Bull. Environ. Contam. Toxicol. (1997) 58:254–262

Bulletin of Environmental Contamination and Toxicology

PCB and Heavy Metal Contamination and Effects in European Starlings *(Sturnus vulgaris)* at a Superfund Site

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Received: 30 October 1995/Accepted: 15 September 1996

From 1946-1967 industrial activities at the Crab Orchard National Wildlife Refuge (CONWR) included the construction of landfills and dumps for disposal of hazardous waste. Heavy metals such as cadmium, chromium, mercury, and lead and polychlorinated biphenyls (PCBS) are a special concern because of transport to biotic communities (O'Brien and Gere 1988). During routine monitoring, Hite and King (1977) found elevated concentrations of mercury in fish from Crab Orchard Lake, and Kohler et al. (1990) reported PCB concentrations greater than FDA safety limits (2ppm) in 38% of the fish collected in the eastern portion of Crab Orchard Lake. Additionally, livers from hunter harvested white-tailed deer killed on CONWR contained significantly greater concentrations of nickel and lead compared to deer collected off CONWR (Woolf et al. 1983). In 1984, the Environmental Protection Agency (EPA) added 7 sites within CONWR to the National Priorities List under the Comprehensive Environmental Recovery, Compensation and Liabilities Act. These sites contained varying concentrations of PCBS (Aroclor 1254) and heavy metals that were assessed as posing risk to wildlife (O'Brien and Gere 1988).

Because few studies have been completed on the effects of PCB and heavy metal concentrations on avian species among sites on the refuge, and preliminary studies by McKee (1995) indicated possible avian reproductive impairment due to contaminant exposure, additional study to evaluate adverse effects in avian species was initiated, Using the European starling *(Sturnus vulgaris)* as an avian model, this project provides biological and contaminant baseline data for evaluating avian exposure and effects, and provides a benchmark for determining the effectiveness of proposed remedial actions on CONWR.

MATERIALS AND METHODS

Twelve starling nest boxes were constructed at each of 3 study and 2 reference sites. The Area 9 Building Complex (PCB1) and Landfill (PCB2) contain PCB contamination and the Old Refuge Shop (MET) contains heavy metal contamination. Nest boxes were observed for 30 minute time blocks 3 times per week and adult behavior was recorded. Sampling methods included frequency and scan sampling

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as described by Lehner (1979). Reproductive parameters were recorded, eggs and chicks were individually marked, and chick weights were measured on days 3, 9, and 15 post-hatch.

Eggs that failed to hatch were collected 5 days after the last hatching occurred and egg measurements were recorded and embryos examined for abnormalities. Fifteen day old chicks were collected, euthanized with ^{2}, necropsied, and liver, kidney, and whole carcass were collected for EROD activity, metals, and PCB analysis, respectively. Chicks that died prior to 15 days post-hatch were collected, necropsied, and tissues as described previously were collected. All tissues and organs were wrapped in aluminum foil, labeled, and archived at -80°C prior to analysis.

Aroclor 1254 (AR1254) concentrations and the sum of 19 individual CB congener concentrations (CBS 44, 49, 52, 77, 87, 99, 101, 105, 110, 118, 126, 128, 138, 153, 156, 167, 169, 170, 180) were determined in whole carcass homogenates following methods of Tanabe et al. (1987) as modified by Hong and Bush (1990). Samples were homogenized with anhydrous sodium sulfate at a ratio of 6:1, Na_2SO_4 to tissue, and extracted with hexane using a Soxtec continuous extractor. Clean-up was accomplished by solvent partitioning with concentrated H_2S0_4 and the hexane fraction reduced using rotary evaporation, followed by Gel Permeation Chromatography (GPC). A Hewlett-Packard 5II 11 Gas Chromatography equipped with a Ni-63 electron capture detector and DB5 fused silica capillary column was used for extract analysis. A thermal gradient from 60°C to 250°C was used for elution of congeners, and helium and nitrogen were used as the carrier and make-up gas, respectively. Quantification was accomplished by comparing 10 representative peaks of a standard ARl254 preparation to the same 10 peaks in extracted samples containing unknown quantities of PCBS . Lipid conten t was determined gravimetrically by extracting with petroleum ether, reducing to dryness, and weighing remaining lipid.

