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Feminization of Male Common Tern Embryos Is Not Correlated with Exposure to Specific PCB Congeners

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Recent proposals that reproductive impairment in piscivorous wildlife may be caused by exposure to endocrine-disrupting chemicals present in the environment (Colborn et al. 1993, Fry 1995) require further investigation to elucidate critical exposures and cause-effect linkages. Effects reported in some wildlife species include feminization of male embryos and other gonadal pathologies arising during embryonic development (Fry et al. 1987). The environmental contaminants cited as possible causative agents include polychlorinated biphenyls (PCBs) and their phenolic metabolites (OH-PCBs). Among the more potent estrogenic agents are 4'-hydroxy-2,4,6-trichlorobiphenyl (4'-OH-PCB30) and 4'-hydroxy-2,3,4,5-tetrachlorobiphenyl (4'-OH-PCB61) (Korach et al. 1988). However, the parent compounds of these metabolites (PCB30 and PCB61) were not present in Aroclor mixtures, are rare in environmental samples, and have not been reported in wildlife tissues.

This paper reports an exploratory study of wild common terns (Sterna hirundo) at a breeding colony in Buzzards Bay, Massachusetts, USA. Common terns and other piscivorous wildlife in Buzzards Bay are highly exposed to PCBs migrating from a contaminated waste site in the sediments of New Bedford Harbor (Nisbet and Reynolds 1984). PCBs at this site have an unusual congener profile, consisting mainly of less chlorinated congeners (di- to tetra-chlorobiphenyls, hereafter referred to as LPCBs), including some non-Aroclor congeners that have presumably been formed by in situ dechlorination (Brown and Wagner 1990). Laboratory experiments have shown that birds can metabolize LPCBs, and that some phenolic metabolites of LPCBs are strongly estrogenic (Korach et al. 1988). We reasoned that any estrogenic effects of PCBs or their metabolites might be especially prominent in piscivorous wildlife exposed to these environmental residues. We hypothesized: (1) that common terns might

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be affected by their exposure to LPCBs during embryonic development; (2) that OH-PCBs might be the primary causative agents for such effects; and (3) that the birds' exposure to these agents might be correlated with tissue residues of the parent LPCBs. Accordingly, we sought to correlate gonadal pathology in common tern embryos with tissue residues of selected chlorobiphenyls. We used residues in the liver at the time of hatching as indicators of the exposure of the embryo during the period of gonadal development.

MATERIALS AND METHODS

We collected 30 pipped eggs (full-term embryos) of common terns at Bird Island, Massachusetts (41°40'N, 70°43'W) on 26 June 1993. Under the terms of our permits, we did not sample eggs at random, but collected eggs from late nests established at low elevations on the periphery of the nesting area; hence, we may have sampled primarily eggs laid by young parents (Nisbet et al. 1984). The gonads were excised from the embryos and fixed in formalin. The livers were transferred to chemically-cleaned vials, frozen, and stored at about -20 C until analyzed.

Gonads were examined by Fry at the University of California, Davis. Tissues were embedded in paraffin, transverse sectioned at 3-5 um intervals yielding at least 20 sections, and stained with hematoxylin and eosin. The extent of feminization of male gonads was assessed subjectively from the extent of ovarian-like cortex. "Slightly" feminized testes were those of normal oval cross-section, with a squamous epithelial capsule (normal), but with some ovarian characteristics, including eosinophilic cytoplasm with large vacuoles and chromatin condensed into meiotic prophase. "Moderately" feminized testes had oval or slightly flattened cross-section, with at least one cluster of ovarian morphology primordial germ cells (PGCs) in the 10 sections examined. "Severely" feminized testes had oval or flattened cross-section, with 20-50 percent of cortical surface having ovarian morphology, consisting of cuboidal epithelium and connective tissue with embedded clusters of PGCs; "severe" feminization usually consisted of a distinct ovarian cortical ridge running along the length of the ventral surface of the testis.

Feminization was assessed quantitatively by counting PGCs in alternate sections. PGCs are a distinctive cell marker, because the cortical (ovarian-type) PGCs are not located within seminiferous tubules, enter into meiotic prophase during embryogenesis, and remain arrested in prophase with condensed chromatin visible in an enlarged nucleus (Fry et al. 1987). The cytoplasm of cortical PGCs is vacuolar, and usually slightly eosinophilic.

