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Review article

# Analytical methods for the endocrine disruptor compounds determination in environmental water samples

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ABSTRACT

The potential risk of exposure to different xenobiotics, which can modulate the endocrine system and represent a treat for the wellness of an increasing number of people, has recently drawn the attention of international environmental and health agencies. Several agents, characterized by structural diversity, may interfer with the normal endocrine functions that regulate cell growth, homeostasis and development. Substances such as pesticides, herbicides, plasticizers, metals, etc. having endocrine activity (EDCs) are used in agriculture and industry and are also used as drugs for humans and animals. A difficulty in the analytical determination of these substances is the complexity of the matrix in which they are present. In fact, the samples most frequently analyzed consist of groundwater and surface water, including influent and effluent of wastewater treatment plants and drinking water.

In this review, several sample pretreatment protocols, assays and different instrumental techniques recently used in the EDCs determination have been considered. This review concludes with a paragraph in which the most recent hyphenated-instrument techniques are treated, highlighting their sensitivity and selectivity for the analyses of environmental water samples.



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## 1. Introduction

In the last 40 years, the possible consequences of exposure to xenobiotics, that can modulate the human and animal endocrine system, have drawn the international agencies attention including the European Commission, the European Parliament, the U.S. Environmental Protection Agency, the Organization for Economic Cooperation and Development, the WHO International Program on Chemical Safety, several non-governmental organizations and the chemical industry (OECD, 198 9; CSTEE, 1999). Only in 1996, the European Commission gave the first definition of endocrine disruption: "exogenous substance or mixture that alters the function of the endocrine system, causing adverse effects on the health of an organism, or its progeny, or (sub) population (European workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife, Weybridge 2-4/12/1996)".

There are many issues in this research field that can range from human epidemiology difficulties to the many thousands of chemicals that could act as endocrine disruptors. Many natural and synthetic (or semi-synthetic) agents, characterized by different chemical structure, have the potential to destroy the normal endocrine processes that regulate cell growth, homeostasis and development. This Endocrine Disruptor Compounds (EDCs) interferes with the synthesis, storage, release, metabolism and transport by removing or binding the endogenous hormone-receptors [1].

EDCs are a group of persistent and bio-accumulative environmental contaminants (see Supplementary material section S.1 for contamination sources) that are found within several classes of chemicals, whose effects on the endocrine systems (see Supplementary material section S.2 for effects of exposure to EDCs) were ignored till a short time ago. Regarding the association between EDC and human health problems, early studies were carried out on aircraft operators that spayed DDT recordering a decline in sperm counts [2]. Scientific studies carried out for years about the presence of compounds with estrogenic activity in streams have demonstrated the presence of many natural or synthetic chemical compounds able to mimic or to interfere with the normal endocrine processes in animals and humans [3–5]. Over the years there have been recorded numerous environmental phenomena caused by these compounds, such as changes of the reproductive system, reduced eggs hatch and low youthful survival of sea turtles and alligators in Lake Apopka in Florida, occurring after the release of an insecticide containing metabolites of DDT [6,7].

Quantifying and cataloging the EDCs is an extremely difficult and time consuming task that required a lot of efforts by researchers. This type of operation is complicated because there are numerous compounds with this kind of activity and every year the list of compounds known as EDCs grows. Substances endowed with endocrine activity, such as pesticides, herbicides, plasticizers (such as bisphenol A, Table 1), metals, excreted estrogens (Fig. 1), etc., are used in agriculture and industry, or derived by anthrophic activities (e.g. medicines for humans and animals).

A further complication in the determination of these substances is the complexity of the matrix in which they are present, in fact, the samples analyzed often belong to groundwater, surface water, influent and effluent of wastewater treatment plants, but, also, to drinking water. In literature the determinations in aquatic samples, due to an environmental contamination (Fig. 2, and Table 2) of these substances were performed using several conventional analytical methods, such as chromatography techniques or biological approaches (see Supplementary material section S.3 for biological approaches). To this purpose, high sensitivity and selectivity of the methods are mandatory as these compounds exhibit their endocrine activity even at very low levels. Up to now, the methods that have provided the best results were gas and liquid chromatography, coupled to high efficiency tandem mass spectrometers achieving detection limits of 0.1 ng/L showing high selectivity.

In this review, several sample pretreatment protocols and assays and the different instrumental analyses able to the EDCs determination are illustrated.

**Table 1**
Sources of exposure to bisphenol A.

| |
|---|
| Bisphenol A |
| Varnishes and glaze |
| Baby bottle |
| Nail varnish |
| Bottles for water in polycarbonate |
| Flame retardant |
| Jars coating |
| Plates for microwave |
| Adhesives |
| Artificial teeth |
| Returnable containers |
| Dental sealant for children |

**Table 2**
Examples of environmental contaminants that interfere with the endocrine system.

| | |
|---|---|
| Insecticide | Surfactants |
| DDT | Nonylphenol |
| Organophosphates | Nonylphenol acetate |
| Herbicides | Plasticizers |
| Atrazine | Bisphenol A |
| Fungicides | Phytoestrogens |
| Mancozeb | Cumestrol |
| Tributyltin | Genistein |
| Heavy metals | Chemical industrial products |
| Cadmium | PCB And PDB |
| Organictin | Solar shielding |
| Lead | Musk fragranceDioxins and furans |

## 2. EDCs analysis

The final analysis on the presence of compounds with hormonal activity in samples of water, wastewater, influent and effluent, ground and surface water is commonly performed by chromatographic methods such as gas chromatography (GC) and high performance liquid chromatography (HPLC). These separation methods are highly efficient and selective when coupled to high sensitivity detectors, such as mass spectrometer (MS) or tandem mass spectrometer (MS/MS) instead diode array detector (DAD). Undoubtly, the most used, flexible and efficient detector is the mass spectrometer in different operating modes. Among the problems that may complicate the determination of EDCs, the most obvious are the concentrations below the ng/L, the different chemical structures and the physical-chemical characteristics of the analyzed compounds. Among the most reliable and sensitive methods for the analysis of steroid hormones in water samples, recently, GC–MS, HPLC–MS and HPLC–MS/MS are recognized as the best analytical tools in environmental sciences. Chromatographic techniques allow simultaneous screening of steroids and their conjugates, and is not limited by factors such as non-volatility and high molecular