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The WRC operates in terms of the Water Research Act (Act 34 of 1971) and its mandate is to support water research and development as well as the building of a sustainable water research capacity in South Africa.

TECHNICAL BRIEF

Water and health

Pesticides as endocrine disruptors in South Africa

A completed Water Research Commission (WRC) study investigated the link between pesticide use and endocrine disrupting activity in South Africa's water resources.

Background

Globally, evidence has been mounting to support the concern that there are many environmental chemicals interacting with the endocrine systems of organisms, including humans, resulting in modulation/disruption of hormonal control. A key concern is the relative number of studies available on the increasing number of so-called endocrine disrupting chemicals (EDCs) and their potential to interfere with hormone systems.

Today pesticides are present in most compartments of the environment and detected in groundwater as well as surface water, used as sources for drinking water. International reports have linked pesticides to human health concerns.

South Africa has the highest agricultural productivity in Africa. Pesticide use in the country represents 60% of the pesticide market in Africa. About 180 different pesticide active ingredients are commercially available in South Africa, with more than 500 registered trade names in use. Some of these pesticides have been identified as potential EDCs.

South Africa has some of the highest pesticide use on the continent.

Concern has been expressed as with regards to the health of wildlife and humans as a result of exposure to pesticides present in the freshwater aquatic environment. The WRC study used two model species, the amphibian, ***Xenopus laevis*** (African clawed frog) and the freshwater fish, ***Oreochromis mossambicus*** (Mozambique tilapia) to address some of the gaps in our knowledge of locally used pesticides as endocrine modulators.

In this study the focus was on selected chemicals from the insecticide (oestrogenic activity), fungicide (androgenic activity) and herbicide (developmental, thyroid disruption) sub-groupings.

Following laboratory exposure studies, selected field studies (Stellenbosch area, Upper Olifants River and Loskop and Flag Boshielo dams in the Olifants River system) were conducted to link possible endocrine disruption manifestation to laboratory data on pesticide exposures.

Laboratory studies

Two well known ***in vitro*** assays were used to screen selected pesticides for effects on oestrogen binding to the human oestrogen receptor (hER). Oestrogenic activity was confirmed in two of the four pesticides, namely Carbaryl and Endosulfan.

Six herbicide formulations were then used to determine developmental stage dependent lethal concentrations and potential induction of malformations in developing African clawed frog tadpoles. Two important results emerged from these studies:

- That in the case of certain herbicides larval tadpoles showed lower lethal concentrations than the expected environmental concentrations as well as lower lethal concentrations for fish.

- That different stage dependent sensitivity exist for developing tadpoles (larval stage proved to be the most sensitive) when compared with early developing embryos or metamorphosing tadpoles.

The project team then went on to evaluate the effect of herbicides on the thyroid hormonal system using the internationally validated Xenopus metamorphosis assay (XEMA) to screen several herbicide formulations at different concentrations for thyroid disruption. In this study it was shown that four herbicide formulations affected tadpole growth and that herbicides differentially inhibited the natural progression of developmental stages in ***X. laevis*** tadpoles.

Finally, adult male African clawed frogs were exposed to three different, non-lethal concentrations of two selected herbicide formulations. Overall the frogs did not show clear adverse reproductive or thyroid effects following exposure to the herbicide formulations (even though the exposure concentrations were rather high). This preliminary research confirmed that differential sensitivity between life-stages should be considered when doing experimental exposures in the laboratory.

Field studies

Stellenbosch region

In this field study the main focus was to screen for reproductive activity, specifically (anti-) androgenic and oestrogenic activity, but also to include biomarkers related to potential thyroid disruption.

The African clawed frog occurring naturally in local water bodies was used. Using recombinant Yeast assays, ELISA hormone determination kits and a minced testis assay, the project team showed that hormones and hormonal modulation activity were present in several of the selected impoundments.

Anti-androgenic activity was confirmed in five of the ten sites and androgenic activity in three of the ten sites. Steroidogenic inhibition was confirmed in a single site and oestrogenic activity in two of the sites.

However, studying several biomarkers in wild-caught frogs from four selected impoundments showed that predicted gonadal endocrine disruption activity, especially (anti-) androgenic activity, in laboratory studies did not always correlate with biomarkers displaying (anti-) androgen activity in wild caught male frogs.

