEQs were obtained by multiplying the concentrations of the individual PCB congeners by the appropriate avian toxic equivalency factors developed by Van den Berg et al. (1998) and summing the resultant toxic equivalents. Both significant ($p < 0.05$) and marginally significant ($0.05 < p < 0.11$) data are reported. This latitude for statistical significance is given because the relatively low numbers of specimens obtained for some species at some sites resulted in a fairly low power to detect statistical significance.

Results

Contamination levels

Table 1 summarizes the average concentrations of total PCBs, and TEQs by species in the sibling nestlings at each site. The reference site, Goose Pond, has the lowest average PCB concentrations by at least one, and up to three orders of magnitude. Most Goose Pond nests had total egg PCB levels that were below the detection limit (nominally 0.05 ppm wet weight). TEQ concentrations in Goose Pond nestlings' overlapped TEQ concentrations in bluebirds at Conard's Branch and Richland Creek, chickadees at Conard's Branch and tree swallows at Winston-Thomas. The greatest difference between individual TEQ concentrations at Goose Pond and the highest TEQ concentrations at any other site was approximately 30-fold. Of the other five sites, Richland Creek was the least contaminated, based on the average nestling PCB and TEQ concentrations. The birds at Conard's Branch, Winston-Thomas, and Illinois

Table 1. Mean (±SD) concentration of total PCBs (ppm wet weight) and total dioxin TEQs (ppt wet weight) for each species collected at the various PCB study sites

Site		Eastern Bluebird	Carolina Chickadee	House Wren	Red-winged Blackbird	Tree Swallow
Goose Pond	PCB (ppm)	0.006***	0.017	np**	0.007 ± 0.002***	0.14 ± 0.11
	TEQs (ppt)	31***	43		40***	124 ± 95
	# of nests (# siblings)	2 (6)	1 (2)		3 (5)	28 (63)
Richland Creek	PCB (ppm)	0.05	np	np	0.28	ns*
	TEQs (ppt)	65			119	
	# of nests (# siblings)	1 (3)			2 (5)	
Conard's Branch	PCB (ppm)	0.74 ± 0.48	0.73 ± 0.96	1.21 ± 0.38	np	np
	TEQs (ppt)	63 ± 39	135 ± 118	263 ± 57		
	# of nests (# siblings)	7 (20)	3 (7)	3 (8)		
Winston-Thomas	PCB (ppm)	9.4	0.74	2.2	12.1 ± 11.4	16.3 ± 9.8
	TEQs (ppt)	1,458	223	158	1,318 ± 1,139	1,025 ± 601
	# of nests (# siblings)	2 (7)	1 (3)	1 (3)	3 (8)	26 (61)
Illinois Central Seeps	PCB (ppm)	np	1.4	9.5 ± 9.3	Np	np
	TEQs (ppt)		271	799 ± 604		
	# of nests (# siblings)		1 (2)	13 (58)		
Pleasant Run	PCB (ppm)	13.6 ± 9.7	5.12	np	np	ns
	TEQs (ppt)	367 ± 359	127			
	# of nests (# siblings)	3 (4)	2 (9)			

*Not sampled.
**Species not present at this site.
***Red-winged blackbird and bluebird at Goose Pond are presented as half the detection limit because they were ''non-detect''.

Central Seeps tended to have higher contamination levels, with Illinois Central Seeps nestlings usually being the more contaminated (though this did vary by species). The birds (bluebird and chickadee) at Pleasant Run Creek and the house wren at Illinois Central Seeps were the most contaminated of all. The avian community at Pleasant Run Creek appeared to be the most adversely impacted based on limited nesting attempts and overall productivity (Pruit, 2004). One of the shortcomings of this study was that not all species were present at all the sites due to differences in habitat preferences among the species (Table 1). Unfortunately, we were unable to find an appropriate reference site for house wren, which resulted in no low dose values for the species.

