

EXTERNAL HEART DEFORMITIES IN PASSERINE BIRDS EXPOSED TO ENVIRONMENTAL MIXTURES OF POLYCHLORINATED BIPHENYLS DURING DEVELOPMENT

JAMIE C. DEWITT,[†] DEBORAH S. MILLSAP,[‡] RONNIE L. YEAGER,[‡] STEVE S. HEISE,[‡] DANIEL W. SPARKS,[§] and
 DIANE S. HENSEL^{*‡}

[†]Curriculum in Toxicology, University of North Carolina at Chapel Hill, Campus Box 7270, 256 Rosenau Hall,
 Chapel Hill, North Carolina 27599, USA

[‡]School of Public and Environmental Affairs, 1315 East Tenth Street, Room 340, SPEA Building, Indiana University,
 Bloomington, Indiana 47405, USA

[§]U.S. Fish and Wildlife Service, Bloomington Ecological Services Field Office, 620 South Walker Street, Bloomington, Indiana 47403

(Received 25 May 2005; Accepted 8 August 2005)

Abstract—Necropsy-observable cardiac deformities were evaluated from 283 nestling passerines collected from one reference site and five polychlorinated biphenyl (PCB)-contaminated sites around Bloomington and Bedford, Indiana, USA. Hearts were weighed and assessed on relative scales in three dimensions (height, length, and width) for externally visible deformities. Heart weights normalized to body weight (heart somatic index) were decreased significantly at the more contaminated sites in both house wren (*Troglodytes aedon*) and tree swallow (*Tachycineta bicolor*). Heart somatic indices significantly correlated with log PCB concentrations in Carolina chickadee (*Parus carolinensis*) and tree swallow and with log 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalent values in tree swallow alone. Ventricular length was increased significantly in eastern bluebirds (*Sialia sialis*) and decreased significantly in Carolina chickadee and tree swallow from contaminated sites versus the reference site. Heart length regressed significantly against the log PCB concentrations (Carolina chickadee and tree swallow) or the square of the PCB concentrations (red-winged blackbird [*Agelaius phoeniceus*]) in a sibling bird. The deformities that were observed most at the contaminated sites included abnormal tips (pointed, rounded, or flattened), center rolls, macro- and microsurface roughness, ventricular indentations on the ventral or dorsal surface, lateral ventricular notches, visibly thin ventricular walls, and changes in overall heart shape. A pooled heart deformity index regressed significantly against the logged contaminant concentrations for all species except red-winged blackbird. These results indicate that developmental changes in heart morphometrics and shape abnormalities are quantifiable and may be sensitive and useful indicators of PCB-related developmental impacts across many avian species.

Keywords—Passerine Polychlorinated biphenyls Toxic equivalents Heart Development

INTRODUCTION

Polychlorinated biphenyls (PCBs) were produced commercially in the United States for nearly 50 years. They were sold and used as varying mixtures of 209 congeners, all of which are relatively lipophilic and relatively slow to environmentally degrade or be metabolized. Although the sale of PCBs in the United States was banned in 1977 because of health and environmental concerns, PCBs have continued to be released into the environment. Polychlorinated biphenyl releases have declined dramatically in the past several decades, but PCBs remain ubiquitous in environmental media and continue to pose problems from both health and regulatory perspectives [1] (<http://www.atsdr.cdc.gov/DT/pcb007.html>).

Of the different congeners, the coplanar, or laterally (*meta*-, *para*-)substituted, congeners tend to be toxicologically similar to polychlorinated dibenzodioxins (PCDDs) and have potencies that are assessed relative to the “standard” PCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; also known as dioxin) [2]. These normalized dioxin-like potencies are expressed as TCDD toxic equivalents (TEQs). Most dioxin-like effects are thought to be mediated by the interaction of the congener with the aryl hydrocarbon receptor. Such effects include induction of cytochrome P4501A-related enzymes [2], thymic atrophy

and immunotoxicity [3], reproductive and developmental toxicity [4], developmental induction of brain asymmetry [5], cardiac teratogenicity [6–8], and nongenotoxic carcinogenicity [9].

The non-coplanar congeners (*mono*- and *di-ortho*-substituted) do not bind effectively to the aryl hydrocarbon receptor and have toxicological profiles that are less “dioxin-like” [2]. Health effects of PCB-exposed animals, including wild birds, are expressed in multiple organs and have been observed in virtually all species assessed. A subset of these congeners includes potent neurotoxicants affecting neurochemistry and behavior [10,11] and second-messenger systems, such as intracellular calcium [11]. Non-coplanar PCB congeners and PCB mixtures also disrupt the immune system [11] as well as the homeostatic functioning of several hormonal feedback control systems, including the estrogen [11], thyroid [12], corticosteroid [13], and retinoid [14] hormone and growth-regulator feedback systems. Most of these effects have been observed in birds as well as in mammals. The passerine PCB literature is limited and tends to focus on exposure evaluations, although a few studies have documented reproductive [15,16] and behavioral anomalies [17] as well as plumage and hormonal changes in response to PCB exposure in tree swallows [13,14,18].

In addition to differences related to chemical structure, the life stage of the organism at the time of exposure greatly

* To whom correspondence may be addressed
 (dhenshel@indiana.edu).

Heart deformities in passerine birds exposed to PCBs

Environ. Toxicol. Chem. 25, 2006

impacts the toxicity of PCBs. Developing organisms are more sensitive to the effects of PCBs, and these effects can persist into adult life [1,10]. For example, PCBs competitively bind to the serum proteins that carry thyroid hormones, reducing serum thyroid hormone levels. Increased rates of gross and subtle neurological abnormalities are induced by reduced thyroid hormone levels (hypothyroid) during development, because the developing brain is highly sensitive to the level of available thyroid hormone [19].

Several landfills and a former wastewater treatment plant in Bloomington, Indiana, USA, are contaminated with PCBs that originated from a capacitor manufacturing plant that used PCBs (primarily 1242) from 1958 to 1977 [20] (<http://www.state.in.us/idem/>). Of hazardous materials detected at the landfills, only PCBs have been detected at high-enough concentrations to be of concern to state and federal regulatory agencies. Polychlorinated biphenyls were released directly from the plant to the sanitary sewer and leaked from capacitors disposed of at local landfills and dump sites. In addition, historic, on-site disposal of spent hydraulic fluid containing PCBs (primarily 1242) at a manufacturing plant in Bedford, Indiana, USA, is the source of contamination at one of our study sites [21] (<http://www.bedfordpowertraincorrectiveaction.com/docrepository/>). Although many of these sites have been or are being remediated (or are capped) residual PCBs continue to move from the remnants of the contaminated sites into the surrounding environment, entering the food chain and contributing to wildlife exposures. The heart is susceptible to dioxin and dioxin-like chemical exposure [6–8,22–29]. Previous work by our laboratory demonstrated that developmental TCDD exposure increased the incidence and severity of teratogenic heart deformities in chicken embryos [7,22–24] exposed throughout the incubation period. Observed anomalies from these and other studies included increased heart weight [6,8], pericardial edema, cardiomyopathy [6,8,25], abnormal valve and vessel development [6], and decreased contractile response [25,28]. Our preliminary evaluations of heart development in passerines environmentally exposed in ovo to mixtures of PCBs (through maternal deposition in the yolk) indicated that virtually all the passerines (eastern bluebirds, Carolina chickadees, house wrens, red-winged blackbirds, and tree swallows) were sensitive to the cardiotoxic effects of PCBs [15,29]. Therefore, this study was conducted to quantify and provide a detailed evaluation of the PCB-induced cardiac teratogenicity in the five passerine species exposed in ovo under field conditions. Our goals were to characterize systematically the site-associated suite of necropsy-observable cardiac deformities and changes in gross heart shape and size in avian wildlife and to correlate quantitatively each deformity and change with total PCBs and dioxin-like TEQs by species, establishing the relative sensitivity of each passerine species.

