

# Levels Of 17 $\beta$ Steroid And Alkylphenol Estrogenic Endocrine Disrupting Compounds In Nairobi River

Kipyegon A.N<sup>1\*</sup>, Mutembei H.M<sup>1</sup>., Tsuma V.T<sup>1</sup> and Oduma A<sup>2</sup>

Accepted 13 October, 2016

<sup>1</sup>Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi, Kenya.

<sup>2</sup>Department of Animal Physiology and Anatomy, Faculty of Veterinary Medicine, University of Nairobi, Kenya.

## ABSTRACT

Water polluted with endocrine disrupting compounds (EDCs) has been demonstrated to cause reproductive problems in humans and wildlife. Rivers flowing through urban settlements have been shown to contain contaminated discharges from domestic, agricultural and industrial sources. Such discharges are suspected to have high levels of EDCs. The residents living along the riparian of rivers in urban settlements tend to use the water of such rivers for farming to enhance their household incomes. Together with vegetable farming, the residents also keep animals such as pigs. This practice has been noticed within the riparian of the Nairobi River, which is heavily polluted and in some places, resembling sewage sludge. The Nairobi river situation was suspected to expose humans, who consume either the vegetables and/or the animals to effects of EDCs. More direct are the effects of known EDCs, like alkyl phenol and 17 $\beta$  estradiol, on the animals raised using the polluted water. Our previous studies noted that boars raised using the Nairobi River had high prevalence of retained testes and high incidence of testicular histopathology. To test if such effects were caused by EDCs within the water, samples were obtained to determine the levels of these two compounds. Samples were collected from Nairobi River along informal settlements of Kibera, Dandora and Mathare using glass amber bottles and transported to the laboratory at 4°C. Water was then analyzed to determine the pollution levels of two known EDCs (17 $\beta$  steroid and alkylphenol) using Gas chromatography-Mass Spectrophotometry. The levels of alkylphenol and 17 $\beta$  estradiol in the sampled water were between 0.08 to 0.917 $\mu$ g/L and below detection limit (BDL) to 0.3005  $\mu$ g/L for 17 $\beta$  -estradiol alkylphenol, respectively. The mean values were 0.0953 $\mu$ g/L and 0.360 $\mu$ g/L for 17 $\beta$  steroid and alkylphenol, respectively. The detected levels of 17 $\beta$  steroid and alkylphenol point towards a suggestion that the effects observed in the boars raised along such riparian are caused by estrogenic endocrine disrupting compounds and the need to have a policy in place to control effects of such EDCs like 17 $\beta$  estradiol and alkylphenol on humans and/or animals.

**Key words:** Endocrine disrupting compounds, Urban river pollution, 17 $\beta$ -Estradiol, Alkylphenol and Reproduction

\*Corresponding author. E-mail: [kipyegon@uonbi.ac.ke](mailto:kipyegon@uonbi.ac.ke).

## INTRODUCTION

Boars raised along the riparian within the informal settlements of Nairobi have been observed to have an increased risk of testicular retention in adulthood (Kipyegon et al., 2016). Our previous study also revealed an increased incidence of reproductive problems likely to have an impact on spermatogenesis (Kipyegon et al., 2016b). These reproductive abnormalities have been

associated with contaminated river water. Various authors have reported that urban rivers are heavily polluted through release of raw domestic and industrial effluents into waterways (UNEP and IUCN 2002; Johnson and Sumpter, 2001; Clara et al., 2004). The Nairobi river has been demonstrated to contain heavy loads of microbial organisms (Musyoki et al., 2013),



**Figure 1.** Section of study site in Kibera settlement.

heavy metals (Ndeda and Manohar, 2014) and pesticides (Wandiga, 2001). These pollutants have been suspected to be containing endocrine disrupting compounds (EDC) with a potential to disrupt the endocrine function (Falconer et al., 2006), and also cause reproductive problems (Mendes, 2002).

