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ENDOCRINE DISRUPTING CONTAMINANTS IN WATER RESOURCES AND SEWAGE IN HAMADAN CITY OF IRAN

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ABSTRACT

Many chemicals which are released into the environment have the potential to disrupt the function of endocrine systems. They pose a growing threat to human and wildlife health. These chemicals have been characterized as endocrine disrupting chemicals. Some phenolic chemicals, such as bisphenol A, have been measured in surface water, sediments and sewage sludge. The objective of this study was to investigate endocrine-disrupting agents in drinking, surface and groundwaters sources and also sewage in the city of Hamadan, in the west of Iran. Samples from all sources were collected during April-September and October-March, extracted by solid phase method and analyzed by HPLC-UV in the wave length of 270nm for estrol, 210 nm for ethinyl estradiol and 280 nm for other compounds. The results showed that presence of bisphenol A with concentrations ranging 0.002 to 1μg/L, 0-0.002 μg/L in drinking and groundwaterfor nonylphenol and 0.001 - 10ng/L for 17-beta estradiol. Estron was not detected in drinking water during October-March. The concentration levels of pollutants in wastewater were tens times higher than the water. Analysis of different types of water showed that concentration levels of pollutants were higher during April-September comparing with October-March (Pvalue <0.005), which may be due to the changes of environmental conditions. The widespread use of birth control pills formulated with these potent estrogenic chemicals appear to be the major route for the release of estrogenic substances into the aquatic environment.

Key words: Endocrine disrupters, Bisphenol A, 17-beta estradiol, Ethinyl Nonylphenol

INRODUCTION

Nowadays, numerous types of chemical substances exist in large quantities in the environment, which can charge inevitable costs to be paid for technological remediation processes (Hirano *et al*., 2001).

Evidences have indicated the humans, domestic and wildlife species have suffered adverse health consequences from exposure to environmental chemicals that interact with the endocrine system and so they are named "endocrine disrupting chemicals", EDCs (Moreno *et al*., 2001).

Reproductive disorders and endocrine disruption in the aquatic wildlife have been demonstrated in many studies in recent years (Kirby, 2004). For example, some of the most widely reported incidences have been observed in fish. This is probably not co-incidental; it seems likely that, on average, aquatic species receive a higher exposure to most pollutants than do terrestrial organisms, for the simple reason that the aquatic environment is the ultimate sink for most wastes. Thus, very high volumes of domestic and industrial effluents are intentionally discharged into the rivers and inshore marine waters. Also many chemicals (of both natural and man-made origin) are accidentally washed from the surrounding lands into the aquatic environment (Sumpter, 2001).

The general presumption for wildlife is that exposure to environmental estrogen happen via food and water. Food, foodstuff containers and potables may be important sources of some environmental estrogens such as phytostrogens, phthalate esters and bisphenol A (BPA). For example flavonoids are some phytochemicals which naturally occur in plant tissues. They are

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members of the large flavonoid family which share with steroidal estrogens with the ability to bind with the estrogen receptor (Breinholt and Chtistian, 1998).

In addition, there are other more important routes of human exposure to such chemicals from substances such as lotions, cosmetics, detergents and shampoos which are usually used. Recent data have suggested that these products are likely containing estrogenic compounds (Hester and Harrison, 1999).

BPA has been reported to be able to bind with estrogen receptors (ERα and ERβ) and play either estrogenic or anti-sterogenic roles in-vitro. BPA may be glucuronidated by a liver enzyme that is called uridine diphosphate-glucuronosyl transferase (UGT). Temporal increases in the incidence of certain cancers in hormonally sensitive tissues in many parts of the industrialized conuntry are often cited as evidence that general population exposures to EDCs have had adverse impacts on human health (Tsutsumi, 2005).

Man-made chemicals endocrine disrupters pose the potential to modulate endocrine function and thus adversely affect human reproductive development (Foster *et al*., 2000).

