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EXPOSURE AND EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN IN TREE SWALLOWS (*TACHYCINETA BICOLOR*) NESTING ALONG THE WOONASQUATUCKET RIVER, RHODE ISLAND, USA

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Abstract—Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in tree swallows (*Tachycineta bicolor*) nesting along the Woonasquatucket River northwest of Providence (RI, USA) in 2000 and 2001 were some of the highest ever reported in avian tissues. Mean concentrations in eggs ranged from 300 to >1,000 pg/g wet weight at the two most contaminated ponds, Allendale and Lyman. Mean egg concentrations at Greystone, the upstream reference pond, were 12 and 29 pg/g. Positive accumulation rates and concentrations in diet samples from 12-day-old nestlings indicated that the contamination was accumulated locally. Concentrations in diet of between 71 and 219 pg/g wet weight were more than 6 and 18 times higher than concentrations considered safe for birds (10–12 pg/g). Hatching success was negatively associated with concentration of TCDD in eggs. Only about half the eggs hatched at Allendale compared with >77% at Greystone. The national average for hatching success in successful nests is 85%. No other contaminants, such as polychlorinated biphenyls and mercury, were present in any sample at concentrations known to affect avian reproduction. Three bioindicators, half-peak coefficient of geometric variation, ethoxyresorufin-*O*-dealkylase activity, and brain asymmetry were assessed relative to TCDD contamination.

Keywords—Hatching success Dioxins 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Tree swallows Woonasquatucket River

INTRODUCTION

From at least 1921 to 1940, a stretch of the Woonasquatucket River northwest of Providence (RI, USA), hereafter called the Centredale Manor Restoration Project Superfund Site, was the location of textile manufacturing. Between 1943 and 1971, the Superfund Site was used for chemical manufacturing and drum recycling. Aerial photographs taken in the 1960s and 1970s showed areas of uncovered, outdoor drum storage in the central area of the Site. In the early 1970s, the mill buildings that housed the former textile and chemical companies on the property were demolished.

In 1977, the Rhode Island Department of Health, Division of Air Pollution Control, responded to complaints of fumes at the property and discovered >50 abandoned drums. In the early 1980s, additional abandoned drums were identified at the site by the Rhode Island Department of Environmental Management Division of Air and Hazardous Waste Management. One drum apparently contained polychlorinated biphenyls (PCBs), whereas some drums might have contained an acid or caustic material (on the basis of the presence of polyliners), solvents, and ink wastes. A notice of violation was issued for violations of the State Hazardous Waste Management Act. In February 1982, approximately 300 drums were removed under the supervision of Rhode Island Department of Environmental Management.

Sediments at the Superfund Site are highly contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 1,2,4,5,7,8-hexachloroxanthene (HCX) [1]. In 1997, sediment samples were collected from six locations along the Woonasquatucket River and ≥5,000 pg/g of TCDD were detected at two of the six locations [1]. The nominal Superfund cleanup level is 1 pg/g [1]. Manufacture of the antibacterial pesticide hexachlorophene could be the source of the dioxin and furan contamination [1]. Other contaminant sources are the largely unregulated use, storage, and disposal of hazardous substances on the property from at least 1921 until 1977.

Swallows, especially tree swallows (*Tachycineta bicolor*), are now being widely used as a study species to quantify distribution and effects of local sediment contamination [2–4]([5] http://www.epa.gov/region01/qe/thesite/restofriver-reports.html)[6–15]. Tree swallows will readily use nest boxes, so study areas can be established at specific locations of interest. They feed near their nest box (within 400 m [16]) on emergent aquatic insects [17], so residues in their tissues reflect sediment contamination for those chemicals that transfer into the biota [18]. They also will nest close to one another; therefore, adequate sample sizes can be obtained. Additionally, data are now available on exposure and effects of contaminants in tree swallows at a number of locations across North America for PCBs [2,3,6,14], other organochlorines [9,10,19], metals [4,7,11,13], and polycyclic aromatic hydrocarbons [7].

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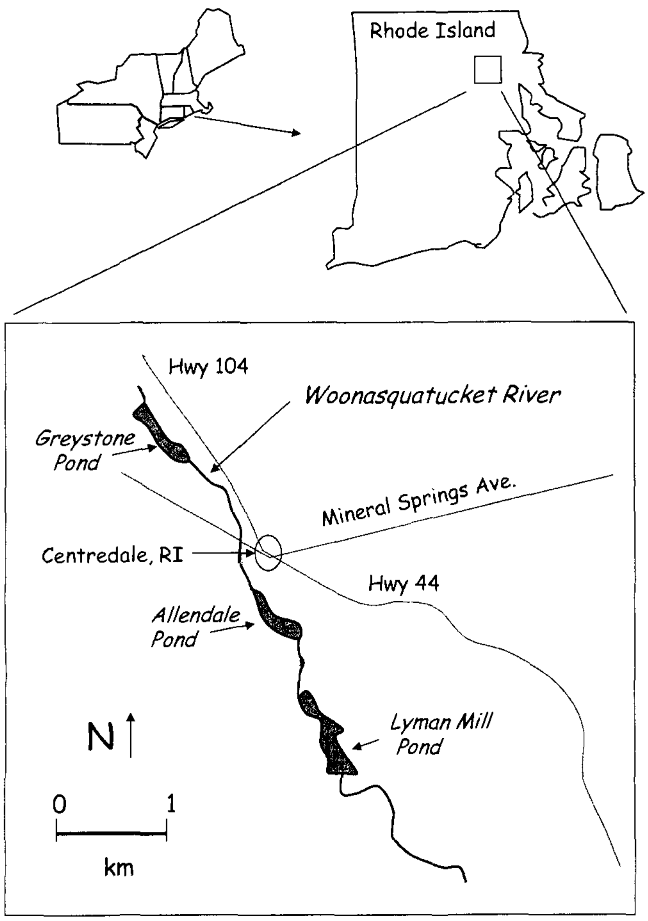

Fig. 1. Study ponds on the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001.

The objectives of this study were to determine whether dioxins and furans were accumulating and causing reproductive or physiological impairments in tree swallows nesting along the Woonasquatucket River.

METHODS

Site description and sample collection

Fifty-nine swallow boxes were attached to posts in early spring 2000 along the shoreline of Allendale Mill Pond (29 boxes), hereafter called Allendale, and Greystone Mill Pond (30 boxes), hereafter called Greystone (Fig. 1). An additional 30 boxes were put up on Lyman Mill Pond, hereafter called Lyman, in 2001. Predator guards were attached to poles after nest building and egg laying commenced. Allendale (4636100 Northing [N], 293900 Easting [E]) is an impounded pond on the Woonasquatucket River downstream of the intersection of Highways 44 and 15 in Centredale, Rhode Island, and is the location where the contamination is thought to have originated [1]. Lyman (4635000 N, 294100 E) is the first impounded pond downstream of Allendale and is known to be contaminated with TCDD as well. Greystone (4637800 N, 293000 E) is the first pond upstream of the Superfund Site.

Each nest box was visited approximately once per week beginning in early May. The stage of nest building and the number of eggs and young present in the box were recorded during each visit. Egg samples were collected at approximately midincubation in 2000 and as each clutch started to hatch in 2001. Clutches of five eggs or fewer had only two eggs collected; clutches of six eggs or more had three eggs collected. Egg samples were collected from the first five to seven clutch initiations at each pond. Eggs from clutches that failed to hatch were also collected. It was not possible to analyze egg samples from every clutch because of the expense of the chemical analyses. A 9- to 13-day-old tree swallow nestling was collected from five nest boxes per pond where sibling eggs had previously been collected. Food samples were collected from the stomachs of tree swallow nestlings on the day they were collected, sacrificed, and pooled by pond into one sample for contaminant analysis. In 2001, nestlings in five boxes were ligatured [20], and food items from their throats were collected for insect identification, done by Lotic (Unity, ME, USA). The insects were identified to the lowest taxonomic level necessary to determine whether the insects were aquatic or terrestrial in origin. All samples were collected under appropriate state and federal collecting permits.

Sample preparation

Eggs or nestlings were removed from the nest box and weighed. Contents of eggs from an individual nest box were emptied into a chemically clean jar; eggshells were discarded. Nestlings were weighed and then decapitated with a sharp pair of scissors [21]. Contents in the upper gastrointestinal tract were removed with forceps, weighed, and placed in a chemically clean jar. The liver was removed and weighed, and a small piece ($\leq$0.2 g) was placed in a cryotube, covered with one or two drops of glycerin, and snap frozen in liquid nitrogen for later ethoxyresorufin-*O*-dealkylase (EROD) analysis. The carcass remainder, including the head and the remaining portion of the liver, was then placed in a chemically clean jar. The egg, carcass remainder, and diet samples were frozen later that day and maintained frozen until shipped to the analytical laboratory following chain of custody procedure. The EROD liver samples were maintained in liquid nitrogen or an ultracold freezer until shipped on dry ice to Patuxent Wildlife Research Center (Laurel, MD, USA).

In 2001, sample collection and preparation were similar to 2000 except that two 12-day-old nestlings were collected from five nest boxes per pond. The livers from both young were excised, a small portion ($\leq$0.2 g) of the liver from the first nestling (a-nestling) was removed for EROD analysis (as above), and that liver remainder and the entire liver from the second nestling (b-nestling) were pooled by nest box and analyzed for trace elements. The head from the b-nestling was preserved in 10% buffered formalin for later sectioning to assess brain asymmetry. A blood sample was collected immediately following decapitation and frozen in liquid nitrogen for analysis of deoxyribonucleic acid (DNA) content by flow cytometry. Blood samples were maintained in liquid nitrogen or an ultracold freezer until shipped on dry ice to Texas A&M University (College Station, TX, USA).

Bioindicators

Ethoxyresorufin-*O*-dealkylase activity was assayed in 12-day-old liver microsomes [2] by the bicinchoninic acid protein method of Burke and Mayer [22] as adapted to a fluorescence microwell plate scanner [23]. Hepatic microsomes were prepared from homogenates of thawed liver samples by differential centrifugation. The 11,000 *g* supernatant was centrifuged at 40,000 rpm for 60 min to obtain the microsomal pellet. Each 100,000 *g* pellet was resuspended in 2.0 ml/g tissue weight of 0.05 M Na/KPO_4 and 0.001 M disodium ethylenediamine tetraacetate, pH 7.6. The 260-μl total assay volume contained microsomes equivalent to 0.65 mg of liver, 2.5 μM substrate, and 0.125 mM nicotinamide adenine dinucleotide phosphate-H in 0.066 M Tris buffer, pH 7.4. Protein concentrations were determined by a 50% reduced volume assay [24], and activities were calculated (pmol product/min/mg microsomal protein).

Blood for flow cytometry was collected in heparinized capillary tubes, aspirated into freezing media (Hams F10 media, Sigma Chemicals, St. Louis, MO, USA) with 18% fetal bovine serum and 10% glycerin), inverted several times, and then frozen in liquid nitrogen. Nuclear preparation and staining followed the method of Vindelov et al. [25]. Nuclear suspensions were prepared following lysis with detergent and trypsin digestion. Nuclear suspensions were treated with RNAse and stained with propidium iodide. Nuclear DNA content was analyzed in approximately 10,000 cells per individual on a Coulter Elite (Fullerton, CA, USA) flow cytometer by quantification of nuclear fluorescence. The mean half-peak coefficient of variation (HPCV) in the G1 peak was quantified. This value expresses the amount of variation in the DNA content of the cells measured. Somatic chromosome damage is suggested when the HPCV in a population is significantly greater than the HPCV in a reference population [26,27].