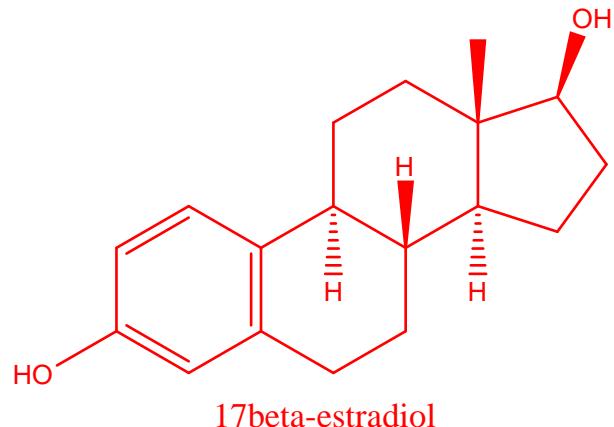
The observed male reproductive abnormalities in the boar accessing the polluted Nairobi River are suspected to be caused by estrogenic compounds in such water (Kipyegon et al., 2016b). The present study therefore sought to determine the levels of two known estrogenic endocrine disrupting compounds (Alkyl phenol and 17 $\beta$  estradiol) in the Nairobi urban river flowing through informal settlements. Large quantities of Natural and synthetic estrogens have previously been detected in surface water (Jafari et al., 2009; Knez, 2013) and wastewater (Ying et al., 2012). This paper tries to shed light on the extent of the contamination of the Nairobi River with alkyl phenol and 17 $\beta$  estradiol endocrine disrupting compounds in order to try and elucidate the main cause of the observed effects of the Nairobi river water on reproduction and testicular function. This data would be used to inform policy on governance of the pollution to avert reproductive effects in animals and humans.

## MATERIALS AND METHODS

The study was carried out in the settlements dependent upon the tributaries of Nairobi River for mixed agricultural farming; vegetable and rearing of domestic animals like pigs. The water of the tributaries is heavily contaminated

with household, industrial and farm wastes. The sampling sites were selected based on; the physical appearance of the water (looking like sewage sludge) and presence of mixed farming activities that included rearing of pigs that accessed the contaminated river (Figure 1). In each site 2.5 L of river water were collected into amber Winchester bottles previously rinsed with hexane. The samples were kept at 4°C and transported to the laboratory for solid phase extraction (SPE) and analysis. Suspended matter was removed through filtration using a 1  $\mu$ m GFC (Whatmann, USA) followed by 0.45  $\mu$ m (MN GF5) membrane filters. The filtered samples were then extracted following the procedure described previously (Zhang et al., 2006). The cartridges were dried under vacuum and wrapped in hexane-rinsed aluminum foil for storage at -18°C until use. The C-18 cartridges were removed from the freezer and left to thaw for 2 h in a fume hood.

The analytes were eluted into autovials using 5 ml of methanol at flow rate of 1mL/min. GC-MS analysis was carried out using Agilent 6890N gas chromatograph interface with a 5973C mass selective detector equipped with an Agilent 7683B auto sampler and a DB-5 fused silica capillary column of 30 m x 0.25  $\mu$ m i.d. x 0.25  $\mu$ m film thickness coated with cross-linked 5% phenyl dimethyl polysiloxane. Oven temperature was maintained initially at 70°C for 1min, increased at 15°C/min to 175°C, then at 2°C/min to 215°C, at 10°C/min to 265°C and finally at 20°C/min to 290°C and held for 8 min. The carrier gas was helium (99.999% purity) at a flow rate of 1.0 ml/min. Injection volume was 1 $\mu$ L, injected in split less mode at injection temperature of 250°C. Identity of



**Figure 2.** Structure of 17 beta-estradiol.

**Table 1.** Summary of descriptive statistics findings for 17 $\beta$ -estradiol and alkyl phenol.

Location	17 $\beta$ -estradiol ( $\mu\text{g/L}$ )	Alkyl Phenol ( $\mu\text{g/L}$ )
Kibera	0.3005 $\pm$ 0.02*	0.4428 $\pm$ 0.2*
Dandora	0.0807 $\pm$ 0.05*	0.08 $\pm$ 0.01*
Mathare	BDL	0.9174 $\pm$ 0.32*

\*Means significantly high  $P \leq 0.05$ .

alkyl phenol and 17 $\beta$  estradiol in the samples was confirmed by the retention time and abundance of quantification/confirmation ions in the authentic standards. Confirmation of identity of the analytes was done using NIST/EPA/NIH MASS SPECTRAL LIBRARY (NIST 05) and NIST MASS SPECTRAL SEARCH PROGRAM Version 2.0d. The data was stored in Microsoft Excel programme and calibration standard series used were evaluated for laboratory reproducibility and acceptability. Results are presented in table 1 as mean of triplicate analysis with standard error deviation. The significance level was tested using Student T-test at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

