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Review Research Paper

Persistent and Endocrine Disrupting Organic Pollutants: Advancements and Challenges in Analysis, Health Concerns and Clinical Correlates

Muhammad Sajid*, Chanbasha Basheer*†, Kothandaraman Narasimhan, Abdelbaset Buhmeida**, Mohammed Al Qahtani** and Mahmoud Shaheen Al-Ahwal****

*Department of Chemistry, King Fahd University of Petroleum and Minerals, Dhahran, 31261, Kingdom of Saudi Arabia
**Center for Excellence in Genomic Medicine Research, Faculty of Medicine, King Abdulaziz University, Jeddah, 21589, Kingdom of Saudi Arabia
†Corresponding author: Chanbasha Basheer

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ABSTRACT

Persistent and endocrine disrupting organic pollutants pose serious health risks to humans and wildlife due to their long half-lives, bioaccumulation and toxicity. These compounds have a negative impact on human health and induce a variety of carcinogenic and non-carcinogenic disorders. Extremely low concentrations of these compounds can induce adverse health effects in human. Efficient analytical methods are always demanded in order to extract and determine such low concentrations in environmental and biological samples. In the last decade, studies mostly focused on profiling and identification of persistent and endocrine disrupting pollutants in tissues and body fluids using various analytical methods. However, investigation of trace level pollutants associated with various disorders requires complex analytical protocols. Biological matrix presents serious challenges in the extraction of these pollutants mainly because of its complexity and inherent limitations of extraction procedures. We outline advantages and pitfalls of recent extraction approaches. Analysis of these compounds is carried out by using gas and liquid chromatography methods coupled with different detectors. We have reviewed the current status of advancements in the area of analytical chromatography and accomplishments and weaknesses have been pointed out. At the end, we encapsulate some health effects and clinical correlates of persistent and endocrine disrupting organic pollutants.

INTRODUCTION

Persistent organic pollutants (POPs) represent a toxic class of organic chemicals which have potential to travel in lipids, accumulate in tissues and induce a variety of health complications. Because of very long half-lives, they have managed to sustain in the environment for years and that is the reason why some POPs can still be found in the environment, although their use was banned in the early 1970s. The causalities caused by these chemicals are blown out throughout the history, but the most terrible aspect arises from the fact that these series of events have not stopped yet. A more recent story is published about excessive use of endosulfan based pesticides in an Indian state, Kerala, where almost 45000 people have been reported to suffer from different diseases, being a victim of haphazard usage of such chemicals in pesticides (Saseendran 2014).

More chemicals are being added to the famous "dirty dozen" which were confirmed as POPs by Stockholm Convention and now this number is preceded to 23. This is a significant increase over a period of one decade. Moreover, a number of other chemicals have been reported to possess the persistent nature and the ability to accumulate within biological matrix and have not been regulated yet. Stockholm convention categorizes these 23 POPs under different annexes like A, B and C. POPs in annex A are banned from production, use and applications. POPs belonging to Annex B are also banned for production and use, but there exist certain exemptions. POPs in annex C are produced as a consequence of some unintended processes. It is pretty important to note that these 23 compounds have been regulated, but there are hundreds of other organic compounds which show similar characteristics, but have not been paid that much attention with regard of regulations. For example, some organo-metals such as methylmercury and other derivatives are highly persistent and tend to bioaccumulate in the food chain and human body (Carpenter 2011).

POPs present a global challenge and hundreds of reports are published every year describing the current status, concentrations in different matrix and health effects. Primary sources of POPs, although have been banned, but they have heavily contributed in developing secondary sources which include seawater, sediments, aquatic organisms, other water bodies, and vegetation soils. These secondary sources are expected to release POPs for next hundred years or even more. POPs have been extensively reviewed from different

aspects in the past few years.

