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Organochlorine Pesticide Residues in Helmeted Guineafowl (*Numida meleagris*), South Africa

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Ecosystems are under increasing threat of development and habitat degradation due to continued population growth and urbanisation. Toxic substances are utilized in agriculture, crop storage, mining and human and animal disease vector control (Koeman and Pennings 1970; Bouwman *et al.*1992). In Southern Africa anthropogenic persistent organochlorines (OCs) have been found to bioaccumulate in Little Stints (*Calidris minuta*) and Threebanded Plovers (*Charadrius tricollaris*) (Lötter and Bouwman 1997), Cape Griffon Vultures (*Gyps coprotheres*) (Van Wyk *et al.* 1993), and Pied Kingfishers (*Ceryle rudis*) (Evans and Bouwman 1993).

The present study focused on the Helmeted Guineafowl (*Numida meleagris;* Order: Galliformes, Family: Numididae). This bird is indigenous to Africa, wide spread throughout South Africa, well known, terrestrial and often associated with agriculture. The diet of the Helmeted Guineafowl consists of various animals and plants (Crowe and Elbin 1984) ranging from insects, snails, ticks, millipedes, grass, seeds (Grafton 1971), bulbs, tubers and berries (Maclean 1993). Being associated with agricultural areas where pesticides are utilized to control and combat insect pests, Helmeted Guineafowl, as a non-target organism, can be exposed to a variety of pesticides as well as other agricultural chemicals (Blumton *et al.* 1990) and could be at risk of raised pesticide levels, impaired reproductive success or death

Media reports on the deliberate poisoning of Helmeted Guineafowl with pesticide treated maize kernels, the results from a preliminary study by the authors, as well as complaints received from farmers of declines in Helmeted Guineafowl numbers, are of great concern. This could be the result of ingesting seeds treated with toxic chemicals, or as a result of secondary effects (in the case of birds of prey) by consuming contaminated prey such as seed-eating birds and mammals (Blus *et al.* 1984; Driver *et al.* 1991). Frank and Braun (1990) reported that in the past, raptors and fish-eating birds contained very high levels of organochlorine pesticide residues, while seed- and insect-eaters have had relatively low organochlorine residues. It was thus believed that the effects of environmental DDT pollution are not as pronounced in galliforms and anseriforms, as in the predatory birds (World Health Organization 1989). Elliott and Shutt (1993) and Anderson (1985) evaluated the potential use of blood samples to estimate organochlorine exposure and found that the blood levels equilibrate with the levels in the brain and adipose tissues. The purpose of this study therefore was to evaluate the Helmeted Guineafowl as indicator of environmental organochlorine pollution, and to report the levels and distribution of the pesticides in blood and tissues.

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MATERIALS AND METHODS

During July to November 1994 and July 1995, Helmeted Guineafowl were collected by shooting on organised hunts in agricultural areas of the North West Province (NWP) (n=17) and the Western Cape Province (WCP) (n=21). The crops in the NWP are mostly maize, potatoes and sunflowers. The planting season ranges from October to March. The pesticides used over the last 10 years include organochlorine compounds such as gamma-BHC and endosulfan. The produce of the WCP includes grapes, wheat and deciduous fruit such as apples and pears. Pesticides such as DDT and dieldrin are generally no longer in legal use, although they are sometimes used on very small scale (personal communication with farmers).

