Effects of Polychlorinated Dibenzo-\(p\)-dioxins and Dibenzofurans on Nesting Wood Ducks (\textit{Aix Sponsa}) at Bayou Meto, Arkansas

Donald H. White\textsuperscript{1} and David J. Hoffman\textsuperscript{2}

\textsuperscript{1}National Biological Service, University of Georgia, Athens, Georgia; \textsuperscript{2}National Biological Service, Patuxent Environmental Science Center, Laurel, Maryland

Wood ducks (\textit{Aix sponsa}) nesting along Bayou Meto downstream from a hazardous waste site in central Arkansas were contaminated with polychlorinated dibenzo-\(p\)-dioxins (PCDDs) and dibenzofurans (PCDFs). Residues in eggs, based on 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin equivalents (TCDD-EQ), ranged up to 611 parts per trillion (ppt), and egg arithmetic means were 90-fold higher at the site nearest the point source compared with a reference site. We monitored productivity of wood ducks in artificial nest boxes at three sites on the bayou and at a reference site on a separate drainage during 1988–1990. Productivity was suppressed (\(p<0.05\)) at the bayou sites compared with the reference site, and egg TCDD-EQs were inversely correlated (\(p<0.001\)) with productivity in corresponding nests. The threshold range of toxicity, where reduced productivity was evident in wood ducks based on TCDD-EQs, was > 20 to 50 ppt. Oxidative stress and teratogenic effects occurred in ducklings at the more contaminated nesting sites nearest the point source. These findings suggest that wood ducks may be more sensitive to PCDD and PCDF contamination than other aquatic birds and could serve as an indicator species for monitoring biological impacts from these contaminants. — Environ Health Perspect 103(Suppl 4):37–39 (1996)

Key words: polychlorinated dibenzo-\(p\)-dioxins, dibenzofurans, reproductive impairment, \textit{Aix sponsa}, contaminants, Arkansas, wood ducks

\textbf{Introduction}

Bayou Meto, a major drainage system in central Arkansas, is contaminated with polychlorinated dibenzo-\(p\)-dioxins (PCDDs) and dibenzofurans (PCDFs). The U.S. Environmental Protection Agency (U.S. EPA) identified a former chemical plant that manufactured the herbicide 2,4,5-T as the source of contamination and subsequently included the area on the national priorities list of hazardous waste sites in 1982. Preliminary investigations by the Arkansas Game and Fish Commission (AGFC) revealed elevated levels of PCDDs in waterfowl collected downstream from the chemical plant. Residues of 2,3,7,8-tetra-CDD in wood duck (\textit{Aix sponsa}) carcasses ranged up to 510 parts per trillion (ppt) wet weight, but no PCDFs were found in wood ducks from a reference site on a separate drainage (GA Perkins, SC Yaich, unpublished report). Field studies have implicated PCDDs as contributing factors in reproductive failure of certain aquatic birds (\textit{1,2}), and we reported similar negative effects in wood ducks nesting at Bayou Meto, Arkansas (\textit{3}). Here we review our earlier results and provide additional evidence of PCDD and PCDF effects on wood ducks based on liver enzyme and thiol status assays.

\textbf{Materials and Methods}

Bayou Meto flows southeast through agricultural lands in central Arkansas for about 110 km; its banks consist of a narrow strip (100–200 m wide) of bottomland habitat that is homogeneous throughout its length. The adjacent croplands are a major wintering area for waterfowl, mainly mallards (\textit{Anas platyrhynchos}). Wood ducks nest in natural cavities in large trees along the stream. To facilitate monitoring of wood duck productivity, we erected 30 wooden nest boxes with metal predator guards at each of three sites 9, 17, and 50 km downstream from the point source and at one site on the White River National Wildlife Refuge (reference site) 111 km away. These four sites were the same where wood ducks were collected earlier by AGFC, so we had prior knowledge of PCDD and PCDF contamination there.

Refer to our previous report (\textit{3}) for detailed methods and procedures. Briefly, during 1988 to 1990 we visited nest boxes weekly beginning in mid-February each year. When a new nest was found, one fresh egg was taken for chemical analysis. These were kept refrigerated until the end of the nesting season (mid-July) each year and then prepared for analysis. Triangle Laboratories, Inc. (Durham, NC) performed the chemical analyses of egg contents according to U.S. EPA Method 8290 (\textit{4}). Although specific isomers were quantified, we only report homologue totals here. Because Bayou Meto flows through croplands, samples also were analyzed for organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) as possible contributors to reproductive impairment. Eggs in nests that failed to hatch were collected and examined for embryonic anomalies. In addition, during the nesting season of 1989, we collected one duckling on the day of hatch from 16 nests at sites 1, 2, and 3 for liver enzyme and thiol status assays (\textit{5}); these assays indicate oxidative stress from contaminants such as PCDDs (\textit{6,7}).