Organ somatic indices: evaluation by site

Tables 2–6 summarize the mean organ somatic indices (+SD) for each organ within each species for which there is a statistically significant difference between at least one contaminated site and the respective reference site. Of the organs evaluated in this paper, brain, bursa, heart, kidney, liver, lungs, pancreas, spleen, stomach and thyroid showed a significant or a marginally significant difference in organ somatic index by site in at least one of the 5 species evaluated. Included in the tables are the results of the cross-correlations using the Least Squared Means within PROC GLM of SAS, which corrects for the unbalanced design. All comparisons in the LS Means evaluation are for the contaminated sites against the reference site.

Of the somatic indices evaluated, differences between the reference and contaminated sites for brain, pancreas and thyroid were significant (or marginally significant) for four species each (brain: BB [, CC [, HW ∴, RW [; pancreas: BB ∴, HW ∴, RW [, TS [; thyroid: BB [, CC [, HW [, RW ∴). Bursa, heart, kidney (left, right or total), and liver somatic indices were significantly (or marginally significantly) affected between sites for three species each (bursa: BB [, CC [, TS [; heart: CC [, HW ∴, TS ∴; kidney: BB ∴, CC ∴, HW ∴; liver: CC [, HW [, RW []). Lung and spleen were significantly affected between sites for two species (lung: RW [, TS ∴; spleen: CC ∴, HW []). Stomach had marginally significant increases at two of the contaminated sites for chickadee only. Adrenal somatic indices (left, right or total) were not significantly affected between sites for any of the five species using SAS PROC GLM.

Table 2. Means (±SD) of eastern bluebird organ somatic indices that are significantly different and marginally significantly different between sites, analyzed by LS Means *t*-test within PROC GLM

Organ SI	Goose Pond (ref)	Richland Creek	Conard's Branch	Winston-Thomas	Pleasant Run
Brain	0.03185 ± 0.0023	0.03305 ± 0.0002	*0.03395* ± 0.0026*	0.03408 ± 0.0014	**0.03620** ± 0.0045**
Bursa	0.00077 ± 0.00033	*0.00119* ± 0.00020*	0.00088 ± 0.00031	0.00100 ± 0.00031	0.00087 ± 0.00035
Rt Kidney	0.0068 ± 0.0009	0.0059 ± 0.0004	0.0062 ± 0.0013	*0.0055* ± 0.0007*	*0.0053* ± 0.0004*
Total Kidney	0.0130 ± 0.0025	0.0110 ± 0.0008	0.0126 ± 0.0025	*0.0107* ± 0.0015*	*0.0105* ± 0.0008*
Pancreas	0.0050 ± 0.0007	0.0049 ± 0.0005	*0.0044* ± 0.0008*	*0.0043* ± 0.0006*	**0.0040** ± 0.0004**
Lt Thyroid	0.000040 ± 0.000021	**0.000072** ± 0.000011**	0.000056 ± 0.000024	0.000059 ± 0.000025	0.000041 ± 0.000007

*Italics indicate a mean marginally significantly different from the reference site using LS Means comparisons ($0.05 < p < 0.11$).
**Bold indicates a mean significantly different from the reference site using LS Means comparisons ($p < 0.05$).

Using the results of the PROC GLM ANOVA and LS Means cross-tabulations comparisons between reference sites and each contaminated site for each species, the chickadees had the largest number of significantly affected somatic indices (8 organs), followed by house wrens (7 organs, melding together left, right and total kidneys), bluebirds and red-winged blackbirds (5 organs each), and tree swallows (4 organs).

Table 7 presents the linear regression equations for the best significant or marginally significant correlation between sibling PCB or TEQ concentrations (or their transforms), averaged by site, and the mean organ somatic indices for each organ that was included in Tables 2–6. Because tree swallows only nested at two sites, and any two points (i.e. site averages) determine a line, these linear regressions have no meaningful *p* value or r^2 for the tree swallow data. Therefore, only the PCB regression lines are presented in Table 7. Of the organ somatic indices evaluated for which there were significant correlations, only the bluebird brain somatic indices seemed to be differentially affected by PCBs (which increased the brain somatic index) and TEQs (which decreased the brain somatic index). For all other organ somatic indices for which a significant (or marginally significant) correlation could be determined, thyroid (red-winged blackbird) and kidney (bluebird only) somatic indices correlated similarly with both PCBs and TEQs. Bluebird pancreas, chickadee and red-winged blackbird brain, and chickadee spleen only correlated with PCBs. Chickadee bursa was the only organ somatic index that correlated exclusively with TEQs.