For brain asymmetry, each brain was removed from the 10% formalin buffer solution in which it had been stored, stained with thionine, imbedded in paraffin, and then sectioned both horizontally and vertically into 16 sections. The height and width of the left and right sides for each section were measured (mm), and the absolute difference between the right and left side was calculated. An average absolute difference for all sections combined was calculated for each individual.

Two out-of-basin reference locations were used for the three bioindicator assessments: the upper Mississippi River (zone 15, 639433 E, 4843747 N) in Houston County, eastern Minnesota, USA, and Threemile Pond (zone 18 639803 E, 4666369 N), Berkshire County, western Massachusetts, USA. These out-of basin reference locations were used to further assess normal levels for the bioindicators.

Statistical analysis

Data were analyzed with one-way analysis of variance (ANOVA) to compare year-pond means for concentrations of organic chemicals, trace elements, and bioindicators. Contaminant concentrations were log transformed prior to analysis to satisfy the homogeneity of variance (Levene's and Bartlett's tests) requirement of ANOVA. Geometric means (antilog) ± 95% confidence interval (CI) are presented in tables and text. In a multivariate context, an analysis of similarity (ANOSIM) [28], which is a nonparametric permutation test based on ranks of proportions, was used to compare the pattern of dioxin and furan congeners among ponds, years, and matrix (eggs or nestlings). For this test, the proportion that each congener made up of the total concentration was calculated and then ranked. A similarity matrix was constructed and used to compare congener patterns [28]. The ANOSIM test statistic varies between a + 1 and a − 1. Results near zero indicate that the predetermined groups are as similar within the groups as between the groups (i.e., no difference among groups). A value near 1.0 indicates that the groups differ from one another; that is, within-groups are more similar to one another than between-groups. Only chemicals found above the detection limit in ≥50% of samples were analyzed statistically. Half the detection limit was substituted for samples that had no detectable concentrations.

Accumulation rates (mass of contaminant in the nestling minus the mass in the egg divided by the age of the nestling) for each egg–nestling pair were calculated. Contaminant mass was calculated by multiplying the concentration in the sample by the sample weight. Accumulation rates were rank transformed to satisfy ANOVA assumptions.

The association of dioxin and furan congener concentrations with one another was evaluated by Pearson's correlation coefficients. Additionally, the association between TCDD concentrations in nestlings and the two brain asymmetry measurements and TCDD concentrations in eggs, nestlings, diet, and accumulation rate were also assessed with Pearson's correlation coefficients. Correlations of TCDD among egg and nestling concentrations and accumulation rate were correlated on the basis of data from individual clutches. Concentrations in pooled diet were correlated with means for the above three measurements for each year-pond because only one value per year-pond was available for diet because of pooling.

Daily survival estimates of eggs were calculated for the egg laying and incubation period and for nestlings for the first 12 days of age by the Mayfield method [29,30]. Survival rates were compared among ponds with the use of contrasts according to Hensler and Nichols [31]. Nest attempts that were lost to predation or disturbed by humans were not included in the Mayfield analyses. We assumed that predation and human disturbance were independent of chemical concentration. Concentrations of TCDD in nests that had hatching failures, which is defined as clutches that contained more than one dead embryo or more than two eggs that failed to hatch for any reason, were compared with successful nests by one-way ANOVA. Probit analysis was used to estimate an LCE50 (concentration in eggs where hatching was reduced by 50%) for comparison with other published data.

Bioindicators were compared among locations without additional transformations. Ethoxyresorufin-*O*-dealkylase was compared among locations for each year individually because EROD assays for each year were done on different days. Pearson correlations were run for HPCV, EROD, and TCDD in 2001. Whole body weights, liver weights, and liver weight to whole body weight ratios were compared among ponds in 2001 by one-way ANOVA. Body and liver weights were not compared in 2000 because different ages of nestlings were collected in that year.

Chemical analyses

Polychlorinated dibenzo-*p*-dioxins (n = 7 congeners) and polychlorinated dibenzofurans (n = 10 congeners) were analyzed in tree swallow egg, nestling carcass remainder, and diet samples in 2000 and 2001 by Battelle (Columbus, OH, USA). Additionally, the concentrations of total PCBs and 107 PCB congeners were measured in five 12-day-old nestling carcasses per pond in 2000 and in one randomly chosen nestling carcass from Allendale, Greystone, and Lyman in 2001. Total PCBs were quantified by both Aroclor analysis and by summing the PCB congener concentrations. Total PCBs and congeners were analyzed in five egg samples in 2001; these were not the same egg samples in which the dioxin and furans were analyzed. Battelle Marine Sciences Laboratory (Sequim, WA, USA) analyzed trace elements in five nestling liver samples per pond in 2001. Organic pesticides were analyzed in five carcass remainders (entire carcass less the upper gastrointestinal tract contents and the liver) by Battelle (Duxbury, MA, USA).

Chemicals analyzed included the following dioxin and furan congeners: TCDD; 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD); 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD); 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxins (HpCDD); octachlorodibenzo-*p*-dioxins (OCDD); 2,3,7,8-tetrachlorodibenzofuran (TCDF); 1,2,3,7,8-pentachlorodibenzofuran (PeCDF); 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF); 1,2,3,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF); 1,2,3,4,7,8,9-HpCDF; and octachlorodibenzofuran (OCDF). Nominal limits of detection were 1 pg/g wet weight for TCDD and TCDF, 10 pg/g for OCDD and OCDF, and 5 pg/g for the other dioxin and furan congeners. Also analyzed was 1,2,4,5,7,8-hexachloroxanthene (HCX) with a nominal detection level of 50 pg/g wet weight. Organochlorine chemicals analyzed were aldrin; α-, β-, γ-, and δ-benzene hexachloride (BHC); α- and γ-chlordane; technical chlordane; 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane (*p,p*′-DDD); 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene (*p,p*′-DDE); 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p*′-DDT); dieldrin; endosulfan I; endosulfan II; ensodsulfan sulfate; endrin; endrin aldehyde; endrin ketone; heptachlor; heptachlor epoxide; methoxychlor; and toxaphene. Nominal levels of detection for organochlorines were 0.005 μg/g wet weight, except for chlordane (0.7 μg/g wet wt) and toxaphene (0.1 μg/g wet wt). Generally, detection limits for egg and diet samples were slightly higher than for nestlings because of smaller sample mass.

The following PCBs were analyzed; congeners separated by a slash coeluted in 2000 but were quantified separately in 2001. Congeners followed by 2001 in parentheses were only analyzed in 2001. Ballschmiter and Zell (BZ) numbers for the congeners analyzed were 1, 3, 4/10, 6, 7/9, 8/5, 12/13, 16/32, 17, 18, 19, 21, 22, 24/27, 26, 25, 28, 29, 30 (2001), 31, 33/20, 40, 41/63/71, 42, 43, 44, 45, 46, 47/75, 48, 49, 50 (2001), 51, 52, 53, 56/60, 59, 61 (2001), 62 (2001), 63, 64 (2001), 65 (2001), 66, 69 (2001), 70/76, 73 (2001), 74, 77, 81, 82, 83, 84, 85, 86 (2001), 87/115, 88 (2001), 89, 91, 92, 93 (2001), 95, 97, 98 (2001), 99, 100, 101/90, 102 (2001), 105, 106 (2001), 107, 108 (2001), 109 (2001), 110, 113 (2001), 114, 115 (2001), 116 (2001), 117 (2001), 118, 119, 123, 124, 125 (2001), 126, 128, 129, 130, 131, 132, 134, 135/144, 136, 137, 138/160/163, 141, 143 (2001), 146, 147 (2001), 149, 151, 153, 154 (2001), 156, 157/156, 158, 161 (2001), 163 (2001), 164 (2001), 167, 168 (2001), 169, 170/190, 169, 171, 172, 173 (2001), 174, 175, 176, 177, 178, 180, 183, 184, 185, 187/182, 189, 191, 193, 194, 195, 197, 198, 199, 200, 201, 203/196, 205, 206, 207, and 209. Nominal level of detection for PCB congeners was 0.0002 μg/g wet weight.

Trace elements analyzed with nominal detection limits (μg/g dry wt) in parentheses included aluminum (4), antimony (0.1), arsenic (0.1), barium (0.04), beryllium (0.02), cadmium (0.7), chromium (1.0), cobalt (1.0), copper (0.3), iron (1), lead (0.1), manganese (0.1), mercury (0.01), methylmercury (0.01), molybdenum (1.0), nickel (1.0), selenium (2.0), silver (0.2), thallium (0.1), vanadium (0.6), and zinc (0.4). Concentrations of organic and inorganic chemicals are presented on a wet weight basis. Trace element concentrations can be converted to dry weight by dividing the concentration by 0.3 (proportion dry mass of livers) [4].

Dioxin, furan, and HCX analyses were done using modified U.S. Environmental Protection Agency (EPA) Method 1613, revision B [32]. In brief, tissues for dioxins, furans, and HCX were homogenized and mixed with Hydromatrix® (Charlotte, VA, USA) drying agent and then extracted by accelerated solvent extraction. Each extract was acid washed and then split for separate cleanup and analysis for dioxin, furan, and HCX congeners and for PCB congeners. For dioxin, furan, and HCX congeners, samples were processed through acid/base silica, alumina, and carbon cleanup columns. For PCB congeners, cleanup included acid/base silica and alumina followed by additional acid/base silica cleanup. Extracts were analyzed by gas chromatography/high-resolution mass spectrometry. For PCBs and chlorinated pesticide samples, modified U.S. EPA Method 1613, revision A [33,34] were used. Samples were freeze-dried, ground, and then extracted. The extract was processed through an alumina column and high-performance liquid chromatography cleanup and then analyzed by gas chromatography with electron capture detection. For trace elements, aluminum, chromium, iron, manganese, silver, vanadium, and zinc were analyzed by inductively coupled plasma atomic emission spectroscopy according to modified U.S. EPA Method 200.7 [35] and SW6010B [36]. Selenium was analyzed by flow-injection atomic spectroscopy following a modification of SW846 Methods 7062 [36] and 7742 [36]. Antimony, arsenic, barium, beryllium, cadmium, cobalt, copper, lead, molybdenum, nickel, and thallium were analyzed by inductively coupled plasma according to U.S. EPA Methods 1638 [37] and 1640 [38] as modified and adapted for analysis of solid sample digestates. Mercury and methylmercury were analyzed by cold vapor atomic absorption and cold vapor atomic fluorescence [39]. In brief, liver samples were frozen, freeze-dried, and then homogenized. An aliquot was digested in nitric and hydrofluoric acids. Blanks, spikes, and duplicates were analyzed according to Battelle [40] on 5% of all samples. Samples were not corrected for percent recovery; however, isotope dilution techniques correct for sample lost during extraction and cleanup for dioxin, furan, HCX, and PCB congeners.

Toxic equivalents

Toxic equivalents (TEQs) were calculated with the World Health Organization [41] toxic equivalent factors (TEFs). The concentration of each congener in the sample was multiplied by the TEF for that congener and then summed. The TEQ values are reported separately for the dioxin and furan congeners and the PCB congeners.

