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Evaluation of Endocrine Disruptor Levels in Kafue Lechwe (*kobus leche kafuensis*) Samples from the Blue Lagoon National Park of Zambia

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Abstract The concentrations of endocrine disruptors were determined in 36 liver tissue, serum and whole blood sample extracts drawn from 15 Blue Lagoon National Park Kafue lechwe. Out of 10 analytes evaluated, 89% of the sample extracts showed very high dieldrin concentrations of between 0.08–100 μg/mL in serum, 0.08–24.8 μg/mL in whole blood and 0.08–4.6 μg/g wet weight in liver tissue extracts. pp-DDE was detected in 83% of the sample extracts at 0.006–5.1 μg/mL in serum, 0.006–8.5 μg/mL in whole blood and 0.006–0.12 μg/g wet weight in liver tissue extracts. There was strong correlation between pp-DDE and dieldrin in all the three matrices. Deltamethrin and endosulfan detected at 50% frequency each. Percent recoveries in spiked laboratory blanks ranged between 60–100% while calculated detection limits ranged from 0.004 to 0.21 μg/mL for all the endocrine disruptors evaluated.

Keywords Kafue lechwe · Blue Lagoon · Dieldrin · Zambia · pp-DDE

The blue Lagoon National Park is located west of Lusaka, the capital city of Zambia in the Central Province 15°21′–43′S, 27°15′–27°32′ E (World Wildlife Fund 1978). It was converted into a National Park in 1973 having previously been a private ranch where large herds of cattle were kept. It covers an area of 45,000 hectares and lies at 970–1,010 m altitude and on the North bank of the Kafue Flats. It is home to a sizeable number of the Kafue lechwe (*kobus leche kafuensis*) which is also found in the Lochinvar National Park that lies adjacent to it. Despite, the evidence that there has been an environmental impact due to a hydropower station, the Kafue Gorge, which is 250 km downstream and a dam in the area that feeds the power station when the water level is low in the river, there is no data pertaining to the extent of contamination by noxious chemicals on the National Park. Some of these chemicals are suspected to disrupt the endocrine system in wildlife and humans.

Water from the Kafue river which meanders through the Blue Lagoon National Park has been shown to contain lipophilic pollutants (Norrgren et al. 2000) i.e., pesticides such as DDT, PCBs and dieldrin in high concentrations. Lipophilic sediment extracts from the Kafue river have also been determined to have biological activity inducing dioxin-like substances near the town of Kitwe (Norrgren et al. 1998) in the Copperbelt Province of Zambia. It is difficult to discern the source of these chemicals because information pertaining to pesticide registration and usage in Zambia is incomplete, except for banned and severely restricted chemicals provided by the United Nations Prior Informed Consent (PIC) circulars (Orme and Kegley 2004). Large scale agricultural activities which employed large quantities of pesticides, fish kills during large scale pest control programs which use endosulfan (Chisupa 2005) against tsetse flies have been reported in addition to bird and snake kills during aerial and ground pest control activities in Zambia. These could have been potential sources of endocrine disrupting chemicals which found their way into the food chain through species such as the Kafue lechwe that depends on the Kafue river water for

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their survival. Not withstanding the volatility and mobility of pollutants such as Persistent Organic Pollutants (POPs) which have a direct impact on wildlife and humans especially in developing countries (Alsebae 1993).

A study of the Kafue Lechwe sample extracts from the Lochnivar National Park (Sichilongo and Torto 2006) which lies adjacent to the Blue Lagoon National Park showed presence of endocrine disruptors in the samples. As this preliminary study was being carried out, samples were being collected from the Blue Lagoon National Park so that a qualitative comparative outlook could be established. Another study (Syakalima et al. 2006) of pesticide/herbicide pollutants in the Kafue river showed high concentrations of organochlorine compounds i.e., heptachlor, pp′-DDE, cypermethrin, chlordane, toxaphene, terbufos, kelthane, endosulfan, dieldrin, pp′-DDD, pp′-DDT, atrazine, disulfoton, d-trans-allethrin and endrin especially in fish tissue. The samples examined in this study included sediments, water and fish collected from the Kafue River. Due to a wider spectrum of pesticides/herbicides in fish, researchers in this study speculated bioaccumulation as being responsible for the high concentrations. A myriad of similar studies on endocrine disruptors and related compounds in wildlife and human samples (Wang et al. 2008; Wang and Wang 2007; Holland 2003; Cheng et al. 2008) have been done globally. The purpose of this study was to determine endocrine disruptors in Kafue Lechwe (*Kobus leche kafuensis*) sample extracts of liver, whole blood, and serum from the Blue Lagoon National Park.