We were unable to get ducklings from the reference site for this purpose. For comparing cumulative effects of PCDDs and PCDFs in samples among sites, we calculated 2,3,7,8-tetra-CDD toxicity equivalents (TCDD-EQ) (\textit{8}) using factors developed by U.S. EPA (\textit{9}).

We combined data from all years because no differences were detected in residue means, hatching success, or

\textit{Environmental Health Perspectives}
duckling production within sites among years. For among site comparison, we used chi-square contingency tables (nest success and hatching success) and analysis of variance with Tukey’s multiple comparison procedure (residue and duckling production). We used correlation analysis to test relationships between egg residues and reproductive variables. We used t-tests to compare liver enzyme levels in ducklings; data from sites 1 and 2 were combined and compared with site 3. Null hypotheses for all tests were rejected at $p \leq 0.05$.

**Results and Discussion**

Data are scarce on PCDD and PCDF contamination in wild birds. Most reports are of residues in eggs of aquatic birds from the Great Lakes (10–12) where elevated concentrations up to 1200 ppt have been found. Some eggs from our three bayou sites, especially sites 1 and 2 (9 and 17 km downstream), had elevated PCDD and PCDF residues compared to those from the reference site, and generally, as distance from the point source increased, residues in eggs decreased (Table 1). On average, the highly toxic 2,3,7,8-TCDD isomer accounted for 70% of the total TCDD detected. Variable residue concentrations were not unexpected because wood ducks are highly mobile and may cover a wide range of habitats. Even resident species with extremely small home ranges accumulate pollutants differently (13). Only a few of our samples contained detectable levels of DDE, dieldrin, and heptachlor epoxide (all $< 1$ ppm), and we believe these concentrations are biologically insignificant. No other OCs or PCBs were detected.

All nests at site 3 and the reference site hatched at least one egg, but nest success at sites 1 and 2 was reduced (Table 2). Hatching success was lowest at site 1 nearest the point source and increased downstream, as did duckling production. In contrast, mean egg TCDD-EQs (representing cumulative toxicity) were 90-fold higher at site 1 than at the reference site and were negatively correlated ($p < 0.001$) with hatching success and duckling production. In other words, as toxicity in eggs decreased, productivity increased, regardless of where the eggs were laid. We arbitrarily grouped eggs TCDD-EQs to establish a threshold range of toxicity where some reduction in productivity was evident. Nest success was normal until egg TCDD-EQs exceeded 20 ppt (Table 3). Hatching success was reduced in clutches with TCDD-EQs of $> 5$ to 20 ppt, but these nests still produced nearly as many live ducklings as the optimal group. However, nest success, hatching success, and duckling production all dropped 30 to 40% when egg TCDD-EQs were in the range of $> 20$ to 50 ppt, compared to the optimal group.

Because 2,3,7,8-TCDD is a known teratogen (14), we examined 250 failed eggs for overt embryonic anomalies. Only about 35% ($n = 87$) contained discernable embryos, and the rest were desiccated or added. Six eggs from a 10-egg clutch in 1989 had full-term embryos with lower bill deformities; at least two of these also exhibited subcutaneous edema of the head and neck. A deformed duckling from this clutch had a TCDD-EQ value of 42 ppt. Similar effects were produced in chickens when the eggs were injected with 10 pg/g 2,3,7,8-TCDD (15).

Alterations in hepatic glutathione status, as well as increased activities of associated enzymes, have been related to

### Table 1. Arithmetic mean PCDD and PCDF residues in wood duck eggs from Bayou Meto, Arkansas. Sites 1, 2, and 3 are 9, 17, and 58 km downstream from the point source of contamination; site 4 is the reference site on a separate drainage (1988–1990).

