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TECHNICAL BRIEF

EDCs and drinking water – reducing the risk

This study investigated ways in which to reduce estrogenic activity through drinking water treatment.

EDCs in drinking water: The concern

Some man-made chemicals, as well as naturally occurring estrogens, might alter the normal regulatory function of the immune, nervous and endocrine systems in humans or disrupt the endocrine system by mimicking natural hormones or inhibiting hormonal action. Such endocrine disrupting chemicals (EDCs), which have also been implicated in the occurrence of certain human disorders and diseases, can enter the water environment through direct discharge into water, through the use of pharmaceuticals and chemicals in households, agriculture and industry, through accidental spills and releases of compounds and, indirectly, through diffuse sources such as storm water runoff. Natural hormones, including estrogens, can be released into the environment via sewage effluent and effluent from sources such as animal feedlots.

A number of EDCs have in fact been detected in South African surface water and effluent discharges. Significant estrogenic activity has also been demonstrated in both surface waters and domestic/industrial effluent discharges through the use of ***in vitro*** and ***in vivo*** biological/biochemical techniques. The presence of EDCs and estrogenic activity in sources of drinking water is therefore a matter of grave concern and poses the question of how effectively the potentially harmful chemicals are removed by conventional water treatment methods. Owing to the wide structural diversity of EDCs, it is possible that more than a single treatment process will be required for their removal.

Water treatment: how much of a remedy?

It was, therefore, deemed necessary to acquire a better understanding of the effect of drinking water treatment processes on the removal of estrogen mimicking substances from source water.

This goal was pursued by conducting a pilot investigation into the occurrence and fate of estrogen mimicking substances in both source and treated drinking water at three drinking water treatment plants (A, B and C) in the Gauteng Province, using various biological/biochemical techniques and chemical analyses, primarily to detect estrogenic activity. Techniques and analyses were selected to both reveal changes in estrogenic activity during various treatment steps (e.g. flocculation, sand filtration, chlorination) and to enable recommendations to be made regarding the most appropriate combination of tests to be applied by treatment plants in order to detect estrogenic activity in drinking water. The detection of possible thyroid, immune, neurological or anti-androgenic effects of EDCs did not form part of the study.

The battery of selected biological/biochemical techniques included techniques established by different South African as well as international laboratories, and comprised the following:

In vitro* tests:** These included a recombinant yeast estrogen screen (YES), an estrogen receptor-mediated chemical activated luciferase gene expression (ER-CALUX) assay, a ***Xenopus laevis liver slice assay and a primary rainbow trout hepatocyte (PRTH) assay.

In vivo* test:** Testing involved the use of juvenile zebrafish ***(Brachyodanio redo), 16 to 24 days old, exposed to water for 14 days whereafter vitellogenin (VTG) in whole body homogenates was measured directly with an enzyme linked immunosorbant assay (ELISA) and gel electrophoresis, and indirectly with an alkali-labile phosphate (ALP). Under normal circumstances, no VTG is present in the blood of males. The male does, however, have a functional VTG gene that can be expressed in the presence of estrogen. The presence of VTG in the blood of males thus indicates the exposure to exogenous estrogens or estrogen mimicking substances.

Chemical analyses: These were used to determine atrazine and p-nonyl phenol residues in water. In addition, water was analysed for the estrogens l7α- and ß-estradiol, estrone, 17α-ethinylestradiol and estriol.

Efficacy of some existing treatment plants

The bioassays clearly showed estrogenic activity in source and treated drinking water. Chemical analysis indicated that triazines and nonyl phenol were occasionally present in water samples, whilst concentrations of the estrogenic compounds, 17α- and β- estradiol, estrone, 17α-ethinylestradiol and estriol, were below the detection limit of 1.0 ng/ℓ in all the water samples.

The results obtained with the YES and ER-CALUX assay showed reduction in and/or removal of estrogenic activity by the water treatment processes, despite both assays indicating some increase in activity after the addition of flocculants. The most significant reduction in estrogenic activity was observed in the final waters, after chlorination.

A reduction in concentration of triazines and p-nonyl phenol in final waters was also noticed during 50 to 75% of the sampling occasions.

The data obtained with the YES and ER-CALUX and PRTH assays indicated higher estrogenic activity in both source and treated water of Treatment Plant B, whilst the YES and ER-CALUX assays showed the lowest estrogenic activity in the water of Treatment Plant C. The PRTH assay, on the other hand, showed the lowest estrogenic activity in the water of Treatment Plant A.

Factors such as heavy rain, high algal loads and changes in flocculant type did not appear to have impacted on estrogenic activity. What did appear to be an important factor in the efficiency of estrogenicity reduction or removal was the source water quality. However, linking specific water quality constituents to estrogenic activity was not possible with the limited number of organic chemical analyses carried out. Nevertheless, since nonyl phenol is an estrogen mimicking compound, it is expected that this chemical contributed to some of the effects observed. Certain endocrine disrupting metals, namely cadmium, lead and mercury, were present above the detection limits in water from Treatment Plants A and B and could also have contributed to observed estrogenicity.

Suitability of tests

The YES, ER-CALUX and PRTH assays proved to be the most suitable for the detection of estrogenicity in drinking waters. Of the four assays used, only the liver slice assay could not be included in a recommended battery of tests on drinking water; it would have to be adapted for use with concentrated samples before further consideration.

The ***in vivo*** tests did not perform well in the study. The zebrafish ELISA did not show estrogenic activity in any of the water samples. Although the zebrafish ALP assay detected estrogenicity in some samples, results were erratic and several samples in fact showed reduced VTG. Gel electrophoresis as applied in this study also could not detect VTG in any of the fish exposed to water samples.

Recommendations for further work

Besides lacking in sensitivity and reliability, the ***in vivo*** tests, as used in this investigation, are time consuming and currently not to be recommended for drinking water testing. Nevertheless, ***in vivo*** tests have an important role to play in environmental water monitoring and, for this reason, the zebrafish ELISA should be further explored. In doing so, some important considerations would be standardisation of the exposure protocols (fish handling, fish age, etc), investigation of procedures to process fish for shipment and normalisation of VTG concentration with respect to total protein determination to reduce possible masking effects.

With regard to the three promising ***in vitro*** assays:

- Efforts should be made to establish the PRTH assay locally. This test has proved a valuable member of the battery of tests performed by Environment Canada to detect toxicity and estrogenicity.
- Likewise, the ER-CALUX assay is not available in South Africa. An alternative mammalian cell test is urgently required as part of the battery of bioassays to be used for drinking water testing. The T47D-Kbluc assay, currently being introduced into the University of Pretoria laboratories, might prove to be a suitable alternative.
- Detection limits and limits of quantification for the whole YES method are not well established. The role of the concentration factor used in the test needs to be investigated and better defined.
- With regard to optimising the preparation of sample extracts for ***in vitro*** tests, the relative merits of XAD-7 and C18 extracts need to be extensively investigated to allow firm recommendations to be made.

With the presence of estrogenic activity in drinking water clearly established, two distinct research priorities have emerged:

- Links to the occurrence of specific chemicals need to be established through extensive chemical monitoring.
- The risk attached to the drinking of water that exhibits estrogenicity needs to be comprehensively assessed.

Further reading:
To obtain the report, *An Investigation of the Estrogenic Activity in Water from Selected Drinking Water Treatment Processes* (**Report No: 1532/1/08**), contact Publications at Tel: (012) 330-0340; Fax: (012) 331-2565; E-mail: orders@wrc.org.za; or Visit: www.wrc.org.za