RESULTS

Chemical contamination—Dioxin, furan, HCX, and PCBs

Tree swallow eggs from Greystone had significantly lower mean concentrations of TCDD (<30 pg/g wet wt) than eggs from Allendale in 2000 (mean = 826 pg/g; maximum value 1,533 pg/g) and 2001 (mean = 314 pg/g; maximum value 2,299 pg/g), and Lyman (mean = 1,013 pg/g; maximum value 1,949 pg/g) in 2001 (Table 1). Maximum concentrations at Greystone were 52.3 pg/g (2000) and 284.9 pg/g (2001). Concentrations of TCDD in eggs were similar among Allendale in 2000, Allendale in 2001, and Lyman in 2001. Nestling carcasses demonstrated the same pond differences and similarities as eggs (Table 2); Allendale and Lyman had significantly higher nestling carcass concentrations of TCDD (>500 pg/g) than Greystone (<10 pg/g) in both years. Maximum

Table 1. Dioxin, furan, and total polychlorinated biphenyl (PCB) congener geometric mean concentrations and their associated toxic equivalents (TEQs) in tree swallow eggs collected along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001

		Concentration (pg/g wet wt) and 95% CI[c]				
		2000		2001		
Chemical[a]	p Value[b]	Allendale ($n = 13$)	Greystone ($n = 7$)	Allendale ($n = 13$)	Greystone ($n = 6$)	Lyman ($n = 9$)
2,3,7,8-TCDD	<0.001	825.5 A 720.6–945.7	17.21 B 11.73–25.25	313.6 A 243.1–404.6	29.43 B 16.0–54.1	1,013.3 A 885.1–1,160
1,2,3,7,8-PeCDD	0.001	13.74 A 11.9–15.9	6.09 AB 5.04–7.35	6.25 AB 4.83–8.097 1 ND[d]	2.96 B 2.00–4.37 1 ND	9.11 A 7.78–10.7
1,2,3,4,7,8-HxCDD	<0.001	13.65 A 12.0–15.5	5.88 B 4.85–7.14	7.69 AB 6.61–8.95	4.64 B 4.10–5.25	8.63 AB 7.45–10.0
1,2,3,6,7,8-HxCDD	0.023	59.02 A 49.9–69.8	31.97 AB 24.2–42.2	46.88 AB 39.39–55.78	25.47 B 23.3–27.8	59.06 A 49.4–70.6
1,2,3,7,8,9-HxCDD	<0.001	9.15 A 7.93–10.6	3.72 C 3.24–4.27	6.53 ABC 5.85–7.29	4.20 BC 3.90–4.51	6.83 AB 6.00–7.79
1,2,3,4,6,7,8-HpCDD	0.007	149.2 AB 126.3-176.3	77.91 B 63.8–95.1	145.4 AB 113.5–186.1	243.2 A 185.4–318.9	292.9 A 234.9–365.1
OCDD	<0.001	107.9 B 86.8–134.2	63.83 B 52.4–77.7	167.7 AB 121.7–231.1	429.6 A 294.2–627.3	567.6 A 399.6–806.1
2,3,7,8-TCDF	0.008	15.94 A 14.2–17.9	11.69 AB 9.92–13.8	12.13 AB 10.7–13.7	6.42 B 4.70–8.77	13.12 A 11.9–14.5
1,2,3,7,8-PeCDF	0.091	3.97 3.53–4.47	2.59 2.15–3.11	3.59 3.21–4.01	1.71 1.27–2.32 1 ND	2.76 1.93–3.96 2 ND
2,3,4,7,8-PeCDF	0.067	7.35 6.30–8.58	4.79 4.06–5.64	3.72 2.83–4.90 2 ND	2.44 1.89–3.16	4.32 3.10–6.02 1 ND
1,2,3,4,7,8-HxCDF	0.028	5.47 AB[e] 4.87–6.14	3.74 B 3.17–4.41	4.41 AB 3.54–5.48 1 ND	3.70 B 3.43–3.98	8.17 A 6.91–9.67
1,2,3,6,7,8-HxCDF	0.005	5.82 AB 5.15–6.58	3.14 B 2.68–3.68	3.78 AB 3.10–4.61 1 ND	2.97 B 2.71–3.25	6.44 A 5.76–7.21
1,2,3,7,8,9-HxCDF		11 ND	6 ND	11 ND	6 ND	7 ND
2,3,4,6,7,8-HxCDF	0.270	3.13 2.77–3.53	1.82 1.54–2.14	2.55 2.14–3.05 1 ND	1.80 1.65–1.97	2.51 1.82–3.45 2 ND
1,2,3,4,6,7,8-HpCDF	0.001	18.53 B 15.7–21.9	10.90 B 8.75–13.6	22.13 AB 16.42–29.83	32.3 AB 27.0–38.6	57.0 A 45.0–72.1
1,2,3,4,7,8,9-HpCDF	0.004	0.94 B 0.80–1.10 3 ND	0.81 B 0.68–0.95 1 ND	1.02 AB 0.81–1.28 3 ND	1.70 AB 1.30–2.22	2.80 A 2.06–3.80
OCDF	0.002	3.77 BC 3.11–4.57	2.46 C 2.07–2.93	4.26 ABC 2.68–6.75 2 ND	11.67 AB 8.22–16.5	19.6 A 14.3–26.9
Total PCBs (μg/g)		NA	NA	1.00 ($n = 3$)[f] 0.87–1.15	NA	1.13 ($n = 2$)[f] 0.95–1.34
TEQs[g]						
WHO dioxin/furan	<0.0001	960.0 AB 847.2–1,072.6	52.2 C 40.6–64.0	502.6 BC 343.2–663.94	94.2 C 46.34–142.4	1,126.4 A 973.0–1,281.0
WHO PCBs		—	—	220.9 ($n = 3$)[f] 167.0–274.8	—	143.8 ($n = 2$)[f] 119.5–168.1

[a] TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; WHO = World Health Organization.

[b] Analysis of variance. $df = 4, 42$; alpha = 0.05 for Bonferroni mean separations except where noted.

[c] Means sharing the same letter are not significantly different among year-ponds. NA = no samples analyzed.

[d] Number not detected. If no notation, then all samples had detectable concentrations.

[e] Alpha = 0.075 for Bonferroni mean separations.

[f] Sample sizes for total PCB, PCB congener, and TEQ analyses only.

[g] Means for TEQs are arithmetic not geometric (pg/g wet wt) for dioxin, furan, and PCB congeners.

— = TEQ not calculated because no samples were analyzed for PCB congeners.

concentrations of TCDD in nestling carcasses were 795 and 1,703 pg/g wet weight (Allendale 2000 and 2001, respectively), 1,610 pg/g (Lyman 2001) and 7.5 and 23.1 pg/g (Greystone 2000 and 2001, respectively).

The remaining six dioxins and 9 of 10 furans were detected in >50% of egg and nestling samples (Tables 1 and 2). These other dioxin and furan congeners tended to be present at much lower concentrations than TCDD except for 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, and 1,2,3,4,6,7,8-HpCDF in eggs at Greystone (Tables 1 and 2). Although con-

Table 2. Dioxin, furan, 1,2,4,5,7,8-hexachloroxanthene (HCX), and total polychlorinated biphenyl (PCB) congener geometric mean concentrations and their associated toxic equivalents (TEQs) in tree swallow nestling carcasses (n = 5 nestlings per pond) collected along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001

		Concentration (pg/g wet wt) and 95% CI[c]				
		2000		2001		
Chemical[a]	P Value[b]	Allendale	Greystone	Allendale	Greystone	Lyman
2,3,7,8-TCDD	<0.001	572.0 A 485.2–674.3	5.73 B 5.30–6.20	990.0 A 863.7–1,171	9.32 B 7.21–12.06	838.1 A 558.5–1,258
1,2,3,7,8-PeCDD	<0.001	2.77 AB 2.42–3.17	1.49 BC 1.34–1.66	3.38 AB 3.11–3.69	1.03 C 0.72–1.51 1 ND[d]	3.93 A 3.57–4.31
1,2,3,4,7,8-HxCDD	<0.001	2.52 AB 2.16–2.94	1.37 C 1.25–1.51	3.46 A 3.22–3.72	1.74 BC 1.55–1.96	3.72 A 3.28–4.21
1,2,3,6,7,8-HxCDD	0.001	7.89 AB 6.51–9.55	4.73 B 4.15–5.39	12.11 A 10.84–13.54	5.07 B 4.36–5.90	11.09 A 8.96–13.72
1,2,3,7,8,9-HxCDD	0.002	1.14 AB 0.78–1.68 1 ND	0.82 B 0.77–0.88	2.89 A 2.59–3.25	0.92 B 0.75–1.13	1.94 AB 1.67–2.25
1,2,3,4,6,7,8-HpCDD	0.013	17.53 AB 13.23–23.22	12.27 B 9.99–15.06	37.99 A 30.12–47.91	15.49 AB 11.93–20.12	32.17 AB 26.35–39.28
OCDD	0.026	11.69 AB[e] 9.22–14.82	9.26 B 7.17–11.96	34.57 A 24.37–49.05	23.09 AB 15.28–34.91	30.57 AB 23.06–40.52
2,3,7,8-TCDF	0.004	4.79 AB 4.06–5.66	3.94 B 3.63–4.28	7.17 A 6.54–7.85	4.47 AB 3.75–5.33	7.59 A 7.04–8.18
1,2,3,7,8-PeCDF	0.008	0.98 AB 0.82–1.19	0.77 B 0.69–0.86	1.54 A 1.39–1.69	0.92 AB 0.812–1.05	1.45 A 1.25–1.68
2,3,4,7,8-PeCDF	0.055	1.51 1.30–1.76	0.91 0.83–1.00	2.92 2.40–3.56	1.13 0.98–1.29	1.67 1.00–2.80 1 ND
1,2,3,4,7,8-HxCDF	<0.001	1.13 B 0.93–1.37	0.89 B 0.84–0.96	2.92 A 2.40–3.56	1.09 B 0.92–1.30	2.47 A 2.32–2.62
1,2,3,6,7,8-HxCDF	<0.001	1.27 AB 1.03–1.56	0.85 B 0.79–0.92	2.239 A 2.11–2.70	0.86 B 0.73–1.02	2.21 A 1.96–2.50
1,2,3,7,8,9-HxCDF		5 ND	5 ND	5 ND	5 ND	5 ND
2,3,4,6,7,8-HxCDF	<0.001	0.65 AB 0.50–0.84 1 ND	0.30 C 0.28–0.33 1 ND	1.15 A 1.06–1.24	0.45 BC 0.38–0.53	0.85 AB 0.77–0.93
1,2,3,4,6,7,8-HpCDF	0.008	2.38 AB 1.73–3.27	1.41 B 1.27–1.58	5.52 A 4.72–6.45	1.39 B 0.81–2.39 1 ND	4.562 AB 4.16–5.00
1,2,3,4,7,8,9-HpCDF		4 ND	5 ND	0.35 0.30–0.42 1 ND	4 ND	0.32 0.29–0.35 2 ND
OCDF	0.007	0.56 AB 0.54–0.58	0.31 B 0.27–0.35	2.19 A 1.36–3.52	1.32 AB 0.77–2.26	1.11 AB 0.85–1.46
HCX	0.005	5 ND	5 ND	25.9 A 19.3–34.8	0.33 B 0.15–0.75 4 ND	8.58 A 3.09–23.9 1 ND
Total PCBs (μg/g)	<0.001	0.41 A 0.36–0.47	0.11 B 0.10–0.12	0.71 —	0.21 —	1.72 —
TEQs[f]						
WHO dioxin/furan	<0.001	612.3 AB 520.6–704.1	12.8 B 11.9–13.6	1,063.4 A 887.9–1,241.9	18.6 B 18.76–451.4	1,069.9 A 812.5–1,327.4
WHO PCBs		133.4 111.5–155.3	32.8 31.3–34.3	152.0 —	27.1 —	179.4 —

[a] TCDD = tetrachlorodibenzo-p-dioxin; PeCDD = pentachlorodibenzo-p-dioxin; HxCDD = hexachlorodibenzo-p-dioxin; HpCDD = heptachlorodibenzo-p-dioxin; OCDD = octachlorodibenzo-p-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; WHO = World Health Organization.