Cadmium, chromium, and lead concentrations in kidney and feathers were quantified following EPA methods 213.2, 218.2, and 239.2 (USEPA 1987), respectively, using atomic absorption spectrophotometry. Samples were prepared according to EPA Method 200.3 (McDaniel 199 1). Duplicate aliquots were dried and digested with nitric acid and metal concentrations were determined using a Perkin-Elmer Model 4100ZL Graphite Furnace Atomic Absorption Spectrometer .Mercury concentrations in kidney were determined using cold vapor spectrometry as described by EPA Method 245.7 (Lobring and Potter 1991).

Ethoxyresorufin O-deethylase (EROD) activity i ıliver tissue was quantified following methods of Mazel (1971) and Lubet et al. (1985). Microsomal fractions were obtained by centrifugation followed by O-dealkylation of ethoxyresorufin and measurement of resorufin formation using an Hitachi F-2000 Fluorescence spectrophotometer.

For all analyses, duplicates, blanks, and spiked samples were analyzed with every

Table 1. Starling nest construction and productivity at study and reference sites, 1995.

Site[1]	# Nests Initiated	# Nests Full Cup	# Nests with Eggs	# Nests with Chicks	# Nests with 15 Day Chicks
REF 1	13	8	6	5	2
REF2	15	8	7	5	2
MET	13	6	6	6	3
PCB1	18	12	11	11	3
PCB2	11	1	1	1	1

[1]REF 1, REF2 = references sites, MET=Old Refuge Shop, PCBl=Area 9 Building Complex, and PCB2=Area 9 Landfill.

Table 2. Number of biological samples collected from study and reference sites, 1995.

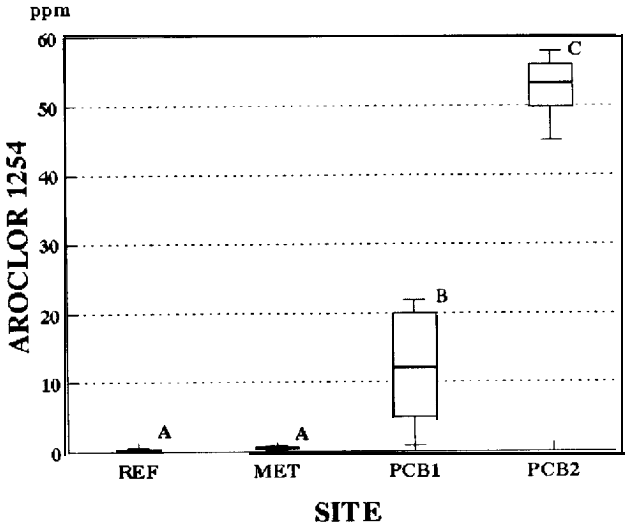
Site[1]	Eggs	Pre-15 Day	Chicks P(M)[2]	15 Day
REF1	7	8	**8(0)**	4
REF2	11	6	4(2)	7
MET	1	7	0(3)	14
PCB1	5	14	10(16)	4
PCB2	0	0	0(0)	3

[1]REF1, REF2 = references sites, MET=Old Refuge Shop, PCB1 =Area 9 Building Complex, and PCB2=Area 9 Landfill.
[2]Chicks Predated(Missing)

