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**Effects of Polychlorinated Biphenyls (PCBs) on
Reproduction, Physiological Processes, and Biomarkers
in Tree Swallows (*Tachycineta bicolor*)**

by

Amy Lynn Yorks

Dissertation submitted to the Faculty of the Graduate School
of the University of Maryland in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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ABSTRACT

Title of Dissertation: Effects of Polychlorinated Biphenyls (PCBs) on
Reproduction, Physiological Processes, and Biomarkers in
Tree Swallows (*Tachycineta bicolor*)

Amy Lynn Yorks, Doctor of Philosophy, 1999

Dissertation directed by: Katherine S. Squibb, Assistant Director, Toxicology
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Tree swallow (*Tachycineta bicolor*) nestlings were studied at ten sites exhibiting a range of polychlorinated biphenyl (PCB) contamination. Nesting success, offspring survival, and growth and developmental parameters were recorded. In addition to determining PCB concentrations in eggs, twelve-day-old nestlings, and food items, hepatic cytochromes P450-associated monooxygenase activities (BROD and EROD) were quantified as a biomarker of exposure. Eggs and nestlings from one site were dosed with model cytochromes P450 inducers, β -naphthoflavone (BNF), 3-methylcholanthrene, PCB 126, and various Aroclors. A subset of microsomal samples was examined by protein immunoblotting. Heart and skin samples from nestlings were examined immunohistochemically to determine relative amounts and cellular location of CYP1A. Effects on gender and gonadal development of nestling offspring were assessed. A range of PCB contamination was seen in composite sediment (0.02-16ppm) and egg (0.7-

9.7ppm) samples from the field sites. While there were only subtle differences between reproductive and growth parameters from nestlings at various sites, body burdens of PCBs were significantly different and correlated strongly with PCB levels in sediment, eggs, and food sources. PCBs were deposited in Tree swallow eggs and accumulated in nestlings. There was no evidence of abnormal gonadal development or anatomical gender alteration due to PCB exposure in nestling Tree swallows. Although BNF induced cytochromes P450 as assessed by enzyme activity and protein immunoblotting, PCBs were ineffective inducers except in the case of pipped embryos which were treated *in ovo* with PCB 126 and exhibited dose-related enzyme induction. BROD and EROD activities correlated significantly with each other ($r > 0.8$). Differences between sites and treatments show that there is a cytochrome P450 biomarker response in Tree swallows which is indicative of contaminant exposure and effects. Tree swallows appear to be resistant to the reproductive and physiological effects of PCBs and cytochrome P450 induction at field sites may be due to parallel contaminants. Due to their resilience to PCB contamination, Tree swallows did not prove to be a good sentinel species. However, Tree swallows are good indicators of PCB exposure through chemical analyses of tissues.

TABLE II. Site names, abbreviations, locations, and levels of contamination

Site	Location	Level of PCB Contamination
Fort Mifflin, FM	Near Philadelphia International Airport, at the confluence of the Schuylkill and Delaware Rivers, PA 39°55'N; 75°10'W	U.S. Army Corps of Engineers dredge spoil site
John Heinz National Wildlife Refuge, JH	Along Darby Creek, near Philadelphia International Airport, PA 39°50'N; 75°15'W	PCBs detected in fish (memo from Tom Walker, assistant refuge manager)
Valley Forge National Historical Park, VF	Along Valley Creek, west of Norristown, Montgomery and Chester Counties, PA 40°6'N; 75°27'W	Downstream of a Superfund site historically contaminated with PCBs
Texas Eastern Transmission Corporation - Bechtelsville natural gas pumping station, BCH	Bechtelsville, Berks County, PA 40°15'N; 75°40'W	Underwent PCB remediation in 1995
Texas Eastern Transmission Corporation - Bernville natural gas pumping station, BRN	northwest of Reading in Berks County, PA 40°20'N; 76°00'W	Underwent PCB remediation in 1996
Blue Marsh Lake, BML	northwest of Reading in Berks County, PA 40°20'N; 76°00'W	U.S. Army Corps of Engineers recreational area, Control site in Pennsylvania
Patuxent Wildlife Research Center, PWRC	Prince George's County, MD 39°10'N; 76°50'W	Control site in Maryland; Manual dosing of nestlings

Goose Pond, GP	Monroe County, IN 39°07'N; 86°24'W	Control site in Indiana
Winston-Thomas sewage treatment plant, WT	Near Bloomington, Monroe County, IN 30°07'N; 86°32'W	Superfund site highly contaminated with PCBs
Special Area 13, upper Hudson River, HR	Near Fort Edward, Saratoga County, NY 43°15'N; 73°35'W	River and sediment highly contaminated with PCBs from two General Electric plants; Capped deposit of contaminated dredged River sediment