Normal male PGCs are positioned within the seminiferous tubule, have nuclei in interphase, and do not have eosiniphilic cytoplasm. PGCs were counted in alternate sections, to avoid double counting of cells which were bisected in sequential sections. The index of feminization used is the number of cortical PGCs per 10 sections. This index usually ranges from 0-3 in testes of normal male embryos, 1-5 in "slightly" feminized testes, 2-10 in "moderately" feminized testes, and 20->100 in "severely" feminized testes (Fry, unpublished data).

Livers (130-389 mg, wet weight) from male embryos were analyzed for organochlorines by Lynn at Mississippi State Chemical Laboratory. In addition to a set of 21 pesticides and pesticide metabolites, we selected 32 PCB congeners for analysis. Twenty-one congeners (IUPAC numbers 8, 16, 17, 20, 22, 25, 28, 29, 31, 33, 41, 48, 49, 52, 53, 67, 70, 72, 84, 87, 91) were selected because they had been detected in common terns from Bird Island (Aquatec, Inc., South Burlington, Vermont, unpublished report prepared for Commonwealth of Massachusetts, 1990) and because they met structural criteria for ability to be metabolized and for binding of metabolites to the estrogen receptor. These criteria were derived from data in Korach et al. (1988) and McKinney and Waller (1994) and included 2-5 chlorine substitutions, one or both para-positions unsubstituted, and one or both ortho-positions substituted on the opposite ring. Six congeners (nos. 9, 10, 30, 39, 61, 80) were added because they met these structural criteria, although they had not been detected in common terns from the site. Five congeners (nos. 66, 74, 118, 138, and 153) were selected because they are common in wildlife samples and may reflect general environmental contamination.

Liver samples were extracted via a micro method described in Section 5,A(2) of USEPA (1980). Contaminants were extracted by grinding liver tissue with acetonitrile, partitioned into hexane, and cleaned up sequentially on solid phase columns packed with florisil and silicic acid. The silicic column was first eluted with 20 mL petroleum ether which was analyzed for HCB and mirex residues. The second fraction was obtained by eluting the column with an additional 150 mL of petroleum ether and contained the 34 PCB congeners of interest. The third fraction was obtained by eluting the column with 20 mL of a solution of 1% acetonitrile, 19% hexane, and 80% methylene chloride and was analyzed for the remaining pesticides and metabolites of interest.

Samples were analyzed on a Varian 3400 gas chromatograph equipped with 8200 autosampler, 1077 splitless injector and an electron capture detector (nitrogen makeup gas).

The GC column used in all assays was a DB-5 fused silica capillary (60 m x 0.25 mm id, 25 um film thickness). The column temperature program was 50 C for 2 minutes, ramp @ 25 C/minute to 150 C, hold for one minute, ramp @ 4 C/minute to 280 C and hold for 5 minutes. The injector was held at 250 C with the detector at 300 C. Samples were identified by comparison to the retention times of certified individual congener standards (AccuStandard, Inc., New Haven, CT) on the 60 m column. The congeners were quantified using a three point calibration curve. The quality controls (QC) for this set of fifteen samples were limited by sample size, but a reagent blank and matrix spike (chicken liver) QC samples were analyzed. No detectable peaks were observed at the retention time of PCB29 in the blank and the recovery from the chicken liver spike was 104% for PCB29. The presence of PCB congeners was confirmed in 1/15 samples by GC/MS analysis; however no effort was made to identify any individual congener. The lower limit of quantitation for this set was determined to be 1 ppb for the PCB congeners.

RESULTS AND DISCUSSION

The sample included 15 male and 15 female embryos. The female gonads showed no gross or microscopic abnormalities. The male gonads appeared grossly normal, but histopathological examination revealed evidence of feminization in 11/15. Four cases were classified as "severe", two as "moderate", and five as "slight" based on the extent of ovarian-like cortex. Among these 11 cases, the numbers of PGCs per 10 sections ranged from 1 to 177 with a median of 17. Both the frequency and severity of these conditions were higher than in other wildlife samples examined by Fry (Fry et al. 1987). However, the sample collection was not necessarily representative of the local breeding population.