Collection of birds by rifle in the agricultural areas of the NWP had to be handled with care, as some of the farmers also kept livestock on their farms. To prevent the unnecessary risk of losing cattle, the shoots had to be carried out under supervision of the farmer. The farmer was also asked to assist in the shooting. The fresh carcasses were labelled and blood was collected from the heart with a syringe (Henny and Meeker 1981). Before it could coagulate, the blood was transferred into a Coulter glass micro-pipette with an accurate volume of 44.7 µl end to end (Evans *et al.* 1995). Tissue samples (liver, kidney, breast muscle, heart muscle and brain) accurately weighed between one and two grams, were obtained from each specimen. The tissues were then frozen in glass and stored until extraction and analysis (Bouwman *et al.* 1990). A single-step extraction and clean-up procedure for determining blood pesticide levels was carried out on silica gel/celite columns eluted with ethylether/hexane mixtures (Bouwman *et al.* 1989; Bouwman *et al.* 1990). Extraction and clean-up procedures for determining tissue pesticide levels (liver, kidney, breast muscle, heart muscle, brain and fat) was previously described by Bouwman *et al.* (1990). The extracts were analyzed by gaschromatography, using a Varian 3300 with an electron capture detector (^{63}Ni), linked to a Varian Star Chromatography Workstation, version 3. A 30m X 0.25mm J & W fused silica column with a DB-1701 stationary phase with a film thickness of 0.25 µm was used. Nitrogen with a set flow rate of 35 cm sec^{-1} was used as a carrier gas and 30 ml min^{-1} as a make-up gas. Blood and tissues were analyzed for 14 organochlorine residues (excluding the internal standard, aldrin): alpha-BHC, gamma-BHC, beta-BHC, heptachlor epoxide, alpha-endosulfan, gamma-chlordane, alpha-chlordane, DDE, dieldrin, beta-endosulfan, DDD, endrin, DDT and endosulfan sulphate. Recoveries for all varied between 65-107% (depending on fortification level), by adding standard mixtures in hexane to frozen samples, evaporating the hexane with a stream of nitrogen while the sample remains frozen, thawing of the sample, incubating for six hours in darkness, re-freezing for a week, and then extracted (Bouwman *et al.* 1990). This fortification procedure therefore eliminated the influence of solvent in the sample, and the effect often obtained with fortification and immediate extraction (Bouwman *et al.* 1990). Calibrations were checked daily with the fortification standards. Results were not corrected for recovery and are reported on wet weight.

RESULTS AND DISCUSSION

DDE, dieldrin, gamma-BHC, heptachlor epoxide, alpha-endosulfan, endosulfan sulphate and gamma-chlordane were detected in Helmeted Guineafowl blood and tissues from the NWP and WCP (Tables 1 and 2). Data for breast muscle and heart muscle are not shown. Alpha-BHC, alpha-chlordane, beta-endosulfan, DDD, endrin and DDT were not detected in any of the tissues of Helmeted Guineafowl from the NWP, whereas beta-BHC was the only pesticide not detected in the blood or tissues from the WCP.

Table 1: The minimum, maximum and mean organochlorine peticide residue levels analysed and the distribution thereof in Helmeted Guineafowl tissue samples from the NWP and WCP.

	BLOOD μg l^{-1}		LIVER μg kg^{-1}	
	NWP	WCP	NWP	WCP
1	*a* ND	*b* 3.0 *d* (1/21) *c* 3.0	ND	2.6 (1/21) 2.6
2	0.07-0.6 (13/17) 0.3±0.1	11.6 (1/21) 11.6	ND	ND
3	38-68.4 (17/17) 51.7±9.4	ND	1.2-3.5 (5/17) 2.1±0.9	ND
4	0.1-0.8 (4/17) 0.4±0.3	1.3 (1/21) 1.3	ND	2.6-10.8 (4/21) 6.4±3.7
5	0.2-0.7 (2/17) 0.5±0.3	2.9-2.9 (2/21) 2.9±0.1	0.1 (1/17) 0.1	0.06-1.1 (5/21) 0.6±0.5
6	0.3-1.0 (9/17) 0.6±0.2	0.02-1.4 (9/21) 0.6±0.5	ND	ND
7	ND	0.2-3.1 (14/21) 1.0±0.7	ND	0.2-20.3 (10/21) 4.4±7.1
8	2.7-66.0 (17/17) 10.1±15.3	0.1-44.7 (16/21) 10.4±13.5	0.6-75.4 (12/17) 9.6±20.9	0.03-370.6 (13/21) 73.1±131.9
9	9.17-48.3 (14/17) 17.8±11.3	4.1-15.5 (18/21) 7.5±2.9	3.6-55.1 (17/17) 10.8±13.5	ND
10	ND	0.4-5.2 (8/21) 2.2±1.6	ND	ND
11	ND	1.8-11.8 (19/21) 4.4±2.6	ND	14.4-30.6 (4/21) 25.1±7.3
12	ND	0.4-5.1 (14/21) 1.4±1.3	ND	0.08-2.4 (11/21) 0.3±0.6
13	ND	1.9-37.4 (6/21) 15.1±14.9	ND	ND
14	0.5-29.9 (11/17) 7.6±9.2	9.8-133 (19/21) 58.27±27.1	0.1-0.7 (2/17) 0.4±0.4	0.2-9.4 (9/21) 2.5±3.4