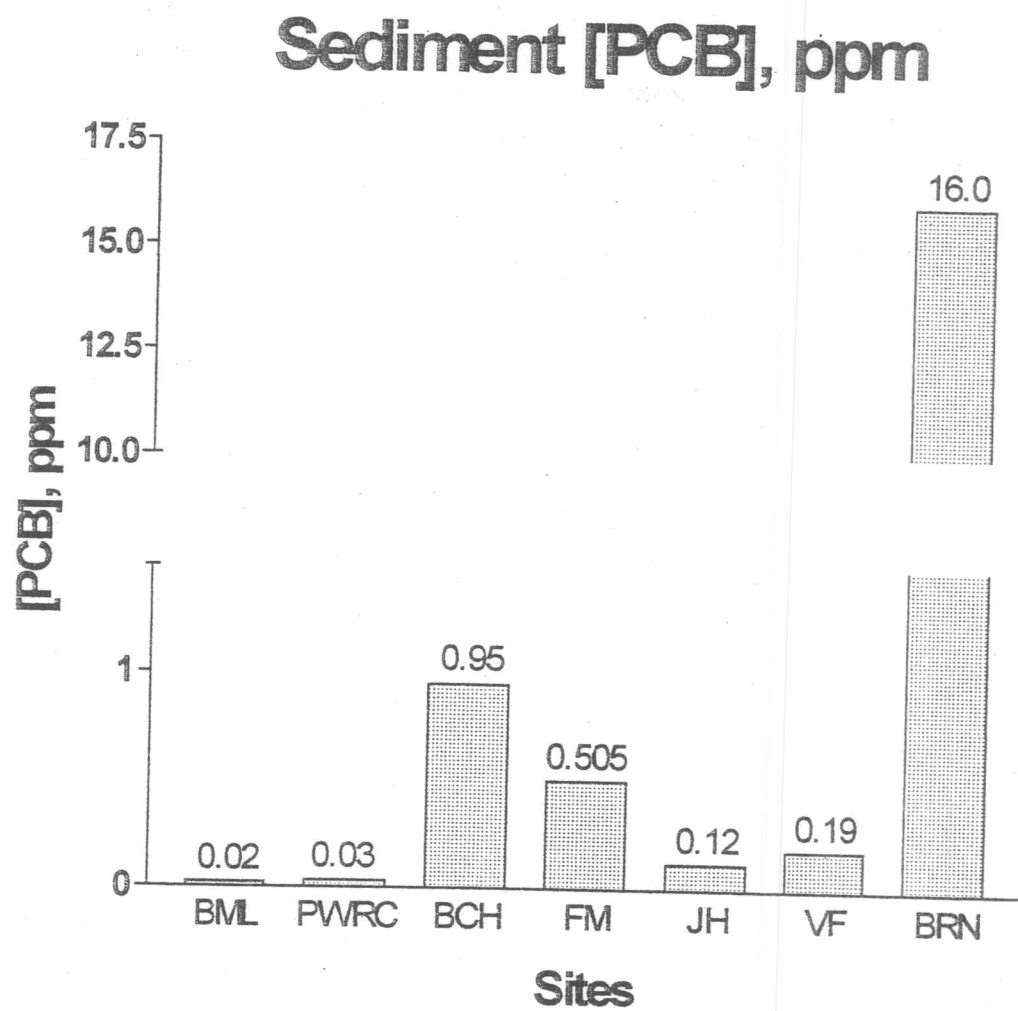


FIGURE 5. Total PCBs in pooled sediment samples collected at sites in 1995.

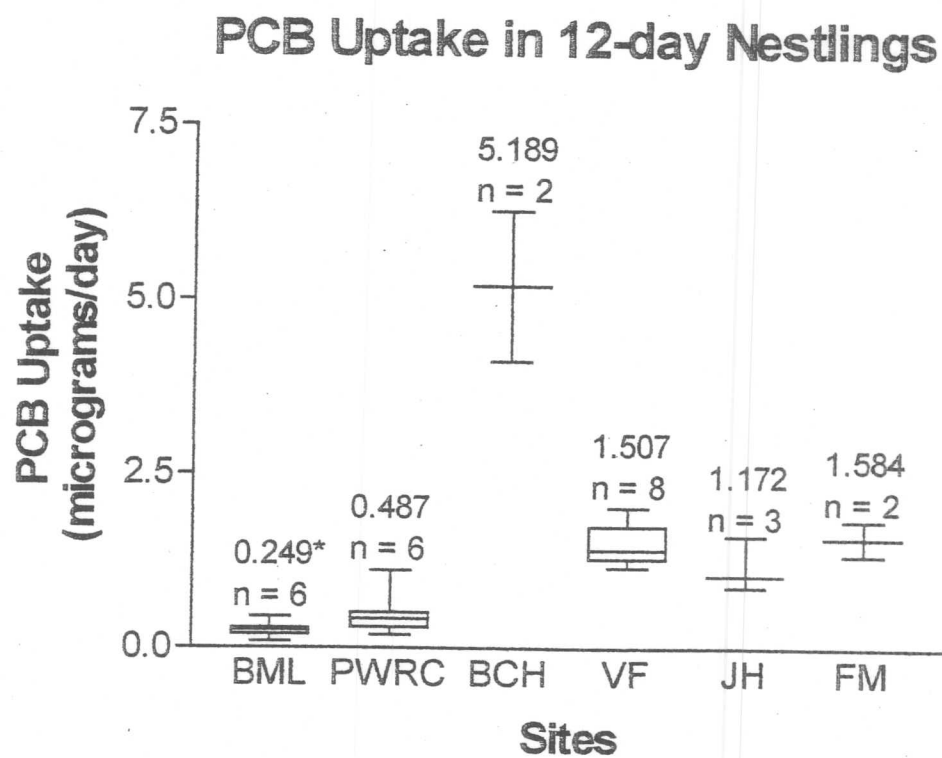
TABLE IX. PCB concentrations in eggs from field sites.

Site	Year Collected	Individual [PCB], ppm	Mean [PCB], ppm Std. Dev. n
BML	1995	1.3, 0.41, 1.6, 1.3, 0.5, 0.58	0.9483 0.5096 6
PWRC	1995	0.37, 0.96, 0.47, 0.7, 0.68, 0.99	0.6950 0.2505 6
BCH	1995 1996	1.2, 6, 0.8, 2.4	2.6000 2.3664 4
BRN	1995	9.7	n = 1
VF	1995 1996	1.4, 1.7, 2.3, 2, 2.2, 1.8, 2.4, 1.3	1.8875 0.4086 8
JH*	1995	2.3, 3.2, 4.4, 2.5, 1.9, 2.9	2.8667 0.8779 6
FM**	1995	4.8, 2.7, 5.5, 6.9, 2.5, 5.2	4.6000 1.7041 6

*Significantly higher than PWRC ($P < 0.05$)**Significantly higher than BML ($P < 0.001$), PWRC ($P < 0.001$), and VF ($P < 0.01$)

TABLE X. Body burdens at each site for all sampling years.

Site	Year Collected	Individual [PCB], ppm	Mean [PCB], ppm Std. Dev. n
BML	1995	0.18, 0.22, 0.08, 0.33, 0.12, 0.20, 0.10, 0.18	0.169
	1996	0.18, 0.15, 0.12, 0.22, 0.12	0.066 13
PWRC	1995	0.68, 0.12, 0.16, 0.14, 0.33, 0.33, 1.20, 0.07, 0.23, 0.55, 0.40, 0.30, 0.15, 0.09, 0.67	0.294
	1996	0.26, 0.27, 0.27, 0.18, 0.00, 0.00, 0.29, 0.18	0.264
	1997	0.44, 0.04	25
BCH	1995	2.30, 3.30, 0.95	2.183
			1.179 3
VF	1995	0.70, 0.87, 1.30, 0.79	0.911
	1996	0.74, 1.00, 0.77, 0.84, 1.00, 1.20, 0.81	0.194 11
JH	1995	1.50, 0.78, 0.66, 0.93, 0.90, 0.84, 0.39, 1.40, 0.67, 0.99, 0.77, 0.38, 0.68	0.743
	1996	0.56, 0.54, 0.54, 0.54, 0.51, 0.66, 0.63, 0.68, 0.63, 0.46, 1.20, 0.71, 0.69, 0.49, 0.70, 0.60, 1.40, 0.61	0.288 31
FM	1995	1.0, 1.1, 1.7, 1.4, 0.8, 0.8, 1.1, 0.7, 1.2, 0.9, 1.0, 1.1, 1.1, 1.7, 1.2, 1.4, 1.7, 1.9, 2.0, 0.7	1.446
	1996	1.10, 2.00, 1.70, 1.50, 1.90, 1.60, 1.50, 1.50, 1.20, 2.00, 1.80, 2.40, 1.30, 1.60, 2.20, 1.90, 1.80	0.442 37
GP	1996	0.09, 0.1, 0.05, 0.05, 0.18, 0.1, 0.1, 0.04, 0.13, 0.11, 0.07, 0.11, 0.21, 0.07, 0.06, 0.07, 0.42, 0.09, 0.05, 0.33, 0.31	0.1305 0.1037 21
WT	1996	24, 14, 4.6, 4.4, 23, 11, 9.8, 27, 16, 24, 11, 6.4, 25, 31, 40, 27, 25, 9.1	18.46 10.20 18
HR	1997	6.8, 17, 10, 22, 9.6, 12, 14, 15, 20, 8.5, 11	13.27 4.832 11