Materials and Methods

Standards were purchased from AccuStandard Inc. New Haven, CT, USA. They were all solutions of 100 μg/mL in methanol. Acetone and n-hexane were HPLC/GC grade from Sigma-Aldrich (Pty) Ltd, Aston Manor, South Africa. All other reagents used in this study were of analytical grade.

A combined team of researchers from the Zambia Wildlife Authority (ZAWA), The School of Veterinary Medicine at the University of Zambia, (UNZA) and this research group (UNZA) collected the samples randomly from the Blue Lagoon National Park. Sample collection in the Blue Lagoon National Park, preceded that in the Lochinvar National Park and took place at a time when there was an outbreak of foot and mouth disease in the Kafue flats. ZAWA had been mandated to destroy the affected animals in the Blue Lagoon National Park. Our group took advantage of this to collect samples during this period because there was an order to crop all infected animals and destroy the carcasses. Samples from eight male and seven female Kafue lechwe were collected. The exact ages of male Kafue lechwes were determined by counting the number of rings on the horns. This was not possible for females since they do not have horns. Blood and serum samples were collected by cutting the jugular vein while liver samples were collected with sterilized forceps. Whole blood and serum samples were stored in sterilized capped vials while liver samples were stored in plastic bags and all were preserved at 4°C. They were transported in this state to the laboratory for extraction.

Whole blood and serum samples were extracted by the following procedure: After bringing to room temperature, 1 mL was pipetted into a clean 250 mL separatory funnel. 0.1 mL of 5 μg/mL γ-chlordane was added as an internal standard followed by 10 mL of n-hexane/acetone mixture in a 1:1 ratio. The funnel was sealed and shaken for 10 min with periodic venting. The organic layer was allowed to separate from the aqueous phase for 10 min followed by collection of the organic layer into a 50 mL volumetric flask by passing through a filter funnel packed with a glass wool plug and 5 g of anhydrous sodium sulphate. The extraction was repeated with two 10 mL portions of the n-hexane/acetone mixture and drained through the filter funnel containing anhydrous sodium sulphate into the 50 mL volumetric flask. A column of florisil (100–200 US mesh) activated at 130° C for 6 h followed by deactivation with 5% water was prepared and used for extract clean up. Elution was accomplished with the n-hexane/acetone mixture and the solution made up to 50 mL with the same solvent.

Liver tissue was allowed to reach room temperature and approximately 2 g was weighed into a clean 10 mL beaker. Five milliliters of n-hexane/acetone (1:1) was added followed by 0.5 mL 0f 5 ppm γ-chlordane as an internal standard. The mixture was homogenized for 1 min with a blender. It was then allowed to separate. The liquid portion was drained and allowed to percolate through a filter funnel containing a plug of glass wool and 5 g of anhydrous sodium sulphate and collected into a 50 mL volumetric flask. The extraction was repeated with two 5 mL portions of the extraction solvent and finally with a 5 mL portion while transferring all the material to the filter funnel containing anhydrous sodium sulphate and allowed to percolate through it. The funnel was rinsed with 10 mL of extracting solvent and solution collected into a 50 mL volumetric flask. A column of florisil was prepared as in whole blood/serum extractions, used to clean the volumetric flask contents, and the solution made up to 50 mL.

The same method used to analyze endocrine disruptors in Kafue lechwes (*kobus leche kafuensis*) samples from the Lochnivar National Park and developed by these authors (Sichilongo and Torto 2006) was used in this study. This method showed percent recoveries that ranged between 60 and 100% with detection limits ranging between 0.004 and 0.21 ppm (w/v) as shown in Table 1.

