Methodological and molecular issues involved in assessment of endocrine disruptive potentials of water contaminants: A mini review

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Abstract- Endocrine disruptive chemicals (EDCs) are known to cause deleterious effects on human beings and wildlife. For decades, effects of EDCs on biological system have questioned conventional concepts of toxicology. In recent years, great attention was given on the development of appropriate modalities for the precise assessment of endocrine disruptive potential of various water contaminants. However, these developments were hindered due to various issues linked to the chemical and biological characterization of EDCs. Thus, the present review discusses the methodological and molecular issues that are associated with the assessment of endocrine disruptive potentials of water contaminants.

Keywords – Endocrine disruptive chemicals, environmental contaminants, toxicology, biological characterization

Abbreviations:

AhR	: Aryl hydrocarbon receptor
AR	: Androgen receptor
BIS	: Bureau of Indian standards
DDT	: Dichlorodiphenyltrichloroethane
EDCs	: Endocrine disrupting chemicals
ER	: Estrogen receptor
FDA	: Food and drug administration
GC-MS	: Gas chromatography-Mass Spectrometry
HPLC	: High performance liquid chromatography
ICP-MS	: Inductively coupled plasma-Mass spectrometry
IDL	: Instrument detection limit
LC-MS	: Liquid chromatography- Mass spectrometry
LLE	: Liquid-liquid extraction
MDL	: Method detection limit
PCBs	: Polychlorinated biphenyls
SPE	: Solid phase extraction
USEPA	: United States environmental protection agency
Vtg	: Vitellogenin

I. INTRODUCTION

Developed countries use advance treatment methodologies (Coagulation/flocculation, sedimentation, ozonation, dual-media filtration, and chloramination, preoxidation (Cl_2) etc. for the removal of EDCs from the water matrix [1,2]. On the contrary, many developing countries do not have enough infrastructures to treat water even with conventional treatment modalities. In India, approximately 90% of wastewater is discharged without treatment [3]. Small scale industries in developing countries do not have appropriate effluent treatment facilities as they operate on low profit margins [4,5]. Many countries, including India, do not have defined stringent safety measures for the regulation of EDCs in the environment. Due to lack of or poor regulatory standards in many countries, household and industrial wastes are disposed into water bodies without proper treatment. This results in the contamination of ground, waste, river, pond and municipal water [3]. Increasing prevalence of EDCs in these water sources has been reported [6]. EDCs include DDT and its metabolites, compounds of industrial origin (dioxins, PCBs, bisphenol A, pesticides, phthalates, parabens, metals, detergents, etc.), natural and synthetic hormones/steroids etc. [7,8,9,10]. Chronic exposure to EDCs can cause reproductive and endocrine disorders (feminization/masculism), cancer, cardiopulmonary diseases and auto immune diseases [11,12,10]. Considering an increased threat pose by EDCs, which elicited international concerns, scientific and regulatory authorities have initiated various programs to strengthen stringent rules for the regulation of EDCs in the environment [13, 14]. In recent times, the greatest challenge is to correlate endocrine disruptive potentials of water contaminants with the development of endocrine related diseases/disorders in human and in wildlife. In-depth knowledge about the assessment methodologies can facilitate better understanding of the adverse health effects induced by EDCs. Herein, we present various methodological and molecular issues related to the assessment of endocrine disruptive potential of water contaminants.

II. ASSESSMENT OF ENDOCRINE DISRUPTIVE POTENTIALS OF WATER CONTAMINANTS

Table 1 presents methodological and molecular issues that are associated with the assessment of endocrine disruptive potentials of water contaminants along with the suggestive plan of action.

Table 1.

