

Endocrine disruptors in water for human consumption: chemical and biological monitoring

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Abstract

Endocrine disrupting compounds (EDCs) are substances that, mimicking the effect of natural hormones or antagonising their action, alter function of the endocrine systems and consequently cause adverse health effects. The problem of Endocrine Disruptors in general and, in particular, their presence in water intended for human consumption has aroused interest from researchers and concern among International organisations for the last decade.

Introduction

The World Health Organization and the Environmental Protection Agency in the United States, the European Union and the Istituto Superiore di Sanità in Italy has promoted research programmes on this subject. The European Union, adopting the precautionary principle, has decided to undertake, through German Research Centres, a monitoring campaign on these compounds in water intended for human consumption, followed by publication of a report presented in February 2003: "Study on Endocrine Disruptors in drinking waters - Final Report ENV.D./ETU/2000/0083 (A. Wenzel, T. Ternes and J. Muller). The study has been focused on the following substances: synthetic and natural estrogens, Alkylphenols, Alkylphenol ethoxylates, Alkylphenol acetic acids, Bisphenol A and organostannic compounds. These compounds have been monitored in water sources exploited by some European water companies. The monitoring involved four water companies and included bibliographic research and collection of data through questionnaires sent to European water companies. The results can be summarised as follows:

- Presence of pesticides with possible active EDCs in groundwater and surface water intended for human consumption, also at concentrations higher than 0.1

µg/L. The most common pesticides in natural waters are Atrazine, Simazine, Diuron, Isoproturon and Lindane.

- Presence of Tributyltin (organostannic compound) in surface water.

- Presence of Bisphenol A and Alkylphenols (Nonilphenols and octilphenols) only in surface water at concentrations equal to some ng/L.

- Presence of traces of natural and synthetic Estrogens in surface water.

The available surveys, aiming at assessing the exposure of population to EDCs, have identified very low concentrations of these compounds in raw surface water, in ground water and in treated water. Furthermore, the data available on EDCs in water for human consumption are scarce and incomplete.

Materials and Methods

Fondazione Amga, established in 2003 to promote and organise scientific and cultural initiatives in the field of environmental protection and water resources, has started, in cooperation with the University of Genoa (Department of Health Sciences - DISSAL), a preliminary study aimed to collect information on these compounds, analyze and describe the analytical methods available for their determination in water for human consumption, gather available data on their presence in aqueous matrix, which has been published in the report "Stato dell'arte sulla presenza nelle acque di sostanze denominate Endocrine Disruptors" [1].

Considering the interest aroused by the initiative, Fondazione AMGA has established a working group, including research centres, universities and water companies, to further investigate on this topic.

The group includes some leading Italian water companies:

- Mediterranea delle Acque S.p.A. – Genova
- Publiacqua – Firenze
- Hera S.p.A. – Bologna
- SMAT – Torino
- ACSM – Como
- Acquedotto Pugliese S.p.A. - Bari
- Research is carried out in cooperation with:

University of Genoa: *Department of Health Sciences, Department of Chemistry and Industrial Chemistry, Department of Biology*

University of Pisa: *Department of Experimental Pathology*

University of Trento: *Department of Environmental and Civil Engineering*

Italian Health Institute - Roma: *Department of Environment and Primary Prevention, Department of Food Safety and Veterinary Public Health.*

Furthermore the Institute for Pharmacological Research Mario Negri (Laboratory of Food Toxicology - Department of Environmental Health Sciences) and the Fraunhofer Institute For Molecular Biology And Applied Ecology (Schmallenberg-Germany) have taken part in the project. The group has the following targets:

- Promotion of interactions between water companies and research institutes to set up reference laboratories on EDCs in Italy
- Development and validation of analytical methods for determination of EDCs in water
- Study of the impact of EDCs on surface water used for production and distribution of drinking water in Italy
- Evaluation of bioassay tests and comparison with chemical methods
- Assessment through case studies representing different real situations (especially surface waters with different impacts) to help water companies to verify effectiveness of their treatment plants and estimate possible weaknesses
- Assessment of effectiveness of drinking water treatment techniques in use in ED removal