Discussion

Of the passerines studied in this paper, there is limited to no similar data for most of them, and only a few much more limited analyses in tree swallow and bluebird. Thus, this paper is the first to test the concept that a single species may be used as a model or indicator species for the other songbirds exposed to contaminants in the wild.

Table 3. Means (±SD) of Carolina chickadee organ somatic indices that are significantly different and marginally significantly different between sites, analyzed by LS Means *t*-test within PROC GLM

Organ SI	Goose Pond (ref)	Conard's Branch	Winston-Thomas	Illinois Central Seeps	Pleasant Run
Brain	0.059 ± 0.004	0.059 ± 0.003	0.060 ± 0.005	*0.067* ± 0.003*	**0.069** ± 0.004**
Bursa	0.0012 ± 0.0005	0.0013 ± 0.0005	0.0014 ± 0.0003	**0.0028** ± 0.00004**	0.0008 ± 0.0003
Heart	0.0098 ± 0.0008	**0.0123** ± 0.0019**	0.0089 ± 0.0006	0.0092 ± 0.0012	0.0095 ± 0.0027
Rt Kidney	0.0075 ± 0.0011	0.0065 ± 0.0005	0.0059 ± 0.0013	0.0065 ± 0.0013	*0.0058* ± 0.0015*
Liver	0.0316 ± 0.0018	**0.0397** ± 0.0042**	0.0330 ± 0.0015	0.0318 ± 0.0023	0.0314 ± 0.0033
Spleen	0.00083 ± 0.00014	0.00058 ± 0.00021	0.00058 ± 0.00003	0.00069 ($n = 1$)	**0.00051** ± 0.0002**
Stomach	0.027 ± 0.001	*0.033* ± 0.005*	*0.035* ± 0.005*	0.029 ± 0.004	0.030 ± 0.003
Lt Thyroid	0.00004 ± 0.000007	0.00006 ± 0.00001	0.00006 ± 0.000006	0.00003 ± 0.00003	**0.00007** ± 0.00001**

*Italics indicate a mean marginally significantly different from the reference site using LS Means comparisons ($0.05 < p < 0.11$).
**Bold indicates a mean significantly different from the reference site using LS Means comparisons ($p < 0.05$).

Table 4. Means (±SD) of house wren organ somatic indices that are significantly different and marginally significantly different between sites, analyzed by LS Means *t*-test within PROC GLM

Organ SI	Conard's Branch (ref)[#]	Winston-Thomas	Illinois Central Seeps
Brain	0.056 ± 0.005	0.053 ± 0.002	*0.054* ± 0.005*
Heart	0.0085 ± 0.0018	**0.0063** ± 0.0008**	**0.0070** ± 0.0013**
Lt Kidney	0.0073 ± 0.0014	*0.0056* ± 0.0005*	**0.0061** ± 0.0014**
Rt Kidney	0.0075 ± 0.0019	0.0066 ± 0.0013	*0.0066* ± 0.0014*
Tot Kidney	0.015 ± 0.003	0.012 ± 0.001	**0.013** ± 0.002**
Liver	0.039 ± 0.003	0.045 ± 0.011	**0.044** ± 0.006**
Pancreas	0.0046 ± 0.0015	*0.0031* ± 0.0010*	0.0043 ± 0.0015
Spleen	0.0007 ± 0.0004	**0.0015** ± 0.0013**	*0.0011* ± 0.0005*
Rt Thyroid	0.00007 ± 0.00004	0.00010 ± 0.00006	*0.00009* ± 0.00005*

[#]Conard's Branch is used as the reference site for the house wrens, despite the moderate PCB contamination at Conard's Branch, because there were no house wrens collected from the nest boxes at Goose Pond.
*Italics indicate a mean marginally significantly different from the reference site using LS Means comparisons ($0.05 < p < 0.11$).
**Bold indicates a mean significantly different from the reference site using LS Means comparisons ($p < 0.05$).

