

94-4N38

NORTH CAROLINA



DIOXINS AND FURANS IN WOOD DUCK EGGS
FROM THE LOWER ROANOKE RIVER, NORTH CAROLINA

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April 1996

U.S. Fish and Wildlife Service / Southeast Region / Atlanta, Georgia

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Abstract: In the springs of 1992 and 1993 ten clutches of wood duck eggs were collected from the lower Roanoke River below a kraft process pulp paper mill that had historically bleached with molecular chlorine. In addition, one clutch was collected from Pocosin Lakes National Wildlife Refuge (PLNWR), a site with no known history of dioxin contamination. Embryos only, with no yolk sacs, and a limited number of companion yolk sacs were analyzed for the seventeen 2,3,7,8-substituted polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD) isomer was found in all eggs sampled from the Roanoke River (0.32 to 26.6 pg/g wet weight) but was not detected in the sample from PLNWR (<0.15 pg/g). The 2,3,7,8-tetrachlorinated dibenzofuran (2,3,7,8-TCDF) isomer was detected in all samples from the Roanoke River (0.92 to 393 pg/g) as well as the sample from PLNWR (1.6 pg/g). We calculated 2,3,7,8-TCDD equivalencies (TEQs) using the international toxicity equivalency factors (I-TEFs). TEQs were calculated for embryos and yolk sacs; a regression curve was derived to predict yolk sac TEQs in the samples for which only embryos were analyzed. Total TEQs approximated for two whole eggs (22 and 82 pg/g) were within a range reported in the literature as deleterious to wood ducks (20-50 pg/g).

Liver monooxygenase activities of three dealkylases, benzyloxyresorufin-O-dealkylase (BROD), ethoxyresorufin-O-dealkylase (EROD), and methoxyresorufin-O-dealkylase (MROD), were induced in some clutches for the Roanoke River but not in all clutches. The hatching success of two nests were below 75%, the remainder ranged from 75 to 100%. Three clutches had 10-day survival rates below 75%, the others ranged from 75 to 100%. No relationship was found between hatching success or survivorship and PCDD/PCDF burdens. One embryo had multiple deformities including being a conjoined twin, a bill deformity and exencephaly. Although the deformities observed are consistent with those that can be caused by dioxin, available data do not indicate that dioxins were a causative factor.

Data indicate that enticing wood ducks to nest boxes within two kilometers downstream of the mill may not be prudent. The areal extent of habitat adjacent to the mill where concentrations of PCDDs and PCDFs may cause eggs to exceed reproductive effects levels should be determined along with a characterization of the diversity and abundance of waterfowl and other avian species at risk in the immediate vicinity.

Key Words: dioxins, furans, polychlorinated-dibenzo-dioxins, pulp and paper mills, biomonitoring, monooxygenases, wood duck, *Aix sponsa*, Roanoke River, North Carolina

Preface

This report is the final documentation of work performed under Environmental Contaminants study identifier 94-4N38. The investigation represents a cooperative effort among the staffs of the National Biological Service's North Carolina Cooperative Fish and Wildlife Research Unit, North Carolina State University, and the U.S. Fish and Wildlife Service's Raleigh Ecological Services Office and Roanoke River National Wildlife Refuge. Questions, comments, and suggestions related to this report are encouraged. Inquires should be directed to the Service at the following address:

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Acknowledgements

This study was designed and conducted by Diane Beeman, a PhD candidate under the direction of Dr. W. J. Fleming at the North Carolina Cooperative Fisheries and Wildlife Research Unit at North Carolina State University, Raleigh. Monooxygenase activities were determined in the laboratory of Dr. Mark Melancon at Patuxent Environmental Sciences Center. The authors thank the staff of both Roanoke River National Wildlife Refuge and Pocosin Lakes National Wildlife Refuge for their help in conducting this study. In addition the authors wish to thank the cast of thousands who have helped in nest checks, egg collection and duckling care. Ms. Este Stifel, Mr. Jerry Holloman, Mr. Dennis Luszc, and Mr. Tim Kubiak provided useful review comments on a September 1995 draft.

Introduction

The lower Roanoke River, located in eastern North Carolina, extends from the Virginia border to the Albemarle Sound. The portion of the river from Plymouth, North Carolina to the Sound is one of numerous areas in the southeast with a history of contamination by polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) due to chlorine bleaching during the pulp kraft paper making process (U.S. Environmental Protection Agency [hereafter U.S. EPA] 1992a). The lower Roanoke River and western Albemarle Sound have been the subject of a State-issued fish consumption advisory since 1990 due to PCDD and PCDF contamination of resident fish (North Carolina Division of Environmental Management 1991).

Dioxins and furans are present as trace impurities in some commercial products and industrial wastestreams and can enter the environment from various sources. They are not intentionally generated, but rather are unwanted impurities resulting from incineration of wastes and the manufacture of certain chlorinated organic compounds, such as the wood preservative pentachlorophenol and the herbicide 2,4,5-T (Eisler 1986; U.S. EPA 1992b). Dioxins and furans are also known to be generated as a by-product of the pulp and paper mill bleaching process which uses molecular chlorine (U.S. EPA 1988, 1992b). There are 75 individual PCDD isomers differing primarily in the number and location of chlorine atoms per molecule with the more well known and most toxic isomer being 2,3,7,8-tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD) (Figure 1). The PCDDs are chemically stable and bioaccumulate in animal tissues. The biological effects that have been reported include carcinogenicity, wasting syndrome, thymus atrophy and immunotoxic effects, reproductive impairment including fetotoxicity and teratogenicity, and cytochrome P-450 induction (Eisler 1986; U.S. EPA 1992b; Birnbaum 1994). In addition to PCDDs there are 135 isomers of PCDFs, a structurally similar group of compounds. The uniqueness of these, too, is dependent on the number and location of chlorine atoms per molecule. Although much less emphasis has been placed on these compounds, they have been shown to induce physiological changes similar to PCDDs (U.S. EPA 1992b) and to be potent inducers of cytochrome P-450 activities (Tysklind et al. 1994).

Most field studies evaluating the effects of PCDDs and PCDFs on wildlife have concentrated on top predators because they are especially at risk for biomagnification or secondary poisoning. This is because these compounds are highly lipophilic, or fat-soluble, and tend to bioaccumulate and be passed through food chains. Reproductive failure of herring gull (*Larus argentatus*) colonies in the Great Lakes region has been retrospectively correlated with TCDD levels in their eggs (Gilbertson 1983). Subcutaneous edema was reported in laboratory hatched great blue heron (*Ardea herodias*) chicks from a PCDD contaminated site in British Columbia, but no abnormalities were found in control chicks (Hart et al. 1991). In addition, various growth parameters of great blue heron chicks were inversely correlated with 2,3,7,8-TCDD levels, and liver microsomal ethoxyresorufin-O-dealkylase (EROD) activity was positively correlated with 2,3,7,8-TCDD concentrations in eggs.

In the lower Roanoke River, the only evaluations of PCDD and PCDF accumulation by birds has thus far been limited to great blue herons (Beeman et al. 1993a). Low levels of PCDDs and PCDFs were found in eggs of these piscivores at a rookery about 20 kilometers up-stream of the paper mill in Plymouth which historically utilized molecular chlorine in their paper making process. A lack of heron rookeries downstream of the mill inhibited analyses closer to the suspected source.

The wood duck (*Aix sponsa*) is found in bottomland hardwoods throughout the Roanoke River floodplain. It is an ideal study animal as it can be enticed to nest in artificial nest boxes. In addition, unlike the mallard (*Anas platyrhynchos*), female wood ducks gain fat reserves for reproduction at the

nesting location instead of prior to migrating (Drobney 1982). Therefore residue levels in eggs will largely reflect those accumulated in the vicinity of the nesting area. In addition, the species has been shown sensitive to the affects of PCDDs and PCDFs in the field. Nest success, hatching success and duckling production of wood ducks were reduced at sites contaminated with PCDDs and PCDFs downstream from a former chemical plant in Arkansas that manufactured the herbicide 2,4,5-T (White and Seginak 1994; White and Hoffman 1995). There was a significant inverse correlation between egg PCDD/PCDF burdens and hatching success and duckling production.

Biomonitoring environmental contamination is possible using cytochrome P450 associated monooxygenase activities. Cytochrome P450 refers to a group of enzymes that biotransform endogenous and exogenous organic compounds. These transformations can result in detoxification of parent compounds or their activation to a more toxic metabolite (Stegeman et al. 1992). The reactions catalyzed by these enzymes are generally referred to as mixed-function oxidase (MFO) or monooxygenase reactions. Cytochrome P450 proteins are present in all tissues, but as might be expected from their role in detoxification, they are especially prominent in the liver (Melancon 1995). Bluebirds (*Sialia sialis*) and tree swallows (*Iridoprocne bicolor*) inhabiting areas treated with dioxin contaminated sludge exhibited no adverse reproductive or growth effects. The only effect seen was significant hepatic enzyme induction in bluebird nestlings on the treated area (Martin et al. 1989). This cannot be considered an adverse response; rather, this appears to be an adaptive response that is reversible and not harmful to the nestlings. Cytochrome P450 associated monooxygenase activities of piping black-crowned night herons (*Nycticorax nycticorax*) were significantly associated with total PCB burdens (Rattner et al. 1993). Residue analyses for PCDDs and PCDFs are expensive; therefore, a biomarker of exposure is desirable. Liver cytochrome P450-related monooxygenases are inducible by 3-methylcholanthrene, a model for dioxin-like induction (Beeman et al. 1993b). Therefore the cytochrome P450 related monooxygenases have potential as biomarkers for dioxin exposure.