In the last two decades, a great deal of research effort has been devoted to the identification, occurrence, fate and effects of organic contaminants found in municipal wastewaters. Among these organic chemicals, some contaminants that have the potential to disrupt the normal functions of the endocrine system in wildlife, fish, and humans have drawn special attention. They encompass a wide range of industrial and house-hold chemicals such as polychlorinated biphenyls (PCBs), chlorinated insecticides, alkyl phenols and their ethoxylates and BPA (Lee *et al*., 2005).

Bisphenol A is an estrogenic endocrine-disrupting chemical with two unsaturated phenol rings which, is widely used in the production of polycarbonate plastics and epoxy resins, which are used in dentistry, food packaging, and as lacquers to coated food cans, bottle tops, and water pipes (Tsutsumi, 2005).

Estrogens which are produced by women excretion are extremely potent endocrine receptor modulators, while the other compounds interfere with normal hormonal activity by mimicking and blocking the action of natural human hormones (Lagana *et al*., 2004). Estrogens included in this work were the female hormones 17β-estradiol (E2), estrone (E1) and the synthetic contraceptive additive 17α-ethynylestradiol (EE2) that has potency similar to natural hormones. 17β-estradiol (E2) is the one that display the highest estrogenic capacities (Smeets *et al*., 1999).

Given the lack of information in Iran about surface water, groundwater, drinking water and wastewater contamination by EDCs, endocrine disrupting phenolic and estrogenic compounds in water sources and wastewater in Hamadan city, west of Iran have been studied and analyzed.

MATERIALS AND METHODS

Chemicals

Pure standards of ethinyl estradiol, 17 beta estradiol and bisphenol A were obtained from Sigma- Aldrich; standards of estron and nonylphenol were obtained from Merck Co. Stock solutions of the standards were prepared in methanol and stored at -18 ˚C in dark room.

General remarks

The major challenge is that wastewater has to face a complex and variable mixture of numerous organic and inorganic substances that could be extracted with the analytes of interest. This makes quantification difficult, because of matrix-induced signal suppression effects or isobaric spectral interferences from the complex sample extracts. Also, the low concentration levels of endocrine disrupters in sewage treatment plant (STP) effluents and in natural water, except for BPA and nonylphenol (NP), require extremely sensitive and selective analytical methods (Lagana et al., 2004). To solve both problems of interferences and signal suppression, a solid phase extraction (SPE) procedure was used to either rinse away or retain a portion of the causative matrix components from the sewage extracts.

Different analytical methods have been developed for analyzing Endocrine Disrupters (EDCs) from water and wastewater samples. A number of methods based on liquid chromatographic separation and mass spectrometric, fluorimetric and electrochemical detection, using simpler and faster sample treatment, have been

recently reported. Solid-phase extraction is the technique commonly used for bisphenol extraction from environmental aqueous samples prior to LC analysis because of its advantages over liquid-liquid extraction such as: high pre-concentration factors, low consumption of organic solvents, elimination of emulsions and easy autoimmunization (Moral *et al.*, 2005).

Sample collection

Various types of environmental water and wastewater samples, including drinking water, groundwater, surface water, and wastewater were collected from municipal wastewater network, river, and municipal drinking water network located in the city of Hamadan in the west of Iran. 150, 100, 10 and 100 samples were randomly collected from wastewater, drinking water, Ekbatan dam water and groundwater, respectively, in April- September and October-March in 2006-2007. The sampling sites were chosen after consulting the Hamadan Department of Environment and Water & Wastewater company. Samples were collected in brown glass bottles and stored at 4°C in the dark box until analysis.

Extraction and HPLC analysis

The samples before passing through a C18 solid phase extraction (SPE) cartridge were passed through a fiber glass filter paper (0.1 μm) which was hold in a Buchner funnel to remove particulates. Then, pH was adjusted at 2 by sulfuric acid. Prior to the extraction step, each SPE column was conditioned by rinsing successively with 9 mL hexane, 9 mL acetone and 3 mL deionized water. Extraction flow rate was 10-15 mL/min. Then cartridges were centrifuged under 2000ram for 10 minutes and double washed with 5 mL acetone and dried under nitrogen. The phenolic compound was eluted with 2×2.5 mL ethanol and the solvent was evaporated to 0.5 mL with a gentle stream of nitrogen (Penalver et al., 2002). The extracts were stored in glass vials at 4°C until analyzed by HPLC-UV.

