

Organochlorines in Crocodile Eggs from Kenya

J. U. Skaare,¹ K. Ingebrigtsen,¹ A. Aulie,² and T. I. Kanui³

¹Department of Pharmacology and Toxicology, Norwegian College of Veterinary Medicine / National Veterinary Institute, P.O. Box 8146, Dep. 0033 Oslo 1, Norway; ²Department of Physiology and Nutrition, Norwegian College of Veterinary Medicine, P.O. Box 8146, Dep. 0033 Oslo 1, Norway, and ³Department of Animal Physiology, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

The extensive use of persistent organochlorine chemicals (OCs), both in agriculture and industry, has lead to widespread pollution of the environment, and residues of for instance DDT and PCBs are found at all levels of the food chain. Most industrialized countries have imposed restrictions on their use and disposal. In most developing countries, however, persistent organochlorine pesticides (OCPs) are still in use. Few data are available on the occurrence and levels of OCs in the Kenyan aquatic ecosystems. Crocodiles are among the top predators in aquatic ecosystems and should, therefore, accumulate lipophilic persistent pollutants.

The accumulation of persistent lipophilic chemicals in yolk and their subsequent elimination via eggs constitute an important excretory pathway for these compounds in avian species. Hence, the egg levels of OCs reflect the total body burden of the laying bird and may indicate the extent of the general contamination of a population with such compounds. As crocodiles are egg layers and at the end of the aquatic food chain, accumulation in and excretion via eggs of OCs are likely to take place also in this species. The aim of the present study was to generate data on the pollution levels of OCPs in an aquatic environment in Kenya by analysis of eggs from the Nile crocodile. In addition, an autoradiographic study of the distribution of ¹⁴C- DDT in crocodile hatchlings was included.

Send reprint requests to J.U. Skaare at the above address.

MATERIALS AND METHODS

Eggs from the Nile crocodile (*Crocodylus niloticus*) were obtained from Lake Baringo, Kenya. They were kept in wet sand for 8 weeks and thereafter kept frozen at -20 °C until analysis. For the chemical analysis the eggs (8) were macerated in a glass tube using a scalpel. With slight modification, the method described by Brevik (1978) was applied for extraction, clean-up and the final analyses of the samples. The modified method has been described in detail by Skaare *et al.* (1988), where results on quality assurance testing of the analytical method are included. The limit of PCB quantification was 0.02 mg/kg fat. The PCBs were determined with a gas chromatograph via pattern recognition using the commercial standard Aroclor 1260R. The sum of as many peaks as possible was taken for comparison. The method of the US Food and Drug Administration (Sawyer 1978) was followed for quantification. Pesticides analysed included both o,p' and p,p' isomers of DDT (1,1-(2,2,2-trichloroethylidene)-bis-(4-chlorobenzene) and its metabolites TDE and DDE, hexachlorobenzene (HCB), γ -hexachloro-cyclohexane (HCH) lindane, α -HCH, β -HCH, aldrin, dieldrin, endrin, oxychlordane and transnonachlor. The limits of quantification were 0.01 mg/kg fat.

Eggs (6) from a crocodile farm in Mombasa, Kenya, were hatched and kept under laboratory conditions at the Norwegian College of Veterinary Medicine (Aulie *et al.* 1989). The mean weight of the hatchlings was 62 g. At 4 weeks of age two of the animals weighing 71 and 94 g were used for the whole-body autoradiography study. ¹⁴C-DDT was obtained from The Radiochemical Centre, Amersham, England. The specific activity was 29.7 uCi/mmol, and the radiochemical purity 99%. The animals were given a solution of the test substance in peanut oil by gavage (5ml/kg). The dose administered was 1 mg/kg corresponding to 83.4 uCi/kg. Two weeks later the animals were euthanized by an intraperitoneal injection of pentobarbital, embedded in a gel of carboxymethylcellulose (1%), frozen at -75 °C in a bath of hexane and solid carbon dioxide, and submitted to whole-body autoradiography (Ullberg 1954, 1977).

RESULTS AND DISCUSSION

The main organochlorine contaminants found in the eggs were, in order of magnitude, p,p'-DDE, p,p'-DDT, p,p'-TDE and dieldrin (Table 1). Quantifiable amounts of PCBs, HCHs, aldrin, endrin, oxychlordane and transnonachlor were not found. Total DDT included about 80 % p,p'-DDE. About equal concentrations of p,p'-TDE and p,p'-DDT were found in the samples. The mean extractable fat % was 12.09 ± 1.66 .

Table 1. Levels (mg/kg wet weight, mg/kg fat weight in parentheses) of total DDT (p,p'-DDT+1,11 p,p'-DDE+1.11 p,p'-TDE) and dieldrin in 8 crocodile eggs¹.

Total DDT	0.55 ± 0.20	(4.43 ± 1.19)
DDT/DDE	0.08 ± 0.02	
Dieldrin	0.03 ± 0.02	(0.21 ± 0.13)

¹The results are expressed as means \pm s.d.