MATERIALS AND METHODS

Field collection

Approximately 30 to 60 nest boxes per site (standard size for bluebirds and tree swallows, hole dimensions of 3.5 cm [width] × 5.7 cm [height]) were placed adjacent to contaminated waterways at five contaminated sites (Conard's Branch [CB], Illinois Central Springs [ICS], Pleasant Run [PR], Richland Creek [RC], and Winston-Thomas wastewater treatment plant [WT]) at or downstream of sites where the U.S. Environmental Protection Agency is requiring extensive remediation of soil, water, and sediment because of PCB contamina-

tion. Four of these sites—CB, ICS, RC, and WT—are in Bloomington, Indiana, USA (ICS and WT), or in the surrounding county (CB and RC; Monroe County, IN, USA). Pleasant Run is in Bedford, Indiana, USA, in Lawrence County. A reference site (Goose Pond [GP]) was located in an adjacent watershed in Monroe County, Indiana, USA, with no known sources of PCB contamination. Nest boxes were monitored daily during nesting and active-laying periods throughout the spring and summer of 1995 and 1996. Passerine species used for the present study included eastern bluebird (*Sialia sialis*), Carolina chickadee (*Parus carolinensis*), house wren (*Troglodytes aedon*), red-winged blackbird (*Agelaius phoeniceus*), and tree swallow (*Tachycineta bicolor*). The number of nests sampled by species and site and the mean total PCB and TEQ concentrations for each are listed in Table 1. Red-winged blackbird eggs and nestlings were collected from nests near the nest boxes at GP, RC, and WT. Egg laying and hatching dates were recorded to monitor reproductive and hatching success (unpublished data). Nestling birds were collected before fledging, which was approximately day 7 for red-winged blackbird, day 11 for house wren, and day 14 for eastern bluebird, Carolina chickadee, and tree swallow. Nestlings were rapidly transported to the laboratory at Indiana University (Bloomington, IN, USA) in ventilated plastic trays placed inside coolers for protection and to minimize heat stress. Nestlings were in transit no more than 60 min.

Laboratory protocols

At the laboratory, nestlings were weighed individually on a balance (accuracy, ±0.001 g; Mettler Model PM4000; Mettler-Toledo, Columbus, OH, USA) and killed by decapitation. One sibling nestling from each nest was placed in a chemically clean glass jar after death and frozen for later chemical analysis (Table 1). These individuals were sent either to the U.S. Fish and Wildlife Service's Patuxent Analytical Control Facility (Laurel, MD, USA) or to the Geochemical and Environmental Research Group at Texas A&M University (College Station, TX, USA), which is under contract to the Patuxent Analytical Control Facility. These samples were analyzed for total PCBs and more than 70 different PCB congeners, including congeners with di-, tri-, tetra-, penta-, hexa-, hepta-, and octachlorine substitutions. Specific congeners are referred to according to the International Union of Pure and Applied Chemistry (IUPAC) standard numbering system for PCBs. For example, IUPAC congener PCB 126 is 3,4,5,3',4'-tetrachlorobiphenyl, and PCB 153 is 2,4,5,2',4',5'-hexachlorobiphenyl. Chemical extractions of samples followed procedures developed by the National Oceanic and Atmospheric Administration Status and Trends Program [30] with minor revisions [30,31]. Quantification of PCBs was performed by capillary gas chromatography with electron-capture detection [32]. Polychlorinated biphenyl congeners 77, 126, and 169 were analyzed by high-resolution gas chromatography/high-resolution mass spectrometry using U.S. Environmental Protection Agency method 8290 [33]. The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin TEQs were determined using toxic equivalency factors for each congener as listed in Van den Berg et al. [34] (Table 1).

Immediately after death, the liver and brain were rapidly removed, covered with 10% neutral-buffered formalin in glass vials, and archived at 4°C. After at least two weeks, fixed organs were necropsied and weighed on a semiultra balance (accuracy, ±0.00001 g; Mettler Model AE240). Bodies were

Table 1. Mean (\pm standard deviation) polychlorinated biphenyl (PCB) concentration (ppm) and dioxin toxic equivalents (TEQs; ppt) in passerine nestlings collected from reference (GP) or PCB-contaminated sites

Sites ^a	Eastern bluebird		Carolina chickadee		House wren		Red-winged		Tree swallow	
	PCB (ppm)	TEQ (ppt)	PCB (ppm)	TEQ (ppt)	PCB (ppm)	TEQ (ppt)	PCB (ppm)	TEQ (ppt)	PCB (ppm)	TEQ (ppt)
GP	0.006 \pm 0.000 (2) ^b	62 \pm 0	0.02 \pm 0	43 \pm 0	NP ^c	NP	0.007 \pm 0.002 (4)	81 \pm 0	0.14 \pm 0.11 (28)	124 \pm 95
RC	0.05 \pm 0	65 \pm 0	NP	NP	NP	NP	0.28 \pm 0.01 (2)	119 \pm 26	NS ^d	NS
CB	0.73 \pm 0.47 (7)	63 \pm 39	0.59 \pm 0.84 (4)	114 \pm 105	1.2 \pm 0.4 (3)	263 \pm 57	NP	NP	NP	NP
WT	9.4 \pm 2.8 (2)	1,458 \pm 1,257	0.74 \pm 0.00 (1)	223 \pm 0	2.2 ^e \pm 2.4 (2)	161 \pm 4	12.1 \pm 11.4 (3)	1,318 \pm 1,139	16.4 \pm 9.6 (27)	1,036 \pm 584
ICS	NP	NP	1.4 \pm 0.0 (1)	271 \pm 0	9.5 \pm 9.3 (14)	799 \pm 604	NP	NP	NP	NP
PR	13.6 \pm 9.7 (3)	367 \pm 359	5.1 \pm 1.7 (2)	127 \pm 87	NS	NS	NP	NP	NS	NS

^a GP, Goose Pond (Monroe County, IN, USA); RC, Richland Creek (Monroe County, IN, USA); CB, Conard's Branch (Monroe County, IN, USA); WT, Winston-Thomas (Bloomington, IN, USA); ICS, Illinois Central Springs Bloomington, IN, USA; PR, Pleasant Run (Bedford, IN, USA).

^b Numbers in parentheses indicates numbers of individuals chemically analyzed from that site.

^c NP = not present at site.

^d NS = species present at site but not sampled.

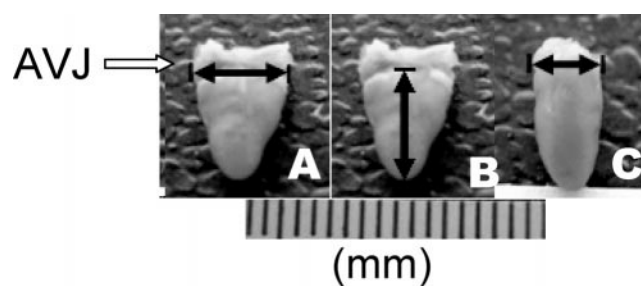


Fig. 1. Representative passerine hearts illustrating heart measurements. **A.** Width refers to the heart width, measured at the widest part of the ventricles. Measurement is taken across the heart just under the atria near the point of the atrioventricular junction (AVJ). **B.** Length refers to ventricular height, measured from where the ventricles begin (just under the atria near the AVJ) to the apex (tip of the heart). **C.** Depth refers to the lateral ventricular depth, measured from the lateral perspective, where the ventral side of the heart near the AVJ transects the width measurement.

placed individually into 120-ml plastic containers, covered with 10% neutral-buffered formalin, and archived at 4°C.

External heart morphology

Mechanical calipers (accuracy, ± 0.05 mm) were used to measure each of three morphological features of the heart—width, length, and depth—using standardized protocols developed from classic morphometric techniques (Fig. 1). Each measurement was made to the nearest 0.05 mm. Two measurers were co-trained to ensure that intersample measurement variation was no greater than 5% on any individual measurement in the training set. Training sets consisted of a sample of at least 10 hearts from each species, covering a range of sites, when appropriate. After the training period, both measurers carried out the full set of measurements independently and were blind to the sample site. Resulting measurements from each measurer were averaged for the final measurement used in subsequent analyses. For all measurements, the caliper point rested slightly on the region of the heart associated with the appropriate morphological measurement and was slowly closed until both points were just touching the predetermined landmark. The caliper points lightly touched the hearts at the point of measurement, ensuring that the heart could easily slide out of the calipers, thus preventing artificial compression of the hearts.