The chromatographic system consisted of a P4000 with a UV-Fluorescence detector (Waters Company, USA). Separation was achieved on a LiChrospher 100 RP-18 column with 15cm × 3.9 id., 4μm) and eluting liquid was water and acetonitril and metanol 13:30:57 with a flow rate 0.8mL/min. The detection wavelengths were 270 nm for 17-estrdiol, 240 nm for estrol, 210 nm for ethinyl estradiol and 280 nm for others (Ying *et al.,* 2003; Iwasaki *et al.*, 2004).

RESULTS

Results of the present study demonstrated that sewage samples have positive effects for the selected EDCs (Table 1). The results showed that concentration levels were from 35 ng/L for 17-beta estradiol to 0.003 μg/L for nonylphenol.

Table 1: Concentration of estrogenic compounds in sewage (n=150)

Compound	Unit	Mean	SD
April-September			
17-beta estradiol	ng/L	35	5
Esteron	ng/L	20	4
Ethinyl estradiol	ng/L	12	3
Bisphenol A	μg/L	6	2.5
Nonylphenol	μg/L	0.7	0.02
October-March			
17-beta estradiol	ng/L	12	4
Esteron	ng/L	6	1.5
Ethinyl estradiol	ng/L	2	2
Bisphenol A	μg/L	3	3
Nonylphenol	μg/L	0.003	0.0005

The results of water samples are shown in Tables 2-5. Values obtained for all water samples showed that contaminates were in concentrations levels of 10 ng/L for 17-beta estradiol to 0.0009 µg/L for nonylphenol. Table 2 showed the concentration of those pollutant compounds in surface water. The results show that concentration of pollutants in April- September was higher than October-March period ($P_{value} < 0.005$) and concentration levels of bisphenol A and 17 beta estradiol were 1 µg/L and 9 ng/L, respectively, which are more than the others.

Table 2: Concentration of estrogenic compounds in surface water (n=100)

Compound	Unit	Mean	SD
April-September			
17-beta estradiol	ng/L	10	4.5
Esteron	ng/L	9	3.5
Ethinyl estradiol	ng/L	2	1.2
Bisphenol A	µg/L	1	0.5
Nonylphenol	µg/L	0.002	0.002
October-March			
17-beta estradiol	ng/L	3	1
Esteron	ng/L	2	0.3
Ethinyl estradio	ng/L	0.01	0.008
Bisphenol A	µg/L	0.01	0.005
Nonylphenol	µg/L	0.0009	0.00003

Table 3 demonstrates the concentration levels of estrogenic compounds in water of Ekbatan Dam. The concentration levels of pollutants in April- September were higher than those levels in October- March which may be due to dilution by rain water. The results also showed that concentration of bisphenol A and nonylphenol were more than other pollutants which were 0.01 and 0.02 µg/L, respectively.

Table 3: Concentration of estrogenic compounds in water of Ekbatan Dam (n=10)

Compound	Unit	Mean	SD
April-September			
17-beta estradiol	ng/L	7	3
Esteron	ng/L	6	2.7
Ethinyl estradiol	ng/L	2	1.2
Bisphenol A	µg/L	0.01	0.01
Nonylphenol	µg/L	0.2	0.03
October-March			
17-beta estradiol	ng/L	1	0.6
Esteron	ng/L	0.9	0.7
Ethinyl estradiol	ng/L	0.004	0.001
Bisphenol A	µg/L	0.001	0.0006
Nonylphenol	µg/L	0.002	0.001

Table 4 shows the level of pollutants in groundwater sources. The results showed that concentration of 17-beta estradiol ranges between 0.3 to 0.2 ng/L, which were the highest among the other pollutants concentrations, but it was lower comparing to in surface and dam waters; also nonylphenol concentration was less than other pollutants concentration.

