Bull. Environ. Contam. Toxicol. (1985) 34:121–125

Bulletin of Environmental Contamination and Toxicology

DDE Residues and Artificial Incubation of Loggerhead Sea Turtle Eggs

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In an earlier study (Clark and Krynitsky 1980), loggerhead (*Caretta caretta*) eggs were collected at Merritt Island, Florida, and frozen after periods of artificial incubation that ranged from 43 to 52 days; both DDE and PCB's (polychlorinated biphenyls) declined significantly during this 10-day interval.

The present study was undertaken to verify and quantify this decline for the entire incubation period. Eggs were analyzed for DCBP (4,4'-dichlorobenzophenone) because it is a breakdown product that is likely to be present if DDE was metabolized. The data collected also allowed us to measure changes in egg weight, embryo size, and weight of eggshell that occurred during incubation.

MATERIALS AND METHODS

On 20 June 1979, a clutch of 109 loggerhead eggs was collected by personnel of the Merritt Island National Wildlife Refuge immediately after the clutch was laid. At that time, 8 eggs were frozen and the remaining eggs began artificial incubation. Samples of 8 eggs were removed and frozen at intervals of about 10 days until hatching (66–70 days after laying). Seven samples (56 eggs) were obtained at 0, 9, 20, 30, 40, 50, and 61 days. Forty-eight turtles hatched and were released, 3 eggs failed to hatch, 1 egg was accidentally broken, and 1 egg was lost. For incubation, eggs were placed in plastic containers, covered with beach sand, moistened regularly, and kept in a building that was open to the ambient temperature and humidity of the beach. Sample eggs were wrapped individually in aluminum foil, sealed in plastic bags, and frozen. Eggs were packed in Dry Ice and shipped to the Patuxent Wildlife Research Center where they were weighed and opened, and the crown-rump length or straight-line carapace length of each visible embryo (≥20 days incubation) was measured with a vernier caliper. Contents of each egg were placed in separate tared glass jars that had been cleaned with acetone and hexane, and the jars and contents were weighed. Subsequently, each egg was homogenized in a blender and analyzed for organochlorines. Eggshells were dried in a desiccator with calcium sulfate at room temperature and then weighed. The first eggshells (0 days incubation) to be dried were not confined in individual containers

and as they dried they shattered and fragments were mixed. Therefore we have no data on weight of eggshells before incubation began.

For each chemical analysis, a 10-g portion of the homogenized egg was mixed with anhydrous sodium sulfate and extracted for 7 hrs in a Soxhlet apparatus. Extraction, sample cleanup, and separation of organochlorine pesticides from PCB's were performed as described by Cromartie et al. (1975), except that the SilicAR separation was collected in four fractions (Kaiser et al. 1980). The fractions were analyzed on a Hewlett Packard Model 5713 or 5840 gas-liquid chromatograph equipped with an electron-capture detector, automatic sampler, digital processor, and a 1.83 m by 4 mm glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcoport at 190°C. Samples were analyzed for *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, 4,4'-dichlorobenzophenone (DCBP), dieldrin, endrin, heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-nonachlor, toxaphene, and PCB's. Recovered PCB's resembled Aroclor 1260. Residues in 10% of the samples were confirmed by gas-liquid chromatography-mass spectrometry. Average percentage recoveries from spiked chicken egg tissue ranged from 92 to 110%, except for *trans*-nonachlor which was 49%. Residue data were not adjusted for these recoveries. Analytical sensitivity was 0.005 ppm for pesticides and 0.025 ppm for PCB's. The residues reported were corrected for background residues found in procedural blanks.

Geometric means are given for residues because the data were positively skewed. Arithmetic means are given for other variables. One-way analysis of variance (ANOVA) was used to determine whether differences existed within groups of means, and Tukey's multiple comparison procedure (Neter and Wasserman 1974) was used to make pairwise comparisons.

Residues are reported as total µg per egg, rather than as ppm, to avoid the effect of changes in water content of eggs during incubation on ppm values.

RESULTS AND DISCUSSION

Of the 56 eggs analyzed, 2 contained *cis*-chlordane, 16 contained oxychlordane, 40 contained PCB's, and 55 contained DDE. No other residues were detected. Levels of *cis*-chlordane and oxychlordane were near the detection limit (0.005-0.008 ppm wet weight). For DDE the overall geometric mean, 95% confidence interval (CI), and range were 0.099, 0.083-0.119, and 0.056-0.15 ppm. These DDE values (Fig. 1A) exclude the single egg in which no DDE was reported. We believe this zero value is an error because all other logerhead eggs in this and the previous study (Clark and Krynitsky 1980) contained DDE. This egg was also eliminated from the ANOVA for DDE. The PCB results were judged unreliable because of a failure of instrumentation and therefore are not presented.

