

In part two of the series on endocrine disrupting chemicals (EDCs) Lani van Vuuren looks at the South African EDC Research Programme and the need for building an armoury of knowledge to contain and manage these potentially harmful substances.

DCs can enter the water environment by direct discharge into water, the use of pharmaceuticals and chemicals in household and in agriculture and industry, accidental spills and releases of compounds and indirectly through diffuse sources, such as stormwater runoff. In turn, natural hormones, including estrogens, can be released into the environment via sewage effluent and from sources such as animal feedlots.

Concerns over the potentially harmful effects of EDCs on humans and wildlife have been rising worldwide over the last two decades. In an attempt to answer some of these concerns the South African EDC Research Programme was initiated under the leadership of the Water Research Commission (WRC) in 1999. The main objective of this programme is to investigate the occurrence, magnitude and affect of EDCs in water systems and to determine to what extent the population and the environment are at risk by being exposed to these chemicals.

Prior to the start of the programme few of the exposure routes of potential EDCs, and hardly any of the suspected health effects had been investigated. Also, the capacity to study these chemicals was virtually non-existent.

The first WRC-funded study on EDCs was published in 2000 and was an evaluation of these substances found in South Africa. The next two projects, undertaken by Prof JH van Wyk from the Department of Zoology at the University of Stellenbosch, were on the development of bioassays using egg-lying species such as freshwater fish, frogs and turtles. Around this time the WRC became involved in the Organisation for Economic Cooperation and Development (OECD). At that time most developed countries were starting to initiate extensive EDC research and monitoring programmes, and passing laws to mandate environmental protection agencies to start evaluating and developing screening programmes for these chemicals.

The first Global Water Research Coalition (GWRC) workshop on EDCs was hosted by the WRC in 2002. Workshop participants included representatives from research organisations in the US, Australia, France, Germany, The Netherlands, and the UK. The resultant workshop report, *State of the Science Report on Endocrine Disrupting Chemicals in Water Systems*, included knowledge gaps and research needs for the GWRC, a priority list of EDCs, occurrence of EDCs in water systems and an overview of sources and biological testing methods for EDCs.

"The study of EDCs requires a multidisciplinary approach," explains WRC Research Manager Annatjie Moolman. "As a result, a strategy research plan has been developed. The early years of the programme focused much on capacity building, both of selected laboratories to undertake the research as well as honing the skills of a network of researchers and scientists in the field. The latter has been done under the guidance and leadership of EDC Programme Manager Ansie Burger."

BUILDING CAPACITY

Assistance was provided to the following universities and research organisations: University of Pretoria (UP), University of Stellenbosch, University of Fort Hare, University of Venda, Medunsa, Tshwane University of Technology, the Agriculture Research Council, CSIR, South African Bureau of Standards and Ampath, a pathology laboratory.

Various research institutions were encouraged to build human resource capacity by training students of especially previously disadvantaged backgrounds. "The aim was to create centres of excellence where EDC research was already being undertaken," says Moolman.

Scientists generally use two types of tests to evaluate whether water contain EDCs. First, researchers can directly measure the concentrations of individual compounds in a sample using analytical methods (the results of these tests, however, will not directly show whether exposure to that material will disrupt the endocrine system).

Second, scientists test a sample to determine whether exposure to that sample could affect a subject's endocrine system. Laboratory technicians perform this type of test, called a bio-assay, either *in vitro* or *in vivo. In vitro* test methods use cell cultures to determine, for example, whether a sample contains compounds that can bind with a hormone receptor. In turn, *in vivo* tests expose a population of animals, such as fish, to water so that technicians can observe the biological response.

After compiling a list of priority compounds in collaboration with stakeholders such as the Department of Water Affairs & Forestry and the GWRC, specific methods for activity testing and chemical analyses were evaluated. Methods for activity testing were validated and verified and selected on grounds of sensitivity, selectivity, repeatability and robustness. "These tests were mainly biochemical and not easy to conduct and would only represent one receptor mode of action, for example, estrogenic activity. This means that assay would not given any indication of the effect on the thyroid, immune or nervous systems, for example," notes Moolman.

The WRC took the initiative to use and evaluate different methods during a limited surveillance survey involving four test sites in Gauteng, KwaZulu-Natal, and North West. The sites were selected based on the suspicion of the presence of EDCs. EDC activity was detected at all the sites and the presence of EDCs was confirmed by chemical analysis.

PILOT STUDY

The initial study was followed up by a pilot study conducted in an urban nature reserve in Gauteng. This study, led by Prof Riana Bornman of UP's Department of Urology, represented a first attempt at using local endemic vertebrate species in and around a study site as bio-indicators in investigations into environmental estrogenic activity. At that stage, this was one of the most comprehensive EDC studies to be undertaken in the world. Both bio-assays and chemical residue analyses were used to determine whether sufficiently high levels of EDCs exist in the general environment to exert adverse health affects on aquatic and/or animals and humans.