*Average PCB uptake per day at site

FIGURE 12. Box and whisker column graph of the average PCB uptake per day in Tree swallow nestlings at each site. Whiskers show the range of data.

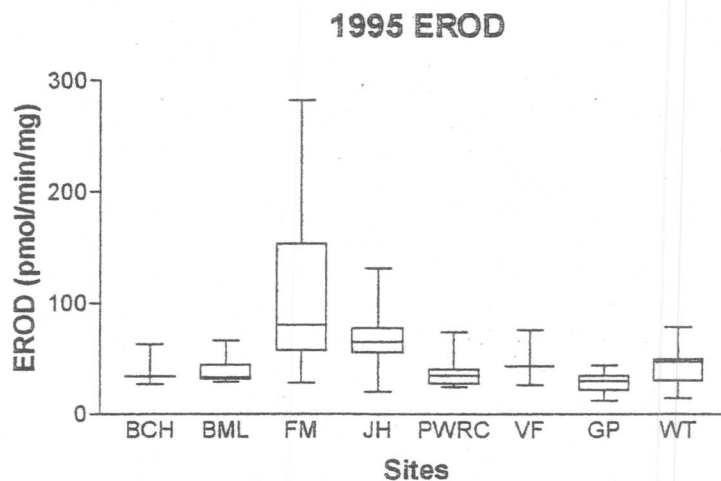


FIGURE 17. EROD activity for environmentally exposed nestlings collected in 1995.

Horizontal lines within the box which extends from the 25th to the 75th percentile represent the medians for each site. Whiskers show the range of data. FM EROD values were significantly larger than BML and WT ($P < 0.01$), PWRC and GP ($P < 0.001$). JH EROD values were significantly larger than GP ($P < 0.01$).

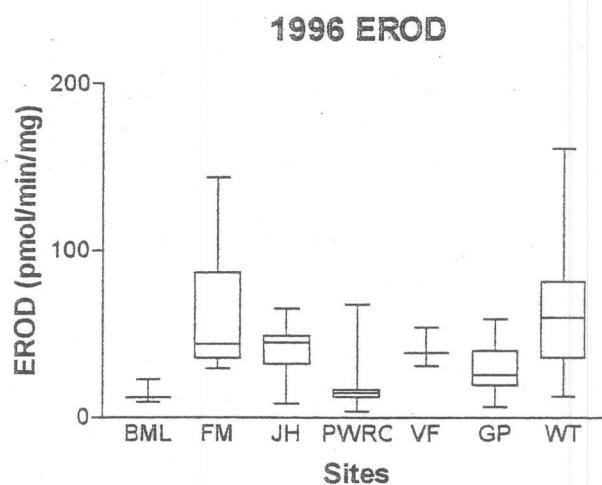


FIGURE 19. EROD activity for environmentally exposed nestlings collected in 1996.

Horizontal lines within the boxes which extend from the 25th to the 75th percentile are the medians for each site. Whiskers show the range of data. FM and WT EROD values were significantly greater than BML, PWRC, and GP ($P < 0.001$). JH EROD values were significantly greater than BML ($P < 0.05$) and PWRC ($P < 0.01$).

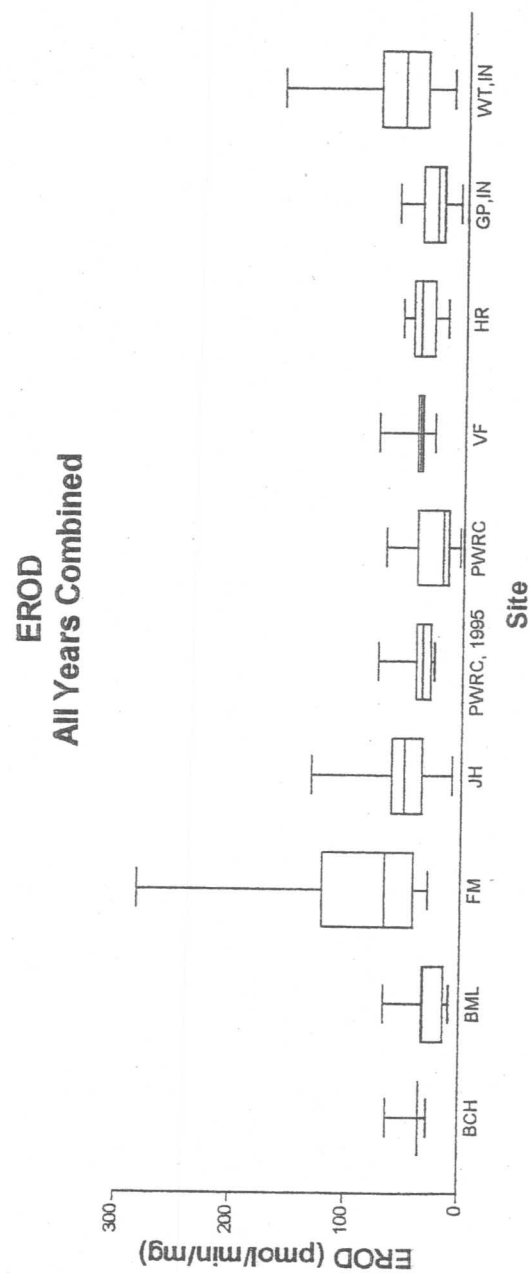


FIGURE 21. EROD data pooled over years. Data at FM was not pooled because of significant differences between years ($P < 0.001$). Whiskers show the range of data.

