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Use of the Nile Monitor, *Varanus niloticus* L (Reptilia: Varanidae), as a Bioindicator of Organochlorine Pollution in African Wetlands

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Considerable quantities of various pesticides, herbicides and fungicides have been used for several decades in Sub-Saharan Africa for locust control programs, protection of cotton fields or eradication of tsetse and malaria vectors (overviews in Dejoux 1988; Ondieki 1996; see also e.g. Davies 1964). The impact of such intensive treatments on local ecosystems remains poorly documented, especially the level of residue concentration in the continental outfalls of great rivers (lakes and plains liable to flooding), and the actual amplification processes that occur in food webs. Progress in this field depends on the use of a bio-indicator specific for humid zones, that would combine three main characteristics: a) this species must be present at the uppermost levels of food chains; b) it must have a broad distribution, so that comparisons between sites should be possible without important methodological adjustments; c) it must be abundant enough to allow significant, regular samplings. Studies conducted hitherto were based on fishes (Koeman and Pennings 1970), or fishing birds (Everaarts et al. 1971), animals that clearly do not occupy the highest level in local food chains. The highest level predator in African continental waters is the Nile crocodile, *Crocodylus niloticus*. Fat and eggs of this reptile have been used for assessing DDT contamination of Lake Kariba, in Zimbabwe (Phelps et al. 1989). However, this species has now disappeared from most of its former distribution area, due to overexploitation. It is strictly protected today under national and international regulations; therefore, the routine capture of a significant number of specimens for analysis is neither possible nor desirable. Local densities of lizard populations have been proposed (Lambert 2005) as a broad-use, low cost bio-indicator for Sub-Saharan Africa. However, if this approach can give some general indication of local ecosystem quality, pending reference densities are available for comparison (a condition difficult to comply with for most species), it does not allow the identification and precise quantification of each pollutant. An alternative solution is offered by the Nile monitor, *Varanus niloticus*. Although this large (up to 250 cm for some 30 kg), strictly carnivorous, aquatic lizard is heavily and regularly exploited for food and leather trade throughout its range, it nevertheless remains relatively common in all non-desert zones of Sub-Saharan Africa (Buffrénil 1993). This note reports the results of the first pesticide analyses made in this species.

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MATERIALS AND METHODS

The biological sample included 50 Nile monitors, *Varanus niloticus* L (24 males and 26 females), with snout-vent lengths ranging from 28.5 cm to 88.2 cm (76.8 to 206.4 cm in total length; weight: 0.500 to 17 kg). Nile monitors have an opportunistic diet: according to individual size, they prey on molluscs, arthropods, numerous vertebrates and their eggs (fishes, reptiles, birds, small or medium size mammals), and carrions (Cissé 1972, Lenz 1995). The preys of Nile monitors seem to be comparable to those of Nile crocodiles of similar body mass (Luiselli et al. 1999). However, each year, during their 4 to 5 month activity period, Nile monitors swallow much larger food amounts than crocodilians, in proportion to their weight. All the specimens of our sample were captured in Chad in 2003 by the professional monitor trappers who supply the local trade in bush meat, and the international trade in exotic leathers (*V. niloticus* is in CITES Appendix II, and Chad has an export quota of 80 000 skins per year). They were autopsied in the field, under the control of local authorities, in the frame of a program on the management of exploited varanid populations. On each specimen some 50g abdominal fat were sampled, and kept in glass bottles at 4 to 5°C. In several reptile species, subcutaneous and intra-abdominal fat deposits proved to have the highest lipid content, and the highest capacity for organochlorine concentration (e. g. Rybitski et al. 1995). Individual ages were determined by the skeletochronological technique (Buffrénil and Castanet 2000). In the whole sample, estimated ages ranged from 18 to 78 months.

All specimens were trapped from mid-September to late December (a period of peak activity for Nile monitors in the Sahelian region), in three broad wetlands and flooded zones where they are regularly harvested (Fig. 1): a) Salamat plain, in south-eastern Chad, within a radius of 5 km around the village of Am Guittey (10° 54' N; 20° 13' E); b) the vicinity of Abba Limane village (11° 09' N; 15° 19' E), in the Chari-Baguirmi district, where the two biggest rivers of the region, the Chari and the Logone, converge; c) the south-eastern bank of Lake Chad, around the island of Koutkou (13° 30' N; 14° 39' E). Lake Chad is a terminal basin for the whole hydrographical network of Chad, northern Cameroon, northern Central African Republic, and north-eastern part of Nigeria. This lake is nearly closed, with no outlet below the altitude 283 m (Carmouze 1976).