Heart morphometrics

Fixed, individual hearts were digitally photographed and assessed for external deformities by the same person. In a given heart, each of 10 deformities was assessed according to a severity scale. These deformities, their associated severity scales, and a description of each deformity are listed in Table 2.

Statistical analysis

All measurements and deformity ratings were initially graphed (Microsoft Excel[®] 2003; Redmond, WA, USA) and then evaluated statistically (SAS System, Ver 8; SAS Institute, Cary, NC, USA). Statistical analysis using SAS included analysis of variance (ANOVA) in the general linear model procedure (PROC GLM) to compare the data by site. (The PROC GLM algorithm corrects for an unbalanced study design.) Individual comparisons within models testing multiple cofactors were evaluated using multivariate ANOVA and the least-squared-means *t* test within PROC GLM. The data were cor-

Table 2. External surface and shape deformities and associated severity scales used to assess hearts from fledgling passerine birds environmentally exposed to polychlorinated biphenyls

Deformity	Scale	Explanation
Center roll(s)	0 = none; 1 = very slight; 2 = slight; 3 = moderate; 4 = severe	Central region of heart stands out from the surface of the heart at the level of the ventricles.
Flattened tip	0 = none; 1 = slight; 2 = significant; 3 = severe	Apex of hearts is flattened rather than slightly rounded for the species.
Pointed tip	0 = none; 1 = very slight; 2 = slight; 3 = significant; 4 = severe	Apex of heart is pointed rather than slightly rounded for the species.
Rounded tip	0 = none; 1 = very slight; 2 = slight; 3 = rounded	Apex of heart is extremely rounded for the species.
Macrosurface roughness	0 = none; 1 = very slight; 2 = slight; 3 = significant; 4 = severe	Overall surface of heart appears grossly bumpy, like cobblestones.
Microsurface roughness	0 = none; 1 = very slight; 2 = slight; 3 = significant; 4 = severe	Overall surface of heart appears uneven and rough, like sandpaper.
Ventricular indentation(s)	0 = none; 1 = very slight; 2 = slight; 3 = significant; 4 = severe	Indentations on the ventral or dorsal surface of the ventricles.
Ventricular notch(es)	0 = none; 1 = very slight; 2 = slight; 3 = significant; 4 = severe	Tip of the right ventricle is shortened from the bottom of the right ventricle to the central face of the left ventricle. This is seen on the lateral aspects of the ventricles.
Visibly thin ventricular walls	0 = no; 1 = obvious	Overall surface of ventricles appears thin.
Shape	0 = normally shaped; 1 = long/pointy shape; 2 = triangular in shape; 3 = C-shaped; 4 = stubby or short (distinct from flattened or rounded tips)	Overall shape of heart.

related with contaminant levels in sibling nestlings using the SAS regression procedure (PROC REG). The *t* test procedure (PROC T-Test) was used to compare group means of the reference site versus the pooled contaminated site data, by species, for the deformity analysis (Fig. 2). Throughout the analysis, statistical significance was defined by $p \leq 0.05$, whereas marginal significance was defined by $0.05 < p < 0.10$.

72

RESULTS

PCBs and toxic equivalents

For all species, the site ranking of mean total PCB concentrations in nestlings was WT > PR > ICS > CB > RC > GP (Table 1). For all species, the order of mean PCBs in nestlings at WT (the one site where all five species were present) was tree swallow > red-winged blackbird > eastern bluebird > house wren > Carolina chickadee. No house wrens were collected at GP; thus, CB was used as the reference site for house wren, for the ANOVA based on overall site contamination. The mean total PCB concentration for eastern bluebird, red-winged blackbird, and tree swallow was two to four orders of magnitude higher ($p \leq 0.05$) at WT or PR than at GP.

In general, TEQs were highest at WT (eastern bluebird, red-winged blackbird, and tree swallow) or ICS (Carolina chickadee and house wren). The order of mean TEQs in nestling birds at WT was eastern bluebird > red-winged blackbird > tree swallow > Carolina chickadee > house wren. The most contaminated nestlings, however, were tree swallows (2,504 ppt), which were more contaminated than the most contaminated eastern bluebird (2,347 ppt) at the same site (WT). The mean ratio of TEQs to PCBs ($\mu\text{g TEQs:g PCBs}$) in all species is highest at our reference site and declines as the total PCB contamination level increases: GP (6,235:1) > RC (863:1) > CB (166:1) > WT (139:1) > ICS (39:1) > PR (26:1).

Heart somatic index

The heart somatic index is the heart weight normalized by body weight. Mean heart somatic indices for all species at all sites collected are summarized in Table 3. Of the five species, only Carolina chickadee, house wren, and tree swallow had changes in mean heart somatic indices that were statistically different between at least one contaminated site and the respective reference site. For both house wren and tree swallow, the mean heart somatic index was statistically significantly smaller at the more highly contaminated sites. House wren heart somatic indices were, on average, 18% (ICS) and 26% (WT) smaller at the two more contaminated sites compared to the reference site (CB). Tree swallow heart somatic indices were, on average, 9% smaller at the more contaminated site (WT) compared to the cleanest site (GP). By contrast, the changes in Carolina chickadee heart somatic indices were not consistent among sites, and the only statistically significant change was an increased mean heart somatic index (26%) at a moderately contaminated site (CB) compared to the reference site (GP). Although not statistically different from the data for the reference site (GP), the red-winged blackbird mean heart somatic index also increased (13%) at a highly contaminated site (WT).

The optimal single variable regression of heart weight and heart somatic index against total PCBs, total PCBs corrected for lipid content, and TCDD TEQs are presented in Table 4. Of the five species, only the tree swallow heart somatic indices