This study was designed to achieve three goals:

1. Determine current dioxin and furan concentrations in wood duck embryos from the Roanoke River near Plymouth and a companion reference site, Pocasin Lakes National Wildlife Refuge;
2. Assess the cytochrome P450-related monooxygenases as an inexpensive screen for dioxin exposure in wood duck embryos; and,
3. Assess the relationship among reproductive variables and dioxin and furan concentrations in wood duck embryos.

Methods

Sample Collection

Prior to the nesting season of 1992, 30 wood duck nest boxes were installed on the north side of the Roanoke River near Plymouth (Figure 2). The boxes were assigned individual identification numbers in descending order from the mouth of Welch Creek (Nest box 30) to an area downstream of the Highway 45 bridge (Nest box 1). At Pocasin Lakes National Wildlife Refuge, existing nest boxes were used. Beginning in late February or early March of 1992 and 1993, nest boxes were checked at 7 to 10-day intervals for nest initiation. Nest initiation date was the date of laying of the first egg or was back-dated according to the normal egg production of 1 egg per day (Leopold 1951). Egg

laying was followed until the clutch was completed. Clutches were collected after a minimum of 15 days of incubation to ensure maximal hatching success (Doty 1972).

Collected eggs were immediately placed in a thermal cooler and transported to Raleigh, North Carolina. Eggs were candled, addled eggs were removed, and the hatching date for the rest of the clutch was predicted according to the method of Miles and Bizeau (1983). The clutch was then artificially incubated in a forced air incubator. Beginning one week prior to the estimated hatch date, the eggs were checked daily for pipping. At the time of pipping, two embryos per clutch were sacrificed by decapitation.

These embryos were removed from the shell, examined for embryonic abnormalities and weighed. The liver from each embryo was weighed and immediately placed in a cryotube containing glycerol and snap frozen in liquid nitrogen. The liver samples were stored at -80° C until assayed for monooxygenase activity.

The sample egg technique (Blus 1982) was employed to determine the degree of correlation between dioxin concentrations in a single embryo from a clutch and the success of the remaining sibling eggs from the clutch. Pipped embryos, rather than fresh eggs, were used for PCDD and PCDF analyses because of the concurrent objective to test the cytochrome P450-related monooxygenases as a screening tool for dioxin exposure. Inherent in application of the sample egg technique is the assumption that intra-clutch variability in organochlorine residues is small relative to inter-clutch variability and that the residues detected in any egg from a clutch accurately reflect residues in the remaining un-analyzed eggs. Although data on the intra-clutch PCDD and PCDF variability in wood ducks are not available, there is a wealth of support for this foundation of the sample egg technique from organochlorine pesticide analyses in a variety of birds (Custer et al. 1990).

Following removal of the liver, the embryo, excluding the yolk sac, was placed in a chemically cleaned jar (I-CHEM Research, New Castle, DE) for PCDD and PCDF analyses. These samples were stored at -20° C until analyzed. Yolk sacs of some samples were also collected for chemical analysis to be used in calculating PCDD and PCDF concentrations in whole eggs.

Embryos not sacrificed at pipping were allowed to hatch. On hatching the ducklings were weighed, examined for abnormalities, web-tagged and placed in a brooder. The ducklings were provided with fresh water, food (Purina Game Bird Starter Chow) and meal worms twice daily. Survivorship was monitored through day ten.

Liver Enzyme Assays

Liver samples were thawed, blotted free of glycerol, weighed and homogenized (one weight to four volumes of 1.15 M KCl in 0.01 M Na/K phosphate buffer, pH 7.4) using a Polytron (Brinkman Instruments, Inc. Westbury, N.Y.; P-20 probe, setting 5, 15-s pulse). The homogenate was centrifuged at 9,000 g for 20 min at 4° C followed by centrifugation of the supernatant at 100,000 g for 60 min at 4° C. The microsome pellet was resuspended in buffer (0.05M Na/K phosphate containing 10⁻³ M disodium EDTA, pH 7.6). Using bovine serum albumin as a standard, microsomal protein was determined (Lowrey 1951).

Monooxygenase activities of three dealkylases, benzyloxyresorufin-O-dealkylase (BROD), ethoxyresorufin-O-dealkylase (EROD) and methoxyresorufin-O-dealkylase (MROD), were measured using the rate of formation of the fluorescent product, resorufin. All activities were assayed in a final volume of 260 ul, containing 66 mM Tris buffer at pH 7.4, NADPH, microsomes and substrate. The concentrations of substrate were BROD: 1 uM; EROD: 0.75 uM; and MROD: 1.25 uM.

The concentration of NADPH was 0.125 uM. Buffer, substrate and microsomes were preincubated at 37° C for 10 min prior to addition of the NADPH. Seven fluorometer readings were recorded per well over a 10.5 min period. Each microtiter plate included a complete range of resorufin standards. Enzyme activity was calculated by linear regression and values were converted to picomoles product per minute per milligram of microsomal protein.

Analytical Chemistry

Triangle Laboratories of RTP, Incorporated, Durham, North Carolina, analyzed each embryo for the seventeen 2,3,7,8-substituted PCDDs and PCDFs. Chemical analyses were performed by high resolution capillary column gas chromatography/mass spectrometry (HRGC/HRMS) according to EPA SW 846 Method 8290. This method includes addition of stable ¹³C-labeled analogues of fifteen of the PCDDs and PCDFs to each sample. Concentrations were corrected for recoveries of the labeled analogues. Although lower limits of detection varied between samples for particular isomers depending on the extent of matrix interferences, limits of detection were 1.0 pg/g wet weight or less for each isomer.

A method blank was extracted with the samples; the 17 analytes were not detected in the blank at detection limits which ranged from 0.2 to 1.0 pg/g. A laboratory control spike and spike duplicate was performed with recoveries of the 17 isomers ranging from 88.6 to 138%; average recoveries for spiked 2,3,7,8-TCDD and 2,3,7,8-TCDF, which comprise the majority of 2,3,7,8-TCDD equivalents in these samples, were 89 and 103%, respectively. In the spike/spike duplicate, relative percent deviations ranged from 0 to 18.1 and were 0.45% for 2,3,7,8-TCDD and 1.94% for 2,3,7,8-TCDF. A duplicate injection of extract for one sample was performed. The relative percent deviation for concentrations of 2,3,7,8-TCDD and TCDF were 1.73 and 1.34, respectively. The QA/QC results were within acceptable ranges for method precision and accuracy. Dry weight values of each congener were converted to wet weight using percent moisture data provided by the contractor. To convert to approximate lipid weight basis, it is necessary to divide the embryo wet weight congener value by .098 as the average percent lipids in these samples is 9.8%.

Because the PCDDs and PCDFs are structurally similar, produce a similar pattern of toxic responses, and their toxicological mode of action is presumed similar (U.S. EPA 1989), toxicity equivalency factors (TEFs) have been derived to approximate the relative toxicity of individual congeners to 2,3,7,8-TCDD. In this process, 2,3,7,8-TCDD, the most toxic congener, is assigned a TEF of 1. All other congeners are assigned a TEF of some fraction of 1 based on their relative potency to 2,3,7,8-TCDD; relative potency is often determined by cytochrome P450 enzyme induction assays with the individual congeners (U.S. EPA 1989). The concentration of a particular PCDD or PCDF congener multiplied by its corresponding TEF results in a "2,3,7,8-TCDD equivalent" concentration, or "TEQ." We calculated TEQs for each congener by the following equation:

$$\text{TEQ} = \text{Congener concentration} * \text{TEF}$$

Where: TEQ = the congener concentration expressed in terms of 2,3,7,8-TCDD toxicity units;

Congener concentration = the concentration of the congener per unit of wet weight; and,

TEF = toxicity equivalency factor; the relative toxicity of the specific congener in terms of the toxicity of 2,3,7,8-TCDD.

Once TEQs are calculated for each of the 17 2,3,7,8-substituted congeners in a sample, they can be summed to give total TEQs; this is an expression of all the measured PCDD and PCDF contaminants in the embryo in terms of 2,3,7,8-TCDD.

Three sets of TEFs were used in this report. The study used TEFs taken from Smith et al. (1990) and, to facilitate comparison with White and Seganik's (1994) work on wood ducks, we also calculated TEQs using the international toxicity equivalence factors (I-TEF/89) (U.S. EPA 1989). Congeners reported below the detection limits were assigned a 0 value for calculating TEQs. Congeners reported as estimated maximal concentration were assigned the estimated maximal concentration when calculating TEQs.

For each manner of calculating TEQs, the relationship between the embryo total TEQs and companion yolk sac total TEQs was determined via linear regression. Embryo and yolk TEQs used for this determination were not only those collected from the Roanoke River but also included data collected from other sites in a similar manner. Regression equations relating embryo total TEQs and yolk total TEQs were constructed to predict the yolk total TEQs for those embryos lacking PCDD and PCDF analysis of the yolk. The regression equation for this relationship for the aryl hydrocarbon hydroxylase (AHH) derived TEQs is:

$$\text{TEQsY} = 3.79 * \text{TEQsE} - 4.75 \quad (n=6, r^2=.999, P<0.00001)$$

Where: TEQsY = yolk total TEQs (picograms per gram wet weight); and,
 TEQsE = embryo total TEQs (picograms per gram wet weight).

For the EROD derived TEQs the relationship is:

$$\text{TEQsY} = 3.79 * \text{TEQsE} - 5.28 \quad (n=6, r^2=.999, P<0.00001)$$

For the I-TEF/89 TEQs the relationship is:

$$\text{TEQsY} = 3.82 * \text{TEQsE} - 7.93 \quad (n=6, r^2=.999, P<0.00001)$$

In order to approximate fresh whole egg total TEQs from the PCDD and PCDF concentrations we measured in embryos and yolk sacs, we used the following equation:

$$\text{WETEQs} = \frac{(\text{ETEQs}) * (\text{EW}) + (\text{YTEQs}) * (\text{YW})}{(\text{EV})}$$

Where: WETEQs = whole egg total TEQs;
 ETEQs = embryo total TEQs;
 EW = embryo weight;
 YTEQs = yolk total TEQs;
 YW = yolk weight; and,
 EV = egg volume.