Chad statistics on pesticide use are particularly incomplete and difficult to access. For this country, FAO data base records for the period 1990 - 1997 (data are not available for subsequent years) declared amounts ranging from 1 metric ton (in 1996) to 57 metric tons (in 1990) of various insecticides. Of course, these data will appear quite unrealistic if one considers the doses per hectare recommended by the programs of cotton protection in Chad (Deguine and Silvie 1988; Silvie and Gozé 1991) and the total area of cotton fields in this country (some 150 000 ha at least), the massive use of insecticides against locusts and tsetse flies (*int. al.* Thiam 1991), and, at last, the very frequent, but quite uncontrolled, use of pesticides for the protection of corn and market gardens.

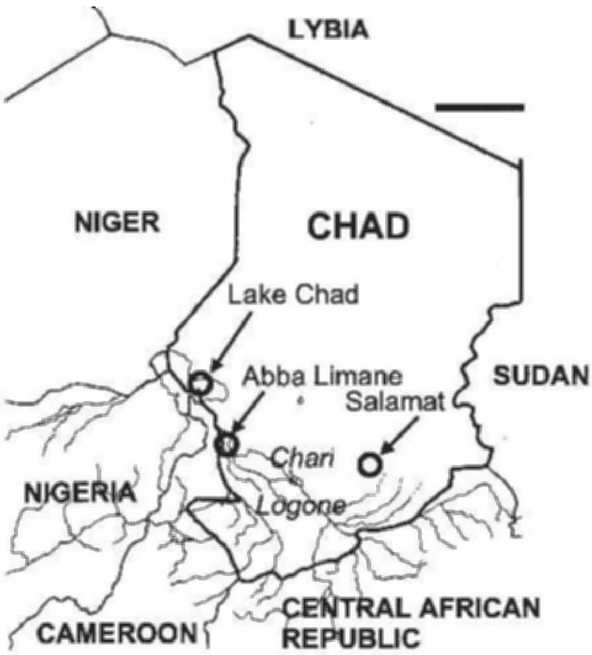

Figure 1. Position of the three capture sites relative to the hydrographical network of Chad. Bar. 200 km.

As a first approach, our analyses were restricted to the organochlorine compounds considered to be most harmful to environment because of their persistence, their liability to bio-magnification and bio-accumulation, and their volatility. Seven of these chemicals, DDT (including all its metabolites), the three forms of Hexachlorocyclohexane (HCHα, β, γ-Lindane), Aldrin-Dieldrin, Endrin and Endosulfan, are either on the list of chemicals that have been commonly used by OCLALAV (*Organisation Commune de Lutte Anti-acridienne et Antiaviaire*, 2003) for locust control since 1950, or on the list of pesticides used during the same period to protect cotton fields (list from the *Direction de la Protection des Végétaux*, N'Djamena 2003). Three other substances that are not on these lists, Heptachlor, Hexachlorobenzene, and Chlordane, were also included in this study because they were (and are likely to be still) very commonly used in Africa.

Organochlorine analysis was performed by gas phase chromatography, with an ion capture detector (Berny et al. 2002 and 2003). Detection limits ranged from 1 to 4 µg/kg, depending on the compound considered. Analyses were conducted in compliance with European Quality Norms (ISO 17025), in the LACMIBIO Laboratory, an institution acknowledged by the French Accreditation Committee (COFRAC).

Selected samples were spiked with 1-50 µg/kg organochlorine (one out of 4 samples, on a random basis) and gave consistent recoveries over the range tested

(85 – 90%). The correlation coefficients were always found to be > 0.99 (calibration curves, five points). Repeatability was determined with 3 determinations of standards and spiked samples and CVs' were found in acceptable ranges (< 10%) both for standards and samples.

RESULTS AND DISCUSSION

All ten organochlorine compounds were actually detected in the sample. Forty six of the 50 monitors (90%) contained traces of at least one of these compounds. Over the 500 possible contamination cases (10 compounds x 50 monitors) the true contamination rate within the sample (n = 91) was 18%. This rate was 16% in the specimens from Lake Chad, 25% in those from the Salamat, and 20% in the monitors from the vicinity of Abba Limane. Relative frequencies of the ten compounds in the whole biological sample, and their frequency in each local sample, are given in Table 1.