An Agilent Technologies 6820 Gas Chromatograph (GC) system fitted with an Electron Capture Detector (ECD) was used to analyze the sample extracts. A Phenomenex Zebron ZB-5 capillary column i.e., 5% phenyl 95% dimethylpolysiloxane stationary phase (non-polar) with a temperature range of −60 to 370°C was used. The carrier gas was nitrogen (N_2) at a flow rate of 10. 9 mL/min measured at the exit of the ECD. The column head pressure was set at 15.5 psi. The GC program used was as follows: The column was held at 55°C for 1 min, ramped to 280°C at 10°C/min and held there for 11 min for a total run time of 34.50 min. The injector and detector temperatures were set at 220 and 280°C, respectively. Five microliters of sample were injected for each run in the splitless mode. The Agilent GC system is equipped with the Cerity software with statistical peak identification functions embedded in it. This was used to automatically identify peaks using standard pesticide solutions while setting the retention time identification window to ±0.1 min. An unknown sample peak that fell within this margin was confirmatory identification for the same in relation to the standard peak.

Statistical analysis of data was accomplished by SPSS Version 12.0.1 for windows, Release 12.0.1(11 Nov 2003). Arithmetic means for each sample were calculated while excluding missing values using SPSS.

Results and Discussion

Throughout the text, the concentrations of endocrine disruptors in liver, serum and whole blood are given uncorrected for percent recoveries. All Kafue lechwe ages are in years.

Table 2 shows the concentrations of ten pesticides determined in 15 Kafue lechwe samples collected from the Blue Lagoon National Park. Perfect negative correlations were observed at p(0.01) for pp-DDE/aldrin and dieldrin/d-t-allethrin ($r = -1.000$). There was also a significant correlation at the same confidence level for dieldrin/pp-DDE ($r = 0.827$), the later being due to the similarity in the frequencies of occurrence between dieldrin and pp-DDE. One way Analysis of Variance (ANOVA) did not show that the differences in the mean concentrations of all endocrine disruptors arose due to age (males only) or sex in addition to random measurement errors at p(0.05). Analysis of arithmetic means for all endocrine disruptors in all liver samples by sex showed that no endrin, pp-DDD or pp-DDT were detected in male liver tissue sample extracts. Endrin was detected in one female liver tissue extracts while aldrin was detected in one male and one female liver tissue samples. The concentration of dieldrin was highest in male Kafue lechwe liver samples averaging 2.89 ± 1.2 μg/g wet weight with a maximum concentration detected in one sample at 4.6 μg/g wet weight. In females Kafue lechwe liver extracts, the concentration of dieldrin averaged at 1.5 ± 1.2 μg/g with the maximum concentration detected in one at 2.6 μg/mL. This pattern of occurrence was very similar to pp-DDE which averaged 2.9 ± 1.2 μg/g wet weight in male liver tissue extracts and 1.5 ± 1.2 μg/g wet weight in female liver tissue thus explaining the significant positive correlation above observed between the two endocrine disruptors.

From Table 3 no heptachlor, aldrin, pp-DDD and pp-DDT were detected in both male and female serum samples from the Blue Lagoon Kafue lechwe. Endrin was detected in one male serum sample at a very high concentration level. Significant correlation at p(0.05) was observed between pp-DDE and dieldrin ($r = 0.717$) again indicating the relationship in the frequency of occurrence and concentration levels in both male and female Kafue lechwe. One way

Table 1 Retention times, regression equations, detection limits and % recoveries evaluated in this study

Pesticide	Retention time (min)	Regression equation	R^2	DL (ppm w/v)	% Recovery
Deltamethrin	12.633	$y = 179.03x - 3.9281$	0.9920	0.09	76 ± 1
Heptachlor	17.364	$y = 7.1571x - 0.0225$	0.9994	0.004	62 ± 9
Aldrin	18.118	$y = 14.275x + 0.1418$	0.9986	0.02	100 ± 7
d-t-allethrin	18.911	$y = 22.95 - 0.2753$	1.000	0.05	100 ± 1
∝-endosulfan	19.478	$y = 5.4605x + 0.5852$	0.9242	0.21	100 ± 1
pp-DDE	20.087	$y = 117.75x + 0.3637$	0.9901	0.006	100 ± 6
Dieldrin	20.244	$y = 5.7857 + 0.2333$	0.9947	0.08	100 ± 8
Endrin	20.747	$y = 3.9832x + 0.1074$	0.9963	0.06	60 ± 12
pp-DDD	20.934	$y = 49.475 + 0.4095$	0.9995	0.02	100 ± 1
pp-DDT	21.654	$y = 22.286 - 0.4587$	0.9921	0.07	84 ± 4