Table 4: Concentration of estrogenic compounds in groundwater samples (n=100)

Compound	Unit	Mean	SD
April-September			
17-beta estradiol	ng/L	0.3	0.1
Esteron	ng/L	0.2	0.05
Ethinyl estradiol	ng/L	1	0.09
Bisphenol A	µg/L	0.9	0.02
Nonylphenol	µg/L	0.001	0.0009
October-March			
17-beta estradiol	ng/L	0.2	0.08
Esteron	ng/L	0.1	0.05
Ethinyl estradiol	ng/L	0.5	0.1
Bisphenol A	µg/L	0.4	0.1
Nonylphenol	µg/L	------	-----

Table 5 shows the concentration of pollutants in drinking water. The results indicat that the value for 17-beta estradiol was the highest and NP, esteron and BPA were not detected in drinking water in October-March period.

Table 5: Concentration of estrogenic compounds in drinking water samples (n=150)

Compound	Unit	Concentration	SD
April-September			
17-beta estradiol	ng/L	0.01	0.005
Esteron	ng/L	0.002	0.0001
Ethinyl estradiol	ng/L	0.001	0.0002
Bisphenol A	µg/L	0.002	0.0003
Nonylphenol	µg/L	------	------
October-March			
17-beta estradiol	ng/L	0.002	0.001
Esteron	ng/L	----	-----
Ethinyl estradiol	ng/L	0.003	0.0004
Bisphenol A	µg/L	----	-----
Nonylphenol	µg/L	----	-----

DISCUSSION

In spite of several investigations which have been done on endocrine disrupting chemicals in water sources in the world, but none of them is from Middle East or Iran. Our study focused on the identification and determination of concentration levels of estrogenic compounds in water and wastewater because the information gathered from the various monitoring programs show that the impact of the human and environmental exposure to EDCs has alarmed scientists and environmentalist about the potentially dangerous consequences of such exposure.

The concentration of estrogenic compound in sewage samples showed that all five studied compounds found in sewage samples and bisphenol A had largest amounts (6±2.5 µg/L) in April-September). Cargouet et al had found estrone, 17 β-estradiol, estriol and 17α ethinylestradiol in wastewater treatment plants and river samples at concentrations ranging from 2.7 to 17.6ng/L, and 1.0 to 3.2ng/L respectively (Cargouet et al., 2004). Ma *et al* showed that estrogenic activity of industrial wastewaters are ranged from 0.1 to 13.3 ng EEQ/L and decreased to the range of 0.03-1.6 ng EEQ/L after treatment, in WWTP influents ranged from 0.3 to 1.7 ng EEQ/L and in the receiving river waters, the estrogenic effect range was 0.1-4.7 EEQ/l (Ma *et al.*, 2007).

Wastewater treatment plants appear to be one of the major sources of pollutants because these

compounds are not totally removed or degraded by biological treatment. A study performed in real samples from sewage treatment plants has evidenced the presence of bisphenol A, estrone and 17ß-estradiol in the water and wastewater treatment plant influents at concentration levels that ranged from 39.6 to 1105.2 ng/L and bisphenol A have been detected at concentrations between 13.3 and 19.2 ng/L in the effluents (Hernando *et al.*, 2004).

The swage treatment plant efficiency in removing BPA is better (90%) than for NP (75%), probably because of the major resistance of NP to microbial degradation occurring during the treatment process. In addition, NP is generated during the break down of products containing alkyl phenol poly ethoxylates which is one of the world's largest groups of surfactants. (Lagana *et al.*, 2004)

This study being performed in real samples from drinking water has evidenced the presence of traces of nonylphenol and estrogenic compounds at concentration levels that ranged from zero to 0.002 µg/L. But it is noteable that the concentration levels in surface water and wastewater are several times drinking water ($P_{value} < 0.005$), The present data showed that the concentration of nonylphenol was from 0.002 to 0.0009 µg/L in surface water samples but 17-beta estradiol values was from 10 to 3 µg/L. The results show that concentration of pollutants were more in April- September comparing to October-March and difference is statistically significant ($P_{value} < 0.005$). Results compared to Peng *et al* findings showed that bisphenol A, 4-nonylphenol were higher than the finding of Peng *et al* study in most surface samples. But in drinking water samples sometime was equal and sometime was less than these findings.