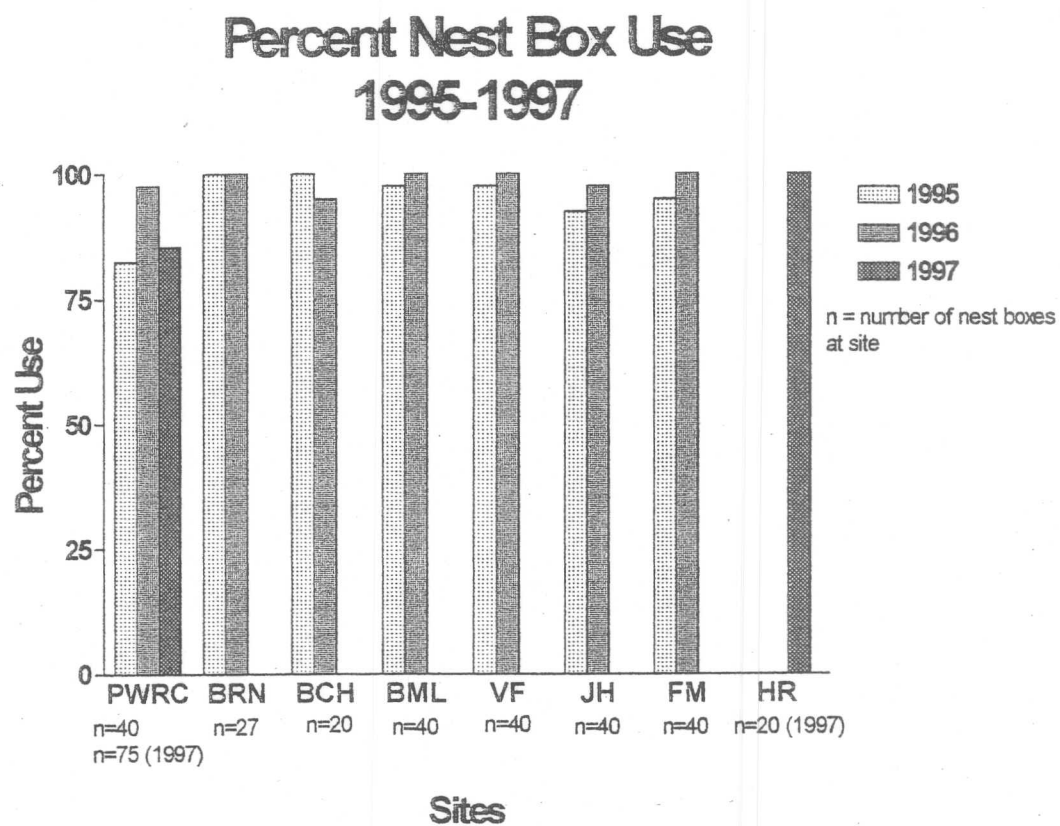


FIGURE 33. Percent of nest boxes used at each site during three breeding seasons.

TABLE XV. Average clutch sizes at study sites for 1995-1997 breeding seasons.

		PWRC	BML	FM	JH	VF	BCH	BRN	HR
1995	Mean	5.47*	4.88	4.52*	5.05	4.40	4.67	3.00	N/S
	SD	0.94	0.64	0.85	0.83	0.55	1.53	0	
	n	17	8	31	20	5	3	1	
1996	Mean	5.22	5.00	4.73	4.96	5.50	5.00	No	N/S
	SD	0.65	1.00	0.60	0.85	0.84	1.41	Nests	
	n	18	9	26	27	6	2		
1997	Mean	5.77	N/S	N/S	N/S	N/S	N/S	N/S	5.61
	SD	0.67							0.78
	n	31							18

N/S-Not sampled that season

*Significantly different ($P < 0.01$)

Yearly Average Clutch Sizes 1995-1997

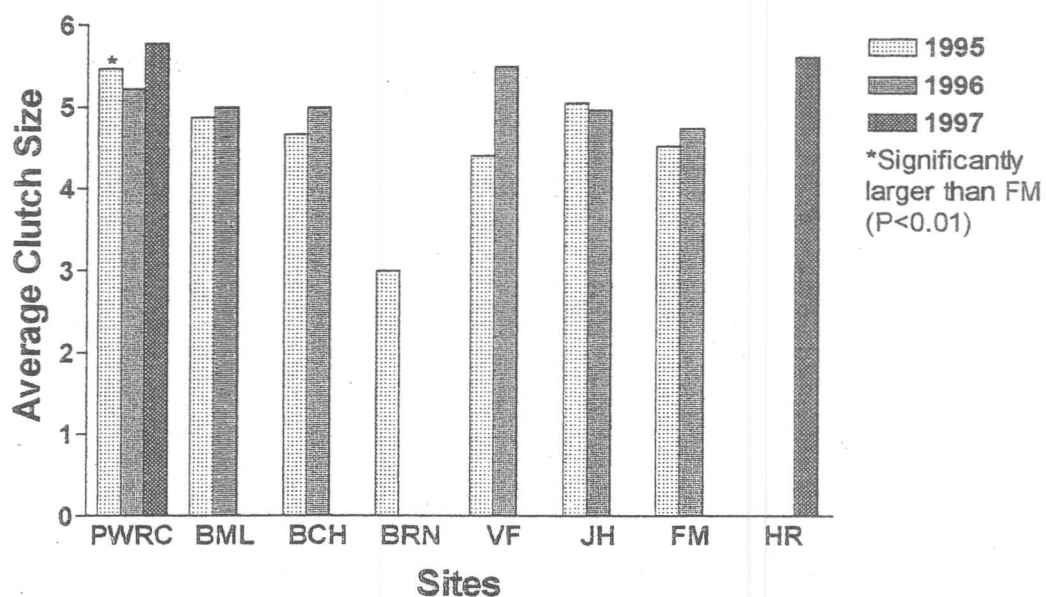


FIGURE 34. Average clutch sizes at study sites for 1995-1997 breeding seasons. In 1995, FM had a significantly smaller average clutch size than PWRC.

TABLE XVI. Predation or abandonment of eggs and nestlings during the 1995-1997 breeding seasons (number of nests depredated/total number of nesting attempts).