Our results clearly demonstrate that no one species full represents the PCB-correlated toxicity pattern observed in other passerine species. Of the species evaluated, the Carolina chickadee and house wren had the most organ somatic indices that varied significantly (or marginally significantly) between the reference site and at least one contaminated site. The house wren data is particularly concerning given that there was no true reference site against which to compare the changes in organ somatic indices. Because it is likely that even stronger differences would have been seen between birds from a true reference site and birds from the contaminated sites, the house wren may well be the most sensitive species of all the passerines evaluated in this study.

Interestingly, tree swallows had the fewest organ somatic indices affected. Because, tree swallows have become the passerine species of choice for evaluating PCB contamination effects in the wild (Bishop et al., 1998a, b; McCarty and Secord, 1999a, b) the lesser sensitivity of tree swallows implies that some species not being used for wildlife and effects monitoring may be affected in ways that are not being evaluated. It is especially notable that Carolina chickadees and house wrens are the species that seem to have relatively lower contamination levels compared to other species at the same site, especially tree swallows and red-winged blackbirds (see Winston-Thomas data in Table 1). Taken together, these results suggest that other species than tree swallows, and ideally more than one species, also need to be monitored and evaluated at contaminated sites.

The second major implication of our results is that no one organ or no selected sub-sets of organs are the best indicator organs for PCB-related effects. Clearly, which organs are affected can vary between species. Depending on the species, almost every organ studied (except the adrenals) is

Table 5. Means (±SD) of red-winged blackbird organ somatic indices that are significantly different and marginally significantly different between sites, analyzed by LS Means *t*-test within PROC GLM

Organ SI	Goose Pond (ref)	Richland Creek	Winston-Thomas
Brain	0.026 ± 0.003	0.029 ± 0.006	**0.032** ± 0.003**
Liver	0.042 ± 0.009	0.040 ± 0.006	*0.048* ± 0.005*
Lungs	0.015 ± 0.004	**0.018** ± 0.003**	**0.018** ± 0.001**
Pancreas	0.0087 ± 0.0021	0.0086 ± 0.0033	**0.0106** ± 0.0022**
Rt Thyroid	0.00010 ± 0.00006	0.00009 ± 0.00003	**0.00006** ± 0.00002**
Tot Thyroid	0.00018 ± 0.00009	0.00017 ± 0.00005	*0.00011* ± 0.00005*

*Italics indicate a mean marginally significantly different from the reference site using LS Means comparisons ($0.05 < p < 0.11$).
**Bold indicates a mean significantly different from the reference site using LS Means comparisons ($p < 0.05$).

Table 6. Means (±SD) of tree swallow organ somatic indices that are significantly different and marginally significantly different between sites, analyzed by LS Means *t*-test within PROC GLM

Organ SI	Goose Pond (ref)	Winston-Thomas
Bursa	0.0013 ± 0.0005	*0.0014* ± 0.0006*
Heart	0.0102 ± 0.0015	**0.0093** ± 0.0015**
Lungs	0.020 ± 0.003	**0.018** ± 0.003**
Pancreas	0.0041 ± 0.0013	*0.0045* ± 0.0014*

*Italics indicate a mean marginally significantly different from the reference site using LS Means comparisons ($0.05 < p < 0.11$).
**Bold indicates a mean significantly different from the reference site using LS Means comparisons ($p < 0.05$).

significantly different between the reference site and at least one contaminated site for at least one species. No organ is consistently affected for all of the species. And no species manifests the site-related differences in all of organs that are affected in at least one species. Further, no two species manifest the exact same pattern of organs affected between sites. Thus, we recommend that complete necropsies are carried out on all field and wildlife specimens.