Assessment of endocrine disruptive potentials of water contaminants		
Methodological issues		
Sr. No.	Issues	Suggestive plan of action
1.	Bioactivity of EDCs	• Implementation of effect-based monitoring methods
2.	Sampling, processing and quantification of EDCs in water matrix and sensitivity of analytical methods	• Better understanding of: a) sampling volume, b) site and timing of sampling, c) sample replicates, d) heterogeneity of water matrix, e) extraction methods (SPE/ LLE), f) degradation of EDCs (steroids, harmones, pharmaceuticals etc.) in the water matrix, g) chemo/ bio-complexes present in water matrix, h) derivatization of samples, i) instrument detection limit (IDL), j) method detection limit (MDL), k) reproducibility of data, l) statistical confidence
3.	Regulatory norms for the assessment of EDCs	• Regulatory norms (based on permissible limits) for chemical characterization of EDCs in water matrix: a) analytical methods for identification of compounds, b) *In vitro* (Reporter constructs) assay using pure compound
		• Regulatory norms for biological characterization of EDCs in water matrix (effects on aquatic fauna): a) *In vitro* (Reporter) assay using water matrix, b) *In vivo* (zebra fish) assay using water matrix
Molecular issues		
1.	Synergistic and inhibitory effects of contaminants present in water matrix (AhR-ER crosstalk's, mimicking effects etc.)	• *In vitro* assays for characterization of EDCs: Better understanding of: a) cell line to be chosen for the crosstalk's studies, b) agonist/antagonist present in cell culture media and other supplements, c) type of trasfection (stable/transient) and transfection efficiencies, d) treatment strategies • *In vivo* assays for characterization of EDCs: Better understanding of: a) In built physiological (hormonal etc.)

		alterations in aquatic fauna, b) gender specific response of aquatic fauna upon exposure to EDCs • Validation of end point indicators (Vitellogenin, ER/AR responsive reporter constructs etc.) for the precise assessment of endocrine disruptive potential of water contaminants; identification and development of new indicators
2.	Bioaccumulation and biomagnifications of EDCs	• Validation using both the analytical and effect-based monitoring methods

A. Methodological issues –

Various analytical methods (GC-MS, LC-MS/MS, ICP-MS, HPLC etc.) have been used to estimate endocrine disruptive potential of water contaminants [15,16,17]. Despite of the efforts in improving the analytical techniques, precise estimation of EDCs in the complex water matrix remains a problem [15]. Suggestive plan of action against each issue has been given in Table 1. It is important to understand that analytical methods can identify EDCs present in the water matrix, but does not guarantee bioactivity of EDCs. Thus the analytical estimates need to be validated through effect-based monitoring methods to determine the bioactivity of EDCs.

Sampling and processing of water sample is one of the most important aspects with regards to the precise estimation of EDCs. Water sources (River, STPs, ponds, etc.) contain higher volume of water. The water may be dynamic and heterogenic in nature. In this regards, sampling of liters of water cannot facilitate a true representation of the whole water matrix. Higher statistical confidence in the qualitative/ quantitative analysis of EDCs present in the water matrix can be achieved by increasing the sample volume, replicates, sites and timings of sampling. Derivatization of complex water samples, appropriate extraction method and proper transport/processing of water samples can further increase the statistical confidence. In addition, biological effects posed by EDCs were found deviate from the normal dose response relationship [18]. Non monotonic dose response exerted EDCs cannot be only predicted by the effects observed at high doses. In order to evaluate these responses analysis using the instruments with higher sensitivity is needed.

Another important concern is the regulatory norms for the assessment of EDCs. Existing norms (e.g., USEPA) defines the maximum permissible limits of the contaminants in different water sources [19, 20]. EDCs are known to cause detrimental effects at a very low concentration, even below the permissible regulatory limits and/or at non-toxic concentrations [21]. Thus, regulatory norms for the chemical and biological characterization of EDCs in the water matrix need to be clearly defined.