R^2 = Pearson's correlation coefficients; *DL* detection limits

Reference: Sichilongo and Torto (2006)

Table 2 Mean concentrations of pesticides in Blue Lagoon Kafue lechwe liver tissues

Sex	Age	1	2	3	4	5	6	7	8	9	10
Male	9	ND	ND	0.74(5.7)	1.1(8.0)	0.52(38)	0.12(22)	ND	ND	ND	ND
Male	12	ND	ND	ND	1.3(6.7)	0.62(1.1)	0.079(1.8)	2.4(0.72)	ND	ND	ND
Male	14	0.59(2.1)	ND	ND	ND	5.4(4.3)	0.093(1.9)	2.7(3.0)	ND	ND	ND
Male	15	ND	ND	ND	ND	ND	ND	4.6(10)	ND	ND	ND
Male	16	ND	ND	ND	ND	0.56(15)	0.067(6.2)	2.1(0.3)	ND	ND	ND
Male	16	0.50(0.6)	0.87(1.2)	ND	ND	3.1(6.5)	ND	4.5(1.2)	ND	ND	ND
Male	19	0.55(1.6)	ND	ND	ND	1.7(0.1)	0.063(5.4)	2.1(8.0)	ND	ND	ND
Male	22	0.57(3.4)	1.2(8.3)	ND	ND	1.6(7.1)	ND	1.8 (6.3)	ND	ND	ND
Female	Adult	0.63(1.4)	1.4(14.4)	ND	0.9(15)	0.51(3.1)	0.063(2.8)	2.6(0.27)	6.3(1.3)	ND	ND
Female	Adult	ND	ND	ND	ND	1.52(1.4)	0.052(1.6)	0.2(1.0)	ND	ND	ND
Female	Adult	ND	1.1(13)	ND	ND	1.1(6.8)	0.068(2.6)	2.5(4.6)	ND	ND	ND
Female	Adult	ND	ND	ND	ND	ND	0.018(9.4)	0.18(22)	ND	ND	ND
Female	Adult	ND	1.1(5.6)	12(13)	ND	ND	0.065(2.7)	ND	ND	ND	ND
Female	Adult	4.9(0.8)	ND	ND	ND	ND	ND	1.2(13)	ND	ND	ND
Female	Adult	ND	ND	ND	ND	0.71(1.1)	0.089(5.7)	2.6(5.1)	ND	ND	ND

Concentrations are in μg/g wet weight at n = 3 for each data point. Values in brackets are relative standard deviations RSDs

1 = Deltamethrin 3 = Aldrin 5 = Endosulfan 7 = Dieldrin 9 = pp-DDD

2 = Heptachlor 4 = d-t-allethrin 6 = pp-DDE 8 = Endrin 10 = pp-DDT

Table 3 Mean concentrations of pesticides in Blue Lagoon Kafue lechwe serum extracts