[b] Analysis of variance, df = 4, 20; alpha = 0.05 for Bonferroni mean separations except where noted.

[c] Means sharing the same letter are not significantly different among year-ponds. NA = no samples analyzed; — = no variance estimate because only one sample per pond.

[d] Number not detected. If no notation, then all samples had detectable concentrations.

[e] Alpha = 0.10 for Bonferroni mean separations.

[f] Means for TEQs are arithmetic not geometric (pg/g wet wt) for dioxin, furan, and PCB congeners.

centrations of the other dioxin and furan congeners varied among study ponds (12 of 15 congeners for eggs and nestlings), the magnitude of the differences among ponds for these other congeners were much less than the differences among ponds for TCDD. In general, concentrations at Greystone were less than or equal to concentrations at the other two ponds; concentrations at Greystone were never greater than concentrations elsewhere. The magnitude of the differences between the highest (Allendale or Lyman) and lowest (Greystone) mean TCDD concentration was 59 times for eggs (17.2 pg/g vs

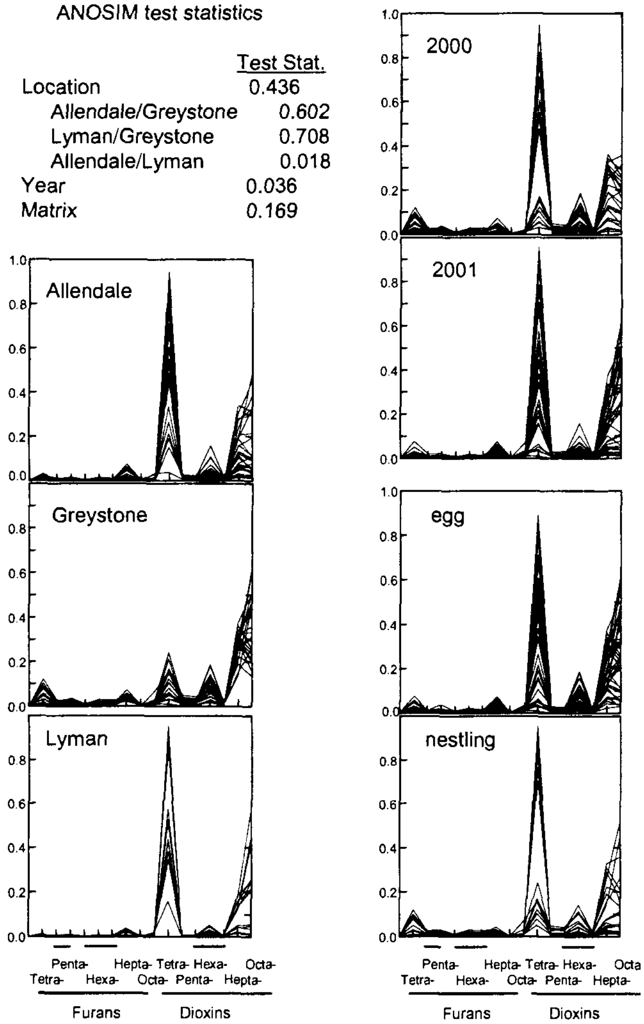

Fig. 2. Analysis of similarity (ANOSIM) for dioxin and furan congeners in tree swallow eggs and nestling carcasses from the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001. Test statistic varies between a − 1 and a + 1, with values near zero indicating no difference. The order and abbreviations of the furan and dioxin congeners are tetra- = 2,3,7,8-tetrachlorodibenzofuran; penta- = 1,2,3,7,8-pentachlorodibenzofuran (PeCDF) and 2,3,4,7,8-PeCDF; hexa- = 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF), 1,2,3,6,7,8-HxCDF, and 2,3,4,6,7,8-HxCDF; hepta- = 1,2,3,4,6,7,8-heptachlorodibenzofuran (HpCDF) and 1,2,3,4,7,8,9-HpCDF; octa- = octachlorodibenzofuran; tetra- = tetrachlorodibenzo-*p*-dioxin; penta- = 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin; hexa- = 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD), 1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD; hepta- = 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin; octa- = octachlorodibenzo-*p*-dioxin.

1,013.3 pg/g) and 173 times for nestlings (5.73 pg/g vs 990.0 pg/g). For the other 15 congeners, the average magnitude of difference between the highest and lowest mean values was a factor of 3.7 for eggs (1.7–8.9 times different) and 3.2 for nestlings (1.7–7.1 times different).

In a multivariate context when the congener pattern is examined in its entirety, the pattern at Greystone differed from the other two ponds and was distinguished by having a lower peak for TCDD (Fig. 2, left side, center); Allendale and Lyman did not differ from one another. The congener pattern was similar between years and between eggs and nestlings (Fig. 2, right panels).

Concentrations of TCDD in tree swallow diet ranged from <1 pg/g to 5.7 pg/g at Greystone and between 71 and 219 pg/g at Allendale and Lyman (Table 3). Because diet samples from 5 to 10 nestlings per pond were pooled, statistical testing was not possible; however, few of the other dioxin or furan congeners were detected at either pond in 2000. In 2001, more dioxin and furan congeners were detected in the diet, with empirically higher concentrations present at Allendale than at either Lyman or Greystone. Sixty-three percent of the diet by frequency was aquatic insects (Table 4). Numerically, Chironomidae and Odonata were the two dominant taxa.

Hexachloroxanthene was not detected in egg samples in 2000 (0 of 20) but was detected in 10 of 28 egg samples in 2001. Hexachloroxanthene was not detected in nestling carcass samples in 2000, but in 2001, Allendale and Lyman had greater concentrations of HCX in carcasses than Greystone (Table 2). Hexachloroxanthene was not detected in the diet samples from Greystone in 2000 or 2001 or from Allendale in 2000. However, HCX was detected in diet samples from Allendale and Lyman in 2001 (Table 3).

Total PCBs averaged 1.05 μg/g (95% CI 0.95–1.16) in eggs and 0.21 μg/g (95% CI 0.17–0.27) in nestlings (Tables 1 and 2). The most prevalent PCB congeners were 90, 118, 129, 153, and 180 in eggs and 90/101, 110, 118, 138/160/163, and 153 in nestling carcasses (Fig. 3). The coplanar congeners (e.g., 77, 126, etc.) were present at ≤0.005 μg/g wet weight in eggs and <0.002 μg/g wet weight in nestlings.

Correlations and accumulation rates

Concentration of TCDD in eggs was highly correlated with the other dioxin congeners except for 1,2,3,4,6,7,8-HpCDD and OCDD (Table 5). Tetrachlorodibenzo-*p*-dioxin in eggs was correlated with the furans as well, except for 1,2,3,4,6,7,8-HpCDF and OCDF. The correlation among dioxin and furan congeners in nestlings (Table 5) was similar to the pattern in eggs, except that TCDD was not correlated with OCDD or OCDF. Hexachloroxanthene in nestlings only correlated with TCDD ($R = 0.731$), not with any other dioxin or furan congeners.

Accumulation rates of TCDD were higher at Allendale and Lyman than at Greystone ([42]; Fig. 4) and were highly correlated with both concentration in eggs ($p = 0.007$, $R = 0.538$, $n = 24$) and nestlings ($p < 0.001$, $R = 0.990$, $n = 24$). The concentrations of TCDD in eggs and nestlings were highly correlated with one another ($p = 0.004$, $R = 0.567$) as well. Concentrations of TCDD in pooled diet were correlated with mean concentrations in nestlings ($p = 0.016$, $R = 0.945$, $n = 5$) and accumulation rate ($p = 0.027$, $R = 0.920$), but not with mean egg concentrations ($p = 0.506$, $R = 0.399$).

Toxic equivalents

In eggs, the TEQs associated with dioxin and furan congeners ($TEQs_{d/f}$) were significantly lower at Greystone than at Allendale in 2000 and Lyman in 2001 (Table 1). For nestlings, $TEQs_{d/f}$ at Greystone were less than at Allendale and Lyman in 2001 (Table 2). Total $TEQs_{d/f}$ and TCDD concentrations were highly correlated ($R = 0.99$ in both eggs and nestlings, Table 5). At Allendale and Lyman, >89% of the total $TEQs_{d/f}$ was associated with TCDD (Table 6). The average percentage of $TEQs_{d/f}$ associated with TCDD was between 42 and 62% at Greystone. The TEQs associated with PCBs were approximately one-half (Allendale) to one-eighth (Lyman) of those associated with the dioxin and furan congeners in eggs and between one fifth and one seventh in nestlings (Tables 1 and 2).

Table 3. Concentrations of dioxin and furan congeners in pooled diet samples from tree swallows nesting along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001

	Concentraton (pg/g wet wt)[b]				
	2000		2001		
Chemical[a]	Allendale	Greystone	Allendale	Greystone	Lyman
2,3,7,8-TCDD	71.4	ND	218.5	5.66	119.5
1,2,3,7,8-PeCDD	ND	ND	1.97	ND	ND
1,2,3,4,7,8-HxCDD	ND	ND	1.41	0.52	0.82
1,2,3,6,7,8-HxCDD	1.48	0.80	7.34	1.85	2.22
1,2,3,7,8,9-HxCDD	ND	0.79	1.83	0.36	0.58
1,2,3,4,6,7,8-HpCDD	ND	ND	55.1	30.3	24.1
OCDD	ND	ND	183.9	62.0	51.1
2,3,7,8-TCDF	0.86	0.70	2.24	0.49	0.92
1,2,3,7,8-PeCDF	ND	ND	1.81	ND	ND
2,3,4,7,8-PeCDF	ND	ND	1.90	0.13	ND
1,2,3,4,7,8-HxCDF	ND	ND	2.81	0.64	0.61
1,2,3,6,7,8-HxCDF	ND	ND	2.54	0.41	0.41
1,2,3,7,8,9-HxCDF	1.40	0.86	1.45	ND	ND
2,3,4,6,7,8-HxCDF	ND	ND	2.26	ND	0.17
1,2,3,4,6,7,8-HpCDF	ND	ND	12.9	4.21	3.20
1,2,3,4,7,8,9-HpCDF	ND	ND	0.94	0.30	0.15
OCDF	ND	ND	1.07	1.83	0.71
1,2,4,5,7,8-HCX	ND	ND	126	ND	202

[a] TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; HCX = hexachloroxanthene.
[b] ND = not detected.