1 = Alpha-BHC; 2 = Gamma-BHC; 3 = Beta-BHC; 4 = Heptachlor epoxide; 5 = Alpha-Endosulfan; 6 = Gamma-Chlordane; 7 = Alpha-Chlordane; 8 = DDE; 9 = Dieldrin; 10 = Beta-Endosulfan; 11 = DDD; 12 = Endrin; 13 = DDT; 14 = Endoslufan sulphate

a = ND (Not detected);
b = range;
c = mean±SD (Standard deviation);
d = number of positive samples

Table 2: The minimum, maximum and mean organochlorine peticide residue levels analysed and the distribution thereof in Helmeted Guineafowl tissue samples from the NWP and WCP.

	BRAIN μg kg^{-1}		KIDNEY μg kg^{-1}	
	NWP	WCP	NWP	WCP
1	*a* ND	*b* 0.01-0.6 *d* (9/21) *c* 0.3±0.2	ND	0.1-0.4 (12/21) 0.2±0.1
2	0.02-0.1 (12/17) 0.1±0.03	0.2-26.9 (5/21) 6.2±11.7	0.1-0.1 (13/17) 0.1±0.03	0.6-0.8 (3/21) 0.7±0.1
3	1.1-6.1 (17/17) 3.2±1.1	ND	2.7-10.8 (16/17) 7.8±1.8	ND
4	0.02-0.1 (5/17) 0.04±0.02	0.1-0.3 (3/21) 0.1±0.1	0.1-0.3 (2/17) 0.2±0.2	ND
5	0.04-0.2 (4/17) 0.1±0.1	0.03-0.5 (3/21) 0.2±0.3	0.01-0.2 (9/17) 0.1±0.1	0.1-3.7 (13/21) 0.5±0.9
6	0.01-0.03 (8/17) 0.1±0.01	0.1-0.4 (3/21) 0.2±0.2	0.01-0.02 (6/17) 0.01±0.01	0.1 (1/21) 0.1
7	ND	0.1-0.8 (13/21) 0.2±0.2	ND	0.2-0.8 (9/21) 0.4±0.2
8	0.1-8.3 (17/17) 0.9±1.9	0.1-106.4 (13/21) 27.8±32.7	0.1-13.3 (15/17) 1.6±3.4	0.2-200.3 (18/21) 47.9±66.1
9	0.2-3.7 (16/17) 1.1±0.9	0.4-3.7 (17/21) 1.0±0.8	1.7-10.9 (15/17) 3.8±2.3	0.9-3.7 (19/21) 1.9±0.8
10	ND	0.03-0.6 (5/21) 0.3±0.2	ND	0.03-1.9 (12/21) 0.5±0.6
11	ND	0.01-4.1 (16/21) 1.3±1.5	ND	0.08-22.2 (12/21) 6.6±7.5
12	ND	0.01-0.3 (2/21) 0.1±0.2	ND	0.1-0.3 (2/21) 0.2±0.1
13	ND	ND	ND	1.0-17.4 (8/21) 7.6±6.0
14	0.03-0.6 (6/17) 0.3±0.2	2.7-8.9 (4/21) 4.7±2.8	0.1-0.4 (5/17) 0.1±0.2	1.9-23.3 (18/21) 5.9±5.0

1 = Alpha-BHC; 2 = Gamma-BHC; 3 = Beta-BHC; 4 = Heptachlor epoxide; 5 = Alpha-Endosulfan; 6 = Gamma-Chlordane; 7 = Alpha-Chlordane; 8 = DDE; 9 = Dieldrin; 10 = Beta-Endosulfan; 11 = DDD; 12 = Endrin; 13 = DDT; 14 = Endoslufan sulphate

a = ND (Not detected);
b = range;
c = mean±SD (Standard deviation);
d = number of positive samples

