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CONCENTRATIONS OF CONTAMINANTS IN MUSCLE OF THE AMERICAN ALLIGATOR IN FLORIDA

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ABSTRACT: Samples of tail muscle from 32 American alligators (*Alligator mississippiensis*) in Florida were analyzed for contaminant concentrations to provide preliminary information on the potential public health hazard of meat consumption. Detectable levels were found for eight metals; copper, zinc, iron, chromium, mercury, lead, cadmium and arsenic. Mean residue was highest for mercury (geometric mean = 0.61 ppm). DDE, DDD, DDT, dieldrin, heptachlor epoxide, lindane, and PCB's were found. Mean residue concentrations were compared by lake. Alligators appeared to be suitable monitors of environmental pollution. Concentrations of contaminants found in these animals probably pose little threat to public health. However, recommendations must await analysis of larger sample sizes and information on amount and frequency of meat consumption. Alligators killed for human consumption should continue to be monitored for contaminant residues.

Key words: American alligator, *Alligator mississippiensis*, toxicology, metals, chlorinated hydrocarbons, muscle tissue, public health.

INTRODUCTION

Agricultural and industrial effluents contribute toxic substances to Florida's wetlands. Many of these substances undergo biomagnification through food chains and may be found in predators associated with wetlands (Ogden et al., 1974). Because American alligators (*Alligator mississippiensis*) are long-lived and represent a high level of the food chain (Delany and Abercrombie, 1986), their potential for biomagnification is high. Peters (1983) found captive alligators capable of accumulating mercury in muscle tissue to concentrations exceeding Food and Drug Administration (FDA) limits for fish for human consumption. Bell and Lopez (1985) found cadmium accumulation in hepatic tissue of alligators exposed to cadmium.

Alligator meat is sold extensively for human consumption. Information on concentrations of contaminants in alligator tissue is needed to determine possible health hazards of meat consumption. Environmental contaminants can affect reptilian health and may interfere with reproduction (Hall, 1980). In addition, alligators usually occupy a small "activity range" (Goodwin and Marion, 1979) and may serve as indicators of local environmental pollution. This paper reports information on the analysis of alligator muscle for contaminant residues.

MATERIALS AND METHODS

Tail muscle was obtained from hunter-harvested alligators from lakes Iamonia, Newnans, Orange, Rodman, George, Apopka, Hancock, and Trafford (Fig. 1) between 26 August and 29 October 1985. Muscle samples were obtained from 32 large alligators ($\bar{x}$ total length = 3.3 m). Alligators ranged in length from 2.9 to 3.8 m and included 31 males and one female. Alligators displayed no obvious signs of toxicosis. Samples were stored in Whirl-Pak plastic bags at −20 C prior to analysis.

Three samples per lake (n = 24) were collected for analysis of metals: copper, Cu; zinc, Zn; iron, Fe; chromium, Cr; mercury, Hg; lead, Pb; arsenic, As; and platinum, Pt. As part of the tissue preparation process, 10 g of tissue were placed in 25- × 200-mm preweighed culture tubes fitted with Teflon-lined plastic screw caps. After the addition of 10 ml of instra-analyzed nitric acid (concentrated), the tubes remained at room temperature for 2 hr and were then heated to 90–95 C for an additional 4 hr. After the tubes cooled to room temperature, 2 ml of hydrogen peroxide (30%) were added slowly and the resulting mixture heated to 90–95 C for an additional 2 hr. Digests were then transferred through prewetted filter paper into 100-ml vol-

umetric flasks and brought up to volume with ultrapure water.

All analyses for metals were conducting using a double-beamed atomic absorption spectrophotometer (Perkin-Elmer model 2380, Perkin-Elmer Corp., Norwalk, Connecticut 06856, USA). For the determination of Hg and As, a hydride generator (MHS-10 Perkin-Elmer, Perkin-Elmer Corp.) was utilized in the flameless and flame modes, respectively. For the determination of Cu, Zn, Fe, Cr, Pb and Cd, an electrothermal graphite furnace (HGA-400 Perkin-Elmer, Perkin-Elmer Corp.) and deuterium arc background corrector were used. All unknowns were compared with commercially-available metal standards (Fisher Scientific, 7464 Chancellor Drive, Orlando, Florida 32809, USA) and concentrations expressed as μg of metal per g wet weight of muscle tissue (ppm). The detection limits for the metals were as follows: Cu, 0.02 ppm; Zn, 0.05 ppm; Fe, 0.30 ppm; Cr, 0.02 ppm; Hg, 0.04 ppm; Pb, 0.02 ppm; Cd, 0.005 ppm; As, 0.02 ppm; Pt, 0.50 ppm.