Comparing our results with those of other studies evaluating effects of PCBs on passerines (most of which have included evaluations of tree swallows) indicates that differences in organ somatic indices are relatively sensitive effects to use in field-based evaluations, and are at least as sensitive as the more typically evaluated reproductive parameters and nestling body weights. The extrapolation of these findings to survival and reproduction is harder to make. However, the somatic indices are not difficult to assess and are indicative of potential gross or biochemical abnormalities that can affect the health and long term viability of the individuals within the affected population.

The PCB concentrations in the passerines in our Indiana sites were equivalent to or higher than concentrations in nestlings from Kidney Island and Arrowhead (bordering Lake Winnebago) in the Green Bay and Fox River region of Wisconsin

Table 7. Significant or marginally significant regression equations linking mean site contaminant concentrations with mean site organ somatic indices by species for endpoints with significant or marginally significant differences between sites

Eastern Bluebird		
Brain SI = 9.89E-4*log PCB + 0.0341	$p = 0.0379$	$r^2 = 0.8082$
Brain SI = 1.27E-3*log PCB−8.11E-10*TEQ^2 + 0.0341	$P = 0.0310$	$r^2 = 0.9690$
Right Kidney SI = −3.47E−4*log PCB + 0.00583	$p = 0.0563$	$r^2 = 0.7539$
Right Kidney SI = −7.16E−4*log TEQ + 0.00749	$p = 0.0737$	$r^2 = 0.7087$
Pancreas SI = −2.81E−4*log PCB + 0.00443	$p = 0.0107$	$r^2 = 0.9153$
Carolina Chickadee		
Brain SI = 5.53E−3*$PCB^{1/2}$ + 0.0566	$p = 0.0616$	$r^2 = 0.7393$
Bursa SI = 2.27E−8*TEQ^2 + 0.000795	$p = 0.0652$	$r^2 = 0.7299$
Right Kidney SI = −6.66E−4*log PCB + 0.00629	$p = 0.0436$	$r^2 = 0.7905$
Spleen SI = −1.16E−4*log PCB + 0.000610	$p = 0.0504$	$r^2 = 0.7703$
House Wren		
No equations are significant or marginally significant.		
Red-winged Blackbird		
Brain SI = 1.70E−3*logPCB + 0.0298	$p = 0.0072$	$r^2 = 0.9999$
Right Thyroid SI = −2.83E−5*log TEQ + 0.000146	$p = 0.0046$	$r^2 = 0.9999$
Right Thyroid SI = −1.34E−5*$TEQ^{1/2}$ + 0.000106	$p = 0.1017$	$r^2 = 0.9747$
Total Thyroid SI = −2.00E−5*$PCB^{1/2}$ + 0.000179	$p = 0.0174$	$r^2 = 0.9993$
Total Thyroid SI = −2.29E−6*$TEQ^{1/2}$ + 0.000193	$p = 0.0312$	$r^2 = 0.9976$
Tree Swallow		
Bursa SI = 7.43E−5*PCB + 0.00134	**	**
Heart SI = −4.60E−4*PCB + 0.00984	**	**
Lungs SI = −9.59E−5*PCB + 0.0201	**	**
Pancreas SI = 2.23E−4*PCB + 0.00427	**	**

**As two points define a line, no *p* or *r*-square values are provided for tree swallows.

(Custer et al., 1998), and the multiple sites evaluated in Severn Sound, along the shores of Lakes Erie and Ontario and along the St. Lawrence River (Bishop et al., 1995). The Indiana birds were, however, equivalently or less contaminated than the tree swallows studied along the Hudson River (McCarty and Secord, 1999a). Both the Hudson River and the Fox River studies evaluated nesting success and mean nestling body weights. In the Fox River/Green Bay study, PCBs had no apparent influence on reproductive success, whereas in the Hudson River study, measurements of reproductive success parameters were altered. Mean body weights did not change significantly between colonies in the Fox River/Green Bay study, and were increased significantly at some of the contaminated sites along the Hudson River. Neither study, nor others to date, evaluated organ somatic index changes in PCB-contaminated versus relatively uncontaminated passerines.