| Site (n) | TCDD | PeCDD | HxCDD | HpCDD | OCDD | TCDF | PeCDF | HxCDF | HpCDF | OCDF |
|---------|------|-------|-------|-------|------|------|-------|-------|-------|------|
| 1 (18)  | 139A | 4.0AB | 6.7CD | 12B   | 59AB | 137A | 35A   | 0.41AB | 0.16A | 0.13A |
|         | (1.6–727) | (ND–24) | (ND–22) | (ND–45) | (16–268) | (7–741) | (1.9–186) | (ND–2.0) | (ND–1.9) | (ND–1.4) |
| 2 (18)  | 33B  | 5.4A  | 9AB   | 28A   | 179A | 49B  | 19A   | 0.36AB | 0.26A | 0.32A |
|         | (2.2–131) | (ND–18) | (ND–84) | (ND–65) | (ND–836) | (3.0–88) | (ND–2.4) | (ND–2.2) | (ND–3.5) |
| 3 (27)  | 8.4C | 3.3AB | 12B   | 25A   | 136A | 16B  | 11B   | 1.1A   | 0.67A | 0.51A |
|         | (ND–39) | (ND–17) | (ND–50) | (4.5–91) | (19–1170) | (ND–98) | (ND–4.8) | (ND–5.7) | (ND–4.3) |
| 4 (23)  | 0.38C | 0.96B | 3.6D | 9.4B | 36B | 1.8B | 0.41C | 0.31B | 0.12A | 0.12A |
|         | (ND–1.4) | (ND–9.8) | (ND–7.1) | (ND–25) | (ND–3.3) | (ND–1.6) | (ND–6.6) | (ND–2.7) | (ND–2.8) |

ND, not detected at limits of quantification (=1 ppt). *Means within a column that do not share a common letter differ ($p \leq 0.05$). **Range of values.

### Table 2. Wood duck productivity relative to 2,3,7,8-TCDD toxicity equivalents (TCDD-EQ) in eggs of corresponding nests at Bayou Meto, Arkansas. Sites 1, 2, and 3 are 9, 17, and 58 km downstream from the point source of contamination; site 4 is the reference site on a separate drainage (1988–1990).

| Site (n) | % hatch 1 egg | % total hatched | X live ducklings | X TCDD-EQ |
|---------|---------------|-----------------|------------------|-----------|
| 1 (18)  | 67B           | 47D             | 5.4B             | 127A (3.7–611)* |
|         | (36–265)      | (12–57)         | (1.9–21)         | (ND–2.3)  |
| 2 (18)  | 72B           | 62C             | 6.4B             | 36B (5.8–129) |
|         | (36–265)      | (12–57)         | (1.9–21)         | (ND–2.3)  |
| 3 (27)  | 100A          | 79B             | 8.8A             | 14B (0.2–91) |
|         | (ND–16)       | (ND–8)          | (ND–4.8)         | (ND–5.8)  |
| 4 (23)  | 100A          | 93A             | 9.3A             | 1.4B (ND–5.8) |

ND, not detected at limits of quantification (=1 ppt). *Average number of ducklings that lived to leave the nest. **Means within a column that do not share a common letter differ ($p \leq 0.05$). **Range of values.

### Table 3. Wood duck productivity at Bayou Meto, Arkansas, and White River National Wildlife Refuge relative to range of 2,3,7,8-TCDD toxicity equivalents (TCDD-EQ) in eggs of corresponding nests (1988–1990).

| TCDD-EQ range | n | % hatch 1 egg | % total eggs hatched | X live ducklings |
|---------------|---|---------------|---------------------|------------------|
| <5 ppt        | 29 | 100A          | 90A                 | 9.3A             |
| >5–20 ppt     | 30 | 90AB          | 71B                 | 7.9AB            |
| >20–50 ppt    | 14 | 71B           | 53C                 | 5.6B             |
| >50 ppt       | 13 | 69B           | 55C                 | 6.0B             |

*Average number of ducklings that lived to leave the nest. **Means within a column that do not share a common letter differ ($p \leq 0.05$).
exposure to oxidative stress by environmental contaminants including certain halogenated hydrocarbons, such as PCDDs and PCDFs (5–7). In our study, there was evidence of increased oxidative stress in duckling livers from the highly contaminated sites (sites 1 and 2) compared with site 3 where contamination was much lower (Table 4). Small sample sizes curbed the analysis, but significant differences were detected between some site comparisons. Gluthathione-peroxidase activity, oxidized glutathione concentration, and the ratio of oxidized versus reduced glutathione were all elevated at combined sites 1 and 2 compared with site 3.

We conclude that wood ducks may be more sensitive to PCDD and PCDF exposure than other avian species and therefore may be a suitable indicator for contamination of this type. Great blue herons (Ardea herodias) reproduced normally with a mean of 92 ppt 2,3,7,8-TCDD in eggs, but not with 252 ppt (16). Kubiak et al. (12) believed PCBs were primarily responsible for reproductive failure in Forster's terns (Sterna forsteri), even though eggs had a median level of 37 ppt 2,3,7,8-TCDD. Exposure to PCDDs decreased egg production in ring-necked pheasants (Phasianus colchicus) (17), but clutch sizes of wood ducks were normal (18) in our study, as was egg size (19). Although most of the PCDD and PCDF homologues in eggs were negatively correlated with reproductive variables, TCDD-EQs provided the highest correlations. Thus, reproductive failure in our study probably resulted from cumulative effects rather than from a single compound, as suggested in other studies (7,12,20,21).