Analysis of variance and Tukey's W procedure were used for comparisons of mean metal concentrations among groups. To stabilize variances, concentrations for Cu, Fe, Cr and Hg were transformed to natural (base e) logarithms. Bartlett's test was used to test homogeneity of variances.

One sample per lake (n = 8) was collected for analysis of residues of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT); 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE); 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD); dieldrin; heptachlor epoxide; lindane; and polychlorinated biphenyl (PCB's). Muscle samples were weighed and homogenized. Each sample was mixed with anhydrous sodium sulfate and extracted with hexane for at least 8 hr in a Soxhlet apparatus. Extracts were evaporated and lipid weights were determined. Extracts were then redissolved in 20 ml of hexane. A 10-ml aliquot containing not more than 0.5 g of lipid was cleaned on a florisil column. Following the clean up procedure, the eluate was concentrated to 5.0 ml and a 4-ml aliquot was placed on a silicic acid column to separate pesticides from PCB's. The column was first eluted with 400 ml of petroleum ether followed by 200 ml of methylene chloride : hexane : acetonitrile (80:19:1). Using this procedure, PCB's were collected in the first 400 ml of eluate and the pesticides were collected in the polar eluate. The PCB and pesticide fractions were evaporated to dryness and redissolved in 10 ml of hexane for analysis by gas-liquid chromatography.

Samples were analyzed on a Hewlett-Packard 5880 (Hewlett-Packard Co., Route 41, Avondale, Pennsylvania 19311, USA) gas-liquid chromatograph equipped with a Nickel 63 electron-capture detector and digital integrator. Pesticides and PCB's were separated on a 60-m glass wide bore open tubular column coated with SPB-5 (Supelco, Inc., Supelco Park, Belefonte, Pennsylvania 16823, USA). The carrier gas flow rate was set at 3 ml/min of 5% methane in argon and the make up flow rate was set at 37 ml/min. Both injector detector temperatures were set at 325 C and 300 C, for PCB and pesticide analysis, respectively. The oven temperature for analysis of PCB's was programmed to begin at 200 C and increase at a rate of 8 degrees per min until it reached 280 C. The oven temperature was held constant at 280 C for 20 min. The oven temperature for analysis of pesticides was programmed to begin at 200 C and increase at a rate of 5 degrees per min until it reached 275 C. The oven temperature was held constant at 275 C for 20 min. Methods were similar to those described by Cromartie et al. (1975).

Recovery of the p,p' isomers of DDE, DDD, and DDT was determined to be 94%, 100%, and 97%, respectively. Recovery of dieldrin, heptachlor epoxide, and lindane were 83%, 79%, and 75%, respectively. Chromatographic profiles of PCB's most closely resembled those of Aroclor 1260. Concentrations of PCB's in muscle fat were calculated by comparing areas of a major peak in the Aroclor 1260 standard to a peak with similar retention time in the sample.

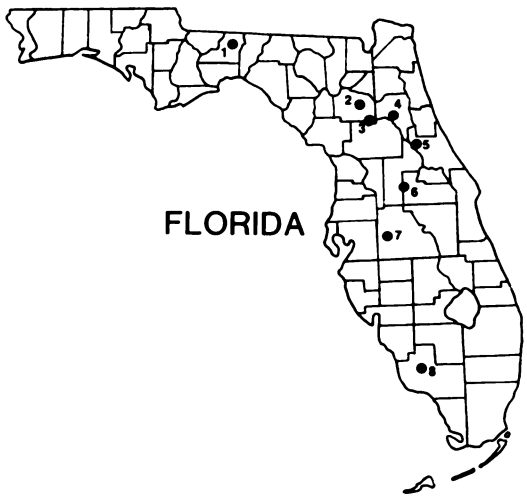

FIGURE 1. Map of Florida depicting sites where alligator muscle samples (n) were collected: 1, Lake Iamonia (Leon County) (4); 2, Newnans Lake (Alachua County) (4); 3, Orange Lake (Alachua County) (4); 4, Rodman Reservoir (Putnam County) (4); 5, Lake George (Volusia County) (4); 6, Lake Apopka (Orange County) (4); 7, Lake Hancock (Polk County) (4); and Lake Trafford (Collier County) (4).