The total DDT and dieldrin concentrations found in crocodile eggs are more than 10 times higher than the corresponding concentrations found in fish in Lake Nakuru in 1975 (Greichus *et al.* 1978) and about half and 5% of the concentration in eggs of free-range hens in Embu and Meru districts (Kahunyo *et al.* 1988; Mugambi *et al.* 1989). DDE and dieldrin are the more persistent metabolites of the insecticides DDT and aldrin, respectively (Hayes 1982). Contamination of crocodile eggs with DDE reflects either earlier exposure of the mother to DDT, which has subsequently been metabolized to DDE and retained in her body fat, or exposure of the mother to DDE as such through consumption of foods of animal origin. Although the present results reveal a low to moderate pollution by DDE and dieldrin of the actual aquatic environment, the excretion of these compounds via eggs implies the exposure of the embryo from an early stage of development. Whether the actual compounds exert any adverse effect on the embryo or the developing hatchling is unknown.

A photopositive of a whole body autoradiogram of a crocodile hatchling is presented in Fig. 1. As seen from the figure high

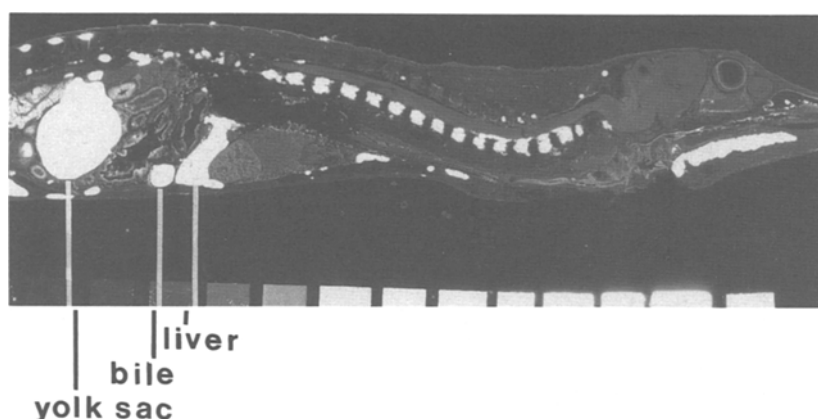


Figure 1. Photopositive of an autoradiogram from a crocodile hatchling two weeks after oral administration of ^{14}C -DDT. Light areas correspond to high levels of radioactivity.

levels of radioactivity are present in the yolk sac, the bile and the liver. The strong radiolabellings in the bile and the liver indicate that the hatchlings possess the ability to excrete substantial amounts of ^{14}C -DDT derived radioactivity via bile. As toxic effects are subject to dose-response relationship, this ability might be of importance to the survival of the crocodile hatchling in a polluted environment.

Acknowledgment. The technical assistance of Ms. A. Polder and Ms. I. L. Gross is highly appreciated. Part of the study was supported by a grant from NORAD KEN-046.

REFERENCES

- Aulie A, Kanui TI, Maloiy GMO (1989) The effects of temperature on oxygen consumption of eggs and hatchlings of the Nile crocodile (*Crocodylus niloticus*). Comp Biochem Physiol 93A:473-475

- Brevik EM (1978) Gas chromatographic method for the determination of organochlorine pesticides in human milk. Bull Environ Contam Toxicol 19:281-6
- Greichus YA, Greichus A, Amman BD, Hopcraft J (1978) Insecticides, polychlorinated biphenyls and metals in African lake systems III. Lake Nakuru, Kenya. Bull Environ Contam Toxicol 7:454-61
- Hayes WJ Jr (1982) Pesticides studies in man. Williams & Williams, Baltimore
- Kahunyo JM, Frøslie A, Maitai CK (1988) Organochlorine residues in chicken eggs: A survey. J Toxic Environ Health 24:543-550
- Mugambi JM, Kanja L, Maitho TE, Skaare JU, Løkken P (1989) Organochlorine residues in domestic fowl (*Gallus domesticus*) eggs from central Kenya. J Sci Food Agric 48:165-176
- Sawyer P (1978) Quantification of polychlorinated biphenyl residues by electron capture gas-liquid chromatography: reference material characterization and preliminary study. J Assoc Off Anal Chem 60:272-281
- Skaare JU, Tuveng JM, Sande HA (1988) Organochlorines and polychlorinated biphenyls in maternal adipose tissue, blood, milk, and cord blood from mothers and their infants living in Norway. Arch Environ Contam Toxicol 17:55-63
- Ullberg S (1954) Studies on the distribution and fate of S³⁵-labelled benzylpenicillin in the body. Acta Radiol Suppl 118:1-110
- Ullberg S (1977) The technique of whole body autoradiography. Cryosectioning of large specimens. Sci Tools, The LKB Instrument J: 1-29

Received November 7, 1990; accepted January 10, 1991.