Table 1 lists the mean concentrations of 24 PCB congeners and three pesticides or metabolites found in most or all of the 15 male embryo livers. PCB congeners nos. 8, 9, 10, 30, 61, and 80 were not detected in any sample, and congeners nos. 39 and 55 were detected in only one sample each. The estimated concentrations of total PCBs were in the range 5-31 ppm, wet weight; the congeners that were analyzed for comprised about 75% of these totals. Nine pesticides (including the estrogenic compounds o,p'-DDT, o,p'-DDD and o,p'-DDE, plus alpha-, beta-, gamma- and delta-HCH, endrin, and gamma-chlordane) were not detected in any sample. Nine others (HCB, alpha-chlordane, cis- and trans-nonachlor, heptachlor epoxide, oxychlordane, p,p'-DDD, p,p'-DDT, and toxaphene) were detected in 2-10 samples at concentrations close to the reporting limits. None of the analytes detected in the

Table 1. Concentrations of PCB congeners and pesticides detected in livers of male common tern embryos, and correlations with degree of feminization of male gonads

PCB congener or pesticide residue[1]	Concentration (ppb, wet wt) mean ±: s.d.(N = 15)	Correlation with number of PGCs/10 sections[2]
16	7 ± 10	-0.231
17	18 ± 11	0.137
20/33[3]	21 ± 13	0.049
22	34 ± 19	0.039
25	23 ± 11	0.078
28	170 ± 100	0.029
29	9 ± 8	0.256
31	81 ± 45	-0.097
41	83 ± 54	0.078
48	220 ± 140	0.029
49	110 ± 90	-0.126
52	38 ± 44	-0.320
63	26 ± 18	0.029
66	320 ± 240	0.135
67	21 ± 16	-0.049
70	98 ± 72	0.097
72	9 ± 10	-0.010
74	170 ± 120	0.077
84	660 ± 500	-0.087
87	95 ± 42	0.125
91	70 ± 44	0.019
110	390 ± 280	-0.087
118/106[3]	1500 ± 970	-0.077
138	2600 ± 1700	-0.058
153	4300 ± 3400	-0.186
Total PCBs[4]	14900 ± 8200	-0.154
Dieldrin	25 ± 14	-0.203
Mirex	25 ± 33	0.201
p,p'-DDE	310 ± 200	-0.010

[1]IUPAC system of numbering congeners
[2]Kendall's rank correlation coefficient, N = 15; all coefficients were non-significant (P>0.05)
[3]Congeners not resolved by the analytical method
[4]Estimated total of all PCBs, including congeners not analyzed for

male embryos was significantly correlated with the number of PGCs counted in the gonads (Table 1).

The male embryos that we examined showed a significant degree of feminization and were highly contaminated with PCBs (5-31 ppm in liver), including some of the LPCBs whose phenolic metabolites are expected on structural grounds to be estrogenic. Nevertheless, we found no significant correlation between our quantitative measure of feminization and the concentration of any PCB congener that we measured. Although it remains possible that one or more OH-PCBs are the causative agents, our results would then indicate that the residual concentrations of the parent PCB congener(s) that we measured in the livers at the time of hatching are not reliable measures of exposure to their metabolites during organogenesis. Further investigation of this hypothesis will require direct measurement of exposure to OH-PCBs. The only other contaminant to which common terns at this site are known to be significantly exposed is mercury (Burger et al. 1992).

Although we failed to find significant correlation of feminization with any individual PCB congener, we nevertheless draw attention to the data for PCB29 (2,4,5-trichlorobiphenyl). This congener showed the highest correlation (Kendall rank correlation coefficient tau = 0.26) with the number of PGCs (Table 1), and was more strongly correlated (tau = 0.34, P = 0.04, not allowing for multiple comparisons) with the subjective measure of severity of feminization. PCB29 is the only congener detected which has chlorine substitutions on only one ring, and is structurally similar to PCB30 and PCB61, whose 4'-OH-metabolites are strongly estrogenic (Korach et al. 1988). PCB29 is present only at trace concentrations in Aroclor mixtures (Schulz et al. 1989), but has been detected in higher proportions in sediment and fish from New Bedford Harbor (1990, Aquatec, Inc., unpublished report 1990), where it has presumably been formed by dechlorination (Alder et al. 1993). We suggest that further investigation of PCB29 and its metabolites may be warranted.

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