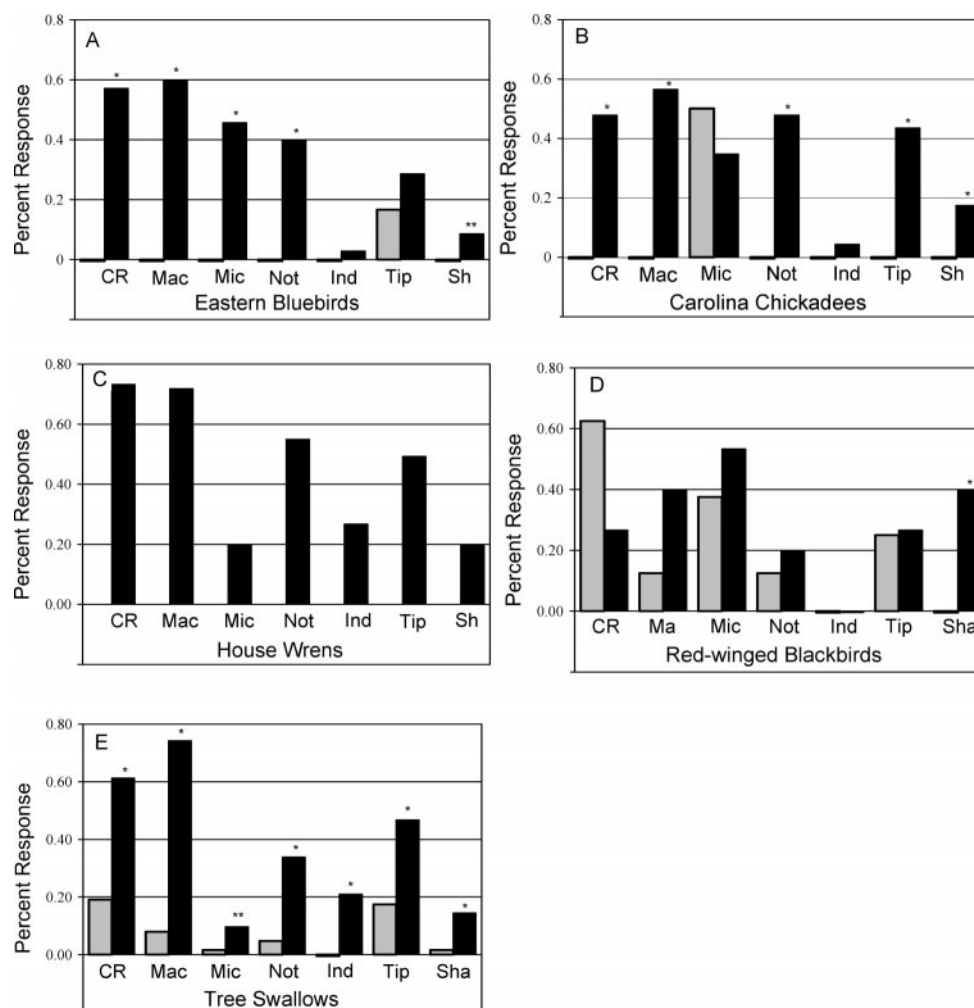


Fig. 2. Percentage of severe deformities at the least contaminated (reference) site (Goose Pond, Monroe County, IN, USA) and at all the more contaminated sites pooled in (A) eastern bluebirds, (B) Carolina chickadees, (C) house wrens, (D) red-winged blackbirds, and (E) tree swallows. CR = center rolls on ventral and dorsal surface of ventricles; Indent = dorsal and ventral surface ventricular indentations; Mac = macrosurface roughness, a cobblestone appearance; Mic = microsurface roughness, sandpaper-like surface; Notch = lateral ventricular notches; Shape = gross shape deformities including C-shaped heart, stubby heart, triangular heart; Tip = abnormal tips including pointed tip, flattened tip, rounded tip. An asterisk indicates a statistically significant difference ($p \leq 0.05$) between percentage response of pooled contaminated sites and percentage response at Goose Pond (t test). Two asterisks indicate a statistically significant difference at the $p \leq 0.01$ level. Dotted fill indicates reference site; solid fill indicates contaminated sites (pooled data).

correlate significantly with the untransformed contaminant concentrations (not shown; PCBs: $p = 0.0014$, $r^2 = 0.0817$; lipid-corrected PCBs: $p = 0.0046$, $r^2 = 0.0644$; TEQs: $p = 0.0010$, $r^2 = 0.0859$). When analyzed using a variety of contaminant concentration transformations in the model (loga-

rithms, squares, and square roots, specifically), the heart weights and somatic indices of Carolina chickadee, red-winged blackbird, and tree swallow were significantly correlated with at least one transformation of the contaminant concentration in a sibling chick. Both red-winged blackbird and tree swallow

Table 3. Mean (\pm standard deviation) heart somatic indices (SI; heart wt adjusted for body wt $\times 1,000$) of passerines collected from reference (GP) or polychlorinated biphenyl contaminated sites

Sites ^a	Eastern bluebird		Carolina chickadee		House wren		Red-winged blackbird		Tree swallow	
	<i>n</i>	Heart SI	<i>n</i>	Heart SI	<i>n</i>	Heart SI	<i>n</i>	Heart SI	<i>n</i>	Heart SI
GP	6	7.8 \pm 0.9	2	9.8 \pm 0.8	—	—	8	8.2 \pm 0.8	63	10.2 \pm 1.5
RC	3	7.7 \pm 0.5	—	—	—	—	5	7.9 \pm 1.1	—	—
CB	20	8.6 \pm 1.9	9	12.3 \pm 1.6*	8	8.5 \pm 1.8	—	—	—	—
WT	7	7.3 \pm 0.8	3	8.9 \pm 0.6	4	6.3 \pm 0.8**	9	9.3 \pm 1.7	61	9.3 \pm 1.5*
JCS	—	—	2	9.2 \pm 1.2	58	7.0 \pm 1.3**	—	—	—	—
PR	4	7.9 \pm 1.1	9	9.4 \pm 2.7	—	—	—	—	—	—

^a Goose Pond (Monroe County, IN, USA); RC, Richland Creek (Monroe County, IN, USA); CB, Conard's Branch (Monroe County, IN, USA); WT, Winston-Thomas (Bloomington, IN, USA); JCS, Illinois Central Springs (Bloomington, IN, USA); PR, Pleasant Run (Bedford, IN, USA).

* Statistically significantly ($p \leq 0.05$) different from GP; ** statistically significantly ($p \leq 0.05$) different from CB.

Heart deformities in passerine birds exposed to PCBs

Environ. Toxicol. Chem. 25, 2006

Table 4. Species-specific optimal single variable linear regressions correlating sensitive developmental heart indicators with total polychlorinated biphenyls (PCBs), lipid-corrected total PCBs (PCB_{lipid}), and tetrachlorodibenzo-*p*-dioxin (TCDD)

Species	Equation	<i>p</i>	<i>r</i> ²
<i>Heart weight</i>			
Eastern bluebird	No correlation was significant or marginally significant.		
Carolina chickadee	Heart wt = -0.02·log PCB + 0.10	0.0152	0.2303
	Heart wt = -0.02·log PCB _{lipid} + 0.09	0.0188	0.2173
House wren	No correlation was significant or marginally significant.		
Red-winged blackbird	Heart wt = -1.2E-4·PCB ² + 0.25	0.0755	0.1650
	Heart wt = -4.2E-4·PCB _{lipid} + 0.25	0.0499	0.1971
	Heart wt = -1.2E-8·TEQ ² + 0.26	0.0937	0.1877
Tree swallow	Heart wt = -0.01·log PCB + 0.14	<0.0001	0.1355
	Heart wt = -0.02·log PCB _{lipid} + 0.16	<0.0001	0.1640
	Heart wt = -0.03·log TEQ + 0.16	<0.0001	0.1621
<i>Heart somatic index (SI)</i>			
Eastern bluebird	No correlation was significant or marginally significant.		
Carolina chickadee	Heart SI = -1.0E-3·log PCB + 0.01	0.0929	0.1179
	Heart SI = -0.002·PCB _{lipid} ² + 0.012	0.0734	0.1327
House wren	No correlation was significant or marginally significant.		
Red-winged blackbird	No correlation was significant or marginally significant.		
Tree swallow	Heart SI = -4.7E-4·log PCB + 0.01	0.0002	0.1105
	Heart SI = -4.3E-4·log PCB _{lipid} + 0.1	0.0002	0.1201
	Heart SI = -9.7E-4·log TEQ + 0.1	<0.0001	0.1201
<i>Length</i>			
Eastern bluebird	No correlation was significant or marginally significant.		
Carolina chickadee	Length = -0.48·log PCB + 7.04	0.0091	0.2605
	Length = -0.49·log PCB _{lipid} + 6.77	0.0071	0.2748
House wren	No correlation was significant or marginally significant.		
Red-winged blackbird	Length = -0.002·PCB ² + 10.022	0.0139	0.2918
	Length = -0.007·PCB _{lipid} + 9.998	0.0139	0.2919
	Length = -2.1E-7·log TEQ ² + 10	0.0190	0.3342
Tree swallow	Length = -0.19·log PCB + 9.15	0.0079	0.0564
	Length = -0.19·log PCB _{lipid} + 8.99	0.0062	0.0599
	Length = -0.41·log TEQ + 10.15	0.0048	0.0632
<i>Width</i>			
Eastern bluebird	Width = -0.002·PCB ² + 6.866	0.1075	0.0651
Carolina chickadee	Width = -0.08·PCB + 5.26	0.0837	0.1244
	Width = -0.36·PCB _{lipid} + 5.29	0.0620	0.1433
House wren	No correlation was significant or marginally significant.		
Red-winged blackbird	No correlation was significant or marginally significant.		
Tree swallow	Width = 4.13E-4·PCB ² + 6.60	0.0453	0.0324
	Width = 1.10E-7·TEQ ² + 6.59	0.0417	0.0335
<i>Depth</i>			
Eastern bluebird	No correlation was significant or marginally significant.		
Carolina chickadee	Depth = -0.09·PCB + 4.24	0.0765	0.1301
	Depth = -0.40·PCB _{lipid} + 4.27	0.0593	0.1462
House wren	No correlation as significant or marginally significant.		
Red-winged blackbird	No correlation was significant or marginally significant.		
Tree swallow	Depth = -0.01·PCB _{lipid} ² + 5.38	0.0003	0.1001
<i>Deformity index</i>			
Eastern bluebird	Deformity index = 1.81·log PCB + 5.71	<0.0001	0.3873
	Deformity index = 1.97·log PCB _{lipid} + 6.95	<0.0001	0.4174
	Deformity index = 2.10·log TEQ + 1.26	0.0079	0.1803
Carolina chickadee	Deformity index = 1.48·log PCB + 6.23	0.0756	0.1309
	Deformity index = 1.64·log PCB _{lipid} + 7.15	0.0448	0.1637
	Deformity index = 4.06·log TEQ - 2.04	0.0417	0.1682
House wren	Deformity index = 1.18·log PCB + 6.75	0.0476	0.0557
	Deformity index = 1.49·log PCB _{lipid} + 7.24	0.0185	0.0801
Red-winged blackbird	No correlation was significant or marginally significant.		
Tree swallow	Deformity index = 1.98·log PCB + 4.45	<0.0001	0.4691
	Deformity index = 1.78·log PCB _{lipid} + 5.99	<0.0001	0.4338
	Deformity index = 3.64·log TEQ - 4.39	<0.0001	0.4042
<i>Macrosurface roughness</i>			
Eastern bluebird	Macrosurface roughness = 0.69·PCB ² + 0.67	0.0001	0.3240
	Macrosurface roughness = 1.52·PCB _{lipid} ² + 0.62	<0.0001	0.3430
	Macrosurface roughness = 1.11·log TEQ - 0.53	0.0034	0.2151
Carolina chickadee	Macrosurface roughness = 0.71·log PCB + 1.91	0.0448	0.1638
	Macrosurface roughness = 0.75·log PCB _{lipid} + 2.33	0.0329	0.1830
House wren	Macrosurface roughness = 0.47·log PCB + 2.2	0.0504	0.0544
	Macrosurface roughness = 0.54·log PCB _{lipid} + 2.40	0.0318	0.0669
Red-winged blackbird	Macrosurface roughness = 0.06·PCB + 0.65	0.0802	0.1604
	Macrosurface roughness = 0.31·PCB ² + 0.51	0.0910	0.1505
Tree swallow	Macrosurface roughness = 0.97·log PCB + 1.47	<0.0001	0.5719
	Macrosurface roughness = 0.87·log PCB _{lipid} + 2.23	<0.0001	0.5257
	Macrosurface roughness = 1.79·log TEQ - 2.88	<0.0001	0.4986