This calculation also corrects residue results for moisture loss (Stickel et al. 1973).

The PCDD and PCDF concentrations were log transformed to stabilize variances. Hatching success and survivorship were arc sine transformed to stabilize variances. Relationships were determined using linear regression analysis.

Results and Discussion

All 10 embryos collected from the Roanoke River had detectable amounts of the 2,3,7,8-TCDD congener (0.32 to 26.6 pg/g wet weight) and the 2,3,7,8-TCDF congener (0.92 to 393 pg/g wet weight) (Tables 1 and 2). Other congeners were detected in 90% of the samples. The sample from Pocosin Lakes National Wildlife Refuge had no detectable 2,3,7,8-TCDD (<0.15 pg/g wet weight), but it did have a low level of 2,3,7,8-TCDF (1.6 pg/g wet weight). For the Roanoke River samples, total AHH based TEQs range from 0.62 to 38 pg/g, EROD based total TEQs range from 0.64 to 45 pg/g and I-TEF/89 based total TEQs range from 1.1 to 69 pg/g (Table 3). The 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners account for between 39 to 96% of the total TEQs based on I-TEF/89s. The AHH and EROD derived TEQs are highly correlated (Figure 3).

The 2,3,7,8-TCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, and 2,3,7,8-TCDF congeners were found in 100% of the yolk sacs (Tables 4 and 5). The presence of other congeners varied. To convert the residue levels to a lipid weight basis, it is necessary to divide by 0.16 as the average percent lipids in these samples is 16. For eggs from which both embryos and yolk sacs were analyzed for PCDDs and PCDFs (n=3), we calculated PCDD and PCDF concentrations in whole eggs (Tables 6 and 7). In these, 2,3,7,8-TCDD ranged from 2.2 to 32 pg/g wet weight and 2,3,7,8-TCDF ranged from 26 to 460 pg/g wet weight.

The total TEQs of yolk (pg/g wet weight) are positively correlated with the total TEQs of embryo (pg/g wet weight) (Figure 4). Using the regressions for the different TEFs, fresh weight whole egg total TEQs were determined for all samples (Table 8) which ranged from 0.22 to 45 pg/g based on AHH TEFs, from 0.30 to 53 pg/g based on EROD TEFs, and from 0.33 to 82 pg/g based on the I-TEF/89s.

Overall, estimated whole egg total TEQs were low; the geometric mean total TEQ for the 10 samples from the Roanoke River was 4.9 pg/g based on I-TEF/89s. Nest 30 (82 pg/g) and Nest 27 (22 pg/g) both have I-TEF/89 derived total TEQs that are greater than the 20 pg/g suggested by White and Seginak (1994) as associated with reproductive impairment in wood ducks. Both of these nests were within 1.5 kilometers of the mill.

Because we express our egg PCDD and PCDF levels as TEQs, we account for the toxicity of all the congeners in terms of 2,3,7,8-TCDD. Many early studies assess only the levels of 2,3,7,8-TCDD. The TEQs in our samples are similar to TCDD levels for bluebird eggs sampled from a dioxin-treated pine plantation (6.6 to 10 pg/g) (Martin et al. 1989). As might be expected, the geometric mean total TEQs reported here are very much lower than 2,3,7,8-TCDD levels reported for herring gull eggs (550 pg/g) in the Great Lakes in the mid 1970s (Gilbertson 1983) and great blue herons in a British Columbia watershed receiving paper mill effluents in 1986 (66 pg/g) (Elliott et al. 1989).

The specific PCDD/PCDF congeners seen in Roanoke River wood ducks embryos are generally similar to other birds sampled from sites with dioxin contamination. However, our specimens exhibited high levels of 2,3,7,8-TCDF (0.92-393 pg/g). Wood duck eggs collected downstream of a hazardous waste site in Arkansas (White and Seginak 1994) exhibit a similar profile (16-268 pg/g 2,3,7,8-TCDF). This contrasts with low levels of 2,3,7,8-TCDF in great blue heron eggs near paper mills in British Columbia (less than detection to 31 pg/g wet weight) (Elliott et al. 1989) and from the Roanoke River approximately 20 km upstream from the area of this study (Beeman et al. 1993a). Levels of 2,3,7,8-TCDF in osprey eggs collected from western Albemarle Sound at the mouth of the Roanoke River (4.53-11.4 pg/g wet weight) (U.S. Fish and Wildlife Service, unpub. data) and osprey eggs collected near pulp mills in British Columbia (1.5-34.5 pg/g wet weight) (Whitehead et al. 1993) are low when compared to the wood duck. Elevated concentrations of 2,3,7,8-TCDF in wood duck eggs may indicate that this species differs from other birds in its ability to metabolize the

2,3,7,8-TCDF congener. Mallard ducks (Norstrom et al. 1976) and herring gulls (Braune and Norstrom 1989) both excrete the 2,3,7,8-TCDF congener more rapidly than the 1,2,3,7,8-PCDF and the 2,3,7,8-substituted PCDDs. We used I-TEF/89s for comparisons with a previously published study on wood ducks, and the I-TEF/89 for 2,3,7,8-TCDF is 0.1. There are differing opinions as to the correct 2,3,7,8-TCDF TEF to use for avian species (Bosveld et al. 1992) with indications that its toxic potential is similar to that of 2,3,7,8-TCDD. Based on the high concentration of 2,3,7,8-TCDF in the wood duck, it might be more appropriate to consider the higher TEFs in future assessments.

Preliminary analysis of the P450 activities indicates only two samples from the Roanoke River were induced when these data are compared to control monooxygenase levels gathered for this and other similar studies (Table 9). The TCDD-equivalents per gram of wet weight embryo were not significantly correlated with the pico moles of product/min/mg of microsomal protein for any of the three dealkylases measured ($n=10$, $p \geq 0.01$). This lack of correlation is not unexpected. Levels of 2,3,7,8-TCDD and the TEQs in wood duck eggs from the Roanoke are lower than those which induce hepatic EROD activity in the pheasant (Nosek et al. 1993). In pheasants the increase in hepatic EROD activity begins between 10 pg TCDD/g egg and 100 pg TCDD/g egg with an ED_{50} of 312 pg/g. Martin et al. (1989) saw no significant increase in hepatic EROD activity in 8-day-old bluebird nestlings dosed in ovo with graded doses of TCDD from 1 to 1000 pg/g egg.

There was no relationship between hatching success and log transformed PCDD and PCDF burdens ($n=10$, $P \geq 0.01$) (Figure 5). Only two nests, numbers 30 and 23, had hatching success of < 75%; the remainder ranged from 75 to 100% with a mean of 86.7% (Table 10). We only collected in-tact nests and intentionally excluded nests with evidence of predator damage. When compared to hatching success calculated as the probability of intact eggs (those not damaged by predators) in successful nests to produce live hatchlings (Nichols and Johnson 1990), hatching success reported here is similar to that in maternally hatched nests. Also, hatching success reported here is within normal ranges reported for wood ducks (Leopold 1951; McLaughlin and Grice 1952; Morse and Wright 1969; Clawson et al. 1979). There was no relationship between survivorship and PCDD/PCDF burden ($n=10$, $P \geq 0.01$). Three nests, numbers 30, 29, and 20, had 10-day survivorship below 75%; the remainder ranged from 75 to 100% with a mean of 76.5%. It is not practical to compare this to the field studies, but like the field studies, we found the majority of mortality occurred within the first two weeks. All of these comparisons must be qualified by the statement that findings are based on laboratory hatching success and survivorship and do not necessarily reflect what could have occurred in the natural situation.

One hundred twenty four ducklings successfully hatched or were sacrificed for monooxygenase analysis. No gross abnormalities were detected in any of these birds. Only 14 eggs did not hatch in the 1992 and 1993 seasons. Of these 14, 9 had evidence of developing embryos. No abnormalities were found in these embryos. The remaining 5 eggs had no evidence of developing embryos. While further collections were being made for a different study in 1994, we did find a single deformed embryo from a nest collected within 1 km of the mill (Figure 6). The embryo was not only a conjoined twin but also had a deformed bill and exencephaly. Subsequent PCDD/PCDF analyses of two sibling embryos from this clutch indicated total TEQs of 1.0 and 1.4 pg/g wet weight (U.S. Fish and Wildlife Service, unpub. data); these values are below those currently thought to be associated with teratogenic effects.

Current data indicate that enticing waterfowl to nests boxes immediately downstream of the mill may not be prudent. These boxes should be removed or moved to a more suitable habitat. Although limited, our data indicate that nest boxes more than 2 kilometers downstream from the mill appear to be at a much lower risk from PCDD and PCDF exposure. Work done with wood ducks raises concerns for other avian species in the lower Roanoke River basin. The

PCDD/PCDF LOAEC (lowest observable adverse effect concentration) in the wood duck is 21 pg TCDD/g egg (White and Seginak 1994). In a review of avian effects of PCDD/PCDFs, Bowerman et al. (1995) report an avian egg NOAEC (no observable adverse effect concentration) value of 2.1 pg TEQ/g egg and an overall avian NOAEC / LOAEC of 7 pg/g egg. As noted, the majority of wood duck TEQs are near the NOAEC (Figure 7), but there have been few studies of other species. Of particular concern due to the biomagnification potential of PCDDs/PCDFs would be piscivorous species.

We have previously assessed the extent of PCDF/PCDD contamination on two heron rookeries located approximately 20 km upstream from Plymouth and found no contaminant levels of concern (Beeman et al. 1993a). Great Blue herons successfully reproduced near a kraft paper mill on Vancouver Island, British Columbia when the mean total TEQs (calculated according to Mason et al. as cited in Elliott et al. 1989) were 79 pg/g wet weight. A reproductive failure occurred in 1987 accompanied by a three fold increase in TEQs, but human disturbance and predation may have affected reproductive outcomes (Elliott et al. 1989). In 1988, TCDD significantly correlated with sublethal effects, such as subcutaneous edema and inhibited growth, in the same colony (Hart et al. 1991).