TABLE 1. Comparison of metal concentrations in alligator muscle samples (n = 3) from various lakes in Florida, 1985.

Metal	Lake							
Cu[a]	Rod[b]	Han	Ora	Apo	New	Iam	Tra	Geo
	0.28	0.34	0.39	0.40	0.41	0.49[c]	1.02	6.03
Zn	Apo	Rod	Tra	Geo	Iam	Han	Ora	New
	14.20	19.00	19.75	22.17	25.25	25.50	27.88	36.00
Fe	Apo	Rod	Han	Iam	Tra	New	Ora	Geo
	4.56	7.42	8.45	8.74	10.26	15.38	17.71	22.76
Cr	Apo	Han	Iam	New	Ora	Rod	Geo	Tra
	0.03	0.05	0.05	0.05	0.05	0.05	0.06	0.11
Hg	Geo	Han	Apo	New	Ora	Tra	Rod	Iam
	0.04	0.10	0.11	0.27	0.37	0.43	0.51	0.61
Pb	Ora	Apo	Rod	Iam	Tra	Geo	Han	New
	0.04	0.07	0.07	0.08	0.09	0.09	0.10	0.12
Cd[d]	Han	Iam	Rod	New	Geo	Apo	Ora	Tra
	0.01	0.01	0.02	0.02	0.03	0.03	0.06	0.06

[a] Metal concentrations are presented as ppm (μg/g) on a wet weight basis.
[b] Iamonia (Iam), Newnans (New), Orange (Ora), Rodman (Rod), George (Geo), Apopka (Apo), Hancock (Han), and Trafford (Tra) lakes.
[c] Means underscored with the same line within a row are not significantly different ($P > 0.05$); geometric means are presented for Cu, Fe, Cr, and Hg.
[d] Lack of variance within samples prohibited statistical comparisons among groups for Cd.

Confirmation of the identity of all chlorinated hydrocarbon compounds was accomplished by the addition of authentic DDE, DDD, DDT, dieldrin, heptachlor epoxide, lindane, and Aroclor 1260 to the sample and subsequent chromatography at various conditions of column temperature and carrier gas flow rate.

RESULTS AND DISCUSSION

Detectable concentrations were found in all samples for seven of nine metals tested (Table 1). As was found only in two samples from Rodman Reservoir (0.16 ppm and 0.07 ppm) and one sample from Lake Iamonia (0.07 ppm). Pt was not detected at values >0.50 ppm. Concentrations of metals commonly associated with environmental contamination (Cr, Hg, Cd, Pb, and As) were relatively low. Mean residue concentrations were highest for Hg (geometric mean = 0.61 ppm) and were <0.2 ppm for other contaminants. Concentrations of Hg in alligators from lakes Newnans, Orange, Trafford, Rodman, and Iamonia were significantly ($P < 0.05$) higher than those measured from lakes George, Hancock, and Apopka. Hg concentrations in alligators from Lake George were significantly ($P < 0.05$) lower than those measured from other lakes. Because sample preparation converts all forms of mercury to the inorganic form it is not known if the residue source was natural or of industrial/agricultural origin. Compared by lake, concentrations of Cu, Fe, and Zn varied considerably. Concentrations of Cu in samples from lake George were significantly ($P < 0.05$) higher than those measured from other lakes and may reflect a contamination problem. Detectable concentrations of DDE, DDD, DDT, dieldrin, heptachlor epoxide, lindane, and PCB's were found in eight samples examined for chlorinated hydrocarbons (Table 2).

These results indicate that wild alligators accumulate metal, pesticide, and PCB residues in muscle tissue. Tissue from large alligators (≥2.9 m, TL) was analyzed to sample animals most likely to have accumulated high contaminant concentrations. Mean age at this size is 20 yr for males

TABLE 2. Concentrations (based on a single analysis) of chlorinated hydrocarbons in alligator muscle samples from various lakes in Florida, 1985.