correlated with a transformation of both PCBs and TEQs, whereas Carolina chickadee only correlated with the total PCB transformation.

Heart morphometrics

Mean heart morphometric data are summarized by species and site in Table 5. Of the heart measurements evaluated, mean ventricular length was significantly changed most frequently across the species (eastern bluebird, Carolina chickadee, and tree swallow). Mean heart width was significantly different (decreased by 9%) for only one species (red-winged blackbird) at a moderately contaminated site (RC) compared to the reference site (GP). Mean heart depth was the most variable measurement of all and was not significantly different between any two sites for any species. Therefore, the remainder of the results presented will emphasize changes in heart length by species.

Mean ventricular length changes by species and site

Mean ventricular length decreased statistically significantly and consistently for both Carolina chickadee and tree swallow at the contaminated sites compared to the reference site (GP). Maximal mean heart length decreased 15% for Carolina chickadee (PR) and 4% for tree swallow (WT) compared to GP. The maximal mean heart length decrease for red-winged blackbirds was also 4% (WT) compared to GP, and this decreased heart length was similarly consistent across both RC and WT but was not statistically significant.

By contrast, mean eastern bluebird heart length increased consistently and, for three sites (RC, CB, and WT), statistically significantly compared to the reference site (GP). The maximal eastern bluebird heart length increase was 15% at a moderately contaminated site (RC). The dose-response curve for eastern bluebird heart length versus contaminant concentration is essentially an inverted U-shape, peaking at the mean site contaminant concentration of 54 ppb of total PCBs and 65 ppt of TEQs. House wren mean heart length also increased somewhat at the more contaminated sites (WT and ICS) compared to the more moderately contaminated site (CB), although these differences (which peak at 3%) were not statistically significant.

Correlations between external heart morphometrics and contaminant

When the heart morphometric measurements were regressed against sibling contaminant values (Table 4), at least one tree swallow correlation was significant for all three measurements (length, width, and depth). Tree swallow ventricular length and width correlated with a transformation (length, log; width, square) of the PCB and TEQ values, whereas depth correlated only with the square of the lipid-corrected total PCB values. Carolina chickadee heart morphometrics regressed significantly (length) or marginally significantly (width and depth) with only the PCB values. Red-winged blackbird length was significantly correlated with both PCB and TEQ values, whereas eastern bluebird heart width was marginally significantly correlated with only the square of the PCB concentration.

Gross heart deformities

External heart deformities that were observed, assessed, and described, as well as the subsequent severity rating indices, are summarized in Table 2. Figure 2 summarizes a subset of these deformities observed at the contaminated sites (pooled)

Table 5. Mean external morphometrics (\pm standard deviation) of hearts from passerines collected from reference (GP) or polychlorinated biphenyl-contaminated sites

Sites	Eastern bluebird (6, 3, 20, 7, NP ^a , 5) ^b			Carolina chickadee (2, NP, 9, 3, 2, 9)			House wren (NP, NP, 8, 4, 59, NS ^c)			Red-winged blackbird (8, 5, NP, 10, NP, NP)			Tree swallow (63, NS, NP, 62, NP, NS)		
	Width	Length	Depth	Width	Length	Depth	Width	Length	Depth	Width	Length	Depth	Width	Length	Depth
GP	7.14 (\pm 0.33)	8.45 (\pm 0.73)	5.5 (\pm 0.26)	5.26 (\pm 0.16)	7.78 (\pm 0.21)	3.96 (\pm 0.27)	—	—	—	7.18 (\pm 0.6)	9.95 (\pm 0.69)	5.66 (\pm 0.28)	6.65 (\pm 0.69)	9.33 (\pm 0.85)	5.35 (\pm 0.62)
RC	7.07 (\pm 0.45)	9.69* (\pm 0.24)	5.82 (\pm 0.24)	—	—	—	—	—	—	6.54** (\pm 0.77)	9.7 (\pm 0.59)	5.21 (\pm 0.56)	—	—	—
CB	6.69 (\pm 0.6)	—	5.2 (\pm 0.48)	5.23 (\pm 0.49)	7.57 (\pm 0.41)	4.39 (\pm 0.32)	6.64 (\pm 0.92)	6.77 (\pm 0.62)	4.21 (\pm 0.6)	—	—	—	—	—	—
WT	6.79 (\pm 0.68)	9.33* (\pm 0.71)	5.4 (\pm 0.27)	5.1 (\pm 0.1)	6.65 (\pm 0.4)	3.97 (\pm 0.23)	4.98 (\pm 0.47)	6.87 (\pm 0.65)	3.78 (\pm 0.41)	7.3 (\pm 0.35)	9.58 (\pm 1)	5.71 (\pm 0.4)	6.69 (\pm 0.89)	8.95* (\pm 0.92)	5.21 (\pm 0.9)
ICS	—	—	—	5.68 (\pm 0.32)	6.98 (\pm 0.74)	4.25 (\pm 0.64)	4.85 (\pm 0.51)	—	—	—	—	—	—	—	—
PR	6.75 (\pm 0.86)	8.88 (\pm 0.9)	5.48 (\pm 0.24)	4.79 (\pm 0.47)	6.63** (\pm 0.79)	3.71 (\pm 0.66)	—	—	—	—	—	—	—	—	—

^a NP = not present at site.

^b Numbers in parentheses indicate number of hearts measured for that species for Goose Pond (GP; Monroe County, IN, USA); Richland Creek (RC; Monroe County, IN, USA); Conard's Branch (CB; Monroe County, IN, USA); Winston-Thomas (WT; Bloomington, IL, USA); Illinois Central Springs (ICS; Bloomington, IL, USA), and Pleasant Run (PR; Bedford, IN, USA).