Eagles from the area around the Great Lakes basin have been slow to recover from the DDT-related decline. This failure has been attributed to elevated TEQs, mostly from the planar PCBs (Bowerman et al. 1995). The NOAEC for eagles has been approximated as 7 pg/g in the egg (Bowerman et al. 1995). The total TEQs for osprey eggs collected from western Albemarle Sound near the mouth of the Roanoke River range from 18.5 to 79.7 pg/g wet weight (U.S. Fish and Wildlife Service, unpub. data) indicating that our identified area of concern for wood ducks (within 2 km from the mill at Plymouth) should not be applied to all avian species.

Based on the results of this work, there may be areas at and adjacent to the mill capable of producing exceedences of wood duck reproductive screening values for PCDDs and PCDFs. Since the mill's waste management practices include the use of an extensive array of settling, aeration and reclamation ponds prior to discharge of treated effluent to the Roanoke River, the extent of PCDD and PCDF contamination in the ponds should be characterized. Effluent was historically discharged to Welch Creek. Fish taken from Welch Creek have generally contained higher PCDD and PCDF levels than fish from the lower Roanoke River (North Carolina Division of Environmental Management 1991). Consequently, Welch Creek's forested wetlands should also be characterized for PCDD and PCDF contamination.

The numbers and species of waterfowl utilizing these areas have not been well documented; however, preliminary indications are that a substantial number of Canada geese (*Branta canadensis*), mallards and wood ducks breed at the settling ponds and that a diversity of dabbling ducks utilize the ponds in winter. The diversity and abundance of waterfowl at risk in the immediate vicinity should be examined. It is recommended that productivity of waterfowl breeding at and adjacent to the facility be examined. Depending on the results of these investigations, further evaluation of PCDD and PCDF accumulation may be warranted. Once the extent of any contamination of the ponds and Welch Creek wetlands is known and the waterfowl resources at risk are better known, additional management options can be more fully addressed. This information is viewed as essential for informed natural resource management at the mill and surrounding environs.

Conclusions and Recommendations

1. Two nests from the lower Roanoke River, 20% of the clutches sampled, had levels of 2,3,7,8-TCDD equivalents which were at or above those reported by White and Seginak (1994) to be associated with reproductive impairment in wood ducks.
2. Preliminary analysis suggest that for the levels of PCDDs and PCDFs found, the cytochrome P450-related monooxygenases are not a reliable screen for dioxin exposure in wood duck embryos.
3. There was no relationship between hatching success or survivorship and residue analysis derived total 2,3,7,8-TCDD equivalents.
4. The areal extent of habitat where concentrations of PCDDs and PCDFs may cause wood duck eggs to exceed the 20 pg/g screening value for reproductive effects should be determined. Based on the samples in this study, the areas most likely to cause exceedences of the screening values are the portion of the Roanoke River within 2 km downstream of the mill, the wastewater management ponds associated with the mill, and the forested wetlands around Welch Creek.
5. Analyses of PCDD and PCDF concentrations in wood duck forage and sediments should be conducted. These data will allow calculation of bioaccumulation factors (BAFs) from the sediments to forage and from forage to wood duck embryos. The BAFs would have utility in assessing the areal extent of habitat where sediment PCDD and PCDF concentrations could be expected to pose a risk to wood duck development.
6. The diversity and abundance of waterfowl at risk in the immediate vicinity should be examined. Available data indicate significant numbers of ducks and geese at the mill may be at risk from PCDD and PCDF contamination. A habitat evaluation of these areas, along with the characterization of wildlife resources, will assist with design and construct of potential management options.
7. Current data indicate that enticing waterfowl to nests boxes immediately downstream of the mill may not be prudent, and it is recommended these boxes be removed. Our data indicate that nest boxes further than 2 km downstream of the mill appear to be at a much reduced risk from PCDD and PCDF exposure. Once the area of contamination (Recommendation 4) and resources are risk (Recommendation 6) are well established, additional management options can be more fully addressed.
8. These data demonstrate bioaccumulation of PCDDs and PCDFs in wood ducks, a species harvested for human consumption. Health agencies should consider characterizing the risks associated with consumption of resident waterfowl in this area.
9. This particular mill has recently stopped bleaching with molecular chlorine and this might be an ideal opportunity to monitor the trends in PCDDs and PCDFs with a shift in the bleaching process.

Literature Cited

- Beeman, D.K., T. Augspurger and W.J. Fleming. 1993a. Productivity and PCDD / PCDF levels in eggs from two great blue heron rookeries near a pulp and paper mill on the Roanoke River, North Carolina. Poster #P118, Society of Environmental Toxicology and Chemistry 14th Annual Meeting. Houston, TX.
- _____, M.J. Melancon and W.J. Fleming. 1993b. The cytochrome P450 monooxygenase system of the wood duck embryo as a potential biomarker for dioxin exposure. Poster #613, Society of Environmental Toxicology and Chemistry 14th Annual Meeting. Houston, TX.
- Birnbaum, L.S. 1994. The mechanism of dioxin toxicity: relationship to risk assessment. Environ. Health Perspect. 102: 157-167.
- Blus, L.J. 1982. Further interpretation of the relation of organochlorine residues in brown pelican eggs to reproductive success. Environ. Pollut. (Series A) 28: 15-33.
- Bosveld, A.T.C., M. van den Berg and R.M.C. Theelen. 1992. Assessment of the EROD inducing potency of eleven 2,3,7,8 substituted PCDD/Fs and three coplanar PCBs in the chick embryo. Chemosphere 25:911-916.
- Bowerman, W.W., J.P. Giesy, D.A. Best and V.J. Kramer. 1995. A review of factors affecting productivity of bald eagles in the Great Lakes region: implications for recovery. Environ. Health Perspect. 103:51-59.
- Braune, B.M. and R.J. Norstrom. 1989. Dynamics of organochlorine compounds in herring gulls (*Larus argentatus*): III. Tissue distribution and bioaccumulation in Lake Ontario gulls. Environ. Toxicol. Chem. 8:957-968.
- Clawson, R.L., G.W. Hartman and L.H. Fredrickson. 1979. Dump nesting in a Missouri wood duck population. J. Wildl. Manage. 43:347-355.
- Custer, T.W., G. Pendleton and H.M. Ohlendorf. 1990. Within- and among-clutch variation of organochlorine residues in eggs of black-crowned night-herons. Environ. Monit. Assess. 15: 83-89.
- Doty, H.A. 1972. Hatchability tests with eggs from captive wood ducks. Poultry Science 51: 849-853.
- Drobney, R.D. 1982. Body weight and composition changes and adaptations for breeding in wood ducks. Condor 84: 300-305.
- Eisler, R. 1986. Dioxin hazards to fish, wildlife, and invertebrates: a synoptic review. U. S. Fish Wild. Serv. Biol. Rep. 85 (1.8). Laurel, MD. 37 pp.
- Elliott, J.E., R.W. Butler, R.J. Norstrom and P.E. Whitehead. 1989. Environmental contaminants and reproductive success of great blue herons *Ardea herodias* in British Columbia, 1986-87. Environ. Pollut. 59: 91-114.
- Gilbertson, M. 1983. Etiology of chick edema disease in herring gulls in the lower Great Lakes. Chemosphere 12: 357-370.
- Hart, L.E., K.M. Cheng, P.E. Whitehead, R.M. Shah, R.J. Lewis, S.R. Ruschowski, R.W. Blair, D.C. Bennett, S.M. Bandeira, R.J. Norstrom and G.D. Bellward. 1991. Dioxin contamination and growth and development in great blue heron embryos. J. Toxicol. Environ. Health 32: 331-334.

- Leopold, F. 1951. A study of nesting wood ducks in Iowa. *Condor* 53: 209-220.
- Lowrey, O.H., N.H. Rosenbrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- Martin, S.G. J.W. Duncan, D.A. Thiel, R.E. Peterson and M.L. Lemke. 1989. Evaluation of the effects of dioxin-contaminated sludges on eastern bluebirds and tree swallows. Nekoosa Paper, Port Edwards, WI.
- McLaughlin, C.L. and D. Grice. 1952. The effectiveness of large scale erection of wood duck boxes as a management procedure. *Trans. North Am. Wildl. Conf.* 17:242-259.
- Melancon, M.J. 1995. Bioindicators used in aquatic and terrestrial monitoring. Pages 220-240 *In* D.J. Hoffman, B.A. Rattner, G.A. Burton, Jr. and J. Cairns, Jr. (eds.). Handbook of Ecotoxicology. Lewis Publishers, Ann Arbor, MI.
- Miles, J.E. and E.G. Bizeau. 1983. Techniques for predicting the hatching dates of wood duck clutches. *J. Wildl. Manage.* 47: 204-209.
- Morse, T.E. and H.M. Wright. 1969. Dump nesting and its effects on production in wood ducks. *J. Wildl. Manage.* 33:284-293.
- Nichols, J.D. and F.A. Johnson. 1990. Wood duck population dynamics: a review. Pages 83-105 *In* L.H. Fredrickson, G.V. Burger, S.P. Havera, D.A. Graber, R.E. Kirby and T.S. Taylors (eds.). Proc. 1988 North Am. Wood Duck Symp. St Louis, MO.
- Norstrom, R.J., R.W. Risebrough and D.J. Cartwright. 1976. Elimination of chlorinated dibenzofurans associated with polychlorinated biphenyls fed to mallards (*Anas platyrhynchos*). *Toxicol. Appl. Pharmacol.* 37:217-228.
- North Carolina Division of Environmental Management. 1991. 1990 update: Fish tissue dioxin levels in North Carolina. Water Quality Section, Raleigh. 39 pp.
- Nosek, J.A., J.R. Sullivan, S.R. Craven, A. Gendron-Fitzpatrick and R.E. Peterson. 1993. Embryotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the ring-necked pheasant. *Environ. Toxicol. Chem.* 12: 1215-1222.
- Rattner, B.A., M.J. Melancon, T.W. Custer, R.L. Hothem, K.A. King, L.J. LeCaptain, J.W. Spann, B.R. Woodin and J.J. Stegeman. 1993. Biomonitoring environmental contamination with pipping black-crowned night heron embryos: induction of cytochrome P450. *Environ. Toxicol. Chem.* 12: 1719-1732.
- Smith, L.M., T.R. Schwartz, K. Feltz and T.J. Kubiak. 1990. Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachloro-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran in Lake Michigan sediment and biota. The question of their relative toxicological significance. *Chemosphere* 21: 1063-1085.
- Stegeman, J.J., M. Brouwer, R.T. Di Giulio, L. Förlin, B.A. Fowler, B.M. Sanders and P.A. Van Veld. 1992. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. Pages 235-335 *In* R.J. Huggett, R.A. Kimerle, P.M. Mehrle, Jr. and H.L. Bergman (eds.). Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. Lewis Publishers, Ann Arbor, MI.