Other chemical contaminants

Only 7 of 22 other organochlorine chemicals were detected in tree swallow egg or nestling samples (Table 7). Of the seven organochlorine chemicals detected, none varied among study ponds in eggs. In nestlings, *p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDT varied among study ponds, with Lyman having significantly higher concentrations than Greystone (Table 8). Alpha chlordane, chlordane, and dieldrin did not vary among study ponds in nestling samples (Table 7). Concentrations in both tissue types were <0.5 μg/g wet weight except for total PCBs, which averaged 1.1 μg/g wet weight in eggs and 0.6 μg/g wet weight in nestling carcasses.

Trace elements

Twenty of 21 trace elements were detected in tree swallow nestling livers (Tables 8 and 9). Only beryllium was detected in <50% of samples (7 of 15 livers). Cadmium, manganese, and thallium differed among study ponds, with Allendale having significantly higher concentrations than either Greystone or Lyman (Table 8).

Nesting success

The percentage of eggs that hatched was significantly higher at Greystone than at Allendale during 2000 and 2001 (Table 10). Only 47 and 51% of eggs hatched at Allendale compared

Table 4. Invertebrate composition of diet in five tree swallow broods that nested along the Woonasquatucket River, Providence County (RI, USA) in 2001

Location of brood[a]	No. invertebrates[b]	Taxa	Habitat
Greystone	23	*Einfeldia,* Chironomidae	Aquatic
	1	Coenagrionidae, Odonata	Aquatic
	4	Formicidae, Homoptera, Coleoptera	Terrestrial
Lyman	14	*Enallagma,* Odonata	Aquatic
	13	Chironomidae	Aquatic
	1	*Perithemus,* Odonata	Aquatic
	3	Lepidoptera, Homoptera, Diptera	Terrestrial
Lyman	4	*Ischnura,* Odonata	Aquatic
	2	Chironomidae	Aquatic
	1	Calliphoridae	Terrestrial
Lyman	3	Chironomidae	Aquatic
	2	Odonata	Aquatic
	8	Homoptera, Hemiptera, Hymenoptera, Diptera, Arachnida	Terrestrial
Allendale	1	*Perithemis,* Odonata	Aquatic
	9	Hymenoptera, Calliphoridae, Diptera	Terrestrial

[a] Each sample listed is an individual brood.
[b] One food bolus was analyzed from each brood.

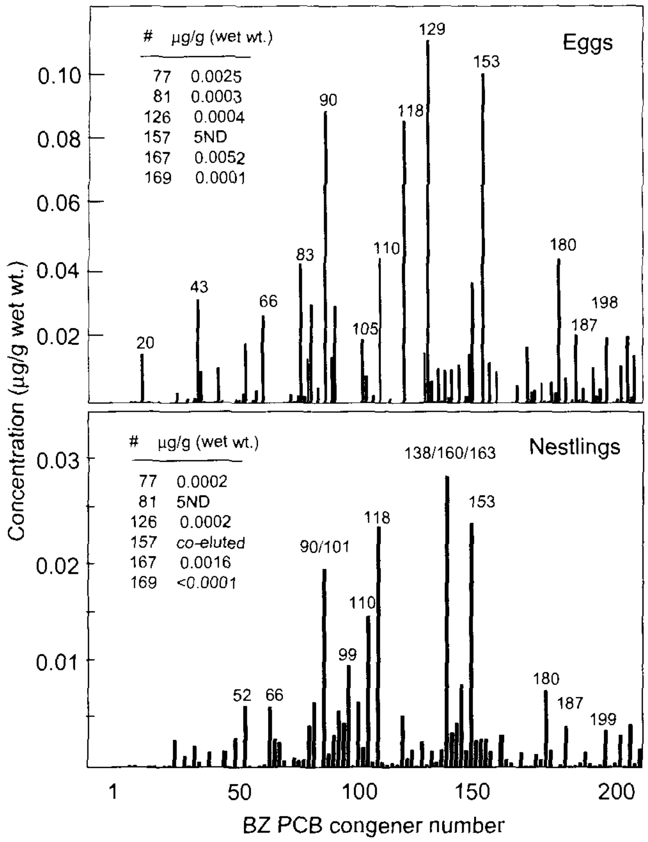

Fig. 3. Concentrations of polychlorinated biphenyls (PCBs, µg/g wet wt) congeners by Ballschmiter and Zell (BZ) numbers in tree swallow eggs (**upper panel**) and nestling carcasses (**lower panel**) from along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001. Major peaks are identified, and concentrations of the non-ortho and mono-*ortho* congeners are listed. Number in front of ND is number not detected. Note the different scales on the *y*-axes.

with 92 and 77% at Greystone for the two years, respectively. Seventy percent of the eggs hatched at Lyman. Forty-seven percent and 57% of clutches were total failures at Allendale (Table 11), and 27% were total failures at Lyman. Zero percent and 17% were total failures at Greystone. Mean concentration of TCDD in clutches that had hatching failures (mean = 520.8 pg/g wet wt, 95% CI 404.0–671.4, $n = 23$) were significantly higher ($p = 0.004$, $df = 1, 46$) than concentrations in successful clutches (mean = 124.8 pg/g wet wt, 95% CI 84.9–183.4, $n = 25$; Fig. 5). The estimated value at which hatching success of the clutch was reduced to 50% was 1,694 pg/g TCDD (probit analysis, Fig. 5). Total hatching failures became common beginning at 180 pg/g TCDD.

The survival of nestlings was significantly less at Allendale than at Greystone in 2000, but differences were not seen among ponds in 2001 (Table 10). Overall, the percentage of the nestlings that hatched and survived until 12 days of age was 89%.

Bioindicators

In 2000, EROD activity was significantly induced at Allendale compared with the two out-of-basin reference areas, but Allendale was not statistically different from Greystone (Table 12). This lack of difference between Allendale and Greystone in 2000 was because one bird was induced at Greystone and one bird was not induced at Allendale. Both Allendale and Lyman had significantly induced EROD in 2001 compared with Greystone and the out-of-basin reference site. Ethoxyresorufin-*O*-dealkylase activity was similar between Greystone and the out-of-basin reference locations in eastern Minnesota and western Massachusetts in 2000 and 2001.

The HPCV in DNA content of 10,000 red blood cells per individual did not differ among the three Woonasquatucket River ponds, but the eastern Minnesota reference location had significantly greater HPCV than Allendale ($p = 0.002$, $df = 3, 19$; Fig. 6). The eastern Minnesota site did not differ from either Lyman or Greystone. HPCV and EROD were not correlated ($p = 0.838$, $n = 12$) nor were HPCV and TCDD concentrations ($p = 0.562$, $n = 11$).

The absolute difference in brain height ($p = 0.148$, $df = 2, 7$) or width ($p = 0.666$, $df = 2, 4$) between the right and left halves of individual brains of 12-day-old nestlings did not differ among ponds. Additionally, concentration of TCDD in the nestling carcass and the absolute difference in size between the right and left sides were not correlated either for height ($p = 0.229$; Fig. 7) or width ($p = 0.752$).

Neither body weight ($p = 0.972$, $df = 2, 12$), liver weight ($p = 0.542$), nor liver to body weight ratios ($p = 0.391$) were significantly different among the three ponds in 2001. Mean weights ± 1 SE ($n = 15$) were 23.5 ± 0.35 g (whole body), 1.08 ± 0.03 g (liver), and 0.046 ± 0.001 (liver to body ratio).

DISCUSSION

Chemical contamination

Concentrations of TCDD in tree swallow eggs at Allendale (mean > 300 pg/g) and Lyman (mean > 1,000 pg/g) were consistently higher than at the upstream reference pond (Greystone, mean < 30 pg/g) and are hundreds of times higher than reported elsewhere for tree swallows. Ankley et al. [12] reported that tree swallow eggs contained 5.3 pg/g TCDD at locations along the Fox River and lower Green Bay, Lake Michigan (MI, USA). Only 13% of tree swallow clutches contained detectable concentrations (≥1 pg/g) of TCDD from locations along the Wisconsin River, in central Wisconsin (USA) downstream of historic pulp and paper mills, or along the Housatonic River (Massachusetts) [6,8].

Tree swallows at Allendale and Lyman also had much higher concentrations of TCDD in eggs than piscivorous birds in TCDD-contaminated habitats. Great blue herons (*Ardea herodias*) nesting in British Columbia in the vicinity of paper mills contained geometric mean concentrations between 4 and 209 pg/g wet weight TCDD in eggs [43]. Double-crested cormorants (*Phalacrocorax auritus*) nesting at two colonies in the late 1980s within 10 to 15 km of pulp and paper mills in the Strait of Georgia (British Columbia, Canada) contained between 30 and 74 pg/g wet weight TCDD in eggs [44]. Osprey (*Pandion heliaetus*) nesting along the Wisconsin River in central Wisconsin contained between 59 and 108 pg/g wet weight TCDD in eggs [45]. Osprey nesting downstream of pulp and paper mills in the Fraser River basin (BC, Canada) contained average concentrations of TCDD between 5.5 and 47 pg/g wet weight; those nesting in the Columbia River basin (WA, USA) contained between 9.5 and 21 pg/g wet weight TCDD in eggs [46]. Forster's terns (*Sterna forsteri*) in Green Bay contained only between 5 and 20 pg/g wet weight TCDD in eggs [12].

The preponderance of TCDD relative to the other congeners was unique and was probably related to the chemical manufacturing that was thought to have occurred at the Superfund Site [1]. A common by-product of the manufacture of hexachlorophene in the 1960s and 1970s was TCDD and HCX [1]. In contrast, at pulp and paper mill sites in British Columbia,

Table 5. Correlation matrix of dioxin and furan congeners and World Health Organization (WHO) toxic equivalents (TEQs) from tree swallows nesting along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001; R values, p values, and sample sizes are provided in the upper right half of the table for eggs and the lower left half for nestlings