Table 1. Relative frequency (in %) of specimens in each local sample that presented detectable contamination by one of the ten organochlorine compounds.

	Salamat n = 9	**Abba Limane** n = 5	**Lake Chad** n = 36	**Total sample** N = 50
Hexaclorobenzene	0%	40%	14%	14%
HCHα	22%	20%	14%	16%
HCHβ	89%	0%	30%	38%
HCHγ (Lindane)	11%	0%	11%	10%
Heptachlor	67%	20%	11%	22%
Aldrin / Dieldrin	0%	0%	14%	10%
DDT total	22%	80%	44%	44%
Endrin	11%	0%	0%	2%
Chlordane	22%	40%	14%	16%
Endosulfan	22%	0%	8%	10%

The pesticide most frequently encountered in the sample was DDT (detected in 44% of the 50 monitors), especially in Abba Limane vicinity (80%). The second compound in frequency was HCHβ (38% of the whole sample; 89% in the sample from Salamat), and the third was Heptachlor (22% of the whole sample; 67% in the Salamat). All the other compounds were only marginally present (less than 20% of the specimens).

The mean concentrations of the various compounds proved to be highly variable from one site to another (Table 2). However, all concentrations were low; and remained far from the Maximal Residue Limits (MRL) tolerated in animal fat intended for human consumption (for example: 20 µg/kg for Lindane, 200 µg/kg for Heptachlor, 1 mg/kg for DDT). Of course, such concentrations were still farther from levels that could be toxic for the animals or their eggs. In the three local samples, considerable differences existed in the concentration of the various pesticides between individuals, a situation likely to explain the large standard deviations for each site. However, statistically significant differences (two-tailed *t* test) were observed only for three pairs: Salamat versus Lake Chad for Heptachlor and HCHβ; Abba Limane versus Lake Chad for HCHα.

Among the 36 specimens from Lake Chad, there was no difference in contamination rates or mean pollutant concentrations related to gender; moreover, pesticide concentrations proved to be independent from the animals' basic biological parameters such as size, weight, and age. Conversely, there was a significant difference ($P = 0.0124$) in HCHβ concentration between male and female specimens from the Salamat; the concentration of this compound in females being 4.5 times higher than males. Moreover, in the same region, both genders displayed an important, though not significant, difference in mean Heptachlor concentration (4.675 ± 2.708 µg/kg for males; 21.900 ± 7.625 µg/kg for females). These results are but tentative only, because of the limited number of specimens included in the study.

Table 2. Mean concentrations (in µg/kg ± SD) of the ten organochlorine compounds in each local sample.

	Salamat n = 9	**Abba Limane** n = 5	**Lake Chad** n = 36
Hexaclorobenzene	-	*1.360 ± 0.833*	*0.386 ± 0.165*
HCHα	*0.83 ± 0.550*	4.300 ± 4.300	*0.500 ± 0.235*
HCHβ	21.580 ± 5.669	-	3.506 ± 1.173
HCHγ (Lindane)	*0.622 ± 0.622*	-	*0.358 ± 0.180*
Heptachlor	14.240 ± 5.151	2.380 ± 2.380	*0.456 ± 0.233*
Aldrin / Dieldrin	-	-	2.350 ± 0.712
DDT total	1.433 ± 0.954	7.580 ± 2.449	5.161 ± 1.386
Endrin	*0.244 ± 0.244*	-	-
Chlordane	*0.311 ± 0.311*	*1.760 ± 1.133*	*1.469 ± 0.643*
Endosulfan	1.244 ± 0.826	-	*0.611 ± 0.349*

Hyphens (-), indicate that no specimen in a sample had detectable contamination for a given compound. Italics: computed means below detection threshold due to individual variation.

Body burdens measured in Nile monitors suggest that wetland pollution by organachlorine residues is relatively low in Chad. In the absence of other data on Nile monitor contamination; there is no direct comparative element. To our knowledge, the closest study was made some 15 years ago, and deals with DDT contamination of the Nile crocodiles (*Crocodylus niloticus*) inhabiting the region of Lake Kariba, in Zimbabwe (Phelps et al. 1989). DDT residues were present in the fat of all examined crocodiles (contamination rate: 100%), with concentrations ranging from 8.47 to 46.79 mg/kg. Such a level of contamination is by no means comparable to that observed in our monitors (for DDT: 0.047 mg/kg in the most heavily contaminated specimen). For the reasons mentioned above (cf. Biological Material), this difference cannot be merely explained by a higher position of the crocodiles (at least, specimens more than 2 m long) in food chains: in the present state of soft water biocenoses in numerous African regions, adult Nile monitors must be considered as top predators, therefore representing the peak level of pollutant bio-magnification.