The findings of a study into the effect of endocrine disrupting chemicals (EDCs) on eland in an urban nature reserve were the first indication of mammalian wildlife being affected by environmental pollution of EDCs in South Africa.



The small reserve receives effluent from sewage treatment plants, industries and informal settlements in the catchment area, making it an ideal site to study the presence of EDCs and the possible effects on the environment and species living there. Previous and ongoing studies in this area also provided valuable background information on the study site.

Water and sediment samples were collected from the two dams in the reserve, a channel and wetland every two months over a period of two years. Analytical chemistry and bio-assays were then performed to test for estrogenisity. Roots from aquatic plants in the area were also examined. Sharptooth catfish, African clawed frogs, freshwater snails, eland and striped mice were evaluated as possible biomarker species for EDC exposure.

The study concluded that wildlife in the area is already affected. Wildlife exposure seems to manifest in feminisation of fish and amphibian species, and on conditions such as intersex (forming of eggs in the testes). Other findings include high chemical residue levels in water, sediment and tissue of the animals studied, skewed sex ratios, reduced biodiversity, and gonadal malformations of fish, snails, mice and eland, among others.

"It is highly unlikely if at all possible that such a diversity of effects in a range of animals could be coincidental," noted Prof Bornman at a workshop to present the findings to stakeholders in 2007. These findings were also the first indication of mammalian wildlife being affected by environmental pollution of EDCs in South Africa. Another world first was the health risk assessment undertaken at the reserve to determine the potential risk to the human population.

GLOBAL RECOGNITION

The WRC-led pilot study has been recognised abroad for its innovative approach to researching EDCs. At a workshop of the Water Environment Research Foundation (WERF) held in San Francisco, in the US, last year, participants agreed that it makes more sense to integrate occurrence, fate and transport, the analytical methods (including chemical target analyses and bio-assays), and health effects (environmental and human) in water-related projects. They maintained that this approach would give a better understanding of the impact of different chemical groups and would stimulate information development for communication, but also the development of new technologies for water treatment.

The WRC has further funded a pilot investigation to assess the present of estrogenic activity during the various processing steps of drinking water treatment. The study, which was recently published, investigated three drinking water plants in Gauteng. Source water and water from selected treatment processes were tested. A battery of *in vivo* and *in vitro* biological and biochemical techniques were applied, while chemical analyses were used to established EDC residues in water.

The bio-assays showed estrogenic activity in source and treated drinking water, while chemical analysis also indicated the occasional presence of EDCs in water samples. Water treatment processes removed some of the EDCs, with final water (after chlorination) showing most reduction in EDCs.

These results are similar to findings of studies globally. An important factor in the efficiency of water treatment plants to reduce or remove estrogenicity is the source water quality.

THE WAY FORWARD

Despite the progress made through the EDCs Research Programme to date, many uncertainties remain, especially regarding the potential health risk of EDCs to the human population. Scientists working on the programme say they have hardly discovered the tip of the iceberg, and much more work needs to be undertaken. "Most of the EDC research we are funding at present is still aimed at refining scientific measures to improve the quality and accuracy of the research, to enable us to prove beyond a doubt the potential risk and effects of exposure to EDCs," notes Moolman.

More research is also required to uncover the different exposure routes to EDCs. Moolman reports that South African researchers are also involved in projects funded by the National Research Foundation and the Medical Research Council looking beyond water sources to other potential exposure routes, such as food.

In lieu of this uncertainty, policy-makers are advised to take a precautionary approach when dealing with the issue of potential EDCs. Scientific research into the potential affects of EDCs will continue for many years into the future, notes Moolman. "Meanwhile the growing body of evidence suggests that there are chemicals present in the environment that are potentially harmful to not only present generations of humans and wildlife, but also perhaps future generations. We need stricter legislative control over these substances and we need to start looking for safer alternatives to avert risks of serious or irreversible harm to humans and the environment."

FURTHER READING

 The Use of Sentinel Species to Determine the EDC Activity in an Urban Natural Reserve (WRC Report No: 1505/1/07)

 An Investigation of the Estrogenic Activity in Water from Selected Water Treatment Processes (WRC Report No: 1532/1/08)

The Development and Validation of Bioassays to Detect Estrogenic and Anti-androgenic Activity Using Selected Wildlife Species (**WRC Report No: 926 & 1253/1/05**) Health Risk Assessment Protocol for Endocrine Disrupting Chemicals

(WRC Report No: KV 206/08). To order these reports, contact Publications at Tel: (012) 330-0340; Fax: (012) 331-2565 or E-mail: <u>orders@wrc.org.</u> <u>za</u>. Selected reports can also be downloaded electronically from the WRC website: <u>www.wrc.org.za</u>.