- Stickel, L.F., S.N. Wiemeyer and L.J. Blus. 1973. Pesticide residues in eggs of wild birds: adjustment for loss of moisture and lipid. *Bull. Environ. Contam. Toxicol.* 9: 193-196.
- Tysklind, M., D. Tillitt, L. Eriksson, K. Lundgren and C. Rappe. 1994. A toxic equivalency factor scale for polychlorinated dibenzofurans. *Fund. Appl. Toxicol.* 22: 277-285.
- U.S. Environmental Protection Agency. 1988. U.S. EPA / paper industry cooperative dioxin screening study. EPA-440/1-88-025. Office of Water Regulations and Standards, Washington, D.C. unpagued.
- _____. 1989. Interim procedures for estimating risks associated with exposure to mixtures of chlorinated dibenzo-p-dioxins and-dibenzofurans (CDDS and CDFS). EPA/625/3-89/016. Risk Assessment Forum, Washington, D.C. 69 pp.
- _____. 1992a. National study of chemical residues in fish, volume I. EPA 823-R-92-008a. Office of Science and Technology, Washington, D.C. 166 pp.
- _____. 1992b. National study of chemical residues in fish, volume II. EPA 823-R-92-008b. Office of Science and Technology, Washington, D.C. unpagued.
- White, D.H. and D.J. Hoffman. 1995. Effects of polychlorinated dibenzo-p-dioxins and dibenzofurans on nesting wood ducks (*Aix sponsa*) at Bayou Meto, Arkansas. *Environ. Health Perspect.* 103:37-39.
- _____. and J.T. Seginak. 1994. Dioxins and furans linked to reproductive impairment in wood ducks. *J. Wildl. Manage.* 58:100-106.
- Whitehead, P.E., J.E. Elliott, R.J. Norstrom, C. Steeger, J. Van Oostdam and G.E.J. Smith. 1993. Chlorinated hydrocarbon levels on eggs of osprey nesting near pulp mills in British Columbia, Canada. *Dioxin* 93. Vienna, Austria.

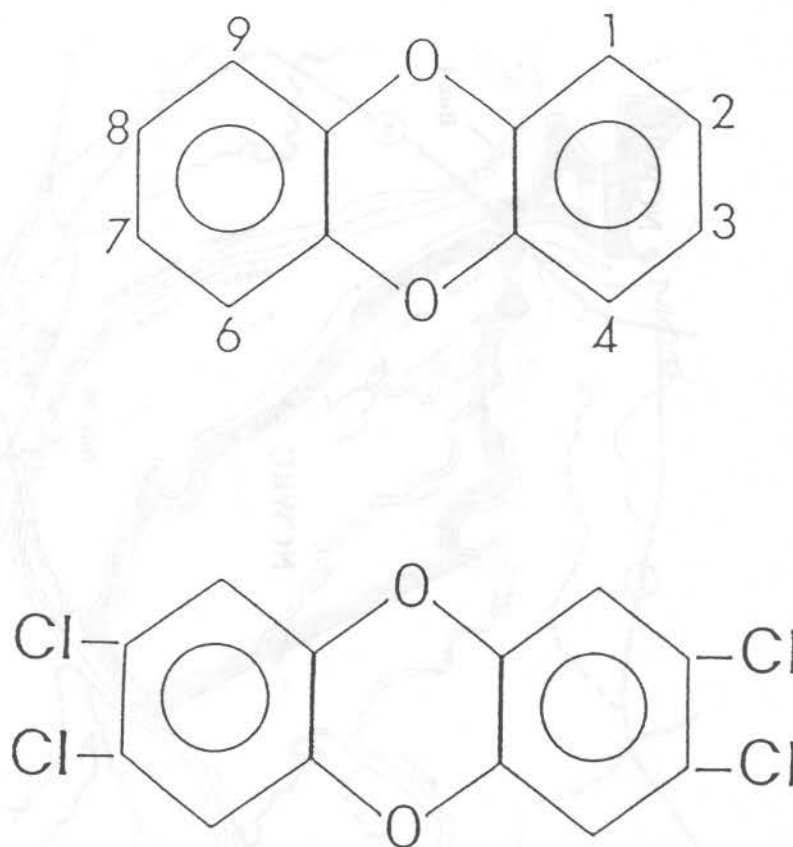
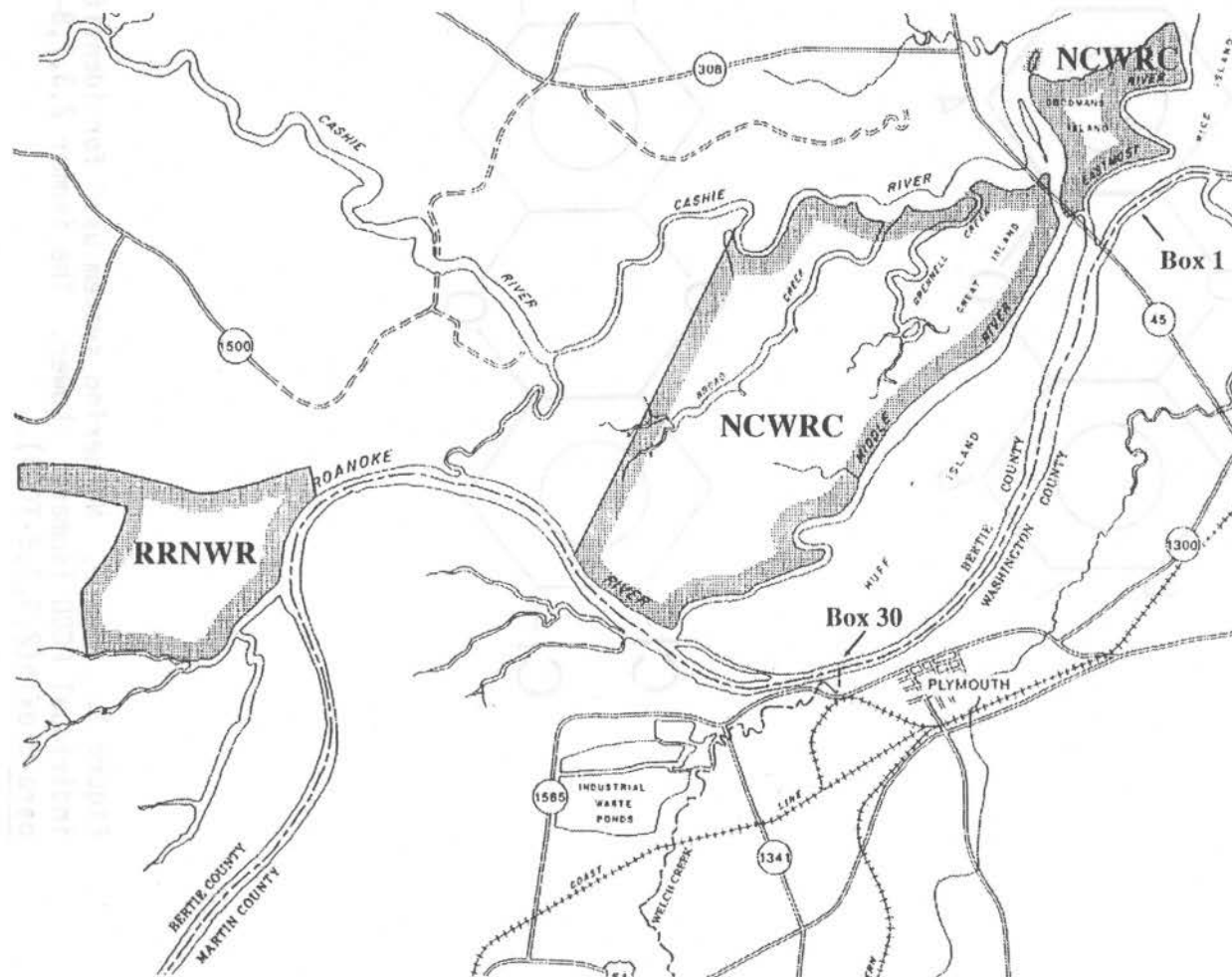


Figure 1. Upper. Numbering system used for identification of individual PCDD isomers. Lower. The isomer 2,3,7,8-tetrachlorodibenzo-para-dioxin(2,3,7,8-TCDD).

From: Eisler (1986).