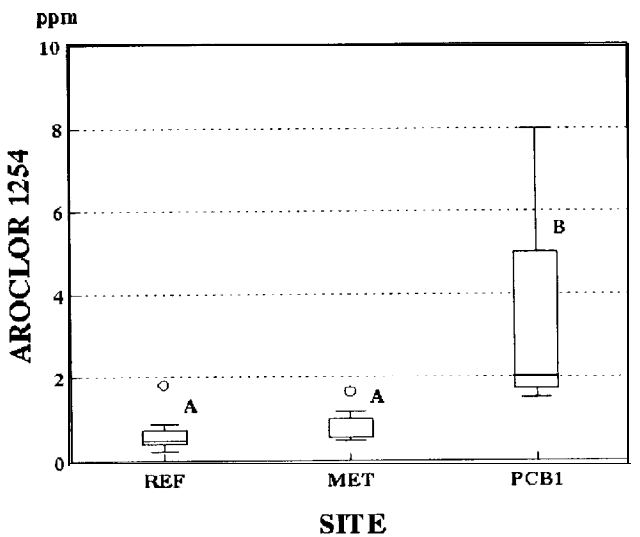
batch. Behavioral, reproductive, physiological and contaminant data were compared among sites using an ANOVA or Kruskal-Wallis test as appropriate. Student's T-test or Mann-Whitney U test were used to compare results between sites. The data from the 2 reference sites or 2 PCB sites were combined when no differences were observed. Results of statistical analysis of lipid adjusted PCB concentrations did not differ from non-lipid adjusted analysis, therefore we have chosen to present only non-lipid adjusted results, Additionally, a concentration of one-half the minimum detection limit (MDL) was used for statistical analysis when samples had concentrations less than the MDL.

RESULTS AND DISCUSSION

From 35 completed nests, 91 samples (67 chicks and 24 eggs) were collected (Tables 1 and 2). Although 11 nests were initiated at PCB2, only one nest was completed.



(a) 15 Day Old Starling Chicks



(b) Pre-15 Day Old Starling Chicks

Figure 1. Whole carcass Aroclor 1254 concentrations (ppm, wet wt) in starlings collected from study and reference sites, 1995. No pre-15 day old chicks were collected from PCB2. CONT=Reference sites, MET=Metals site, PCB1 =Area 9 Building Complex, and PCB2=Area 9 Landfill. Sites with same letters were not different ($p < .05$). Circles represent mild outliers (Q3 + 1. 5(IQR) or Q 1-1.5(IQR))

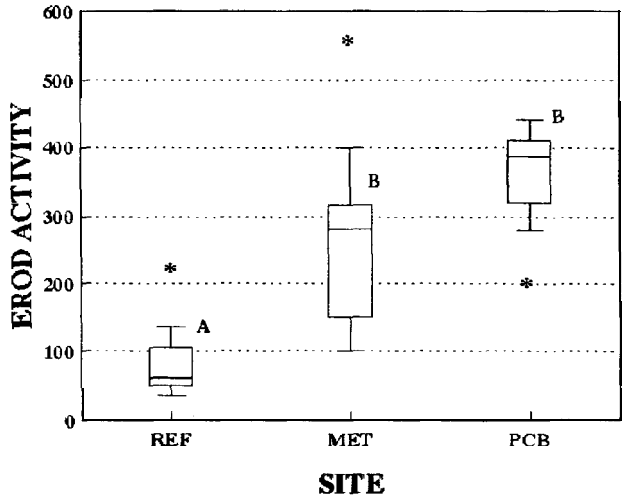


Figure 2. Liver EROD activity (pmole/mg protein/min) in 15 day old chicks collected from study and reference sites, 1995. CONT=Reference sites, MET=Metals site, PCBl=Area 9 Building Complex, and PCB2=Area 9 Landfill. Sites with same letter were not different ($p < .05$). Asterisks represent extreme outliers (Q3 + 3(IQR) or Q 1- 3(IQR)).

This is consistent with Koval et al. (1987) who reported nest abandonment in PCB-treated (Aroclor 1254) Mourning Doves *(Zenaida macroura).* Although PCB concentrations were detected in all chicks from all sites, AR1254 and the sum of CB congeners were greater ($p < .05$) in 15 day old and pre-15 day old chicks collected at PCB sites compared to those collected at reference and metals sites (Figure 1). Statistical analysis of individual congeners and ARl254 resulted in similar conclusions, therefore discussion is limited to AR1254. Mean (±S.E.) ARl254 concentration at PCB2 was 52.45±3.47 (wet wt) compared to McKee's (1995) reported 2.65±0.33 ppm (wet wt) at the same site. Stickel et al. (1984) associated mortality of immature female starlings with whole body ARl254 concentrations of 72-1,120 ppm (wet wt). Thus acute mortality at PCB1 and PCB2 was not expected, although concentrations at PCB2 approached this range. EROD activity in 15 day old chicks was greater ($p < .05$) at PCB and metals sites compared to reference sites and was correlated ($r = .45$, $p = .01$) with PCB concentrations (Figure 2). Greater EROD activity, but low PCB concentrations at the metals site suggest that other EROD inducers may be present. AR1254 and sum of CB congener concentrations in eggs did not differ among sites,