Following the report ENV.D.1/ETU/2000/0083 [2], the study has been focused on the following compounds: Alkylphenol (e.g.: Nonylphenol, Octylphenol), 17 Alfa-Ethinyloestradiol, 17 Beta-Estradiol, Estrone, Bisphenol A. The National Health Institute (ISS) has defined a method for preparation of samples for chemical and biological analyses, based on solid-phase extraction (SPE) of 1-liter sample and using HLB extraction columns. After activation of columns through appropriate solvents, the sample is filtered and, using other solvents, what remains on the column is eluted. The eluate is brought to a volume of 500 μ l, ready for chemical and biological tests. This extraction has been performed in the laboratories of the water companies involved in the project and chemical determinations of extracts have been carried out by the ISS using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Some laboratories, such as Laboratori Irade Acqua Gas - Genova and Hera - Bologna, have run analyses in parallel since both equipment and trained personnel were available.

Another method was developed by the University of Genoa - Department of Chemistry and Industrial Chemistry, using the technology: "Passive sampling with Polar Organic Chemical Integrative Sampler (POCIS*)". These samplers, widely used for air sampling, are not very common for water sampling. They are based on the free flow of pollutants from the sampled medium to a sorbent phase of the sampling device. This method enables sampling, selective extraction and *in situ* pre-concentration

of analytes at the same time. The samplers don't alter speciation, sample only dissolved chemicals and simplify the sample preparation procedure. Three different biological tests have been used. University of Pisa has adopted a short term *in vitro* biological test, called Yeast Estrogen Screen assay (YES), using a recombinant yeast *S. cerevisiae* RMY326 strain containing:

- a gene expressing the human receptor α for estrogens also present in other animal species;
- a gene expressing an estrogen response element (ERE);
- a reporter gene that codifies the production of an enzyme, the β -galactosidase

The test indicates the ability of a compound or a mixture to recognize the human receptor α for estrogens, bind to it and activate the ERE, consequently to induce transcription of the reporter gene with production of β -galactosidase, measurable by spectrophotometer. The estrogenic agonist activity elicited by the xenobiotic is calculated in percentage of that induced by E2 10 nM and can be expressed in Equivalents of Estradiol (EEQs) ng/l; the antiestrogenic (antagonist) activity elicited by the xenobiotic is calculated in percentage as inhibition of that induced by E2 1 nM. The test has been used to assess the detection sensitivity and specificity of the analytes under survey and has been applied to analysis of extracts from aqueous matrices. The obtained data allows evaluation of the endocrine mimetic activity of extracts from samples of raw and treated water samples.

The University of Genoa - Department of Biology has adopted a biological test for evaluation of estrogenic activity through E-SCREEN assay. The E-SCREEN (Estrogen screen) assay uses an epithelial cell line of human mammary carcinoma (cells MCF-7) particularly responsive to estrogens. The test is based on determination of proliferative activity (PE = proliferative Effect) induced by a sample compared to that induced from the natural estrogen, the 17 β -estradiol. Compared to the other *in vitro* tests used for assessment of estrogenic/antiestrogenic activity, the E-SCREEN assay presents the highest level of biological complexity, since it allows identification of estrogen-dependent effects (agonist/antagonist) of individual substances and mixtures, together with possible related metabolites, in human cells. The test has been used to assess the detection sensitivity and specificity of the analytes under survey and has been applied to analysis of extracts from aqueous matrices.

The University of Genoa (DISSAL) has used a biological test for assessment of the genotoxic potential of water containing Endocrine Disruptors. The test evaluates the bond between Bisphenol A and DNA through:

- Mixing and incubation of a water sample extract under survey with DNA and metabolic activation system (S-9 mix).
- Extraction of DNA from the reaction mixture.
- Survey of DNA adducts using 32P postlabeling and 32P imaging techniques.
- Quantification of DNA adducts and expression of results (adducts/108 nucleotides).

Discussion

The working group is currently carrying out tests in locations chosen as case studies. From the first results, it appears that surface water is more subject than other sources to contamination of EDcs coming from waste water, landfill leachate, reuse water and agricultural areas. Water treated for human consumption may contain EDcs at very low concentrations (ng/l), but, usually, treatments in use in the water sector produce a dramatic reduction of potential EDcs in raw water.

References

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