Figure 2. Lower Roanoke River near Plymouth, North Carolina, where thirty wood duck nest boxes were installed in the winter of 1992. The boxes were assigned individual identification numbers in descending order from the mouth of Welch Creek (Nest box 30) to an area downstream of the Highway 45 bridge (Nest box 1). Whole clutches of wood duck eggs were collected from these boxes during the springs of 1992 and 1993. Lands managed by the U.S. Fish and Wildlife Service (RRNWR) and North Carolina Wildlife Resources Commission (NCWRC) are delineated.



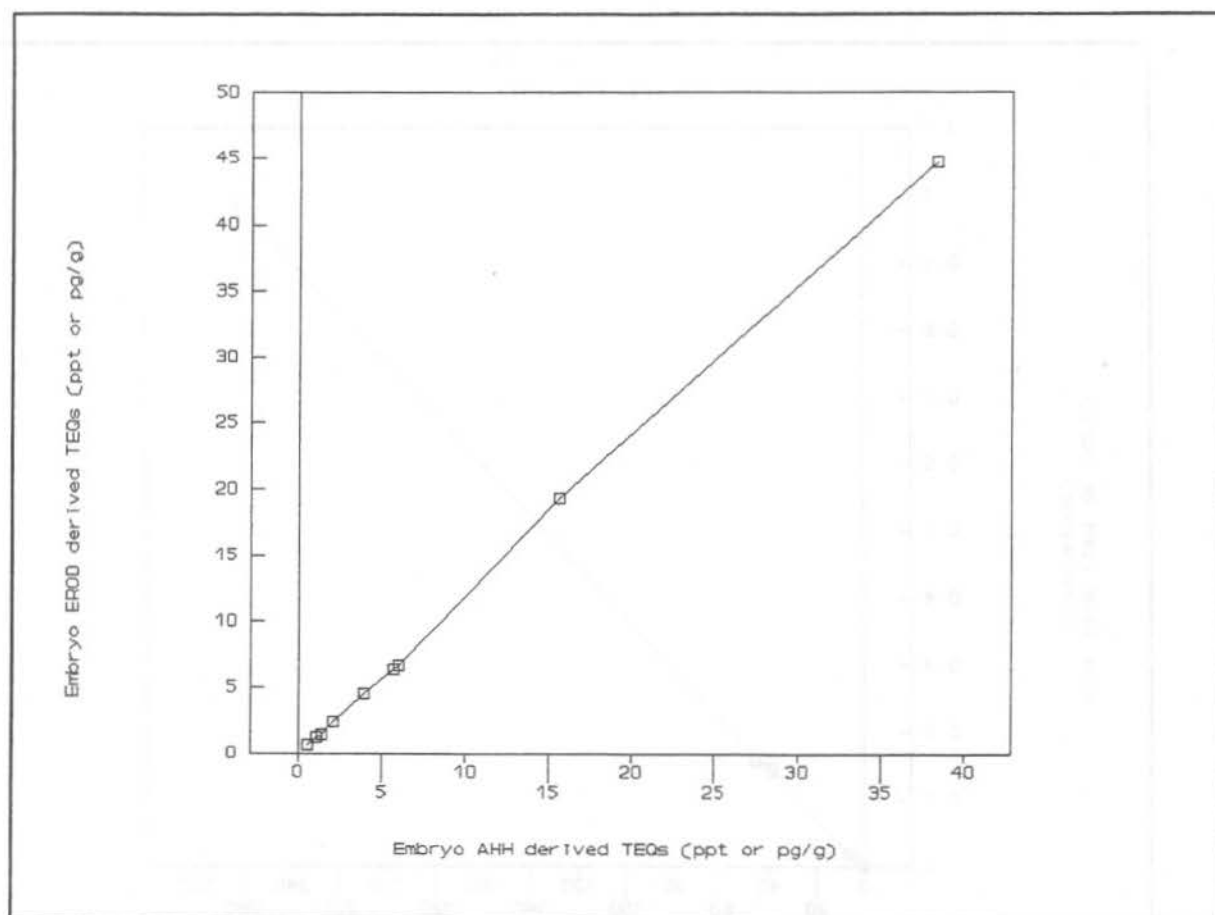


Figure 3. The relationship between AHH derived TEQs and EROD derived TEQs in wood duck embryos.

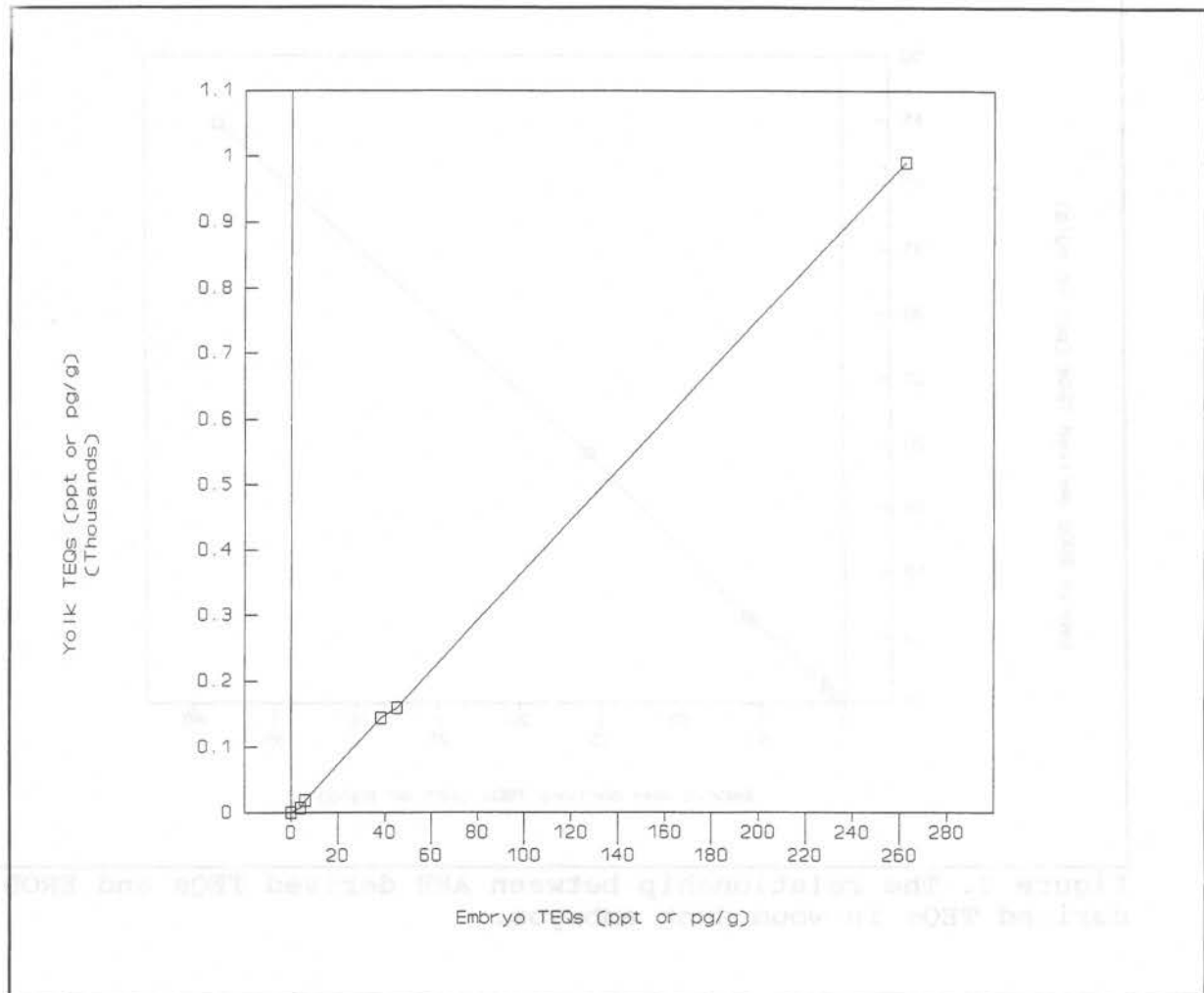


Figure 4. TEQs of yolk sac= $(3.79 \times \text{TEQs embryo} - 4.75)$ defines the relationship between the AHH derived TEQs in wood duck embryos and companion yolk sacs.