The low level of pesticide contamination observed in the monitors agrees with the conclusions drawn by Everaats et al. (1971) from the study of two king-fishers (*Ceryle rudis* and *Megaceryle maxima*) collected in Chad at various levels of the Chari and Logone courses, from the southern border of the country, up to Lake Chad. Although these birds had preyed upon fishes, in regions where massive doses of pesticides (DDT, Endrin, Dieldrin, etc.) were regularly used for cotton protection, they nevertheless had low contamination levels. This situation was interpreted by Everaats et al. (*op. cit.*) as evidence of a natural decontamination of the milieu due to volatilisation of the organochlorine compounds. With an average temperature (mean of maxima at N'Djamena) of 35.6° C (range 31° C in August to 41° C in April), and a relatively short rain season (3 months in the average), the climate of Chad is indeed very propitious to volatilisation. The results of the present study may offer additional example of the consequences of this process.

Three compounds that are not in the list of the pesticides used in Chad since 1950: Hexachlorobenzene, Heptachlor and Chlordane, are no more on the list of pesticides subject to a "provisory authorisation of sell" (so-called APV list, January 1999), that was still in force in 2003 when the samples were collected. The results of this study nevertheless reveal the presence of such compounds in the fat of the monitors from the three capture sites. It is therefore likely that these pesticides have been used, or are still used today, beyond the control of Chadian authorities. Moreover, according to the documents from the Chadian *Direction de la Protection des Végétaux* (i.e. Direction of Plant Protection), and from the OCLALAV, HCH has not been used in Chad since 1978 (liquid form), or 1985 (powder), and Lindane since 1987. Endrin has been subject to experimental studies until 1969, and DDT until 1971 (Silvie and Gozé 1991). The presence of these various compounds in the fat of the Nile monitors could either reflect their high persistence, a rather dubious hypothesis (especially in the case of Endrin, Dieldrin and Chlordane) if one considers the ability of pesticides to volatilise under the very hot and dry climate that prevails in Chad during most of the year (see above), or their use later than indicated on official documents. It is also possible that available lists are incomplete.

The absence of a relation between, on one hand, the age and size of the monitors, and, on the other hand, their contamination level, can be explained to some extent by the eco-physiological features of this species. In the Sahelian zone, Nile monitors replenish their abdominal and caudal fat reserves in four to five months (September to January), when swollen water stretches flood the surrounding savannahs, and when prey items are abundant (Buffrénil 1993). Then, the monitors are extremely active and consume large food amounts every day. When water stretches dry up, and temperature drops, the monitors enter a diapause phase until the middle of the next rain season (August). Energy is then supplied to them by metabolisation of their lipid reserves (Cissé 1972, 1978), and at the end of the diapause phase, the latter have disappeared or are steeply reduced. As a consequence, eco-toxicologic information derived from Nile monitor fat is punctual in time, especially in the Sahelian zone where seasons are sharply defined. The important variability in contamination levels observed between individuals is consistent with the spatial behaviour of Nile monitors: though very mobile, these lizards are sedentary and exploit, according to their size, an activity area of some hectares to some tens of hectares (Lenz 1995). Thus, they are likely to give very localised indications of pollution. With reference to both these characteristics, *Varanus niloticus* could be considered as a high precision bio-indicator, in time and space.

Considering the scarcity of previous data and the absence of relevant comparative elements, this study should be the first step of a long-term follow-up of pesticide contamination in Sub-Saharan wetlands. The results presented here confirm that *Varanus niloticus* is a particularly valuable bio-indicator because of its basic ecological features and geographical ubiquity. In addition, as an ecto-poïkilotherm, it offers an interesting model for comparative analyses of the physiological impact of pollutants. And last, the possibility to determine individual ages with a relatively fine time resolution (one year) opens interesting perspectives for both an assessment of pollutant persistence in local ecosystems, and a precise analysis of bio-accumulation and natural decontamination (if any) processes during ontogeny.

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