Residues of persistent organic pollutants (POPs) are found in most living organisms throughout the world, even in areas far removed from where they are applied (Wassermann *et al.* 1970; Koeman and Pennings 1970; Niethammer *et al.* 1984). Besides being toxic, persistent organochlorines are also extremely stable, dissolve readily in fat and therefore accumulate in animal tissues. From this study it was evident that the qualitative and quantitative distribution of organochlorine pesticides differ between and within Helmeted Guineafowl populations from the two provinces. The results are therefore in agreement with the findings of the study conducted by Ruiz *et al.* (1984) who showed that the distribution pattern of organochlorines between different tissues are different. The distribution of these compounds between organs within individuals should be interpreted by taking into account the overall pharmacodynamics of the compound in the organism and the specific physiological function of each tissue, together with its affinity to different pollutants. Because of lipid metabolism organochlorine residues can be mobilized, resulting in their distribution to, and increased concentrations in other tissues (Ecobichon and Saschenbrecker 1968; Ruiz *et al.* 1984).

Compared to available literature it was found that the mean Helmeted Guineafowl blood DDE residue level was close to that of Cape Griffon Vultures (*Gyps coprotheres*) in South Africa (Van Wyk *et al.* 1993). The blood DDD residue levels were found to be similar to those reported for Pied Kingfishers from the Pongolo Floodplains (Evans and Bouwman 1993) where DDT had until very recently been used for malaria vector control. The blood DDT concentrations for Helmeted Guineafowl from the WCP exceeded the DDT residue levels reported for Cape Griffon Vultures (Van Wyk *et al.* 1993). Dieldrin levels in liver tissues of Helmeted Guineafowl from the NWP were equal to the dieldrin levels reported for Red Kites (*Milvus milvus*) from Spain (Sierra *et al.* 1987). The DDE liver levels for Helmeted Guinefowl from the WCP exceeded the DDE residue levels for Kestrels (*Falco tinnunculus*) from Spain (Sierra *et al.* 1987). The liver DDE pesticide residue level reported for Helmeted Guineafowl by Little *et al.* (1997) were close to the reported DDE resdue level for Red Kites, but exceeded the DDE residue level for Kestrels (Sierra *et al.* 1987). The mean DDE levels obtained for the Helmeted Guineafowl kidney were equal to that of Red Kites, however, the kidney DDE residue levels of birds from the WCP exceeded the DDE levels reported for Red Kites (Sierra *et al.* 1987). The above mentioned results are worrisome as Wiese *et al.* (1969) stated that acute or chronic intoxication of Helmeted Guineafowl as a result of dieldrin application for the control of harvester termites is remote. It also indicates possible continued environmental pollution by dieldrin, from unknown sources.

The presence of DDE, DDD and DDT residues (Tabel 1) in Helmeted Guineafowl from the WCP can be explained by the general findings of Porter and Wiemeyer (1972). DDE is more stable than DDT or DDD and is lost from the animal body very slowly. The results shown in Tables 1 and 2 are in agreement with the known feeding behaviour of the Helmeted Guineafowl (omnivorous). Residues of pesticides such as gamma-BHC and dieldrin in tissue samples of Helmeted Guineafowl could also show its continued illegal use by some farmers. DDT and its metabolites (DDE and DDD), that are also present in Helmeted Guineafowl tissue, indicate exposure to this persistent pesticide from previous (Blus *et al.* 1987) or illegal current use.

The levels, as well as the distribution of the pesticides detected in the various tissues could be explained by the physiological condition of the Helmeted Guineafowl, as well as the physiological functions of the blood and the various organs sampled. Helmeted Guineafowl

collected in the NWP and WCP appeared to be in good condition, except for the subcutaneous and abdominal fat that differed between the two study areas. Helmeted Guineafowl collected in the NWP contained so little body fat, that the sampling of fat tissue was difficult, and in some birds even impossible. The residue levels could furthermore be ascribed to the more intensive agricultural methods used in the WCP, as well as the larger number of pesticides used against a broader variety of pests. The Helmeted Guineafowl from the WCP could thus be more closely associated with the source of pollution, or could be exposed to the pesticide over a longer period of time and to higher pesticide concentrations than the Helmeted Guineafowl from the NWP. The difference in geographical areas and the associated climatic changes, the method of pesticide application, the time (period) of application, the duration of exposure to the pesticides as well as the susceptibility (sensitivity) of the Helmeted Guineafowl from the two study areas could contribute towards the differences in the blood pesticide levels.