Cadmium concentrations in 15 day old chicks were greater ($p < .05$) at the metals site compared to reference and PCB sites (Figure 3). Mean cadmium concentrations were .083±.008, .031±.005, and .0028 ±.002 ppm (wet wt) at metals, PCB, and reference sites, respectively. Concentrations of cadmium, chromium, and lead in feathers were below the detection limits except in chicks collected at the metals site,

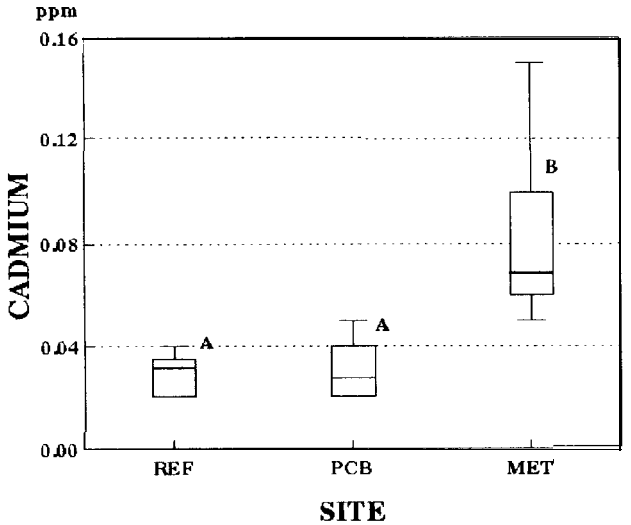


Figure 3. Kidney cadmium concentrations (ppm, wet wt) in 15 day old starling chicks collected from study and reference sites. MET=Metals site, PCB=PCB sites, and REF=Reference sites. Sites with same letter were not different ($p < .05$).

which contained 0.598±0.049 ppm cadmium (Mean±S. E.). White et al. (1978) reported that 130-140 ppm (wet wt) cadmium in combined liver and kidney tissue of adult mallard ducks *(Anas platyrhychos)* resulted in renal tubular necrosis and testicular atrophy. Our study indicated that cadmium is bioaccumulating in starlings at the metals site, however, no evidence of toxic levels was observed. Lead and cadmium concentrations in pre-15 day old chicks and lead in 15 day old chicks were not different among sites. Only 4 and 8 samples had quantifiable concentrations of mercury and chromium, respectively, precluding meaningful statistical comparisons.

Recovery rates for fortified blanks were 109.78, 86.47, 100.49, 84.09, and 114.74 percent for PCBs, lead, cadmium, chromium, and mercury, respectively. These rates were within established control limits.

Mean (±S.E.) hatch rates were 82.5±l1.8, 93.9±3.9, and 77.6±3.2 percent for reference, metals, and PCB sites, respectively. Although previous study by McKee (1995) showed a significant decrease in the percent eggs that hatched at PCB2, we found no difference among sites. Our results were consistent with Kessel (1957) who reported approximately 86% hatching success in starlings nesting in Ithaca, New York. Fledging success (percent of chicks that survived to 15 days), however, was decreased ($p < .05$) at PCB sites compared to reference and metals sites (Figure 4). Mean fledging success at reference sites (78. 8±14.2) was similar to Kessel's (1957) reported rate of 76%, however, the rate dropped to 19.5±9.3% at the PCB sites. Additionally, nest attentiveness behavior (number of times per hour adults came to nest box to feed chicks) was reduced ($p < .05$) from 12.8±l.4 counts/hr at reference sites to 6.2±0.8 counts/hr at PCB sites (Figure 5). No difference in fledgling weight was observed, and incubation behavior did not differ among sites.