Peng *et al* determined estrone in >60% water samples with a maximum concentration of 65ng/L and nonylphenol and bisphenol A was found to be widely present at rather high concentrations in the urban river Guangzhou. BPA was detected in river samples in concentrations of 500 pg/L to 16 ng/L; BPA levels in drinking water ranged from 300 pg/L to 2 ng/L (Peng *et al*., 2008). In river water samples in southern Germany, BPA was found in concentrations range of 500 pg/L up to 16 ng/L, 4-nonyl phenols was from 6 up to 135 ng/L, and the steroids were from 200 pg/L up to 5 ng/L. In drinking water, BPA was found in concentrations ranging from 300 pg/L to 2 ng/L, 4-nonylphenol was from 2 to 15 ng/L, 4-tert-octylphenol was from 150 pg/L to 5 ng/L, and the steroids were from 100 pg/L to 2 ng/L. These results indicate that environmental endocrine-disrupting estrogens are not completely removed in the process of sewage treatment but are carried over into the general aquatic environment. After ground passage, they can eventually be found in drinking water (Kuch and Ballschmiter, 2001).

Effluent from cities and industrial wastewater treatment plants frequently contain bisphenols, because of the incomplete removal of these contaminants during treatment (Moral *et al*, 2005). Estrogens are in fact mainly excreted as conjugates of sulfuric acid and glucuronic acids. In this form they would not possess a direct biological activity, but they can act as precursor hormone reservoirs able to be deconjugated into the parent compounds during the sewage treatment. This event is especially clear for E1, the most abundant estrogen excreted by cycling women, that is even believe to be the by product of biodegradation of E2 in the sewer. Nevertheless, waters analysis data (Tables 2-5) show that tiny level of the most potent estrogen is discharged into receiving waters. However, this low concentration could be important with respect to endocrine activity at sites where sewage treatment plant effluents can make up a considerable proportion of the river flow, even because estrogens may cause deleterious effects on organisms even at such low concentrations (Lagana *et al*., 2004).

The groundwater samples investigated in Spain were found to be free of estrogens. Because of their physical-chemical properties, estrogenic compounds tend to adsorb to the aquatic sediments, thus minimizing leaching through the subsoil into the aquifer (Rodroguez- Mozaz *et al*., 2004) and difference with this study may be due to usage of absorbing well in some areas of the city, rural area and some industries.

Estrogen profile of urine samples indicate, in fact, that women can excrete about 7 µg of E1, 2.4 µg of E2 and 4.6 µg of E3 per day which are discharged

to wastewater collection system. E1 and E2 outlet levels are substantially lower than inlet levels, but they are not completely removed. Among the various groups of endocrine-disrupting, the female sex hormones and the synthetic steroids are considered as the most potent estrogenic compounds. However, these groups of substances have received little attention untill now, perhaps because they have been found in the environment at concentrations as low as ng/L (Lopez and Barcelo, 2001). Based on human daily excretion and other physico-chemical parameters such as dilution factor, sorption to solid matter and also the observations made by other authors, indicate that estrogens are expected to be present in aqueous environmental samples at the ng/l level (Rodriguez-Mosas *et al*., 2004).

A study reported NP levels even up to 330 µg/L in sewage treatment plants and up to 180 µg/L in the receiving waters (Sole *et al*., 2000). However, the normal range observed in most river stretches was between 0.2 and 12 µg/L. Many researches have demonstrated the widespread occurrence of NP in municipal sewage. Therefore, although there was a decrease in the NP concentration to the primary effluent this compound was still easy to detect (at concentrations below µg/L). Discharge of the sewage effluent into rivers are, then, likely to cause contamination of NP in the aquatic environment (Lagana *et al*., 2004).

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