Many organic pollutants, including POPs are well-known to interfere with the endocrine system by imitating, hindering and prompting normal activity of hormones and thus effect the health and reproductive system of humans and wildlife (Schug et al. 2011). These compounds are named as endocrine disrupting compounds (EDCs). A number of these compounds are increasing day by day due to continuous consumption and applications in the industrial sector. These compounds are basically of xenobiotic and exogenous origins which are considered to have an adverse effect on the normal action of the endocrine system and disturb all the functions i.e. synthesis, secretion, transport, and binding of hormones.

EDCs are broadly classified into four categories (i) naturally occurring androgens and estrogens (ii) artificially synthesized androgens and estrogens (iii) phytoestrogens (iv) other industrial compounds (Liu et al. 2009). Synthesized or industrial EDCs are members of different classes of chemical compounds and they have been identified in all industrial products including pesticides, alkyl phenols, personal care products, polychlorinated biphenyls, heavy metals and so on. Synthesized EDCs were basically designed to perform a certain kind of action such, as plasticizer, solvent or pesticide, but later on it was realized that they have functional properties which can result in disruption of endocrine systems.

The studies describing adverse effects of such compounds on human health have been increased in recent years (Sonnenschein & Soto 1998). As a result of rapid industrialization throughout the world, the production of such chemicals and their introduction into the environment has massively increased. Prolonged exposure to trace level concentrations of these compounds can induce very serious health complications in human body and wild life (Bormanet al. 2000). Fig. 1 summarizes the sources, exposure pathways, health effects and analytical strategies for analysis of POPs and EDCs. Table 1 lists some organic pollutants which show different kind of endocrine related activities. In the earlier years, it was thought that the EDCs act through nuclear hormone receptors (genomically), but now it has been recognized that the mechanism of interaction of EDCs with body involves many other pathways (non-genomically) as well (Schug et al. 2011). Endocrine disrupting mechanism of chemicals is described in Fig. 2.

It is a matter of fact that POPs and EDCs occur at trace levels in various environmental and biological samples and thus their detection and removal presents a great challenge to analytical chemists. It remained a major focus of researchers to extract and detect extremely low concentrations of these toxic pollutants. This has been accomplished by developing microextraction procedures for sample preparation in combination with advances in analytical instrumentation. This review is aimed to probe current status and challenges in extraction and detection approaches for analysis of minute concentrations of POPs and EDCs in environmental and biological matrices. Chromatographic methods have been considered in detail and accomplishments, weaknesses and challenges in dealing with such methods will remain a major focus of this review. We have also listed some studies investigating health effects of POPs and EDCs to human and wildlife. A brief detail of clinical correlates of EDCs and database resources used to identify perturbations in gene and pathways by environmental endocrine disrupters is also included.

SPECIFIC CLASSES OF PERSISTENT AND ENDOCRINE DISRUPTING ORGANIC POLLUTANTS

We describe three major classes of persistent and endocrine disrupting compounds; polychlorinated organic compounds represent a very well established class of persistent and endocrine disrupting organic pollutants while other two classes represent some emerging persistent chemicals.

Polychlorinated organic compounds: This class of compounds mainly includes organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDDs). Such compounds are defined by aromatic structures, low water solubility and high chemical stability. PCDFs and PCDDs are obtained as a product of some chemical, photochemical, thermal and enzymatic reactions. These compounds are discharged into the environment via some incineration plants which include clinical, municipal and industrial waste incinerators (Petroviæ et al. 2001).

PCBs are persistent synthetic lipophilic compounds, except few of them. In the past, PCBs have been used in a variety of consumer products including plastics, lubricants, capacitors, vacuum pumps, paints, transformers, flame retardants, adhesives, gas transition tubes, hydraulic fluids, sealants, and heat transfer fluids, but their manufacturing was stopped in the late 1970s. Recent reports reveal their measurable levels in the serum (Rudel & Perovich 2009). PCB concentrations vary from place to place depending on geographical conditions. Higher concentrations are found in urban areas relative to rural areas which show substantial contributions from the local sources situated in these areas (Jones et al. 2006). These compounds have ability to bio accumulate in the food chain and finally reach to the human body. PCBs have also been reported to induce diabetes and

