BONE DENSITY IN FEMALE JUVENILE AMERICAN ALLIGATORS FROM A PESTICIDE POLLUTED LAKE (LAKE APOPKA).

P. Monica Lind^{1,2}, Matthew R. Milnes³, Rebecca Lundberg¹, Louis Guilette^c and Jan Örberg²

 ¹Institute of Environmental Medicine, Karolinska Institutet, Institute of Environmental Medicine, P.O. Box 210, 171 77 Stockholm, Sweden, E-mail address: monica.lind@imm.ki.se
²Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden.
³Department of Zoology, University of Florida, Gainesville, USA.

Introduction

Numerous reports have documented a number of reproductive disorders such as reduced penis size¹, gonad abnormalities², and altered hormone levels in the hatchlings² and juvenile alligators³ of Lake Apopka, Florida (USA). A declining alligator population in Lake Apopka, where successful hatchings fell dramatically from 1983 to 1988, led to studies that found endocrine-disrupting chemicals (EDC) in alligator eggs and serum (e.g. the DDT metabolite *p*,*p*,-DDE, dicofol and polychlorinated biphenyls (PCBs))^{4,5}. A recent report showed that the relationship between the steroid hormone (17b-estradiol and testosterone) concentrations and body size and age differed between Lake Apopka alligators and alligators from Lake Woodruff (control lake)⁶. From results presented the last decade, it is obvious that the bone tissue is a target for a number of EDCs ^{7,8,9}. Since that the reproductive hormones such as estradiol and testosterone plays a vital role in bone tissue homeostasis as well as reproduction, the aim of this particular study was to study bone effects in female juvenile alligators from a lake polluted with EDCs.

Material and Methods

Juvenile female alligators were collected at night by hand from two lakes in central Florida approximately 65 km apart; Lake Apopka (pesticide polluted lake) and Lake Woodruff (reference lake). The mean age of the control animals and the animals from the contaminated lake were similar (5.6 vs 5.2 years). The animals were euthanized with a lethal dose of sodium pentabarbitol (Sigma Chemical, St. Louis, MO, USA) and the tibias were dissected free from soft tissue and stored at -20 °C pending analysis. The bone composition and dimensions were evaluated by peripheral quantitative computed tomography (pQCT, Stratec XCT 960A with software version 5.20 Norland Stratec Medizintechnik, GmbH, Birkenfeld, Germany). The diaphysis of the left tibia was scanned at midshaft using a voxel size of 0.148 x 0.148 mm x 1.25 mm. The scan line was adjusted using the scout view of the pQCT system and an attenuation threshold of 0.93 cm⁻¹ was used to define cortical bone. The total bone mineral density (Tot BMD, mg/cm³) and the cortical thickness (Cort Thk, mm) were analyzed.

Results and Discussion

The results demonstrate differences in bone composition between alligators in Lake Apopka and alligators in Lake Woodruff.

Tibias of alligators from the contaminated lake had higher bone mineral density (BMD) (fig. 1) and thicker cortical bone (fig. 2) than tibias of alligators from the control lake. Effects on BMD indicate interference with bone homeostasis. The pesticide contaminants might inhibit the natural and

ORGANOHALOGEN COMPOUNDS Vol. 56 (2002)

continuous resorption of bone tissue, resulting in increased bone mass. These changes are also in agreement with our own experimental studies with the endocrine disrupting organochlorine 3,3',4,4',5-pentachlorobiphenyl (PCB126). Ovariectomized rats exposed to PCB126 were observed to get both an increased BMD and an increased cortical thickness^{7,8}.

In view of the fact that the alligators have lived in a lake contaminated with environmental estrogens (e.g. DDT and dicofol) and show abnormalities in their reproductive anatomy, the conclusion of this study is that this polluted environment also might have been responsible for the bone effects detected in the alligators.



Figures 1-2. Bone mineral density (BMD, mg/cm^3) and cortical thickness (Cort Thk, mm) of female alligator from Lake Woodruff (control lake) an Lake Apopka (pesticide contaminated lake) measured by peripheral quantitative computed tomography (pQCT). Values are mean \pm SEM, the number of animals are given in parentheses.

Acknowledgements

This work was funded partly by the Swedish Environmental Protection Agency (grant T-482-00-01) and in part by grants to LJG from the US Environmental Protection Agency (no. CR821437 and no. R824760-01-0)

References

- 1. Guillette, L.J., Woodward, A.R., Crain, D.A., et al. Plasma steroid concentrations and male phallus size in juvenile alligators from seven Florida lakes. Gen Comp Endocrinol, 116:356-372(1999).
- Guillette, L.J., Gross, T.S., Masson, G.R., et al. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect. 102:680-688(1994).
- Crain, D.A., Guillette, L.J., Pickford, D.B., et al. Sex-steroid and thyroid hormone concentrations in juvenile alligators (*Alligator mississippiensis*) from contaminated and reference lakes in Florida, USA. Environ Toxicol Chem, 17:446-452(1998).
- 4. Guillette, L.J., Crain, D. A., Gunderson, M., P., et al. Alligators and Endocrine Disrupting Contaminants: A Current Perspective. American Zoology, 40:438-452(2000).
- Guillette, L.J, Brock, J.W, Rooney, A.A, *et al.*, Serum Concentrations of Various Environmental Contaminants and Their Relationship to Sex Steroid Concentrations and Phallus Size in Juvenile American Alligators. Arch Environ Contam Toxicol, 36:447-455(1999)
- Milnes, M. R., Woodward, A. R., Rooney, A. A., and Guillette, J., Louis J. Plasma steroid concentrations in relation to size and age in juvenile alligators from two Florida lakes. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 131:923-930(2002).
- Lind, P.M., S. Larsson, H. Oxlund, H. Håkansson, K. Nyberg, T. Eklund & J. Örberg.: Change of bone tissue composition and impaired bone strength in rats exposed to 3,3['], 4,4['],5-pentachlorobiphenyl (PCB126). Toxicology, 150:43-53(2000).
- Lind, PM., Eriksen, EF, Sahlin, L., Edlund, M., Örberg, J. Effects of the antiestrogenic environmental pollutant 3,3',4,4',5-pentachlorobiphenyl (PCB 126) in rat bone and uterus : diverging effects in ovariectomized and intact animals. Tox Appl Pharmacol 154(3): 236-244(1999).