Brain, heart and liver changes in response to PCB or PCB-related organochlorine exposure have been documented in numerous species across the vertebrate classes. By the mid-1970s, avian wildlife data indicated that PCBs could accumulate in the brain at high concentrations (in the hundreds of ppm) and cause grossly adverse effects, such as severe tremors, convulsions and death (Gilbertson et al., 1991). Other studies on domesticated or laboratory-raised birds (pheasant [*Phasianos colchicus*] and ring dove [*Streptophelia risoria*]) demonstrated that exposure to moderate to high doses of PCBs also correlated with altered behavior. For example, ringdoves fed 10 ppm PCBs were less attentive to their eggs, allowing them to cool more than did the control ringdoves (Peakall and Peakall, 1973). And tested behaviors of chicken hatchlings [*Gallus gallus*] exposed to either PCBs (0.0035–3.5 ppm) or the related chemical 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 2–200 ppt) *in ovo* also changed significantly and in correspondance with the contaminant dose (DeWitt and Henshel, 2000). Recent studies on birds exposed *in ovo* to PCBs and TCDD indicate that embryonic brains are more sensitive to the teratogenic effects of these compounds. Brain malformations and gross asymmetries have been observed in a number of avian species (chicken, heron [*Ardea herodias*], cormorant [*Phalacrocorax auritus*], eagle [*Haliaeetus leucocephalus*], and passerines [the latter birds are a part of this study]) exposed *in ovo* in the laboratory or in the wild to PCBs or TCDD at environmentally relevant, even ''background'' doses as low as 2 ppt TCDD (Henshel, 1998).

Supporting the supposition that PCBs may adversely affect the developing brain both anatomically and physiologically are the observations by McCarty and Secord (1999b) that tree swallows living along the highly contaminated stretches of the PCB-laden Hudson River demonstrate abnormal nest-building behaviors and abnormally poor nest-building skills. Tree swallows that build inadequate nests may be less likely to successfully lay eggs, brood and hatch them, and have viable fledglings, since the eggs will be more likely to get damaged or chilled during the incubation period, that is, if the nests can even hold one egg.

Hearts are also known to be sensitive to the effects of PCBs and like compounds. Cheung et al. (1981) first documented TCDD-induced heart abnormalities in 14 day chicken embryos, where the full length of incubation is 21 days. Later studies evaluating the effects of TCDD and PCBs, on the development of embryonic hearts documented that the dose-responsive TCDD/PCB-induced heart abnormalities, like the brain abnormalities, are quantifiably detectable very early in embryonic development (Henshel et al., 1993). These characteristic heart abnormalities have been observed in tree swallow embryos from Winston-Thomas in a related part of our study (Henshel et al., 1997). Walker et al. (1997) and Walker and Catron (2000) confirmed that approximately 60 ng/g TCDD toxic equivalents (TEQs) caused edema, structural changes, and increased heart weight due to dilation in developing chicken hearts by embryonic day 10 or 12. Malformed and dysfunctional hearts can lead to reduced cardiac fitness and an eventual increase in heart SI as the body tries to compensate for inefficient heart function. Powell et al. (1996) found that PCB126 caused contradictory changes in embryonic chicken heart weight, depending on the dose (decreased at low dose: 0.2 ppb; increased at high dose: 3.2 ppb). Similarly, the chickadees from the moderately contaminated Conard's Branch site had an average increase in heart somatic index, whereas the mean heart somatic indices at all of the more highly contaminated sites (Winston-Thomas, Illinois Central Seeps, Pleasant Run)

were smaller than the mean heart somatic index in the chickadees from the reference site. Bluebird mean heart somatic indices also varied by site in direction from the mean of the birds from the reference site (increased at Conard's Branch, decreased at Richland Creek and Winston-Thomas [data not shown, as none were statistically significant]). By comparison, mean heart somatic indices in both tree swallow and house wren decreased from the reference site to the contaminated sites.

The mean lung somatic index also varied in direction depending on the species, increasing with higher mean site PCBs in chickadee and red-winged blackbird, decreasing in tree swallow and house wren, and having mixed changes in bluebird depending on the site. A reduced (or enlarged – if due to early tissue vasculature damage) lung size could be potentially detrimental to wildlife.

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