Lake	Weight of lipid (g) per g of muscle	DDE	DDD	DDT	Dieldrin	Heptachlor epoxide	Lindane	PCB's
Iamonia	0.10	0.06 (0.6)[a]	0.12 (1.2)	0.10 (1.0)	0.08 (0.8)	0.14 (1.4)	0.04 (0.4)	1.5 (15)
Newnans	0.02	0 (0)	0 (0)	0 (0)	0.05 (2.5)	0.05 (2.5)	0 (0)	0.1 (7)
Orange	0.14	0 (0)	0 (0)	0.42 (3.0)	0.03 (0.2)	0.11 (0.8)	0 (0)	1.4 (10)
Rodman	0.03	0 (0)	0 (0)	0 (0)	0.02 (0.7)	0.09 (3.0)	0 (0)	0.7 (25)
George	0.04	0 (0)	0 (0)	0 (0)	0.12 (3.0)	0.20 (5.0)	0.02 (0.5)	1.5 (33)
Apopka	0.13	0 (0)	0.03 (0.2)	0.16 (1.2)	0.10 (0.8)	0.13 (1.0)	0.03 (0.2)	2.1 (17)
Hancock	0.06	0 (0)	0.02 (0.4)	0 (0)	0.04 (0.8)	0.08 (1.4)	0 (0)	1.1 (19)
Trafford	0.01	0 (0)	0.02 (2.0)	0 (0)	0.02 (2.0)	0.04 (4.0)	0.01 (1.0)	0.2 (23)

Concentrations of chlorinated hydrocarbons are presented as ppm. Limit of detection for all chlorinated hydrocarbons except PCB's is 0.01 μg/g of tissue; limit of detection for PCB's is 0.1 μg/g of tissue. Calculation of PCB's was based on comparison of sample peak area with that of authentic Aroclor 1242; values in parentheses are concentrations in the fat extracted from muscle tissue.

(Florida Game and Fresh Water Fish Commission, unpubl. data). Lower concentrations would be expected for smaller (younger) alligators from the same locations (Bache et al., 1972; Bourne and Bogan, 1972; Veith, 1975). Because contaminants are removed from reptiles when eggs are laid (sources cited in Hall, 1980), the concentration in tissues from adult (>1.8 m) female alligators also may be lower. Freshwater fish are capable of accumulating toxic substances (Winger et al., 1984) and constituted 57% by volume of alligator food in northcentral Florida (Delany and Abercrombie, 1986). Fish may be an intermediate source for some contaminants.

Contaminant concentrations in alligator muscle tissue found in this study probably pose little threat to public health. Concentrations were below established tissue tolerance concentrations for many contaminants in fish and major livestock species (FDA Compliance Policy Guide 7180.07 and 7141.01; and Code of Federal Regulations: 21CFR, 109.30). Final recommendations however, must await analysis of larger sample sizes and additional information on maximum concentrations of contaminants, amount and frequency of meat consumption, meat preparation, and consumer characteristics. FDA "action levels" do not exist for alligator meat for human consumption. Concentrations of Hg and PCB's were sufficiently high in some samples to warrant continued monitoring of alligators killed for human consumption.

Alligators appear to be suitable, long-term monitors of local pollution. Although no universal trend in concentrations of contaminants by lake was found, there were some differences among lakes that may reflect levels and types of environmental contamination. Because concentrations of contaminants in edible tissues may be less than whole-body residues (Schmitt et al., 1981) the environmental implications of concentrations found in this study are difficult to assess. Residues of As, Cd, Pb, and Hg exceeding 0.05 μg/g in whole-body components are considered potentially harmful to aquatic biota (Walsh et al., 1977). The National Academy of Science and National Academy of Engineering (1972) recommended maximum whole-body residue concentrations for DDT, DDD, and DDE (1.0 μg/g), dieldrin and heptachlor epoxide (0.1 μg/g), and total PCB's (0.5 μg/g) in fish for the protection of predators. Concentrations of contaminants in alligator muscle (and/or extracted fat) samples from some lakes exceeded

these concentrations. It is not known what concentrations are toxic to alligators. However, concentrations of heptachlor epoxide and dieldrin (the two most toxic substances) found in this study were approximately an order of magnitude lower than the lowest lethal concentrations reported for these compounds in other reptiles (sources cited in Hall, 1980). Exposure to environmental contaminants may affect crocodilian reproduction (Hall et al., 1979; Stoneburner and Kushlan, 1984) and has been suspected in declines in alligator egg hatchability at Lake Apopka (Florida Game and Fresh Water Fish Commission, unpubl. data). Other residue concentrating tissues need to be analyzed to determine the effects of contaminants on alligator reproduction and health.

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