### REFERENCES

1. Gilbertson M, Etiology of chick edema disease in herring gulls in the lower Great Lakes. Chemosphere 12:357–370 (1983).
2. Gilbertson M, Kubiak T, Ludwig J. Great Lakes embryo mortality, edema and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J Toxicol Environ Health 33:455–520 (1991).
3. White DH, Segnak JT. Dioxins and furans linked to reproductive impairment in wood ducks. J Wild Manage 58:100–106 (1994).
4. Smith LM, Stalling DL, Johnson JL. Determination of parter-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. Anal Chem 56:1830–1842 (1984).
5. Hoffman DJ, Heinz GH, Krynitsky AJ. Hepatic glutathione metabolism and lipid peroxidation in response to excess dietary selenomethionine and selenite in mallard ducklings. J Toxicol Environ Health 27:263–271 (1989).
6. Klassman CD, Bracken WM, Dudley RE, Goering PL, Hazelton GA, Hjelle J. Role of sulphydryls in the hepatotoxicity of organic and metallic compounds. Fundam Appl Toxicol 5:806–815 (1985).
7. Hoffman DJ, Rattner BA, Silo L, Dockery DE, Kubiak TJ. Embryotoxicity, tretatogenicy, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. Environ Res 42:176–184 (1987).
8. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxicity equivalency factors (TEFs). Crit Rev Toxicol 21:51–88 (1990).
9. U.S. EPA. Interim procedures for estimating risks associated with exposure to mixtures of chlorinated dibenzo-p-dioxins and dibenzofurans (CDDs andCDFs). Publ No 625/3–89/016. Washington:U.S, Environmental Protection Agency, 1989.
10. Norstrom RJ, Simon M. Preliminary appraisal of tetra- octachlorodibenzo-dioxin contamination in eggs of various species of wildlife in Canada. In: Human Welfare and the Environment (Mizamoto J, ed). New York:Plenum Press, 1983:165–170.
11. Mineau P, Fox GA, Norstrom RJ, Weseloh DV, Hallett DJ, Ellenton JA. Using the herring gulls to monitor levels and effects of organochlorine contamination in the Canadian Great Lakes. In: Toxic Contaminants in the Great Lakes (Nriagu JO, Simmons MS, eds). New York;John Wiley and Sons, 1984:245–453.
12. Kubiak TJ, Harris HJ, Smith LM, Schwartz TR, Stalling DL, Trick JA, Silo L, Dockery DE, Erdman TC. Micro contaminants and reproductive impairment of the Forster’s tern on Green Bay, Lake Michigan—1983. Arch Environ Contam Toxicol 18:706–727 (1989).
13. White DH, Krynitsky AJ. Wildlife in some areas of New Mexico and Texas accumulate elevated DDE residues, 1983. Arch Environ Contam Toxicol 15:149–157 (1986).
14. Eisler R. Dioxin hazards to fish, wildlife, and invertebrates: a synoptic review. Report No. 85(1.8). Washington:U.S. Fish and Wildlife Service, 1986.
15. Verrett MJ. Witness statement. In: Hearings Before the Subcommittee on Energy, Natural Resources and the Environment of the Committee on Commerce. Washington:U.S. Senate Serial 9–60, 1970:190.
16. Elliot JE, Butler RW, Norstrom RJ, Whitehead PE. Environmental contaminants and reproductive success of great blue herons (Ardea herodias) in British Columbia, 1986–1989. Environ Pollut 59:91–114 (1989).
17. Nosek JA, Craven SR, Sullivan JR, Hurley SS, Peterson RE. Toxicity and reproductive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in ring-necked pheasant hens. J Toxicol Environ Health 35:187–198 (1992).
18. Haramis GM, Thompson DQ. Density-production characteristics of box-nesting wood ducks in a northern green treenette impoundment. J Wild Manage 49:429–436 (1985).
19. Haramis GM. The breeding ecology of the wood duck. In: 1988 North American Wood Duck Symposium: Selected Papers from the Symposium, 20–22 February 1988. St. Louis (Fredrickson LH, ed). St. Louis:North American Wood Duck Symposium, 1990.
20. Flick DF, Firestone D, Hess J, Allen JR. Toxicity of chick edema factors in the chick, chick embryo, and monkey. Poult Sci 52:1637–1641 (1973).
21. Hoffman DJ, Smith GJ, Rattner BA. Biomarkers of contaminant exposure in common terns and black-crowned night herons in the Great Lakes. Environ Toxicol Chem 12:1095–1103 (1993).