	2,3,7,8 -TCDD	1,2,3,7,8 -PeCDD	1,2,3, 4,7,8 -HxCDD	1,2,3, 6,7,8 -HxCDD	1,2,3, 7,8,9 -HxCDD	1,2,3, 4,6,7,8 -HpCDD	OCDD	2,3,7,8- TCDF	1,2,3,7,8- PeCDF	2,3,4,7,8- PeCDF	1,2,3, 6,7,8 -HxCDF	1,2,3, 7,8,9 -HxCDF	1,2,3,4, 6,7,8 -HpCDF	OCDF	2,3,4, 6,7,8 -HxCDF	1,2,4, 5,7,8 -HCX	TEQs WHO
2,3,7,8-TCDD		0.399 0.006 46	0.402 0.005 48	0.312 0.031 48	0.398 0.005 48	0.132 0.371 48	0.104 0.482 48	0.492 <0.001 48	0.386 0.009 45	0.440 0.003 45	0.332 0.023 47	0.491 <0.001 47	0.151 0.305 48	0.126 0.402 46	0.445 0.002 45	0.731 0.016 10	0.999 <0.001 48
1,2,3,7,8-PeCD	0.840 <0.001 24		0.962 <0.001 46	0.851 <0.001 46	0.795 <0.001 46	0.180 0.232 46	−0.024 0.874 46	0.583 <0.001 46	0.691 <0.001 44	0.816 <0.001 44	0.456 0.001 46	0.751 <0.001 46	0.036 0.812 46	−0.083 0.587 45	0.691 <0.001 44	−0.049 0.901 9	0.419 0.004 46
1,2,3,4,7,8-HxCDD	0.832 <0.001 25	0.963 <0.001 24		0.880 <0.001 48	0.849 <0.001 48	0.211 0.149 48	−0.038 0.796 48	0.558 <0.001 48	0.718 <0.001 45	0.801 <0.001 45	0.510 <0.001 47	0.816 <0.001 47	0.072 0.626 48	-0.063 0.675 46	0.747 <0.001 45	0.112 0.758 10	0.422 0.003 48
1,2,3,6,7,8-HxCDD	0.740 <0.001 25	0.919 <0.001 24	0.940 <0.001 25		0.771 <0.001 48	0.410 0.004 48	0.151 0.307 48	0.363 0.011 48	0.797 <0.001 45	0.796 <0.001 45	0.670 <0.001 47	0.847 <0.001 47	0.300 0.039 48	0.171 0.256 46	0.846 <0.001 45	−0.048 0.894 10	0.330 0.022 48
1,2,3,7,8,9-HxCDD	0.786 <0.001 24	0.794 <0.001 23	0.808 <0.001 24	0.855 <0.001 24		0.481 0.005 48	0.210 0.152 48	0.383 0.007 48	0.589 <0.001 45	0.650 <0.001 45	0.662 <0.001 47	0.914 <0.001 47	0.374 0.009 48	0.205 0.171 46	0.878 <0.001 45	0.182 0.615 10	0.413 0.004 48
1,2,3,4,6,7,8-HpCDD	0.496 0.012 25	0.616 0.001 24	0.702 <0.001 25	0.775 <0.001 25	0.661 <0.001 24		0.904 <0.001 48	−0.168 0.253 48	0.179 0.239 45	0.043 0.781 45	0.759 <0.001 47	0.564 <0.001 47	0.930 <0.001 48	0.848 <0.0001 46	0.626 <0.0001 45	0.005 0.989 10	0.133 0.366 48
OCDD	0.168 0.421 25	0.361 0.083 24	0.392 0.053 25	0.475 0.016 25	0.377 0.070 24	0.871 <0.001 25		−0.226 0.122 48	−0.006 0.971 45	−0.149 0.330 45	0.677 <0.001 47	0.327 0.025 47	0.911 <0.001 48	0.914 <0.001 46	0.369 0.013 45	−0.245 0.496 10	0.100 0.499 48
2,3,7,8-TCDF	0.796 <0.001 25	0.792 <0.001 24	0.792 <0.001 25	0.680 <0.001 25	0.736 <0.001 24	0.368 0.070 25	0.606 0.774 25		0.490 <0.001 45	0.585 <0.001 45	0.213 0.152 47	0.432 0.002 47	−0.146 0.323 48	−0.147 0.331 46	0.343 0.021 45	0.540 0.107 10	0.508 <0.001 48
1,2,3,7,8-PeCDF	0.798 <0.001 25	0.813 <0.001 24	0.829 <0.001 25	0.767 <0.001 25	0.765 <0.001 24	0.434 0.030 25	0.166 0.429 25	0.863 <0.001 25		0.877 <0.001 43	0.588 <0.001 44	0.741 <0.001 44	0.151 0.323 45	0.079 0.616 43	0.694 <0.001 43	0.144 0.691 10	0.402 0.006 45
2,3,4,7,8-PeCDF	0.677 <0.001 24	0.688 <0.001 23	0.681 <0.001 24	0.695 <0.001 24	0.785 <0.001 23	0.351 0.093 24	0.105 0.626 24	0.712 <0.001 24	0.832 <0.001 24		0.476 <0.001 45	0.700 <0.001 45	−0.026 0.867 45	−0.102 0.510 44	0.650 <0.001 44	−0.153 0.672 10	0.458 0.002 45
1,2,3,6,7,8-HxCDF	0.766 <0.001 25	0.816 <0.001 24	0.808 <0.001 25	0.751 <0.001 25	0.812 <0.001 24	0.534 0.006 25	0.337 0.099 25	0.802 <0.001 25	0.891 <0.001 25	0.815 <0.001 24		0.830 <0.001 47	0.781 <0.001 47	0.674 <0.001 46	0.798 <0.001 45	−0.037 0.919 10	0.340 0.019 47
1,2,3,7,8,9-HxCDF	0.920 <0.001 25	0.850 <0.001 24	0.864 <0.001 25	0.773 <0.001 25	0.830 <0.001 24	0.592 0.002 25	0.287 0.165 25	0.890 <0.001 25	0.885 <0.001 25	0.716 <0.001 24	0.899 <0.001 25		0.514 <0.001 47	0.353 0.016 46	0.954 <0.001 45	0.610 0.061 10	0.505 <0.001 47
1,2,3,4,6,7,8-HpCDF	0.581 0.003 24	0.739 <0.001 23	0.734 <0.001 23	0.737 <0.001 23	0.738 <0.001 23	0.840 <0.001 24	0.764 <0.001 24	0.570 0.004 24	0.691 <0.001 24	0.537 0.008 23	0.783 <0.001 24	0.743 <0.001 24		0.915 <0.001 46	0.590 <0.001 45	−0.136 0.708 10	0.150 0.309 48
OCDF	0.057 0.787 25	0.322 0.125 24	0.144 0.494 25	0.237 0.254 25	0.450 0.027 24	0.204 0.327 25	0.360 0.077 25	0.158 0.449 25	0.277 0.181 25	0.526 0.008 24	0.443 0.027 25	0.162 0.440 25	0.439 0.032 24		0.419 0.005 44	−0.481 0.159 10	0.123 0.417 46
2,3,4,6,7,8-HxCDF	0.765 <0.001 23	0.789 <0.001 22	0.830 <0.001 23	0.823 <0.001 23	0.921 <0.001 23	0.633 0.001 23	0.348 0.104 23	0.709 <0.001 23	0.799 <0.001 23	0.683 0.001 22	0.830 <0.001 23	0.850 <0.001 23	0.796 <0.001 22	0.309 0.150 23			0.457 0.002 45
WHO TEQs	0.999 <0.001 25	0.841 <0.001 24	0.834 <0.001 25	0.741 <0.001 25	0.787 <0.001 24	0.496 0.012 25	0.168 0.421 25	0.798 <0.001 25	0.800 <0.001 25	0.679 <0.001 24	0.768 <0.001 25	0.921 <0.001 25	0.582 0.003 24	0.058 0.781 25	0.766 <0.001 23		

[a] TCDD = tetrachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; HxCD = hexachlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran; PeCDF = pentachlorodibenzofuran; HxCDF = hexachlorodibenzofuran; HpCDF = heptachlorodibenzofuran; OCDF = octachlorodibenzofuran; HCX = hexachloroxanthene.

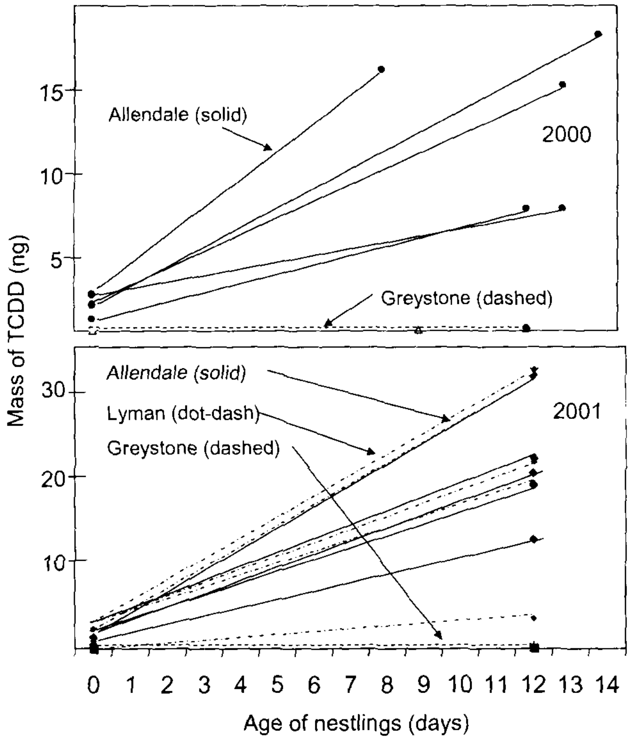

Fig. 4. Accumulation rates (slope of lines) for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in tree swallows nesting along the Woonasquatucket River, Providence County (RI, USA) in 2000 (**upper panel**) and 2001 (**lower panel**). The five dashed lines from Greystone are superimposed on each other along the x-axis of each panel at this scale.

1,2,3,6,7,8-HxCDD was the most prevalent congener in great blue heron eggs [42] followed by 1,2,3,7,8-PeCDD. In bald eagle nestlings (*Haliaeetus leucocephalus*), 1,2,3,6,7,8-HxCDD was also the dominant congener in the yolk sac [47], as it was in whole eggs of double-crested cormorants [44]. The congener TCDD was only occasionally (<15%) most prevalent in those two studies. Where dioxin and furan contamination was a cocontaminant with PCBs, TCDF followed by PeCDF were the dominant congeners in tree swallow eggs [43]. The similar pattern among ponds for the other dioxin and furan congeners argues for a more regional source. The sources for the other dioxin and furan congeners in the Woonasquatucket River are unknown; however, it has been a working industrial river since the first mill was built on its banks in 1809. Also, dioxins and furans are trace impurities in commercial herbicides and chlorophenols and can be produced by photochemical and thermal reaction in fly ash and by municipal and industrial incineration processes [48].

The results obtained with univariate statistical tests for each dioxin and furan congener individually were similar to the results obtained by the multivariate ANOSIM test that compared congener patterns in their entirety. Results of the ANOSIM indicated that the pattern of congeners differed at Greystone compared with the other two ponds, whereas matrices (eggs and nestlings) and years (0.058) did not differ. The portion of TCDD in the total dioxin and furan mixture was the predominant difference among ponds.

Diet

Diet samples at Allendale (71–219 pg/g TCDD) and Lyman (120 pg/g TCDD) were between 6 and 18 times above safe

Table 6. Percentage of World Health Organization dioxin and furan congener toxic equivalents (mean and 1 SE) that were associated with 2,3,7,8-tetrachlorodibenzo-p-dioxins in tree swallow egg and nestling carcass samples from along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001

Year and pond	Mean[a]	n	SE	Minimum	Maximum
Eggs					
2000					
Allendale	94.5 A	13	0.9	88.1	97.7
Greystone	41.5 C	7	6.1	22.3	60.1
2001					
Allendale	89.4 A	13	2.7	63.2	97.9
Greystone	62.3 B	6	9.6	37.8	91.6
Lyman	96.6 A	9	0.4	94.8	98.9
Nestling carcasses					
2000					
Allendale	98.3 A[a]	5	0.2	97.5	98.7
Greystone	45.6 C	5	1.9	40.9	51.4
2001					
Allendale	98.5 A	5	0.2	97.9	99.0
Greystone	55.1 B	5	7.9	29.9	77.4
Lyman	97.8 A	5	0.8	94.5	99.0

[a] Means sharing same letter are not significantly different among year-pond (eggs: $p < 0.001$, $df = 4, 43$; carcasses: $p < 0.001$, $df = 4, 20$).

levels for birds and much higher than reported in avian diets elsewhere. The concentration of TCDD in an avian diet that is considered safe is 10 to 12 pg/g wet weight [48]. Concentrations of the sum of the polychlorinated dibenzo-p-dioxins (PCDD) present in fish from the Strait of Georgia in the 1980s and early 1990s near pulp and paper mills ranged between <0.1 and 90 pg/g wet weight [43]. Concentrations of TCDD in largemouth bass (*Micropterus salmoides*) from Lyman ranged between 100 and 300 pg/g wet weight [49]. The PCDDs

Table 7. Geometric mean concentrations of organochlorine chemicals in tree swallow egg ($n = 8$) and nestling carcass samples ($n = 15$) that did not differ among three ponds along the Woonasquatucket River, Providence County (RI, USA) in 2001

Chemical[a]	Concentration (μg/g wet wt) and 95% CI: Concentration (μg/g wet wt)	95% CI	p Value (among ponds)
Eggs			
p,p'-DDE	0.309	0.251–0.379	0.343
p,p'-DDD	0.006	0.005–0.007	0.728
p,p'-DDT	0.005	0.004–0.005	0.165
Alpha chlordane	0.009	0.006–0.014	0.908
Chlordane	0.457	0.371–0.565	0.945
Dieldrin	0.017	0.015–0.020	0.069
Nestling carcasses			
Alpha chlordane	0.005	0.004–0.007	0.226
Chlordane	0.326	0.285–0.372	0.055
Dieldrin	0.007	0.006–0.007	0.382
Total PCBs	0.64	0.35–1.17	—[b]

[a] p,p'-DDD = 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane; p,p'-DDE = 1,1-dichloro-2,2-bis(p-chlorophenyl)ethene; p,p'-DDT = 1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane; PCBs = polychlorinated biphenyls.