Year	BCH	BML	BRN	FM	JH	VF
1995	0/3	0/8	1/1	5/34	22/36	1/5
1996	2/2	7/12	0/0	39/56	14/35	2/9

Eggs Hatched per Clutch (1995-1997)

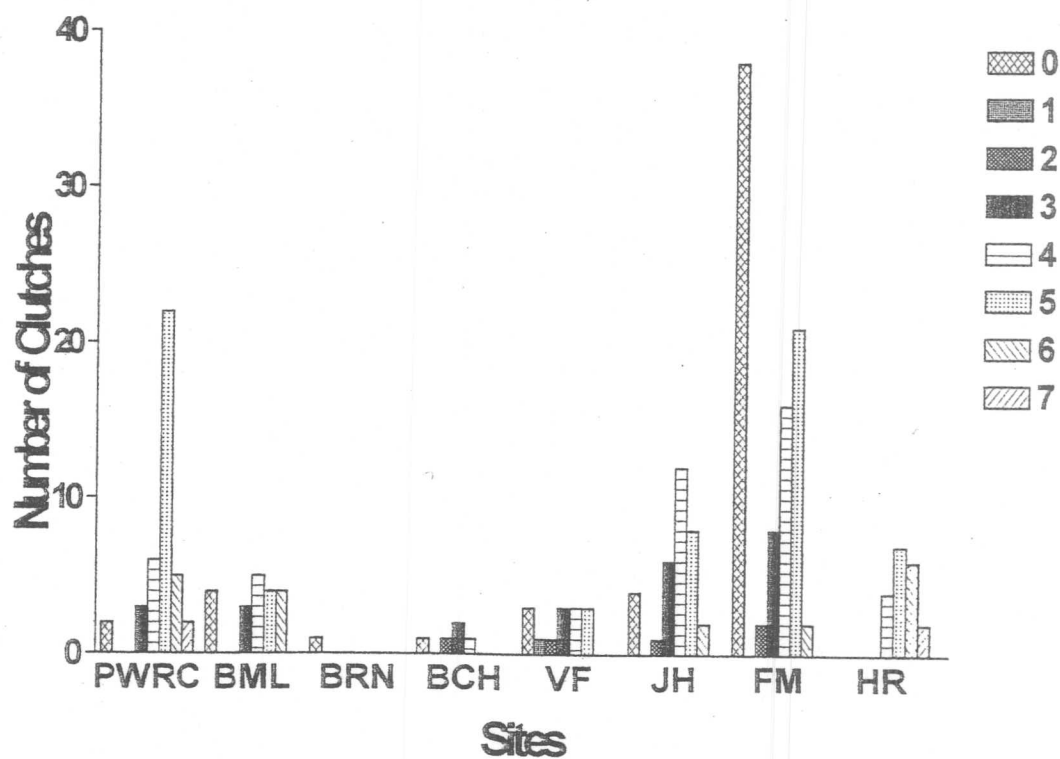


FIGURE 35. Number of eggs which hatched per clutch. Data from HR are from 1997 only; data from all other sites are from 1995 and 1996.

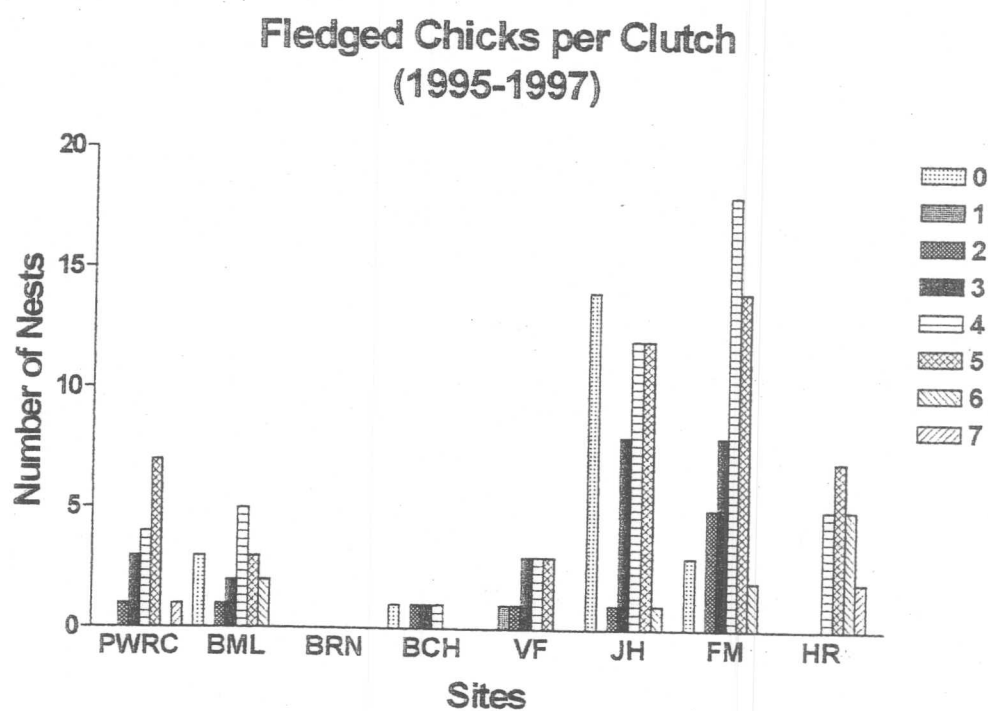


FIGURE 36. Number of eggs which fledged per clutch. Data from HR are from 1997 only; data from all other sites are from 1995 and 1996.

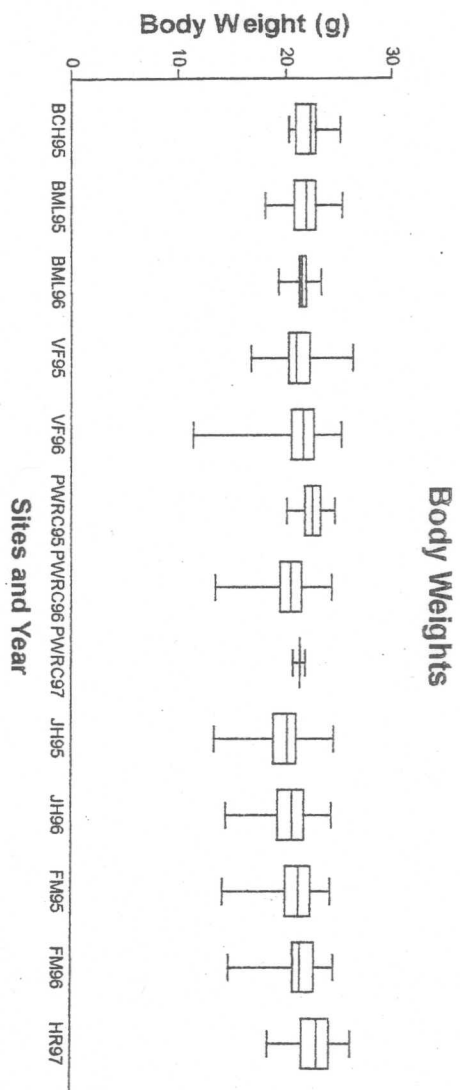


FIGURE 37. Box and whiskers plot of the average body weights at each site per year. The box extends from the 25th -75th percentile with a line at the median. The whiskers show the range of data.