Although frogs from individual sites did show signs of endocrine disruption, the reproductive health implications are unclear. In one site the team also recorded a significant down-regulation of an associated thyroid gene. These findings confirm the importance of presenting both ***in vitro*** and ***in vivo*** endpoints in environmental screening efforts.

Olifants River

This study linked to a larger study concerning the water quality in the Upper Olifants River, led by CSIR. The team used ELISAs to determine human female hormone and synthetic female hormones and contraceptives in the water. The team also screened for (anti-) oestrogenicity and (anti-) andogenicity using recombinant yeast assays.

Finally, juvenile Mozambique tilapia were exposed to water collected at the selected sites, followed by an assessment of changes in expression of six different genes associated with the endocrine system.

The Upper Olifants River catchment showed human oestrogen contamination, suggesting contamination from wastewater treatment plant sources. The ER yeast screen indicated oestrogenic activity at a single location downstream of a wastewater treatment plant, and anti-androgenic activity at two locations within an agricultural region. The latter is likely associated with pesticides.

In spite of the Olifants River catchment being known for the diversity of receiving-effluent types, limited effects were observed in the expression of a number of endocrine-linked genes in juvenile tilapia fish exposed to surface water collected throughout the catchment. The lack of responses in reproductive linked genes is surprising seeing that oestrogen concentrations exceeding the reproduction predicted no-effect concentration for fish were measured at all six the localities sampled.

These results therefore suggest that the fish were insensitive to exogenous oestrogen exposure. A statistical analysis indicated an association of the wastewater treatment works with oestrogenicity and oestrogen concentrations.

Loskop Dam

In the third study, the focus was on two impoundments in the Olifants River catchment, the Loskop Dam and Flag Boshielo Dam. Mozambique tilapia in Loskop Dam is characterised by obesity, lipid peroxidation and yellow fat disease (pansteatitis).

Fish collected from Flag Boshielo Dam (downstream from Loskop Dam) generally does not show the same degree of obesity and incidence of pansteatitis. Since the thyroid hormonal system is known to be an integral part of growth, development and metabolic activity in humans and most vertebrates, including fish, in this study the focus was on the potential disruption of the thyroid as an explanation for the high incidence of obesity and pansteatitis in tilapia.

The team followed a two-tiered approach: the first study was based on adult fish collected from Loskop Dam and two other locations for comparison purposes. To confirm potential contaminant induced thyroid and adrenal modulation and induction of obesity by obesogens, the researchers exposed juvenile tilapia to water collected from Loskop Dam.

The fish collected from Loskop Dam were significantly larger than fish collected from Flag Boshielo Dam and the incidence of pansteatitis was confirmed in Loskop Dam fish. Thyroid status differed between Loskop Dam and Flag Boshielo fish, with the former displaying active thyroids.,

After considering additional data linked to nutritional status, indications are that the higher thyroid activity associated with Loskop Dam fish may be partly due to over-nutrition, rather than causing it. These data therefore does not suggest a direct link between thyroid disruption and incidence of pansteatitis in Loskop Dam tilapia.

Conclusions

The aims of the project were largely reached, however, the linking of endocrine disruption caused by pesticides to ecological risk assessment models proved to be a bridge too far to cross.

Pesticides, including insecticides, fungicides and herbicides, could interact with reproductive as well as thyroid endocrine systems, and a single chemical may interact with more than one system at a time. Modes of action associated with insecticides and herbicides varied and individual chemicals or formulations needs comprehensive testing to predict the potential for endocrine disruption.

Fungicides, on the other hand, were mostly associated with anti-androgenic activity, either by inhibiting binding of male hormone to its receptor or by inhibiting the activity of the enzyme 5α-reductase.

Laboratory studies proved valuable to perform first-tier screening for potential endocrine disruption activity of individual pesticides and mixtures of pesticides. However, in vivo exposure experiments are needed to validate modes of action and understand real health implications.
Mixture exposure experiments, on the basis of the dose addition hypothesis, may be helpful to make accurate predictions if the mode of action on the particular endocrine system is the same. But, when conducting ***in vivo*** exposures this prediction did not always hold.

Field collection of sentinel species proved to be valuable, but remains a complex system to understand and confirm direct links to endocrine disrupting activity being the origin of compromised physiological systems.

Further reading:
To order the reports, ***Pesticides as endocrine disruptors in South Africa: Laboratory and field studies*** (**Report No. 1932/1/14**) contact Publications at Tel: (012) 330-0340, Email: orders@wrc.org.za or Visit: www.wrc.org.za to download a free copy.