^c NS = species present at site, but not sampled.

* Statistically significantly ($p \leq 0.05$) different from GP, ** Statistically significantly ($p \leq 0.01$) different from GP.

Heart deformities in passerine birds exposed to PCBs

Environ. Toxicol. Chem. 25, 2006

Table 6. Summary of external heart deformity no observed adverse effect levels (top lines) and/or lowest-observed-adverse-effect levels (bottom lines) in nestlings for each species based on at least two criteria being considered to be severe

Site	Eastern bluebird		Carolina chickadee		House wren		Red-winged blackbird		Tree swallow	
	PCBs ^a	TEQs ^b	PCBs	TEQs	PCBs	TEQs	PCBs	TEQs	PCBs	TEQs
Goose Pond ^c	0.01	62	0.02	44	Not present				0.04	41
Richland Creek ^c	0.05	65	Not present		Not present		0.006	81	0.09	90
Conard's Branch ^c	0.29	67	Not present				0.28	138	Nest predated	
	0.35	14			1.37	274	Not present		Not present	
Winston-Thomas ^d	7.4	569	0.74	223	0.51	15	2.2	278	4.4	
							9.6	1,143		276
Illinois Central Springs ^d	Not present		1.4	271	1.6	84	Not present		Not present	
Pleasant Run ^e	7.7	159	3.9	66	Did not hatch		Not present		Nest predated	

^a PCB = polychlorinated biphenyl (ppm).^b TEQ = toxic equivalency quotient (ppt).^c Monroe County (IN, USA).^d Bloomington (IN, USA).^e Bedford (IN, USA).

and reference site (GP) for each species. The severity cutoff used for these graphs is three and above for all but the indentation deformity, which was only scaled to two. Therefore, the graphs show the percentage of hearts at the reference or contaminated sites that manifest the listed deformities (center rolls, macro- and microsurface roughness, lateral ventricular notches, tip deformities, or shape deformities, but not ventricular indentations) with a severity of three or above. Three tip abnormalities are pooled for these graphs: Flattened tip, rounded tip, and pointed tip. Tip deformities are judged relative to the normal tip shape for that species. For example, normal Carolina chickadee hearts have relatively pointed tips, whereas in comparison, tree swallow hearts have moderately rounded tips. Of the tip anomalies, the most common across the species was the pointed tip.

The shape abnormalities pooled for the graphs included a C-shaped heart, a short and fat stubby heart (distinct from a rounded or flattened tip heart), and a very triangular shape that was distinct from a pointed tip. Of these shape deformities, the most common across most species was the C-shaped heart. Of the birds from the pooled contaminated sites, 8.3% of hearts (17 of 204) were C-shaped. Red-winged blackbird had the highest occurrence of C-shaped hearts (33.3%), followed by Carolina chickadee (13.0%), house wren (10.1%), eastern bluebird (2.9%), and tree swallow (1.6%).

Across all the species, the deformities that seemed to dominate at the contaminated sites were center rolls, macrosurface roughness, ventricular notching, abnormal tips, and C-shaped hearts. These scaled ratings were then summed into a deformity index and correlated against the contaminant values, including transformations of the contaminant values (Table 4). The deformity index regressed significantly against some measure of contamination for all species except the red-winged blackbirds. For three of four species (eastern bluebird, Carolina chickadee, and tree swallow), both PCBs and TEQs correlated significantly with the deformity index. Of all the specific deformities evaluated, only the macrosurface roughness index correlated significantly (eastern bluebird, Carolina chickadee, house wren, and tree swallow) or marginally significantly (red-winged blackbird) with some measure of nestling contamination for all species (Table 4).

Effective doses

The lowest-observed-adverse-effect levels (LOAELs) were determined for all species. For some species, no-observed-

adverse-effect levels (NOAELs) also were determined based on both PCB concentrations and TEQs (Table 6). The LOAELs were based on the lowest dose at which any two heart deformities with a severity rating of three or more were noted in the same nestling heart. Based on these LOAELs and NOAELs, the relative PCB sensitivity of the five passerine species based on heart malformations was red-winged blackbird > eastern bluebird > tree swallow > Carolina chickadee > house wren. The relative TEQ-related sensitivity of these five passerine species was eastern bluebird > house wren > Carolina chickadee > red-winged blackbird > tree swallow. Because our sample sizes were limited for some species, however, and because the actual field-measured contaminant concentrations were generally higher than a laboratory controlled dosing effort that would include more low and moderate dose ranges, these sensitivity comparisons can only be applied generally.

The incidence of external heart deformities was variable within affected nests (Table 7). At three sites (RC, ICS, and PR), every nest had at least one nestling with external heart deformities. At our reference site (GP), six individual nestlings were affected from each of six different nests (out of a total of 39 nests studied). At our three most contaminated sites (WT, PR, and ICS), we had 73 to 80% of all nestling birds with at least two external heart deformities in the severe range.

DISCUSSION

We evaluated PCB-induced cardiac growth and teratogenicity in five passerine species developmentally exposed to PCBs (eastern bluebird, Carolina chickadee, house wren, red-winged blackbird, and tree swallow). We characterized and quantified necropsy-observable cardiac deformities in five species of passerines, and we calculated the statistically significant relationship between several characteristic cardiac deformities and mean total PCBs and mean TEQs. Based on our data, we demonstrate that all five species develop cardiac malformations in response to in ovo exposure to PCBs. We were not able to fully evaluate all of our red-winged blackbird samples, because even though cardiac anomalies were found within all but a few nests, matching chemistries did not always include complete congener analysis or did not have a sibling analyzed. Nevertheless, the red-winged blackbird seemed to express the most anomalous cardiac response pattern. This may be related to the fact that in the reference site, red-winged blackbirds had a ratio of μg TEQ to g PCB of greater than 11,500. Alter-

Table 7. Incidence of external heart deformities in nestlings for each species based on at least two criteria being considered to be severe

Site	Eastern bluebird		Carolina chickadee		House wren		Red-winged blackbird		Tree swallow	
	Nestlings affected	Nests affected	Nestlings affected	Nests affected	Nestlings affected	Nests affected	Nestlings affected	Nests affected	Nestlings affected	Nests affected
Goose Pond ^a	0/6	0/2	0/2	0/1	Not present		2/8	2/6	5/63	5/29
Richland Creek ^a	2/3	1/1	Not present		Not present		2/5	2/2	Nest predated	
Conard's Branch ^a	6/20	5/7	2/11	2/4	2/8	2/3	Not present		Not present	
Winston-Thomas ^b	7/7	2/2	3/3	1/1	3/4	2/2	4/10	2/4	42/62	25/28
Illinois Central Springs ^b	Not present		2/2	1/1	47/59	14/14	Not present		Not present	
Pleasant Run ^c	5/5	3/3	6/9	2/2	Did not hatch		Not present		Nest predated	

^a Monroe County (IN, USA).

^b Bloomington (IN, USA).

^c Bedford (IN, USA).

natively, the natural shape and developmental pattern of red-winged blackbirds may be very different from those of other bird species and need further study.

The key malformations that appear most consistently at the PCB-contaminated sites include a center roll standing out across the ventral surface of both ventricles, a notch in the side of the right ventricle, a curved C-shaped heart, a cobblestone-like macrosurface roughness, and abnormal heart tip formation. A deformity index developed from the relative scaling of these five abnormalities correlated significantly with some measure of PCB contamination (PCBs or TEQs) for all species except the red-winged blackbird. The macrosurface roughness index alone generically correlated either significantly or marginally significantly with PCBs (all species) and TEQs (eastern bluebird and tree swallow).