Percent of Total Number of Insects which are Aquatic 1996 Samples

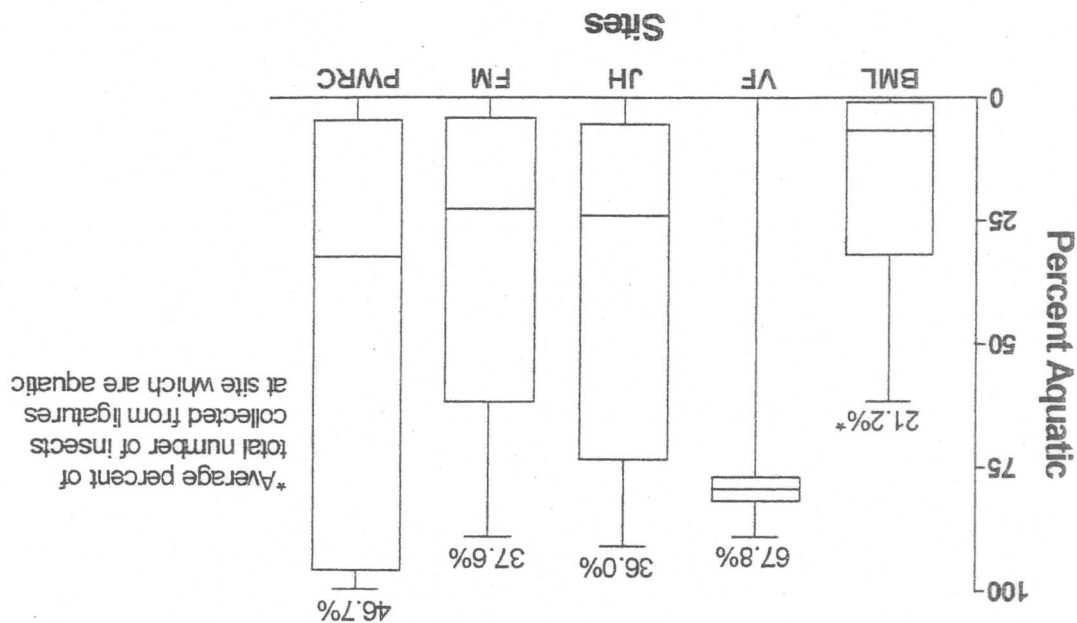


FIGURE 45. Aquatic insects from 1996 samples. The box extends from the 25th to the 75th percentile with a horizontal line at the median. Whiskers show the range of data.

VII. DISCUSSION

A. PCB Residue Analyses

There were significant differences in PCB contamination among study sites as evidenced by sediment levels ranging from 0.02 and 0.03ppm at reference sites to 16.0ppm at contaminated sites. All of the PCBs in environmentally exposed eggs are derived directly from the parents and represent the PCBs initially deposited into the egg. The significant correlation between the levels of PCBs in sediment and eggs ($r = 0.918$) indicates that there is a relationship between the level of contamination in the local environment and the amount of contaminant the female transferred to her offspring. This relationship continued throughout the maturity of the nestlings, manifested as strong correlations of both sediment and egg residues with carcass PCBs ($r > 0.96$ and $r = 0.650$, respectively).

For environmentally exposed nestlings, the likely routes of exposure are direct maternal deposition of PCBs into the egg, "inherited" residues, and the consumption by nestlings of contaminated food materials, often aquatic insects. Ligature samples contained 20 to 70% insects which lived at least some portion of their life cycle in an

aquatic environment. Nestlings consuming contaminated insects increased their PCB body burdens as the residues in the food materials were bioaccumulated. Food materials, analyzed for total PCBs as pooled ligature samples, correlated strongly with sediment and carcass PCB levels ($r > 0.96$). This link of Tree swallows to waterways via the food web supports previous observations that insects play an important role in the transfer of PCB contamination from abiotic to biotic media and between trophic levels (Ankley *et al.*, 1993; Secord and McCarty, 1997).

Tree swallow nestlings collected in Pennsylvania and Maryland contained lower PCB concentrations than corresponding eggs collected from the same nest. The trend follows the typical dynamics of organochlorine pollutants in pre-fledged nestlings relative to eggs (Anderson and Hickey, 1976). The observation is attributed to growth dilution of the PCBs in nestlings in which the body mass of growing nestlings increases more rapidly than the nestlings' net dietary accumulation of PCBs. Other studies evaluating PCBs in Tree swallows have also shown nestling residues to be lower than egg residues (Ankley *et al.*, 1993; Bishop *et al.*, 1995; Nichols *et al.*, 1995). However, one Canadian study revealed sites where Tree swallow nestlings possessed higher residues than eggs collected from the same nest (Shaw, 1984). Higher nestling PCB concentrations were also reported in samples collected in 1994 at two sites along the Hudson River (Secord and McCarty, 1997). These findings suggest, while growth dilution is occurring, Tree swallow nestlings are accumulating PCBs from the breeding territory. The high PCB contamination at the Canadian and Hudson River sites resulted in greater PCB accumulation which counteracted the growth dilution (Table XXXV, Fig.

One of the sites sampled in 1994 along the Hudson River where PCB accumulation masked growth dilution, SA13, was called HR for the purposes of this study and was sampled (nestlings only) in 1997. Carcass concentrations of the 1997 nestlings were approximately 25% of those reported for the 1994 nestlings collected at the same site. The levels were also more than three-fold less than the eggs sampled at SA13 (HR) in 1994 (Table XXXV). This difference in residue concentrations may be attributed to sample collection technique. In 1994, entire Hudson River nestling carcasses were chemically analyzed. In 1997, HR carcasses minus the livers, which were required for biomarker assessment, were submitted for analysis (Lincer and Peakall, 1973). It should be noted that removal of heads (1995 and 1996) and livers (1995, 1996, and 1997) at all of the other sites in this study may partially influence the carcass body burdens with respect to corresponding egg residues.

Stomach contents did not show the same trends and relationships in PCB concentrations as did other media which were more internally consistent. Since there was no leftover sample, the inconsistent data are not reproducible. The reported results may be due to contaminated samples or rapid absorption in the gastrointestinal tract which affected the concentrations.