B. Molecular issues –

Quantification of estrogen receptor (ER)-mediated vitellogenin (Vtg) expression in male fish has been reported as a *in vivo* method for the identification of biologically active EDCs in environmental samples [22,23]). Recent studies examined the inhibition of Vtg expression due to crosstalk's between AhR and ER-mediated signal transduction pathways [24]. Since both the AhR and ER agonists may be present in the complex water matrix [25]), it is important to elucidate the role of crosstalk's on the overall effects of endocrine disruptive potential of water contaminants. In addition, appropriate selection of the cell line for *in vitro* reporter assays is also important. Cell lines with inactive AhR may not elucidate possible crosstalk between AhR with ER and can overestimate the endocrine disruptive potentials of water contaminants.

Vtg is exclusively synthesized in the females in oviparous vertebrates and invertebrates for providing egg yolk proteins as a source of nutrients during embryonic development [26,23,27]. Vtg expression is silent in males but can be elevated upon their exposure to EDCs. Thus, in-built physiological (hormonal) alterations and gender specific responses of aquatic fauna need to be considered while characterizing the endocrine disruptive properties of water contaminants. In addition, end point indicators (e.g., Vtg, transcription response elements of reporter vectors etc.) need to be validated in different *in vitro* and *in vivo* models.

Aquatic fauna are highly motile. Therefore it is difficult to judge the actual volume of dynamic and heterogeneous water sample to which the fish is exposed to over a period of time. In addition, fish motility is affected by temperature, habitat and several other environmental conditions. Thus, the interpretations of bio-concentrating/bio-accumulating properties of water contaminants based on the static water exposure (laboratory data) should be validated with the field data. Although the EDCs have been precisely quantified in water samples, interpretations based on concentrations of EDCs in the water samples does not provide a reliable estimate of their adverse health impact due bio-concentration, bio-accumulation and bio-magnification of EDCs in the biological systems [28]

III. FUTURISTIC DIRECTIONS

To assess the endocrine disruptive potential of water contaminants qualitative and quantitative estimates of water contaminants can be obtained by analytical methods, depending upon the availability of analytical standards. Effect of pure standard synthetic compounds at concentrations similar to the actual concentration present in water matrix can be tested with battery of Tier I screening assays (ER/AR receptor binding assays, fish screening assay, steroidogenesis assay, aromatase assay etc.) recommended in EDSP guidelines [29,19]). This data can be useful in strengthening the regulatory permissible limits against individual compound with endocrine disruptive potential.

Since the aquatic fauna are exposed to whole water matrix and not to the individual pure compounds, overall characterizations of water matrix with regards to its endocrine disruptive potentials have immense importance. In this regards, interpretations based on the individual compound may give erroneous information due to non consideration of inhibitory/synergistic/additive effects induced by the contaminants present in the water matrix. In our recent study (data unpublished), we found that different extraction processes (solid phase, liquid-liquid) may depict different results while assessing the endocrine disruptive potential of water contaminants. Methods that are specially designed to extract EDCs and remove inhibitory compounds (e.g., AhR agonists) from the water samples may be useful in understanding the endocrine disruptive potential of water. On the contrary, liquid-liquid isolation methods facilitate the extraction of both EDCs and inhibitory compounds from the water samples. Thus, comparison of the results obtained by solid phase and liquid-liquid extraction methods can be helpful in understanding the crosstalk of biological pathways related to endocrine disruption with other cellular pathways (e.g., AhR-ER crosstalk). In addition, both Tier I and Tier II (one- or –two generation mammalian reproductive toxicity, avian reproduction, fish life cycle, etc.) screening assays can be implemented for the characterization of water matrix [19] . These results will be helpful in understanding the differences in effective concentrations of EDCs between *in vitro* and *in vivo* settings.

IV.CONCLUSION

Chronic exposure of EDCs is reported to impact deleterious effects on human health and aquatic fauna. Today, precise estimation of endocrine disruptive potential of water contaminants is immensely required to enact strict measures for the regulation of EDCs in different water sources. In this regards, suggested plan of action can enrich our understanding about the methodological and molecular issues that are related to the estimation of endocrine disruptive properties of water contaminants. This will help us to minimize the erogenous results during the experimental analysis.

V. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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