Residues of DDE in loggerhead eggs declined significantly between

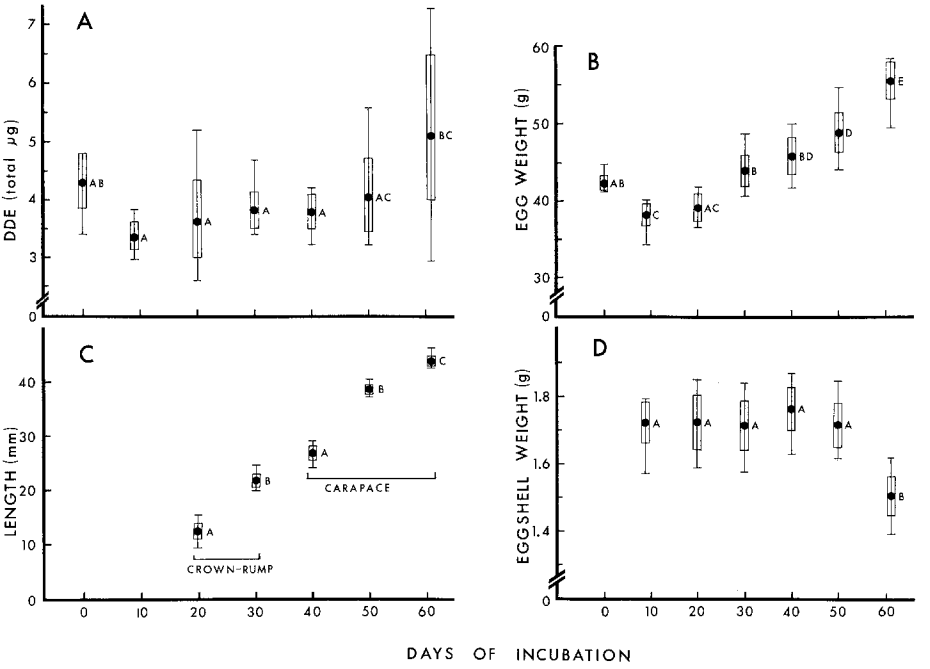

Figure 1. Means, 95% confidence intervals, and ranges of (A) DDE content, (B) weight, (C) embryo length, and (D) weight of eggshells in loggerhead eggs during incubation. Sample sizes were 8 for each except for DDE at 50 days which was 7. Shared letters indicate means that are not significantly different at a minimum of 95% confidence.

43 and 52 days of incubation (Clark and Krynitsky 1980). Hatchlings from chicken eggs injected with DDE contained DCBP as the only metabolite (Abou-Donia and Menzel 1968). Contrary to these earlier findings, DDE in the present loggerhead eggs did not decline during incubation (Fig. 1A), and DCBP was not found in any sample. Either metabolism of DDE did not occur or amounts of DDE broken down were too small to allow detection of a decline or of the metabolite. The marked decline seen in the earlier study must have been due to chance or to unknown causes.

Concentrations of DDE (geometric mean and 95% CI) reported previously in 9 loggerhead eggs from 9 clutches from Merritt Island (Clark and Krynitsky 1980) were 0.047 ppm and 0.024-0.090 ppm. In the present study, concentrations in the 15 eggs at a similar incubation interval (40 and 50 day samples) were higher at 0.091 and 0.084-0.099 ppm. Nevertheless, these amounts are also far below levels thought to be harmful.

After an initial decrease, weights of incubating loggerhead eggs increased steadily and significantly throughout incubation (Fig. 1B). There was no decrease toward the end of incubation as that

reported in artificially incubated snapping turtle eggs, *Chelydra serpentina* (Packard et al. 1982). The average weight of loggerhead eggs at the final sampling was 131% of that at laying. This increase is about 14% more than the maximum reported for snapping turtles (Packard et al. 1982). Lengths of embryonic loggerheads increased significantly at each sampling point (Fig. 1C).

The weight of loggerhead eggshells did not change during incubation except for an average loss of 209.7 mg (12.2%) between the 50 and 61 day samples (Fig. 1D). Data from Bustard et al. (1969) show that yolk plus albumen of 10 loggerhead eggs contained an average 34.8 mg of calcium whereas 9 hatchling loggerheads contained 88.5 mg of calcium (our average of means for hatchlings from 2 types of sand). Those authors believed that the additional 53.7 mg of calcium came from the eggshell. A similar weight of calcium in the eggs we studied would represent 25.6% of the observed decrease in eggshell weight. The remainder of the loss would be attributable to sloughing of the mineral layer, as noted by Ewert (1979).

Acknowledgments. We thank M. J. Packard for comments that were helpful to our data interpretation. We thank R. C. Lee and S. R. Vehrs for providing the incubated eggs, T. B. Wright for preparing the eggs for analysis, and C. M. Bunck for advice on statistical procedures. Finally, we thank R. J. Hall and R. M. Prouty for critical reviews of the manuscript.

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Received February 27, 1984; accepted April 4, 1984