Nestlings accumulated PCBs at all collection sites. Even at sites which were references, with no history of contamination, nestlings accumulated detectable concentrations of PCBs, albeit at levels lower than 0.5 µg/day. This suggests that nestlings accumulated PCBs from at least one source independent of maternal

deposition. Results show PCBs are prevalent and persistent in our environment and must be reckoned with at all levels of taxa.

Nestlings dosed on day 9 in 1995 and 1996 retained the dose administered.

However, eggs which were dosed in 1997 and sacrificed as piped embryos and nestlings did not show elevated PCB levels indicative of treatments, especially those from the PCB 1260 dose groups. Pipped embryos likely metabolized some PCBs, but did not clear their bodies as efficiently as the older nestlings, as evidenced by generally higher body

burdens. Walker *et al.* (1987) reports that monooxygenase activity may be related to age since there is evidence for an increase in enzyme activity during the development of

young animals. They showed lower monooxygenase activities in juvenile kestrels (*Falco tinnunculus*) and sparrowhawks (*Accipiter nisus*) when compared to levels in adult birds. Tree swallow nestlings which came from dosed eggs most likely did not exhibit body

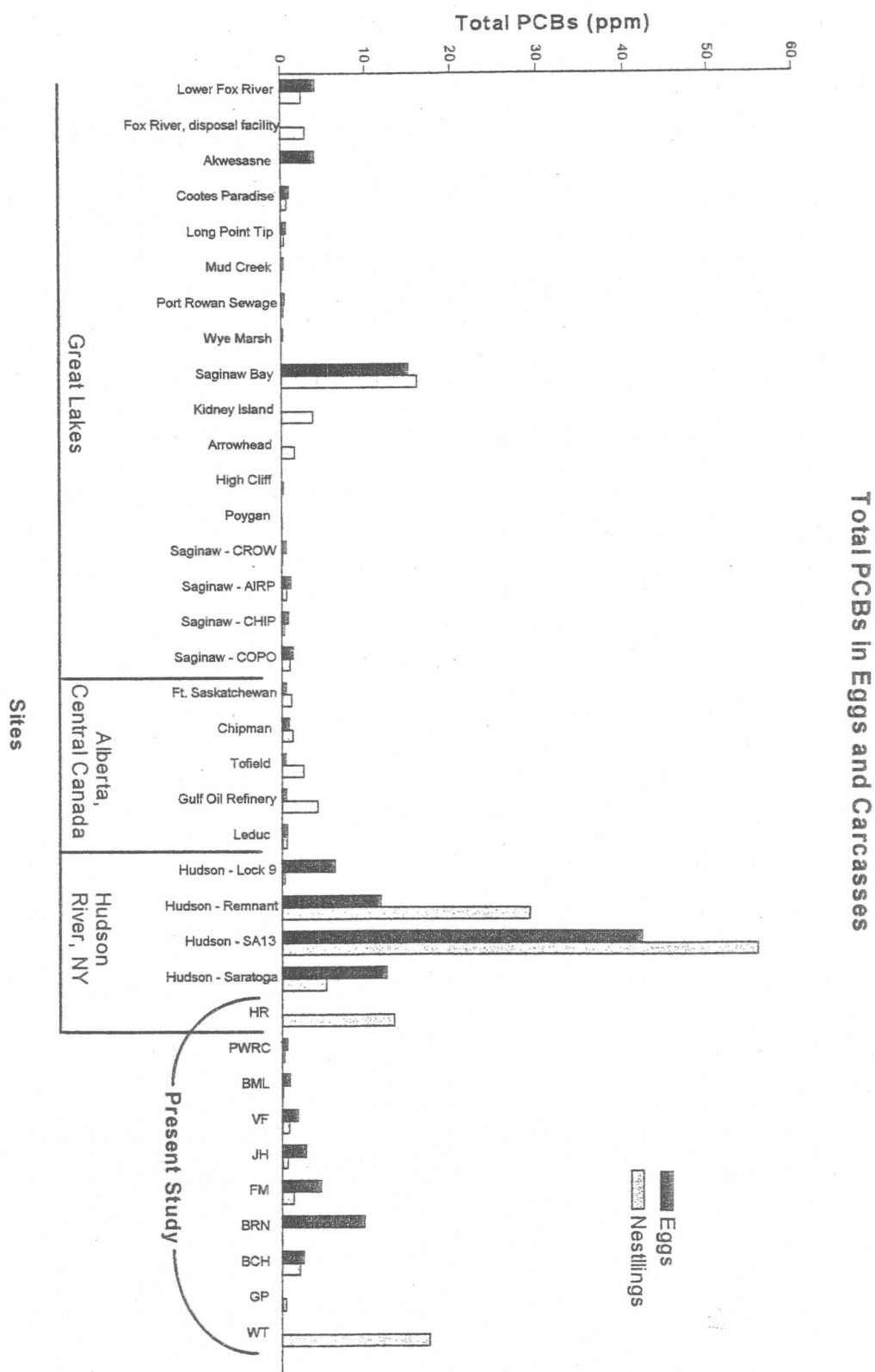
burdens indicative of the dosing regime due to growth dilution, the ability to metabolize and excrete PCBs, or a combination of both processes during the twelve days post-hatch.

TABLE XXXV. Concentrations of total PCBs in Tree swallow eggs and nestlings

(Adapted from Secord and McCarty, 1997).

Concentration (ppm)			
Site	Year(s)	Eggs	Nestlings
Lower Fox River ¹	1988	4.12	2.49
Fox River, confined disposal facility ¹	1988		2.97
Akwesasne ²	1991	4.01	
Cootes Paradise ²	1991	1.02	0.75
Long Point Tip ²	1991	0.69	0.43
Mud Creek ²	1991	0.38	0.16
Port Rowan Sewage Lagoon ²	1991	0.49	0.28
Wye Marsh ²	1991	0.25	0.01
Saginaw Bay ³	1992	15.00	16.00
Kidney Island ⁴	1994 and 1995		mean = 3.77
Arrowhead ⁴	1994 and 1995		mean = 1.59
High Cliff ⁴	1994 and 1995		mean = 0.225
Poygan ⁴	1994 and 1995		mean = 0.085
Saginaw - CROW ⁵	1991	0.56	0.17
Saginaw - AIRP ⁵	1991	1.14	0.62
Saginaw - CHIP ⁵	1991	0.84	0.33
Saginaw - COPO ⁵	1991	1.37	1.03
Hudson - Lock 9 ⁶	1994	6.28	0.38
Hudson - Remnant ⁶	1994	11.7	29.1
Hudson - SA13 ⁶	1994	42.1	55.8

FIGURE 55. Total PCBs in Tree swallow eggs and nestlings (Adapted from Secord and McCarty, 1997).