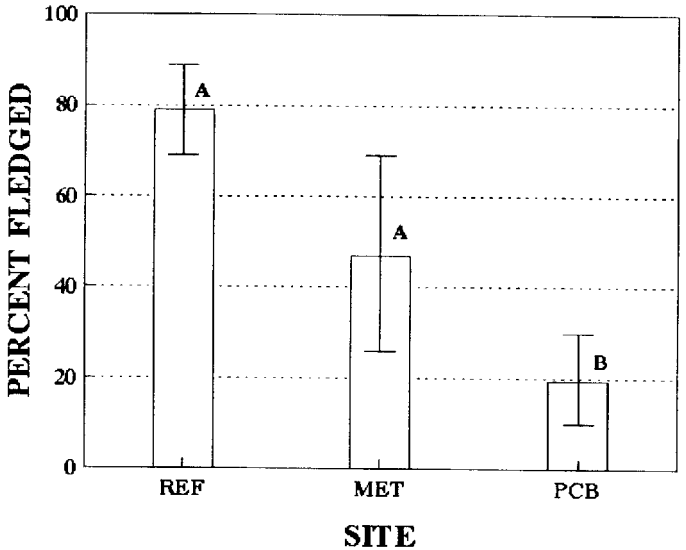


Figure 4. Starling fledging success, 1995. PCB=PCB sites, REF=Reference sites, and MET=Metals site. Sites with same letter were not different ($p < .05$).

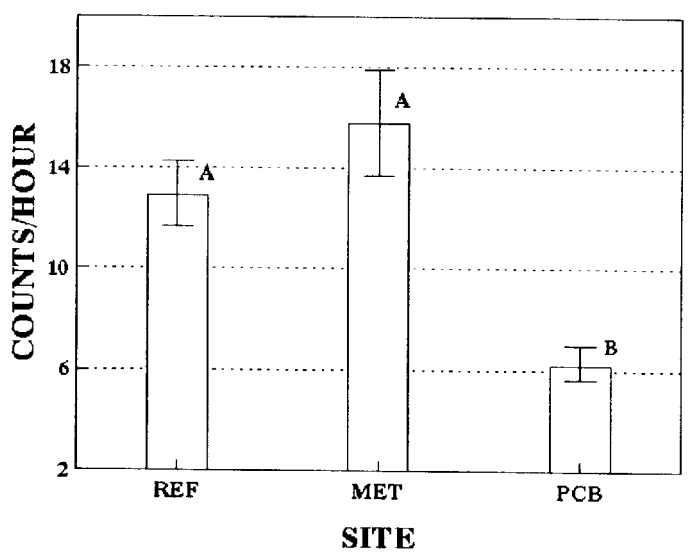


Figure 5. Number of times per hour adult starlings came to nest box to feed chicks, 1995. PCB=PCB sites, REF=Reference sites, and MET=Metals site. Sites with same letter were not different ($p < .05$).

Chicks at PCB1 and MET sites turned pale yellow to white within 1-2 days and died prior to 15 days post-hatch. During necropsy of a 2 day old, pale chick from PCB1 we observed the blood to be pale pink and watery suggesting anemia. Fledging success decreased 86% at PCB1 and 67% at MET from first to second broods, however, high variance at the metals site precluded interpretation of statistical differences. DeHaven and Guarino (1970) reported an 18% decrease in nesting success from first to second clutches in starlings nesting in Denver, Colorado, and attributed this decline to infestation by the chicken mite *(Dermanyssus gallinae).* Although mites were noted at both study and reference nests during second broods, reference nests did not show anemia or high mortality. The cause of the observed early mortality at study sites has not yet been determined and will be part of the focus of the 1996 study.

PCB concentrations were significantly greater at PCB sites compared to reference and metals sites, and cadmium concentrations were greater at the metals site compared to PCB and reference sites. Effects data revealed significantly higher chick mortality, reduced fledging success, and a reduction of nest attentiveness behavior at PCB sites compared to reference sites. It is not known to what extent contaminant concentrations impacted reproductive and behavioral effects demonstrated in our study, however, future investigations will attempt to elucidate this relationship.

Acknowledgments. Financial support for this project was provided by the Hazardous Waste Research and Information Center of the Illinois Department of Natural Resources. We offer special thanks to MaryJo Woodruff who treated this project as her own, to Hui-Lin Ju and the analytical staff for their dedication to the accuracy of these results, and to the staff at Crab Orchard National Wildlife Refuge.

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