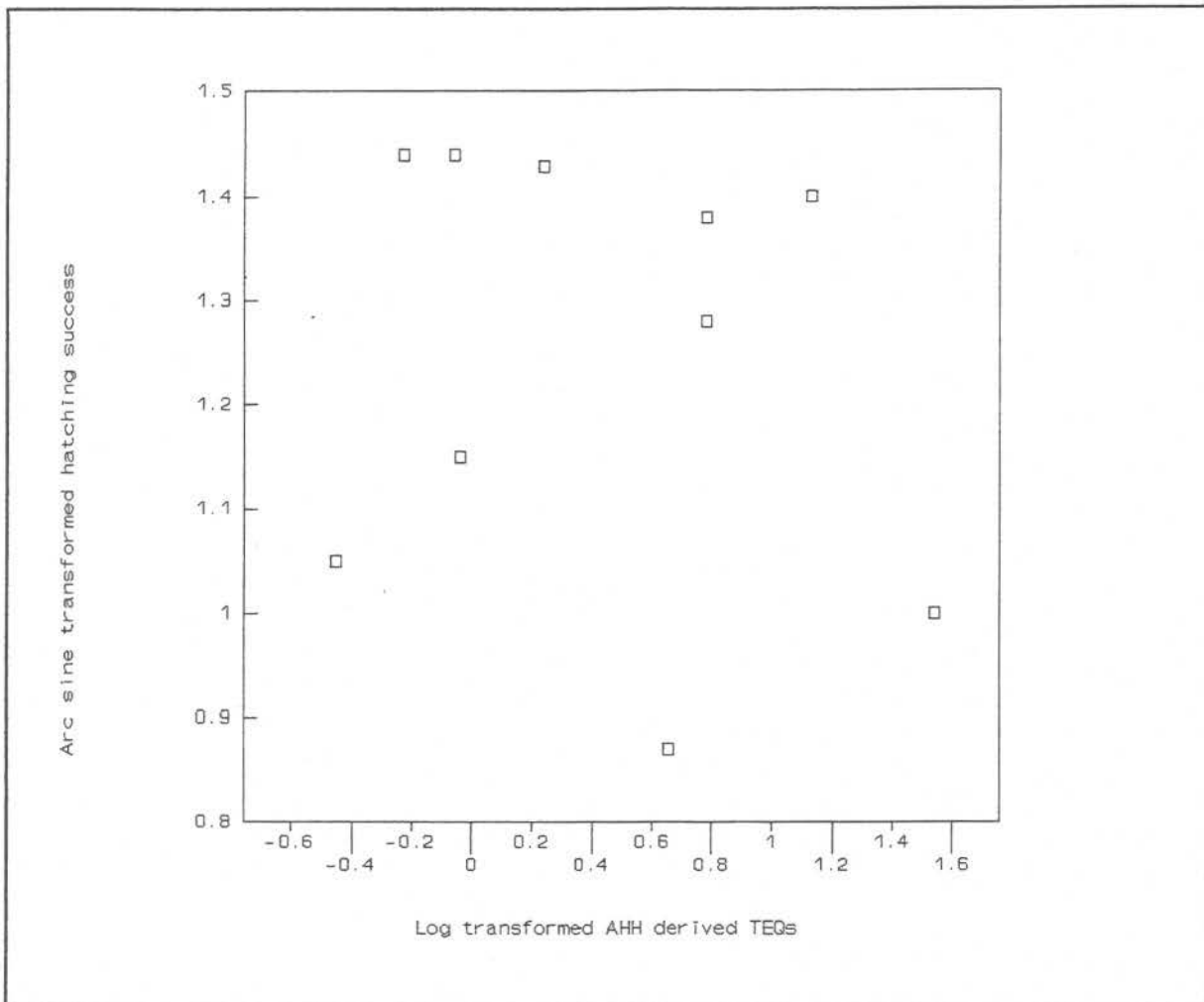


Figure 5. Hatching success and PCDD/PCDF burdens in wood duck eggs collected from the Roanoke River below Plymouth, NC in 1992 and 1993.

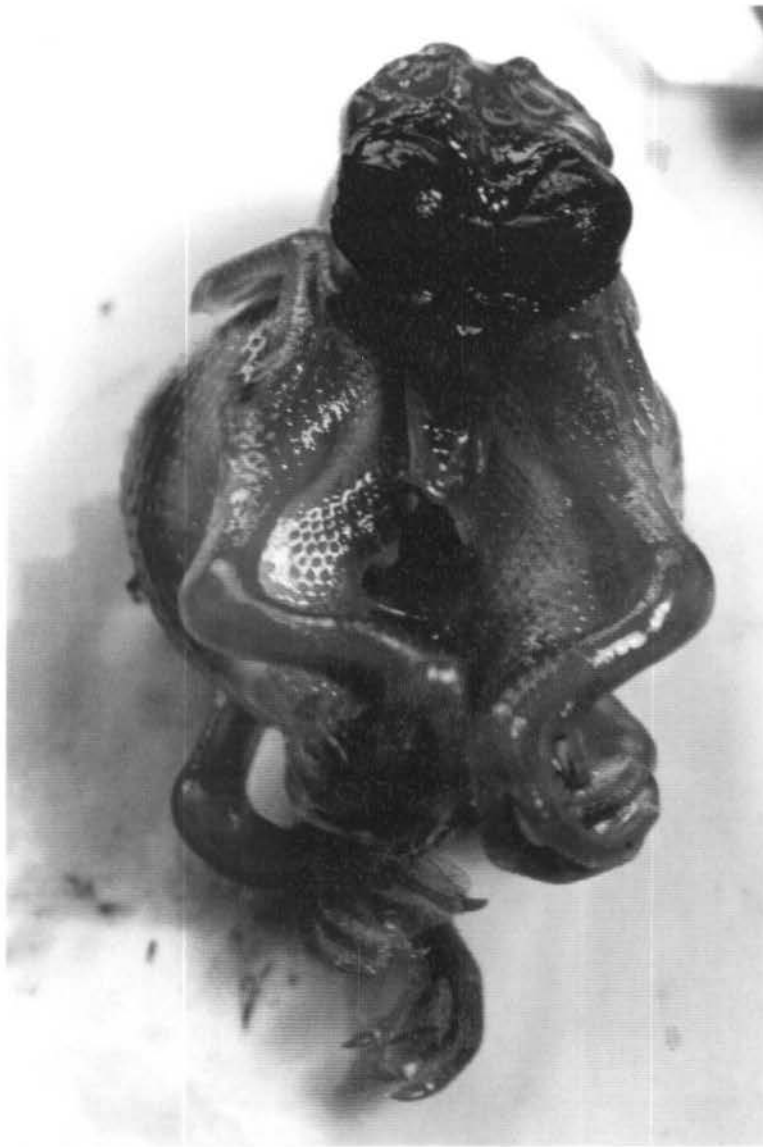


Figure 6. Deformed, unhatched wood duck embryo collected from a nest box on the Roanoke River near Plymouth, North Carolina, 1994.

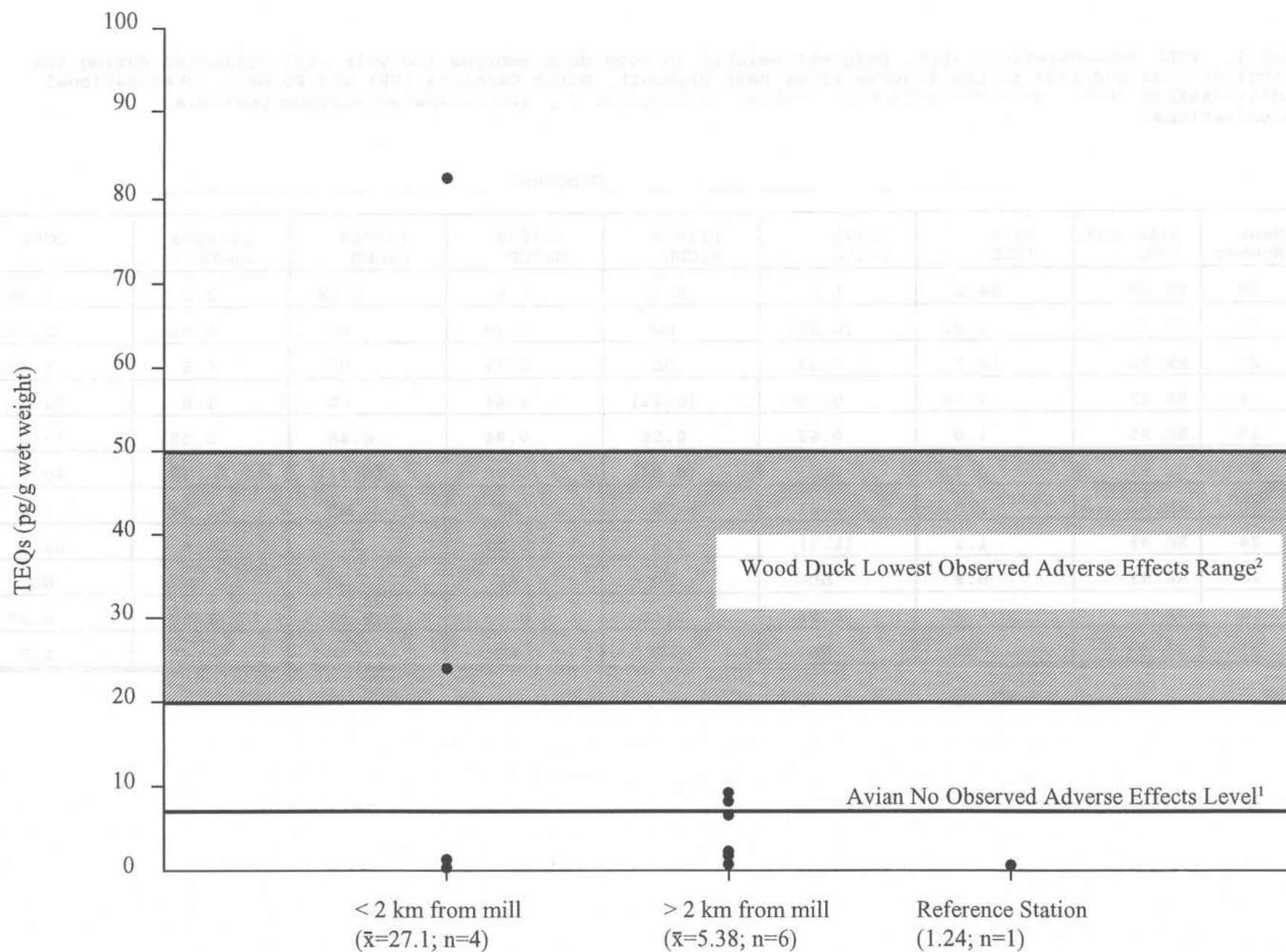


Figure 7. 2,3,7,8-TCDD equivalents (TEQs) in wood duck eggs collected from the lower Roanoke River and Pocosin Lakes NWR (Reference). Data from the Roanoke River are divided into two river segments (< 2km and > 2km from mill at Plymouth, NC) and compared to screening levels for avian effects of dioxins from ¹Bowerman et al. (1995) and ²White and Seginak (1994).

Table 1. PCDD concentrations (ppt, pg/g wet weight) in wood duck embryos (no yolk sac) collected during the springs of 1992 and 1993 at the Roanoke River near Plymouth, North Carolina (RR) and Pocosin Lakes National Wildlife Refuge (PL). ND = Not detected. Values in brackets [] are estimated maximum possible concentrations.