Sex	Age	1	2	3	4	5	6	7	8	9	10
Male	9	5.6(2.8)	ND	ND	ND	41.6(0.88)	1.3(5.6)	34.3(6.3)	ND	ND	ND
Male	14	ND	ND	ND	ND	ND	1.5(4.8)	44.1(0.35)	ND	ND	ND
Male	15	6.5(0.87)	ND	ND	ND	33.2(8.4)	1.5(4.7)	43.3(0.65)	ND	ND	ND
Male	16	ND	ND	ND	15.3(2.5)	27.3(0.67)	2.02(27)	56.4(1.1)	77.1(24)	ND	ND
Male	16	6.8(2.9)	ND	ND	ND	25.3(3.1)	ND	100.1(0.37)	ND	ND	ND
Male	19	6.3(2.7)	ND	ND	27.8(30)	ND	1.0(8.3)	50.2(8.2)	ND	ND	ND
Male	22	63.8(16)	ND	ND	ND	25.6(1.7)	ND	47.7(1.5)	ND	ND	ND
Female	Adult	ND	ND	ND	ND	ND	5.1(10.7)	66.3(27)	ND	ND	ND
Female	Adult	4.9(0.87)	ND	ND	6.8(0.10)	ND	1.6(2.7)	3.2(0.71)	ND	ND	ND
Female	Adult	ND	ND	ND	ND	ND	0.13(54)	7.9(6.6)	ND	ND	ND
Female	Adult	4.6(2.4)	ND	ND	ND	ND	0.04(1.8)	1.0(0.7)	ND	ND	ND
Female	Adult	4.5(0.2)	ND	ND	ND	ND	0.51(1.4)	ND	ND	ND	ND
Female	Adult	4.5(2.8)	ND	ND	7.8(1.2)	ND	0.54(5.2)	13.3(0.96)	ND	ND	ND

Concentrations are in μg/mL at n = 3 for each data point. The values in brackets are relative standard deviations RSDs

1 = Deltamethrin 3 = Aldrin 5 = Endosulfan 7 = Dieldrin 9 = pp-DDD

2 = Heptachlor 4 = d-t-allethrin 6 = pp-DDE 8 = Endrin 10 = pp-DDT

ANOVA at p(0.05) did not attribute the differences in the mean concentrations of endocrine disruptors in serum to age (males) or sex in addition to those solely caused by random errors in the measurements. Analysis of the arithmetic mean concentrations of endocrine disruptors by sex showed that endosulfan was detected in male sample extracts, none was found in all six female serum extracts. The reason for this has to be established in future studies. The concentration of dieldrin was highest in male Kafue lechwe serum samples averaging 53.7 ± 21.5 μg/mL with a maximum concentration detected in one sample at 100 μg/mL. In females Kafue lechwe serum extracts, the concentration averaged at 18.3 ± 27.2 μg/mL with the maximum concentration detected in one at 66.3 μg/mL. Again, this pattern of occurrence was very similar to pp-DDE which averaged 1.5 ± 0.37 μg/mL in male serum extracts and

Table 4 Mean concentrations of pesticides in Blue Lagoon Kafue lechwe whole blood extracts

Sex	Age	1	2	3	4	5	6	7	8	9	10
Male	16	ND	ND	ND	9.4(2.4)	2.8(7.1)	0.97(2.9)	1.7(4.6)	ND	ND	ND
Male	19	ND	ND	ND	ND	ND	4.3(38)	2.7(8.6)	ND	ND	ND
Male	22	4.3(6.9)	ND	ND	ND	ND	7.1(1.0)	1.4(2.4)	ND	ND	ND
Female	Adult	ND	ND	ND	9.8(1.3)	ND	8.5(6.1)	24.8(4.8)	37.5(6.6)	ND	ND
Female	Adult	ND	ND	ND	ND	ND	0.73(3.9)	ND	22.5(8.4)	ND	ND
Female	Adult	5.5(4.2)	ND	ND	9.6(2.1)	ND	1.0(3.4)	2.3(1.4)	ND	ND	ND
Female	Adult	7.4(6.0)	ND	ND	7.9(40)	15(3.4)	1.0(5.0)	19.9(0.4)	ND	6.1(18.3)	ND
Female	Adult	ND	ND	ND	ND	ND	0.74(2.9)	30.5(2.2)	23(2.0)	ND	ND

Concentrations are in μg/mL at n = 3 for each data point. Values in brackets are relative standard deviations RSDs

1 = Deltamethrin 3 = Aldrin 5 = Endosulfan 7 = Dieldrin 9 = pp-DDD

2 = Heptachlor 4 = d-t-allethrin 6 = pp-DDE 8 = Endrin 10 = pp-DDT

1.3 ± 1.9 μg/mL in female serum extracts thus explaining the significant positive correlation above observed between the two endocrine disruptors.