[b] No statistical analysis because only one sample per pond was analyzed for total PCBs.

Table 8. Geometric mean concentrations of organochlorine chemicals in tree swallow nestling carcasses and trace elements in liver samples (n = 5 per pond) that varied among ponds from along the Woonasquatucket River, Providence County (RI, USA) in 2001

Chemical[a]	Greystone	Allendale	Lyman	p Value
	Concentratoin (µg/g wet wt) and 95% CI[b]			
Organochlorine chemicals				
p,p'-DDE	0.017 B 0.015–0.019	0.025 AB 0.024–0.026	0.036 A 0.030–0.044	0.009
p,p'-DDD	0.003 B 0.002–0.003	0.003 AB 0.003–0.004	0.005 A 0.004–0.005	0.017
p,p'-DDT	0.001 AB 0.001–0.002	0.003 A 0.002–0.003	0.0009 B 0.0007–0.001	0.015
Trace elements				
Cadmium	0.009 B 0.008–0.010	0.021 A 0.017–0.028	0.010 B 0.009–0.011	0.006
Manganese	1.14 B 1.06–1.22	1.59 A 1.49–1.70	1.15 B 1.10–1.20	0.003
Thallium	0.005 B 0.004–0.005	0.008 A 0.008–0.009	0.005 B 0.004–0.005	<0.001

[a] *p,p'*-DDD = 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane; *p,p'*-DDE = 1,1-dichloro-2,2-bis(*p*-chlorphenyl)ethene; *p,p'*-DDT = 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane.
[b] Means sharing same letter are not significantly different among ponds.

in swallow food (insects) at Allendale and Lyman were more than four and two times the concentration found in fish in British Columbia, and in the same general range as largemouth bass from Lyman. Because insects generally contain less organic contaminants than fish because of their lower position on the food chain, the elevation of total dioxins in food items from Allendale and Lyman is extreme.

Sediment associations

Sixty-three percent of tree swallow diet was aquatic insects, and aquatic insects accumulate contaminants from sediments [18]. The correlation coefficient between concentrations of TCDD in diet and nestlings was 0.94, indicating food as the primary route of exposure. Additionally, the pattern of high concentrations of TCDD and HCX in tree swallow nestling tissues in Allendale and Lyman, but not in an upstream pond, is similar to the pattern of sediment contamination [1] for TCDD and HCX. Concentrations of TCDD and HCX averaged ~5,000 and 20,000 pg/g in sediments at Allendale and Lyman, but neither was detected in Greystone sediments. Proportionately more TCDD than HCX was found in bird tissues on the basis of the sediment profile. Hexachloroxanthene was detected in 0 of 20 eggs in 2000 and 10 of 28 eggs in 2001. The majority of diet and nestling samples in 2001 had detectable HCX concentrations. Hexachloroxanthene might not transfer into eggs; it might be metabolized while in the egg during incubation, or a shift in diet between the egg-laying and nestling period could account for this difference. No published information on HCX concentrations in bird tissues has been found.

Table 9. Trace element geometric mean concentrations for elements that did not vary among ponds in tree swallow nestling livers (n = 15) from the Woonasquatucket River, Providence County (RI, USA) in 2001

Trace element	Concentration (µg/g wet wt)	95% CI	p Value (among ponds)
Aluminum	0.41	0.251–0.379	0.651
Antimony	0.001	0.0009–0.0016	0.388
Arsenic	0.035	0.031–0.039	0.525
Barium	0.021	0.018–0.025	0.230
Chromium	0.085	0.075–0.095	0.750
Cobalt	0.008	0.008–0.009	0.130
Copper	4.95	4.64–5.28	0.129
Iron	272.1	245.7–301.3	0.109
Lead	0.022	0.017–0.029	0.226
Mercury	0.043	0.040–0.045	0.663
Methylmercury	0.035	0.033–0.036	0.629
Molybdenum	0.57	0.55–0.59	0.308
Nickel	0.017	0.016–0.020	0.386
Selenium	0.53	0.51–0.55	0.276
Silver	0.009	0.007–0.012	0.686
Vanadium	0.006	0.004–0.008	0.620
Zinc	20.2	19.7–20.8	0.757

Nesting success

The probability of an egg hatching from all eggs laid was significantly higher at Greystone (77–92%), the reference pond, than at Allendale (47–51%). Hatching success was reduced at Lyman (70% of eggs hatched), although it was not statistically different from Greystone. The hatching success rate at Allendale was also far below the nationwide average of 87% for hatching success in successful nests (at least one egg hatched) [50]. The 70% hatching success was also below levels found in other tree swallow studies in the northeastern United States during a similar time period, when hatching success averaged 87.2% over three years [6]. Reduced hatching tended to be the result of total clutch failure and not because of partial losses within a clutch. Concentration of TCDD at which total failure became common was 180 pg/g wet weight. Regardless of study pond, clutches that had dead embryos or more than two eggs that failed to hatch had significantly higher concentrations of TCDD (521 pg/g) than clutches in which eggs hatched normally. Percent hatching at Greystone in 2001 was 77%. The TCDD concentration in the two clutches that contributed to this slightly lower hatching rate at Greystone in 2001 were 284 and 130 pg/g wet weight. Both were elevated. Some clutches with high TCDD contamination hatched normally. This is consistent with other field studies [6]. The probability of reduced hatching increases as contaminant concentration increases; it is rarely an absolute threshold.

Table 10. Daily survival probabilities during the incubation and nestling periods for tree swallows nesting along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001

Year and pond	Daily survival probability[a]			Percent successful[b]
	Mean	SE	95% CI	
Incubation				
2000				
Greystone	0.9937 A	0.0036	0.9866–1.0008	92
Allendale	0.9436 B	0.0075	0.9289–0.9582	47
2001				
Greystone	0.9796 A	0.0053	0.9693–0.9900	77
Allendale	0.9497 B	0.0084	0.9332–0.9662	51
Lyman	0.9739 AB	0.0062	0.9618–0.9860	70
Nestling				
2000				
Greystone	1.0000 A	—	—	100
Allendale	0.9727 B	0.0097	0.9535–0.9915	71
2001				
Greystone	0.9904 A	0.0055	0.9796–1.0012	89
Allendale	0.9904 A	0.0055	0.9796–1.0012	89
Lyman	1.0000 A	—	—	100

[a] Means within year sharing same letter are not significantly different. Incubation $\chi^2 = 36.3$, $df = 1$, $p < 0.001$ (2000); $\chi^2 = 9.2$, $df = 2$, $p = 0.010$ (2001). Nestling period $\chi^2 = 7.92$, $df = 1$, $p = 0.005$ (2000); $\chi^2 = 0.00$, $df = 1$, $p = 1.000$ (Greystone vs Allendale, 2001); $\chi^2 = 3.046$, $df = 1$, $p = 0.0810$ (Allendale vs Lyman, 2001); $\chi^2 = 3.046$, $df = 1$, $p = 0.0810$ (Greystone vs Lyman, 2001). — = No variance estimates because all nestlings survived.

[b] Percent success for incubation was calculated by raising daily survival probability to the 13th power. Thirteen days is the length of the incubation period. For the nestling period, the percentage was calculated by raising the daily survival probability to the 12th power.

Most field studies have not found dioxin and furan congener effects on hatching success. An exception is White and Seginak [51], who found reduced hatching success of wood duck (*Aix sponsa*) eggs in Arkansas. Woodford et al. [45] found no effect of dioxin and furan congeners on hatching success of osprey nesting along the Wisconsin River below pulp and paper mills. Mean TCDD concentration ranged between 59 and 108 pg/g wet weight, and they estimated a no-effect level in eggs of 136 pg/g. Elliott et al. [52] found no difference among four great blue heron colonies in average number of young fledged per successful nest and no relationship between dioxin and furan concentrations and hatching success. Mean concentrations of TCDD in eggs at those four colonies ranged between 9 and 210 pg/g wet weight. Both of these studies had concentrations much lower than present in our study and are consistent with our data, with which we estimated a 50% reduction in hatching at approximately 1,700 pg/g wet weight. Concentrations of dioxin and furan congeners also were not related to hatching success of bald eagle or osprey eggs in British Columbia [53,54]. Concentrations were reported from either blood plasma (wet weight) or yolk sacs (lipid weight), so they cannot be compared directly with this tree swallow study. Elliott et al. [53], however, estimated TCDD concentrations in whole osprey eggs of between 2 and 4 pg/g wet weight, so they are considerably lower than even our reference pond at Greystone. On the Housatonic River in western Massachusetts, hatching success was negatively correlated with the sum of the dioxin and furan congeners [6]. The average concentration of the sum of the dioxin and furan congeners in clutches that experienced hatching problems was 451 pg/g wet weight [6], which was similar to the 521 pg/g found on the Woonasquatucket River study ponds.

The estimated LCE50 for tree swallows is approximately

Table 11. Number of nest attempts and number and percentage of nest attempts in which all eggs failed to hatch for tree swallows nesting along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001

Pond	No. nest attempts[a]	No. total failures	Percent total failures
2000			
Greystone	8	0	0
Allendale	23	13	57
2001			
Greystone	12	2	17
Allendale	17	8	47
Lyman	11	3	27

[a] Does not include dump eggs (clutches of 1) or any clutch lost because of human disturbance or predation.

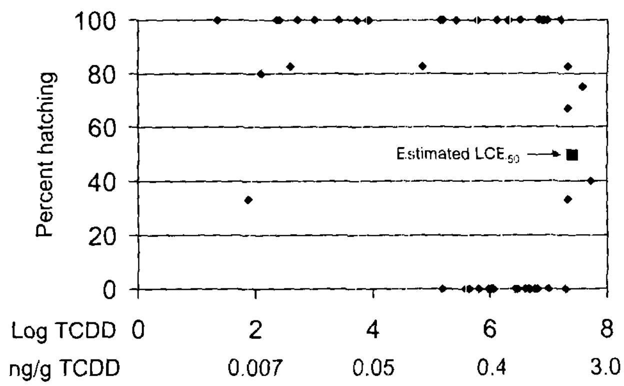

Fig. 5. Plot of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, ng/g wet wt) in eggs, and percent hatching of tree swallow clutches for birds nesting along the Woonasquatucket River, Providence County (RI, USA) in 2000 and 2001. Each point on the graph represents an individual clutch of eggs. LCE50 = concentration in eggs in which hatching was reduced by 50%.