The heart measurement results demonstrate species differences in response to the PCB-associated perturbations in heart growth and development. Whereas Carolina chickadee, red-winged blackbird, and tree swallow all manifested statistically significant decreases in heart length with increasing PCBs, eastern bluebird hearts were, on average, statistically significantly longer at the more contaminated sites compared to those at the reference site. A second, similar anomaly could be observed in the analyses of heart width. Tree swallow hearts were wider at higher levels of PCBs based on the regression analysis, whereas the eastern bluebird and Carolina chickadee hearts were less wide at higher levels of PCBs (these last two correlations were only marginally significant). Hearts are sensitive to the effects of PCBs and other polyhalogenated aromatic hydrocarbons, notably TCDD. Schmittle et al. [35] and Firestone [36] were the first to report that a toxic feed additive (that turned out to be TCDD [36]) induced adverse effects on the developing heart, causing severe hydropericardium in young chickens. Cheung et al. [6] was the first to document TCDD-induced heart malformations in chicken embryos incubated for 14 d of a 21-d incubation period. Malformations noted included ventricular septal defects, aortic arch anomalies, and conotruncal malformations. The median effective dose in the study by Cheung et al. was approximately 1 pM TCDD per egg (0.54 ng of TCDD per 60-g egg; 9 ppt). Other studies evaluating the developmental effects of TCDD and PCBs on hearts have demonstrated that teratogenic changes are detectable as soon as the heart starts to form during embryonic development. Henshel [4,22] as well as Henshel et al. [23] and Dickerson et al. [37] showed that initial heart development was affected adversely by TCDD and that these delays and early heart malformations were observable at 2 and 10 pg TCDD/g egg (~0.1 and 0.5 ng/egg), the lowest doses

tested in each study. Walker et al. [38] examined hearts from 10-d-old chicken embryos exposed to 1 pM TCDD per egg (0.54 ng of TCDD per 60-g egg; 9 ppt) from embryonic day 0 to embryonic day 10. They found that TCDD-exposed hearts had enlarged ventricles, thickened ventricular septa, and thinner left ventricular walls compared to vehicle controls as well as ventricular septal defects. Walker and Catron [8] exposed two different strains of chickens to various doses of TCDD, PCB 126 (3,4,5,3',4'-pentachlorobiphenyl), and PCB 153 (2,4,5,2',4',5'-hexachlorobiphenyl) from embryonic day 0. After embryonic day 10, evaluation of the hearts revealed that TCDD tended to lengthen the ventricles and to increase the left and right ventricular cavities.

Overall, our findings demonstrate that passerines and chickens have similar TEQ sensitivities (chickens, 2–10 ppt; eastern bluebird and house wren, ~15 ppt) and similar patterns of effects on heart development, although the direction of a measured change (length and weight) may vary. After hatching, as the body tries to compensate for inefficient heart function, the heart somatic index increases. Powell et al. [39] observed an apparently contradictory dose-dependent effect on heart somatic indices in hatchling chickens developmentally exposed to PCB 126. At the low dose (0.2 ppb of PCB 126 or 120 ppt of TEQs), the heart somatic index was statistically significantly lower than in the controls, but at the higher dose (3.2 ppb of PCB 126 or 1,920 ppt of TEQs), the heart somatic index was statistically significantly higher than in the controls. Mean concentrations of PCB 126 alone across all species from the present study sites were comparable to the dosing used by Powell et al. (GP, 0.3 ppb; RC, 0.3 ppb; CB, 0.4 ppb; PR, 0.6 ppb; WT, 0.9 ppb; and ICS, 1.1 ppb). with a few individual nests at WT and ICS exceeding 3 ppb. We also observed inconsistent changes in some of the passerine heart somatic indices, especially for the Carolina chickadee. What was more notable, however, was that some species (house wren and tree swallow) had a more consistently decreased mean heart somatic index at the higher exposure sites, and based on the regression analysis, the heart somatic index for both tree swallow and Carolina chickadee decreased with increasing PCBs. Passerines are altricial, and chickens are precocial. All the passerines studied were pre fledgling when they were killed. Thus, the chickens studied by Powell et al. were already moving around more than the passerines were at the time of analysis. It is probable that the passerines were only beginning the stage during which compensatory heart growth would occur in response to inefficient heart function. When the avian heart begins developing, it starts as a three-layered tube that bends and then loops into a S-shaped structure [40]. In chickens,

Heart deformities in passerine birds exposed to PCBs

Environ. Toxicol. Chem. 25, 2006

looping occurs between the second and third days of incubation shortly after the heart begins beating and occurs as a leftward bulging of the right ventricular wall, which eventually forces the entire structure to rotate to the right [40]. Circulation of blood through the embryo and the vitelline vasculature, which transfers nutrients from the yolk to the embryo, is established by the end of incubation day 2 or the beginning of day 3. The looped tube becomes divided into the major regions (atria, ventricles, septum, and valves) between incubation days 3 and 4. Division of the heart into distinct right and left sides occurs between incubation days 3 and 5 [40]. Passerines, with a shorter incubation period, experience these events on a slightly different scale during incubation but at about the same developmental stages as chickens. Given that PCBs were detected in the egg, we know that the deformities we observed in the heart happened concomitantly with PCB exposure throughout the developmental period of the heart.

All the hearts examined for the present study came from nestlings that were alive when they were collected from their nests. This observation implies that the hearts of the nestlings were sufficiently formed to support the nestlings from hatch to near-fledge. We cannot comment on the sufficiency of the hearts to support the nestlings during or after fledge, but the teratogenic changes we observed were not necessarily lethal to these passerine nestlings. The types of deformities we observed (Table 2) suggest that while the major regions of the heart developed, the process of folding into the distinct regions was disturbed. Notably, center rolls, indentations, ventricular notches, and shape and tip malformations were statistically significantly increased in several of the species at the sites with the highest PCB contamination. These deformities would seem to develop from anomalous patterning during the process of folding and shaping the heart.

In summary, the present data provide some key take-home lessons for environmental assessment. Heart formation clearly is very sensitive to PCBs across multiple avian species and is an appropriate endpoint to incorporate into ecotoxicological assessments. A baseline understanding of the characteristic species response, however, should be developed for each species before developmental heart anomaly assessment is incorporated into a suite of PCB-related effects indicators. For the red-winged blackbird, for example, one effect focus probably would be macrosurface roughness, whereas in eastern bluebirds or tree swallows, one also would expect a high incidence of center rolls and ventricular notching. Finally, PCB-related changes in the pattern of heart growth do vary between species, but the most commonly affected heart measurement is length.

Acknowledgement—The present work was supported by the U.S. Fish and Wildlife Service Environmental Contaminants Program and the Department of the Interior Natural Resource Damage Assessment and Restoration Program.