Table 4 shows the concentrations of ten pesticides determined in eight male and female Kafue lechwe whole blood samples collected from the Blue Lagoon National Park. Perfect negative correlations were observed at p(0.01) and two tailed for d-t-allethrin/deltamethrin; endosulfan/d-t-allethrin and dieldrin/endrin (r = −1.000). There were perfect positive correlation at the same confidence level for dieldrin/pp-DDE and dieldrin/endrin (r = +1.000). At p(0.05) and two tailed, perfect positive correlation was observed for pp-DDE/endrin (r = +1.000) The correlation between dieldrin and pp-DDE was observed in liver and serum samples. One way Analysis of Variance (ANOVA) did not show that the differences in the mean concentrations of all endocrine disruptors in whole blood arose due to age (males only) or sex in addition to random measurement errors at p(0.05). No heptachlor, aldrin or pp-DDT were detected in both male and female whole blood samples.

Results showed that, out of 36 liver, serum and whole blood samples from 15 Kafue lechwe samples analyzed, the frequencies of detection of the pesticides were as follows (percentages in brackets): deltamethrin (50), heptachlor

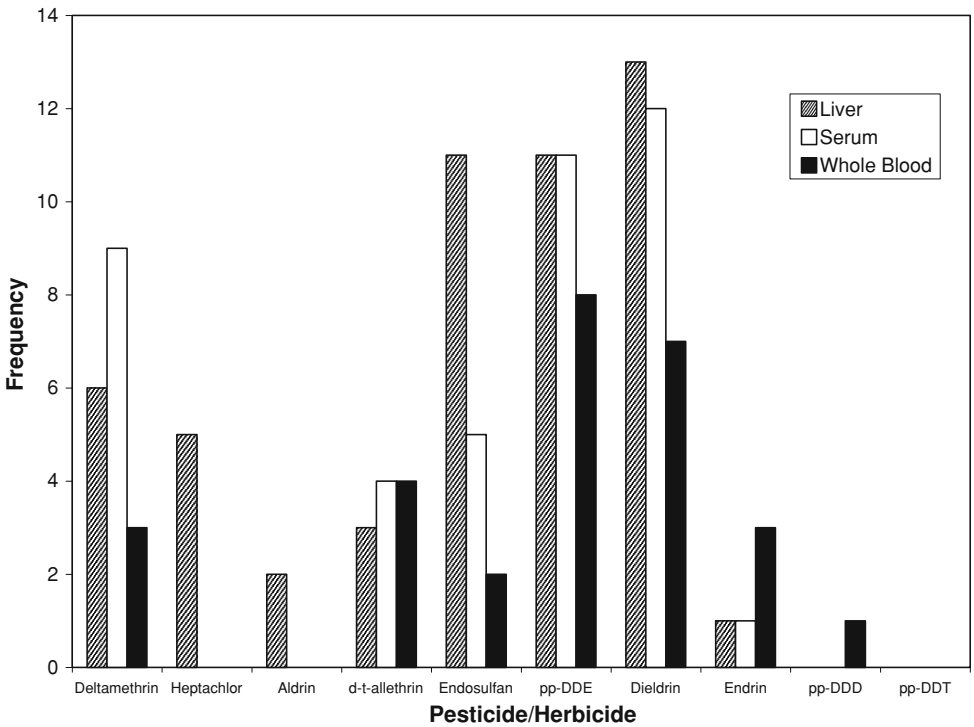

Fig. 1 Comparisons of the frequency of occurrence of pesticides in liver, serum and whole blood from the Blue Lagoon National Park of Zambia

(14), aldrin (6), d-t-allethrin (31), endosulfan (50), pp-DDE (83), dieldrin (89), endrin (14), pp-DDD (3) and pp-DDT (0). The breakdown in occurrence in each matrix can be derived from Fig. 1. Dieldrin was the most frequently detected followed by pp-DDE. Others that were significantly detected were deltamethrin and endosulfan. No pp-DDT was detected in all the samples that were determined and pp-DDD was detected in only one sample. These results were comparable with those obtained from Kafue lechwes (*kobus leche kafuensis*) samples from the Lochnivar National Park by these authors.

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