Table 12. Ethoxyresorufin-*O*-dealkylase (EROD) activity in tree swallow liver samples from the Woonasquatucket River, Providence County (RI, USA), area and at out-of-basin reference areas in 2000 and 2001

Location	EROD activity (pmol/min/mg)[a]	SE	n	Minimum	Maximum
2000					
Greystone	44.6 AB	11.5	5	28.4	90.3
Allendale	66.9 A	16.1	5	4.9	92.0
E. Minnesota	20.9 B	1.9	20	3.4	35.8
W. Massachusetts	20.6 B	2.0	7	12.5	26.8
2001					
Greystone	45.6 B	8.6	5	18.3	65.7
Allendale	210.3 A	44.0	5	49.0	307.9
Lyman	260.3 A	22.5	5	190.0	331.9
E. Minnesota	49.4 B	9.8	14	6.5	129.7

[a] Means sharing same letter are not significantly different. Each year analyzed separately. 2000: $p < 0.001$, $df = 3, 33$; 2001: $p < 0.001$, $df = 3, 25$.

10 times higher than the estimate for chickens (*Gallus domesticus*), but similar to other species. The estimated LCE50 for tree swallows, on the basis of probit analysis was 1,700 pg/g wet weight. The LD50 (dose killing 50% of test organisms) for injected chicken eggs is 150 pg/g [55]. Eggs were injected before incubation into the yolk. Chickens are considered one of the most sensitive avian species [48,56]. The LD50 for TCDD injected into double-crested cormorant eggs is between 5 and 26 ng/g wet weight [57]. These data should be used carefully, however, because the authors were extrapolating beyond the limits of their data. Regardless, the lower range of the Powell's cormorant estimate is slightly higher than we estimated for tree swallows. The LD50 value for pheasant eggs (*Phasianus colchicus*) was between 1.4 and 2.2 ng/g [58], depending on whether the TCDD was injected into the albumin or yolk, which was similar to the concentrations we estimated for tree swallows.

Dioxin and furan congeners seemed to have no effect on nestling survival or growth. Survival of young to 12 days of age was generally good (89%) at all ponds. Survival of young at Allendale in 2000, however, was significantly less than at Greystone in 2000. We do not think it was related to dioxin contamination, however, because nestling survival was equally good at all ponds in 2001, even though dioxin and furan concentrations differed widely among ponds. Additionally, the nestling mortality in 2000 occurred during a cold period and might have been the result of hypothermia or lack of insects because of cold, rainy weather, as has been reported in other studies [6,59]. Glick [60] found a positive linear relationship between the number of flying insects collected and air temperatures between 2 and 24°C. When temperatures were 10 to 13°C, fewer than half the numbers of insects were caught compared with temperatures of 24°C. Feeding by swifts in Finland ceased when the average daily temperature dropped to 12°C [61]. The average minimum daily low temperature in Providence during the period of nestling mortality was 11°C. Additionally, the dam on Allendale breached during this time frame (C.J. Rosiu, personal communication), dropping the water level of the pond. A combination of all these factors might have contributed to poor nestling survival in 2000. Whole body weights and liver weights of 12-day-old nestlings did not differ among ponds in 2001, also indicating no effect of dioxin and furan congeners during the nestling phase on these two measurements.

Other chemicals

Other organochlorine chemicals were either not detected or were present at background levels. Total PCBs averaged ≤1 μg/g wet weight in eggs and nestlings and did not vary among study ponds. These egg concentrations were too low to contribute to poor hatching success [2,6]. On the Housatonic River, reduced hatching was detected beginning at 30 to 50 μg/g wet

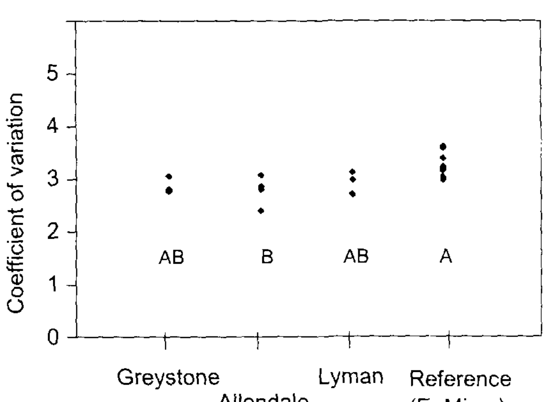

Fig. 6. Half-peak coefficient of variation of deoxyribonucleic acid (DNA) content for tree swallow nestling blood samples. Woonasquatucket River, Providence County (RI, USA) and eastern Minnesota (USA) 2001. Means sharing same letter are not significantly different.

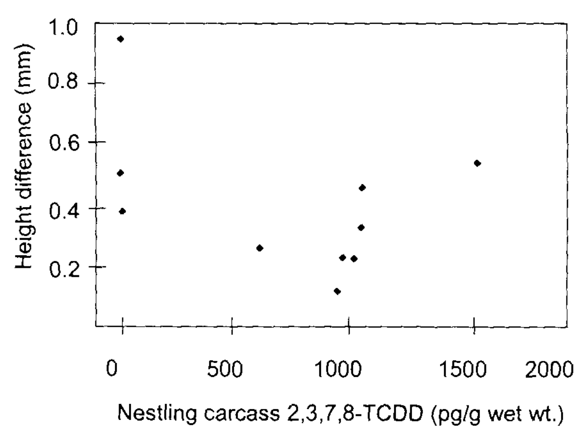

Fig. 7. Measurement of asymmetry for brain height plotted against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) concentration in nestling tree swallow carcasses, Woonasquatucket River, Providence County (RI, USA) and eastern Minnesota (USA) in 2001.

weight total PCBs in eggs [6]. Concentrations of DDE (<0.3 μg/g wet wt) were also below known-effects levels [62,63]. The most sensitive avian species have a threshold of 3 to 6 μg/g wet weight DDE in eggs before hatching success becomes compromised. Few other organochlorine chemicals were even detected.

Trace elements in swallow livers were at background concentrations [4,7]. Mercury (0.14 μg/g dry wt, converted from wet weight with the use of 70% moisture) concentrations along the Woonasquatucket River did not vary among ponds and were the same order of magnitude as tree swallow livers from Colorado (0.067 μg/g dry wt) [4] and Wyoming (0.2 μg/g dry wt) [7]. Mercury concentrations were 18 times less than the 2.5 μg/g dry weight that has been found to be detrimental to reproduction in birds [64]. Cadmium concentrations (0.4 μg/g dry wt) in livers were 10 times lower than the threshold effects level for birds [65].

Toxic equivalents

For dioxin and furan congeners and PCBs, nearly all toxicity was associated with the dioxin and furan congeners (500–1,100 pg/g) in the two contaminated ponds on the Woonasquatucket River. The TEQs associated with PCBs (<220 pg/g) were two to five times lower than those for dioxin and furan congeners. Additionally, >89% of the dioxin and furan toxicity was associated with a single congener, TCDD.

The high TEQs associated with PCBs along the Housatonic and Hudson Rivers without a serious effect on hatching success calls into question whether TEFs developed for PCBs are appropriate to predict hatching effects in field studies. The $TEQs_{d/f}$ on the Woonasquatucket River were associated with reduced hatching success. On the basis of the effect levels generated from the Woonasquatucket River data (LCE50 1,700 pg/g TEQs), catastrophic effects on hatching success would be predicted with the TEQs levels found on the Housatonic and Hudson Rivers. The TEQs in eggs associated with PCBs along the Housatonic River ranged between 6,900 and 22,000 pg/g and did not correlate with reduced hatching success [6] when included in a model that also included TEQs associated with dioxin and furan congeners. On the Hudson River, TEQs associated with PCBs in eggs were estimated to be between 1,730 and 12,700 pg/g [66], again with little effect on hatching success [67]. In contrast, the sum of the dioxin and furan concentrations and the $TEQs_{d/f}$ on the Housatonic River were correlated with reduced hatching success [6]. Hatching success was reduced by ~20% beginning at 1,000 pg/g $TEQs_{d/f}$ on the Housatonic River. These data are consistent with the data from the Woonasquatucket River, where TCDD concentrations of 1,700 pg/g were at the estimated 50% hatching rate. The TEFs developed for PCBs might not be relevant to hatching success because they were generated with the use of biochemical endpoints, in vitro studies, or both and might not adequately account for the toxicokinetics of different mixtures including aryl hydrocarbon-receptor antagonism.

Bioindicators

It was expected that EROD would be induced in tree swallow liver microsomes because induction has been shown in other studies in which tree swallows were exposed to organic contaminants such as PCBs [2,5] and polycyclic aromatic hydrocarbons [7]. Allendale and Greystone in 2000 were not significantly different, but Allendale and Lyman were significantly induced compared with Greystone in 2001. The lack of difference between Allendale and Greystone in 2000 was the result of one nestling that had induced EROD at Greystone and one that lacked a response at Allendale. Laboratory studies have shown that some individuals are not induced even though exposed to aryl hydrocarbon hydroxylase-active chemicals [68].

Neither the coefficient of variation of DNA content nor symmetry of brains varied among study ponds along the Woonasquatucket River. This result was expected with the coefficient of variation of DNA content because dioxins are not known to be clastogenic. We wanted to test this bioindicator in tree swallows along the Woonasquatucket River, however, because of the extremely high level of dioxin contamination at this location. It was surprising that we did not see a relationship between TCDD concentrations and measurements of brain asymmetry. A relationship was reported between dioxin concentrations and brain asymmetry at lower dioxin concentrations [69] in cormorants. Tree swallows might not demonstrate a response between dioxins and brain asymmetry; alternatively, our samples all might have been above some dioxin threshold for asymmetry and all samples were responsive to dioxins. Because our reference pond (Greystone) was still relatively contaminated compared with other locations in the United States, contamination at Greystone might have been sufficient to cause asymmetry; thus, we were not able to detect differences among locations. Additionally, the procedures used by Henshel et al. [69] were different from the methods used here and might have accounted for the different results.

CONCLUSION

Tree swallows nesting on Allendale and Lyman ponds were exposed to and accumulated more TCDD than swallows nesting on nearby Greystone. This is consistent with the U.S. EPA's assessment that TCDD contamination originated from the Centredale Manor Restoration Project Superfund Site on Allendale Pond and is the primary contaminant of concern. Concentrations of TCDD in eggs and nestlings are some of the highest ever reported in a bird species, including birds that feed higher in the food chain, such as great blue herons and osprey. Exposure was the result of eating insects that contained high concentrations of TCDD. Concentrations of TCDD in diet were 6 to 18 times greater than safe levels for bird species and 2 to 4 times higher than in fish collected near pulp and paper mills along the Pacific Coast of Canada. Concentrations of TCDD in eggs and nestlings were markedly higher at Allendale and Lyman (≥90 times higher) compared with the upstream reference area, whereas the rest of the dioxin and furan congeners were more similar among the three locations, differing by only a factor of three. Hatchability of eggs was reduced at Allendale and Lyman compared with Greystone. This is one of the few studies where dioxins have been implicated in reduced hatching success in a field study. Other organochlorine and trace element concentrations were either not detected or present at background or no-effect concentrations.

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