B. Cytochromes P450

Hepatic cytochrome P450 monooxygenase activities were induced at certain sites (i.e. FM, JH, VF) and correlated significantly with carcass PCB residue levels in environmentally exposed nestlings collected from Pennsylvania and Maryland in 1995 and 1996. Though significant, the correlation coefficients were generally between 0.5-0.7. These correlation coefficients suggest that approximately 25 to 50% of the observed variability in cytochromes P450 activity may be attributed to the PCBs and, although hepatic microsomal monooxygenase activities correlated with PCB concentrations, it is not clear that this is a causative relationship because this type of response was not observed during controlled exposures to PCBs. In 1995 and 1996, when the correlations were significant, the samples examined were from sites with a variety of contaminants. The contaminants at the sites in Pennsylvania and Maryland, the most polluted being near populated and industrial areas, most likely represented a mixture of chemicals present in various combinations and concentrations. Results from western blots of hepatic microsomes (anti-BNF) and immunohistochemical staining of skin and cardiac tissues supported enzyme activities but did not provide useful additional information. Nestlings collected at BCH in 1995 were highly contaminated with PCBs (mean body burden = 2.18ppm, n = 3) but did not exhibit induced hepatic microsomal monooxygenase activities. In 1997, when most of the environmentally exposed samples (with the exception of four from PWRC) were collected along the highly and more exclusively PCB-contaminated Hudson River in New York, no enzyme induction was observed and the correlation between log-transformed monooxygenase activity and body

burden was not strong ($r = 0.229$ and 0.191 for BROD and EROD, respectively). Less than 6% of the observed variability in cytochromes P450 activity could be attributed to PCB contamination. It should be noted that the absence of monooxygenase induction is not likely due to inhibition of cytochromes P450 by high levels of PCBs as has been reported by others (White *et al.*, 1997; Monosson and Stegeman, 1991; Abiola *et al.*, 1989). The range of PCB contamination occurring at all other sites lies within the levels observed at WT where cytochromes P450 were induced.

Samples collected and analyzed from Indiana in 1995 and 1996 represent a combination of the characteristics observed at Pennsylvania, Maryland and the Hudson River sites. At WT, where PCB body burdens were higher than at HR and where monooxygenases were induced, evidence exists of confounding contaminants such as other organochlorines including DDE, a breakdown product of DDT (Custer *et al.*, 1996). The enzyme induction observed from samples collected at sites with correlating monooxygenase activity and body burdens most likely was a result of the exposure of the Tree swallow nestlings to an array of contaminants, not just PCBs, accounting for low but significant correlation coefficients.

The controlled exposure study performed in 1996 revealed that BNF, a PAH, induced cytochromes P450 in Tree swallows. PCBs which were administered to other nestling treatment groups in 1996 and in 1995 did not elicit such a response. These monooxygenase activity data from dosed nestlings indicate that the induction observed at the sites in Pennsylvania and WT, where nestlings were exposed to many contaminants, was due to xenobiotics other than PCBs.

Pipped embryos which hatched from eggs dosed in 1997 produced significant induction when dosed with PCB 126 ($r = 0.446$ for BROD, $r = 0.661$ for EROD). This was the only case in this study of hepatic microsomal monooxygenase induction by PCBs in either pipped embryos or nestlings. 1997 nestlings, which received the same doses of PCB 126 during egg injections and were sacrificed on day 12 post-hatch, did not exhibit monooxygenase induction. This may be due to growth dilution or the ability of nestlings to metabolize and/or excrete the PCBs during post-hatch maturation. 1996 nestlings which were treated with PCB 126 on day nine post-hatch received doses lower than those administered to the eggs, which produced cytochrome P450 induction in pipped embryos (0.05mg/kg versus 0.08 and 0.2mg/kg). The original doses of 3-MC (5 and 10mg/kg) administered to eggs were higher concentrations closer to the 1996 treatments, but resulted in embryo mortality within five days of incubation and had to be lowered to insure survival. It is possible that, by using extreme high levels followed by low levels (0.05 and 0.005mg/kg) of 3-MC, efficacious levels were not administered in order to produce the endpoints sought. Results from western blots of hepatic microsomes (anti-BNF) and immunohistochemical staining of skin and cardiac tissues supported enzyme activities, but, as with environmentally exposed samples, did not provide useful additional information.

BROD and EROD activities consistently showed strong correlation. For environmentally exposed Tree swallows and dosed Tree swallows which were sacrificed as nestlings, the correlation coefficient was consistently greater than 0.8. Their close association indicates that both monooxygenase activities are due to the same cytochrome

gender alteration in nestling Tree swallows. Of the 145 nestlings examined (environmentally exposed and nestlings hatched from dosed eggs), the phenotypic sex ratio was 52% female and 48% male. No intersexes were observed. There was complete concordance between the phenotypic sex, as determined by gonadal morphology, and genotypic sex.

Fox (1993) stated that "the ultimate measure of reproductive success is recruitment of offspring into the breeding population". Two breeding adult Tree swallows which were banded as nestlings the previous year were observed in 1996. Interestingly, both of these birds were reared and banded at sites which possessed moderately high levels of PCB contamination. The birds, however, seemed to be unaffected by the contamination at their birth site and returned to the same area the following year to raise their own offspring. It would appear that the PCB contamination at the sites neither adversely affected their migration to and from wintering grounds or hindered reproduction. Since no other banded birds from 1995 were observed even at clean reference sites, this observation may be regarded as anecdotal.

D. Final Conclusions

Tree swallow eggs and nestlings accumulated environmentally derived PCBs from their environments. Despite evidence of PCB accumulation in Tree swallow nestlings through both environmental and controlled exposures, Tree swallows appear to be resistant to the effects of PCBs, and only modest effects on cytochromes P450 and reproductive and growth parameters were observed. Exposure to PCBs did not notably

one clutch per breeding season, have the potential to supply a comparatively larger number of samples over a longer period of time. Wrens prefer a slightly different habitat niche than Tree swallows, are ground-foragers and ingest more grit particles and soil than Tree swallows (to which PCBs may be sorbed), and have been shown to accumulate PCBs (Mayoh and Zach, 1986; Rendell and Robertson, 1990; Elliott *et al.*, 1994). Additional research could determine if wrens simply accumulate PCBs like Tree swallows and are resistant to their effects or if they are more sensitive to PCBs and are capable of being a more readily available and useful biomarker of exposure.