Nest Number	Site and Year	Congener						OCDD
		2378 TCDD	12378 PeCDD	123478 HxCDD	123678 HxCDD	123789 HxCDD	1234678 HpCDD	
30	RR 92	26.6	1.5	0.75	1.5	0.28	2.2	7.26
23	RR 92	2.82	[0.33]	ND	0.39	ND	0.91	2.98
27	RR 92	12.1	0.48	ND	0.74	ND	1.6	4.61
4	RR 92	4.76	0.39	[0.22]	0.54	ND	1.8	13.8
29	RR 93	1.0	0.62	0.56	0.84	0.48	5.05	33.0
27	RR 93	0.32	0.35	[0.24]	0.84	[0.44]	6.39	40.6
20	RR 93	1.5	0.31	ND	ND	ND	[0.34]	1.8
24	RR 93	1.1	[1.1]	1.5	2.93	2.1	16.8	83.5
19	RR 93	0.87	ND	ND	ND	ND	ND	ND
16	RR 93	4.12	0.99	0.40	0.70	0.29	[1.70]	6.27
P1	PL 93	ND	ND	ND	ND	ND	0.45	1.7

Table 2. PCDF concentrations (ppt, pg/g wet weight) in wood duck embryos (no yolk sac) collected during the springs of 1992 and 1993 at the Roanoke River near Plymouth, North Carolina (RR) and Pocosin Lakes National Wildlife Refuge (PLNWR). ND = not detected. Values in brackets [] are estimated maximum possible concentrations.

Nest Number	Site and Year	Congener									OCDF
		2378 TCDF	12378 PeCDF	23478 PeCDF	123478 HxCDF	123678 HxCDF	123789 HxCDF	234678 HxCDF	1234678 HpCDF	1234789 HpCDF	
30	RR 92	393	1.4	4.13	0.50	0.27	ND	[0.47]	ND	ND	ND
23	RR 92	35.1	0.23	0.50	ND	ND	ND	0.33	ND	ND	ND
27	RR 92	122	0.62	1.2	ND	ND	ND	0.34	ND	ND	ND
4	RR 92	39.8	0.27	0.46	[0.09]	ND	ND	0.24	ND	ND	ND
29	RR 93	5.47	0.15	0.30	0.14	ND	ND	0.26	ND	ND	ND
27	RR 93	0.92	0.16	0.25	[0.29]	[0.10]	ND	0.40	0.35	ND	0.88
20	RR 93	15.0	0.21	0.43	ND	ND	ND	0.29	ND	ND	ND
24	RR 93	8.24	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	RR 93	8.77	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	RR 93	40.1	0.60	0.98	0.35	ND	ND	[0.26]	[0.18]	ND	ND
P1	PL 93	1.6	0.38	0.51	0.22	0.12	ND	0.36	ND	ND	0.43

Table 3. Total 2,3,7,8 TCDD equivalents (total TEQs) in wood duck embryos collected from the Roanoke River near Plymouth, North Carolina (RR) and Pocosin Lakes National Wildlife Refuge (PL) during the springs of 1992 and 1993. TEQs are calculated according to the method of Smith et al. (1990) or U.S. EPA (1989). ND = not detected.

		2378 TCDD	TOTAL TEQS	TOTAL TEQS	TOTAL TEQS
Nest Number	Site and Year		AHH	EROD	ITEF/89
30	RR 92	26.6	38	45	69
23	RR 92	2.82	3.9	4.5	6.8
27	RR 92	12.1	16	18	25
4	RR 92	4.76	6.0	6.7	9.3
29	RR 93	1.0	1.4	1.5	2.3
27	RR 93	0.32	0.62	0.64	1.1
20	RR 93	1.5	2.2	2.5	3.4
24	RR 93	1.1	1.4	1.4	3.3
19	RR 93	0.87	1.1	1.2	1.8
16	RR 93	4.12	5.7	6.4	9.4
P1	PL 93	ND	0.40	0.48	0.51

Table 4. PCDD concentrations (ppt, pg/g wet weight) in yolk sacs of wood duck embryos collected during the spring of 1992 at the Roanoke River near Plymouth, North Carolina. ND = Not detected.

Nest Number	Congener						OCDD
	2378 TCDD	12378 PeCDD	123478 HxCDD	123678 HxCDD	123789 HxCDD	1234678 HpCDD	
30	100.0	7.7	5.8	14.0	5.4	28.8	115.0
23	5.6	ND	ND	ND	ND	6.8	19.7
4	14.1	ND	2.6	3.8	ND	22.9	179.0

Table 5. PCDF concentrations (ppt, pg/g wet weight) in yolk sacs from wood duck embryos collected during the spring of 1992 at the Roanoke River near Plymouth, North Carolina. ND=Not detected.

Nest Number	Congener									OCDF
	2378 TCDF	12378 PeCDF	23478 PeCDF	123478 HxCDF	123678 HxCDF	123789 HxCDF	234678 HxCDF	1234678 HpCDF	1234789 HpCDF	
30	1400.0	6.6	19.3	3.2	2.0	ND	3.2	ND	ND	ND
23	62.3	ND	ND	ND	ND	ND	3.0	ND	ND	ND
4	125.0	ND	2.0	ND	ND	ND	ND	ND	ND	ND

Table 6. Calculated PCDD concentrations (ppt, pg/g fresh wet weight) of whole eggs from wood duck embryos and yolk sacs collected from the Roanoke River near Plymouth, North Carolina during the spring of 1992. Fresh weight egg concentrations are determined by the equation ((ppt in embryo)*(weight of the embryo)+(ppt in the yolk sac)*(weight of the yolk sac))/(egg volume). ND=Not detected.

Nest Number	Congener						
	2378 TCDD	12378 PeCDD	123478 HxCDD	123678 HxCDD	123789 HxCDD	1234678 HpCDD	OCDD
30	32	2.2	1.4	3.3	1.1	6.3	24
23	2.2	0.59	ND	0.23	ND	0.67	3.6
4	5.6	0.92	0.71	0.94	ND	4.6	14

Table 7. Calculated PCDF concentrations (ppt, pg/g fresh wet weight) in whole eggs from wood duck embryos collected from the Roanoke River near Plymouth, North Carolina during the spring of 1992. Whole egg concentrations are determined by the equation ((concentration in the embryo)*(weight of the embryo)+(concentration in the yolk sac)*(weight of the yolk sac))/(egg volume). ND=Not detected.

Nest Number	Congener									
	2378 TCDF	12378 PeCDF	23478 PeCDF	123478 HxCDF	123678 HxCDF	123789 HxCDF	234678 HxCDF	1234678 HpCDF	1234789 HpCDF	OCDD
30	460	1.9	5.6	0.83	0.49	ND	0.82	ND	ND	ND
23	26	0.13	0.29	ND	ND	ND	0.47	ND	ND	ND
4	47	0.20	0.63	0.07	ND	ND	0.18	ND	ND	ND

Table 8. Whole egg total 2,3,7,8 TCDD equivalents (total TEQs) in wood duck eggs collected in the springs of 1992 and 1993 from the Roanoke River near Plymouth, North Carolina (RR) and Pocosin Lakes National Wildlife Refuge (PL). Whole egg values are calculated by the following equation: ((embryo TEQs)*(embryo weight)+(estimated yolk sac TEQs)*(yolk sac weight))/(volume). Units are pg/g fresh wet weight.

Nest Number	Site and Year	Total Estimated AHH TEQS	Total Estimated EROD TEQS	Total Estimated I-TEF/89 TEQS
30 *	RR 92	45	53	82
23	RR 92	3.8	4.5	6.7
27	RR 92	14	15	22
4	RR 92	6.1	6.8	9.5
29	RR 93	0.87	0.95	1.5
27	RR 93	0.39	0.40	0.67
20	RR 93	1.7	2.1	2.8
24	RR 93	0.92	0.85	2.6
19	RR 93	0.58	0.64	0.93
16	RR 93	6.0	6.7	9.8
P1	PL 93	0.22	0.30	0.33

* Egg volume for this sample is an estimate based on the average volumes of all eggs analyzed.

Table 9. Microsomal monooxygenase activities from livers of pipping embryos collected in the springs of 1992 and 1993 from the Roanoke River near Plymouth, North Carolina (RR) and Pocosin Lakes National Wildlife Refuge (PL). * indicates values which are induced according to the criterion of being greater than two standard deviations from the control mean.

Nest Number	Site and Year	BROD pmol prod/ min/mg microsomal protein	EROD pmol prod/ min/mg microsomal protein	MROD pmol prod/ min/mg microsomal protein
30	RR 92	21.7	8.8	12.1
23	RR 92	8.9	4.5	7.3
27	RR 92	11.9	6.4	8.5
4	RR 92	9.0	6.4	26.8
29	RR 93	20.6	27.8*	87.6*
27	RR 93	15.6	18.1*	45.6*
20	RR 93	11.4	13.8	35.2
24	RR 93	12.0	11.8	19.0
19	RR 93	7.3	6.6	17.9
16	RR 93	4.6	8.1	24.4
	RR Mean	12.3±5.53	11.23±7.13	28.44±23.97
P1	PL 93	11.6	14.3	31.4
	PL Mean	13.14±4.12	8.23±3.44	21.71±7.57

Table 10. Hatching success and ten day survivorship of clutches of wood ducks collected from the Roanoke River near Plymouth, North Carolina (RR) and Pocosin Lakes National Wildlife Refuge (PL). Clutches were collected during the springs of 1992 and 1993.

Nest Number	Site and Year	Number of Eggs	Hatching Success	Survivorship
30	RR 92	9	71	40
23	RR 92	14	58	100
27	RR 92	11	100	89
4	RR 92	11	100	85
29	RR 93	17	100	38
27	RR 93	18	75	80
20	RR 93	14	100	60
24	RR 93	14	83	100
19	RR 93	16	100	75
16	RR 93	14	92	89
P1	PL 93	11	100	86