The relatively high pesticide residue levels of these terrestrial birds are of great concern as the results obtained contradicts the general assumption that predatory and fish-eating birds have higher residues and are more sensitive when compared with lower residues found in relatively insensitive seed and insect-eating gallinaceous birds (World Health Organization 1989). Cockbill (1979) and Rands (1985) have found that if insects or food is reduced in an area, the organisms that normally feed upon them also diminish in numbers. The ingestion of contaminated food is considered to be the primary route of exposure in birds in agricultural areas (McCaskey *et al.* 1967; Driver *et al.* 1991). Perry *et al.* (1990) reported that insectivorous and omnivorous birds species have declined in numbers as a result of feeding on pesticide-contaminated food in heavily sprayed agricultural areas. Blus *et al.* (1984) reported on insidents of wildlife poisonings as a result of seed treated with toxic chemicals. Declines in Peregrine Falcons (*Falco peregrinus*) and Loggerhead Shrikes (*Lanius ludovicianus*) in the USA in agricultural areas have been attributed to persistent organochlorines as the major causative agent in population declines (Ellis *et al.* 1989). The reduced suitability of farmland as wildlife habitat for many species and the use of pesticides could also have played a role in the decline of Helmeted Guineafowl (personal communication C. Winterbach and L. Pero). The use of organochlorines and organophosphates for insect and rodent control in agricultural lands remain a source of concern relevant to impact on wildlife sources elsewhere (Turnbull *et al.* 1989) as well as in South Africa.

Helmeted Guineafowl from the WCP are at a higher risk from organochlorine pesticides, than Helmeted Guineafowl from the NWP. The different pesticides and different levels found in the birds from the two provinces could therefore reflect the following: a) the respective levels of contamination of their food supply, b) different dietary constituants of the birds in different provinces (indicated by the large amount of body fat reserves and their good condition of the birds in the WCP), and c) the different pesticide application methodologies and regimes used in the different provinces.

Helmeted Guineafowl tissues are generally useful for determining organochlorine pesticides. Blood is an excellent matrix for the evaluation of chronic pesticide exposure, due to the dynamic equilibrium of levels between blood and tissues, as well as the facilitation of non-destructive sampling, multiple sampling and future comparisons. Helmeted Guineafowl were found to be suitable terrestrial indicators of organochlorine pollution as they comply with the characteristics of indicators as suggested by Hellawell (1989). The birds reflect the degree of toxicological and environmental health as pesticide residue levels of agricultural chemicals

used at the time of sampling (e.g. endosulfan and beta-BHC) and in the past (DDE, DDD and DDT), and could be found in blood and tissue samples. Published results illustrate the severe impact of environmental chemicals on bird populations and show that birds in general are more susceptible than mammals. In the interest of conservation and to prevent decreases in bird numbers in future, it would be a great advantage to have the opportunity to recognise threats posed by environmental chemicals at an early stage. One such possibility would be to use birds themselves as indicators of environmental pollution.

The continued presence of these persistent pollutants in biota supports the current worldwide concerns about these chemicals. Many of those that we have found, are also on the first list that are considered for inclusion in the Persistent Organic Pollutants (POPs) convention driven by United Nations Environment Programme (UNEP) (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex and toxaphene), industrial chemicals (hexachlorobenzene, and polychlorinated biphenyls) and unintended byproducts (dioxins and furans) (United Nations Environment Programme 1998). It also shows that the concerns of mainly developed countries, are also valid for developing countries, although the situation in many of these countries are not known. It is also likely that many of the non-pesticide chemicals are also present at levels that could cause biological effects, either individually, or in combination with other chemicals or conditions. It is well known that in many cases the half-lives of chemicals under moderate conditions are longer than those experienced under conditions that characterise those in Africa (e.g. temperature and UV). There are however, possible exceptions, such as the relatively longer lives of birds in the tropics (Francis and Piper 1999). This means that the expected shorter half-lives of chemicals under African conditions, could be compensated by accumulation over a longer period of life, characteristic of many African breeding birds, when compared with their temperate counterparts. There could also be conditions, such as low organic content in arid soils, and consequently low microbial activity, that could result in longer than expected half-lives, compared to more moderate conditions. These considerations stress the need to continue monitoring the environmental behaviour and biological persistence and effects of current and new chemicals.

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