The two target analytes (alkyl phenol and 17 $\beta$ -estradiol Figure 2) were detected in the polluted river water samples except in one sampling point where estradiol was below detectable levels. The four sampling sites recorded varying levels of the endocrine disrupting chemicals tested. The concentration of alkyl phenol in the sampled streams ranged from 0.08 to 0.9174  $\mu\text{g/L}$  whereas those of 17 $\beta$ -estradiol ranged from between BDL to 0.3005  $\mu\text{g/L}$ . Of the two compounds tested, alkyl phenol had the highest significant concentration (0.9174  $\mu\text{g/L}$ ;  $P \leq 0.05$  (Table 1). The mean values were 0.0953  $\mu\text{g/L}$  and 0.360  $\mu\text{g/L}$  for 17 $\beta$  steroid and alkylphenol,

respectively. Whenever detected, the levels were significantly higher than the levels of estrogenic like compounds known to cause detrimental effects (Aerni et al., 2004).enough to cause reproductive problems like retained testes and/or testicular histopathology ( $P \leq 0.05$ ). Thus, the results validated the hypothesis that, indeed the observed changes in the boars, were caused by the EDCs in the polluted water of Nairobi river (Kipyegon et al., 2016).

## CONCLUSION

This study demonstrated the extent of EDCs contamination within the polluted water of Nairobi river and the need to have a policy in place to control effects of such EDCs like 17 $\beta$  estradiol and alkylphenol on reproduction of humans and/or animals.

## REFERENCES

Aerni HR, Kobler B, Rutishauser BV, Wettstein FE, Fischer R, Giger W, Hungerbuhler A, Marazuela MD, Peter A, Schonenberger R, Vogeli AC, Suter MJF, Eggen RIL (2004). Combined biological and chemical assessment of estrogenic activities in wastewater treatment plant effluent. *Anal. Bioanal. Chem.* 378, 688-696.

Clara M, Strenn B, Saracevic E, Kreuzinger N (2004). Adsorption of bisphenol A, 17 $\beta$  estradiol and 17 $\alpha$  ethinylestradiol to sewage sludge. *Chemosphere*, 56(9): 843-851.

Falconer IR, Chapman HF, Moore MR, Ranmuthugala G (2006). Endocrine-disrupting compounds: a review of their challenge to sustainable and safe water supply and water reuse. *Environ. Toxicol.* 21(2): 181-191.

Jafari AJ, Pourkabireh-Abasabad R, Salehzadeh A (2009). Endocrine disrupting contaminants in water resources and sewage in hamadan city of Iran. *Iran. J. Environ. Health. Sci. Eng.*, 6(2): 89-96.

Johnson AC, Sumpter JP (2001). Removal of endocrine disrupting chemicals in activated sludge treatment works. *Environ. Sci. Technol.* 35(1): 4697-4703.

Kipyegon AN, Mutembei HM, Tsuma VT, Oduma JA, Kimeli P (2016). Knowledge and practices of the residents living along the Nairobi River riparian on the use of the contaminated river for farming and its effects on animal reproduction. *J. Agric. Vet. Sci.*, 9(8): 59-61.

Kipyegon AN, Mutembei HM, Tsuma VT, Oduma JA (2016b). Effects of Exposure to Effluent Contaminated River Water on Boar Reproduction. *Int. Journal of Vet. Science* (in press).

Knez J (2013). Endocrine disrupting chemicals and male reproductive health. *Reprod. Biomed. online.* 26(5): 440-448.

Mendes JJ (2002). The endocrine disrupters: a major medical challenge. *Food Chem. Toxicol.*, 40 (2): 781-788.

Musyoki AM, Suleiman M, Mbiti JN, Maingi JM (2013). Water-borne bacterial pathogens in surface waters of Nairobi River and health implication to communities' downstream Athi River. *Int. J. life Pharma. Res.*, 3(1): 2250-0480.

Ndeda LA, Manohar S (2014). Determination of Heavy Metals in Nairobi Dam Water, (Kenya). *J. Environ. Sci., Toxicol. Food Technol.*, 8(5): 68-73.

UNEP & IUCN (2002). Baseline Survey and Environmental Impact Assessment. Nairobi.

Wandiga SO (2001). Use and distribution of organochlorine pesticides: The future in Africa. *Pure Appl. Chem.* 73(7): 1147-1155.

Ying F, Ding C, Ge R, Wang X, Li F, Zhang Y, Zeng Q, Yu B, Ji R, Han X (2012). Comparative evaluation of nonylphenol isomers on steroidogenesis of rat Leydig cells. *Toxicol. In vitro*, 26(7): 1114-1121.

Zhang ZL, Hibberd A, Zhou JL (2006). Optimization of derivatisation for the analysis of estrogenic compounds in water by solid-phase extraction gas chromatography-mass spectrometry. *Anal. Chim. Acta.*, 577: 52-61.