REFERENCES

- Johnson BL, Hicks HE, Cibulas W, Faroon O, Ashizawa AE, DeRosa CT, Cogliano J, Clark M. 2000. Public health implications of exposure to polychlorinated biphenyls (PCBs). Agency for Toxic Substances and Disease Registry, Division of Toxicology, Atlanta, GA, USA.
- Giesy JP, Kurunthachalam K. 2002. Dioxin-like and nondioxin-like effects of polychlorinated biphenyls: Implications for risk assessment. *Lakes Reserv Res Manag* 7:137–181.
- Goff KE, Hull BE, Grasmann KA. 2005. Effects of PCB 126 on primary immune organs and thymocyte apoptosis in chicken embryos. *J Toxicol Environ Health Part A* 68:485–500.
- Hoffman DJ, Rice CP, Kubiak TJ. 1996. PCBs and dioxins in birds. In Beyer WN, Heinz GH, Redmon-Norwood AW, eds, *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. Lewis, Boca Raton, FL, USA, pp 165–207.
- Henshel DS. 1998. Developmental neurotoxic effects of dioxins and dioxin-like compounds on domestic and wild avian species. *Environ Toxicol Chem* 17:88–98.
- Cheung MO, Gilbert EF, Peterson RE. 1981. Cardiovascular teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the chick embryo. *Toxicol Appl Pharmacol* 61:197–204.
- Henshel DS, Hehn B, Vo MT, Steeves JD. 1993. A short-term test for dioxin teratogenicity using chicken embryos. In Gorsuch J, Dwyer J, Ingersoll C, LaPoint T, eds, *Environmental Toxicology and Risk Assessment*, Vol 2. STP 1216. American Society for Testing and Materials, Philadelphia, PA, pp 159–174.
- Walker MK, Catron TF. 2000. Characterization of cardiotoxicity induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related chemicals during early chick embryo development. *Toxicol Appl Pharmacol* 167:210–221.
- Huff J. 1992. 2,3,7,8-TCDD: A potent and complete carcinogen in experimental animals. *Chemosphere* 25:173–176.
- Seegal RF, Schantz SL. 1994. Neurochemical and behavioral sequelae of exposure to dioxins and PCBs. In Schechter A, ed, *Dioxins and Health*. Plenum, New York, NY, USA, pp 409–447.
- Fischer LJ, Seegal RF, Ganey PE, Pessah IN, Kodavanti PR. 1998. Symposium overview: Toxicity of non-coplanar PCBs. *Toxicol Sci* 41:49–61.
- McNabb A, Fox G. 2003. Avian thyroid development in chemically contaminated environments: Is there evidence of alterations in thyroid function and development? *Evolution and Development* 5:76–82.
- Martinovic B, Lean D, Bishop CA, Birmingham E, Secord A, Jock K. 2003. Health of tree swallow (*Tachycineta bicolor*) nestlings exposed to chlorinated hydrocarbons in the St. Lawrence River basin. Part II: Basal and stress plasma corticosterone concentrations. *J Toxicol Environ Health Part A* 66:2015–2029.
- Martinovic BM, Lean DRS, Bishop CA, Birmingham E, Secord A, Jock K. 2003. Health of tree swallow (*Tachycineta bicolor*) nestlings exposed to chlorinated hydrocarbons in the St. Lawrence River basin. Part I: Renal and hepatic vitamin A concentrations. *J Toxicol Environ Health Part A* 66:1053–1072.
- Henshel DS, Sobiech SA, Sparks DW, Mayer CAA, Melancon M, Fox C, Lam Y, Benson K. 1997. PCB effects on passerine productivity reproductive success, growth and development: A multi-species comparison. *Proceedings, SETAC 18th Annual Meeting*, San Francisco, CA, USA, November 16–20, p 150.
- McCarty JP, Secord AL. 1999. Reproductive ecology of tree swallows (*Tachycineta bicolor*) with high levels of polychlorinated biphenyl contamination. *Environ Toxicol Chem* 18:1433–1439.
- McCarty JP, Secord AL. 1999. Nest-building behavior in PCB-contaminated tree swallows. *Auk* 116:55–63.
- McCarty JP, Secord AL. 2000. Possible effects of PCB contamination on female plumage color and reproductive success in Hudson River tree swallows. *Auk* 117:987–995.
- Porterfield SP. 1994. Vulnerability of the developing brain to thyroid abnormalities: Environmental insults to the thyroid system. *Environ Health Perspect* 102:125–130.
- Indiana State Department of Health, Environmental Epidemiology Section. 1994. Final report preliminary data evaluation and pathway analyses report for consent decree PCB Sites. Indiana Department of Environmental Management, Indianapolis, IN, USA.
- Conestoga-Rovers and Associates. 2001. Current Conditions Report. GM Powertrain Bedford Facility. Prepared for General Motors/Encore, Bedford, IN, USA.
- Henshel DS. 1994. Early embryonic teratogenic changes induced by in ovo exposure to TCDD. *The Toxicologist* 14:383.
- Henshel DS, Sparks DW, Mayer CAA, Benson K, Fox C, Lam Y, Sobiech SA, Wagey R. 1997. Preliminary results using the early embryo teratogenesis assay: A comparison of early embryo abnormalities with late embryo and hatchling teratogenic changes. In Dwyer FJ, Doane TR, Hinman ML, eds, *Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment*, Vol 6. STP 1317. American Society for Testing and Materials, Philadelphia, PA, pp 391–401.
- Henshel DS, Hehn B, Wagey R, Vo M, Steeves JD. 1997. The relative sensitivity of chicken embryos to yolk or air cell-injected 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Toxicol Chem* 16: 725–732.

25. Rifkind AB, Hattori Y, Levi R, Hughes MJ, Quilley C, Alonso DR. 1984. The chick embryo as a model for PCB and dioxin toxicity: Evidence of cardiotoxicity and increased prostaglandin synthesis. In Poland A, Kimbrough RD, eds, *The CSH Banbury Report: Biological Mechanisms of Dioxin Action*. Cold Spring Harbor Laboratory, Plainview, NY, USA, pp 255–266.
26. Brewster DW, Matsumura F, Akera T. 1987. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on guinea pig heart muscle. *Toxicol Appl Pharmacol* 89:408–417.
27. Kelling CK, Menahan LA, Peterson RE. 1987. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treatment on mechanical function of the rat heart. *Toxicol Appl Pharmacol* 91:497–501.
28. Canga L, Levi R, Rifkind AB. 1988. Heart as a target organ in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity: Decreased β -adrenergic responsiveness and evidence of increased intracellular calcium. *Proc Natl Acad Sci U S A* 85:905–909.
29. Henshel DS, Sparks DW. 2005. Site specific PCB-correlated interspecies differences in organ somatic indices. *Ecotoxicology* (in press).
30. MacLeod WD, Brown DW, Friedman AJ, Burrow DG, Mayes O, Pearce RW, Wigren CA, Bogar RG. 1985. Standard analytical procedures of the NOAA national analytical facility, 1985–1986: Extractable toxic organic compounds, 2nd ed. NOAA/NMFS/NOAA Technical Memorandum. NMFS F/NWRC-92. National Oceanic and Atmospheric Administration, Silver Spring, MD, USA.
31. Brooks JM, Wade TL, Atlas EL, Kennicutt MC II, Presley BJ, Fay RR, Powell EN, Wolff G. 1989. Analysis of bivalves and sediments for organic chemicals and trace elements. Third Annual Report. NOAA 50-DGNC-5-00262. National Oceanic and Atmospheric Administration, National Status and Trends Program, Silver Spring, MD, USA.
32. Wade TL, Atlas EL, Brooks JM, Kennicutt II MC, Fox RG, Sericano J, Garcia B, DeFreitas D. 1988. NOAA Gulf of Mexico status and trends program: Trace organic contaminant distribution in sediments and oyster. *Estuaries* 11:171–179.
33. Tondeur Y, Beckert WF. 1991. Determination of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in various environmental matrices by high-resolution gas chromatography–high-resolution mass spectrometry. *IARC Sci Publ* 108:211–249.
34. Van den Berg M, Birnbaum L, Bosveld ATC, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Wærn F, Zacharewski T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, and PCDFs for humans and wildlife. *Environ Health Perspect* 106:775–792.
35. Schmittle SC, Edwards HM, Morris D. 1958. A disorder of chickens probably due to a toxic feed—Preliminary report. *J Am Vet Med Assoc* 132:216–219.
36. Firestone D. 1973. Etiology of chick edema disease. *Environ Health Perspect* 5:59–66.
37. Dickerson RL, Hoover JA, Peden-Adams MM, Mashburn WE, Allen CA, Henshel DS. 1996. Alterations in chicken embryonic development as a sensitive indicator of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin exposure. In Bengtson DA, Henshel DS, eds, *Environmental Toxicology and Risk Assessment: Biomarkers and Risk Assessment*, Vol 5. STP 1306. American Society for Testing and Materials, Philadelphia, PA, pp 204–218.
38. Walker MK, Pollenz RS, Smith SM. 1997. Expression of the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator during chick cardiogenesis is consistent with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced heart defects. *Toxicol Appl Pharmacol* 143:407–419.
39. Powell DC, Aulerich RJ, Meadows JC, Tillitt DW, Giesy JP, Stomborg KL, Bursian SJ. 1996. Effects of 3,3',4,4',5-pentachlorobiphenyl (PCB-126) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) injected into the yolks of chicken (*Gallus domesticus*) eggs prior to incubation. *Arch Environ Contam Toxicol* 31:404–409.
40. Bellairs R, Osmond M. 1998. *The Atlas of Chick Development*. Academic, San Diego, CA, USA.