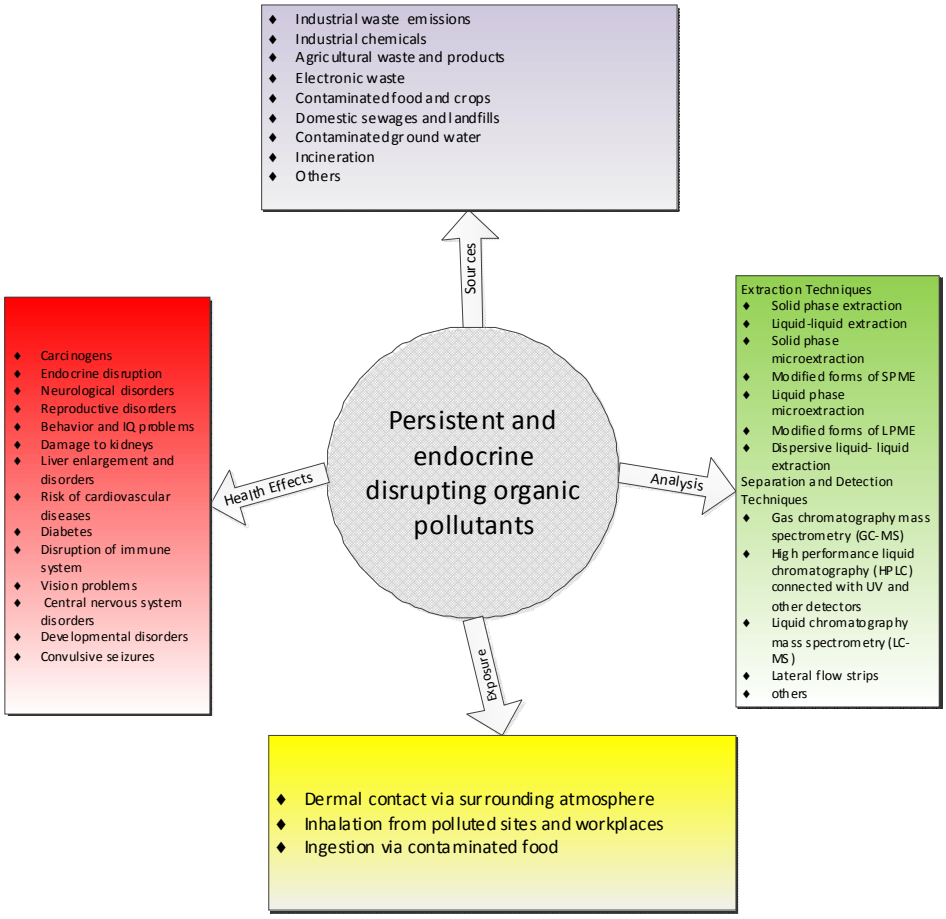

Fig. 1: Summary of sources, health effects, routes of exposure to human and analysis of persistent and endocrine disrupting organic pollutants.

also have a strong effect on thyroid signalling (Turyk et al. 2009). Not only the PCBs, all classes of polychlorinated compounds were studied to determine their possible character as endocrine disrupters and various correlations were observed. Tetrachlorodibenzo-p-dioxin (TCDD) has been reported as an inhibitor of estrogen mediated activity, but their exact mechanism is not known yet (Safe et al. 1991). Another complication about PCBs is that they do not show antiestrogenic activity, but in reality they are estrogenic.

Classification of pesticides is done based on their action with different substances. They are generally classified as insecticides, germicides, fungicides, herbicides, rodenticides, avicides, larvicides, and acaricides. They are composed of organic compounds which are volatile, semi-volatile or non-volatile in nature. Pesticides have widespread applications in the agriculture sector to grow crops and different food stuff. Through food chain, these pesticides find their way to human body and wild animals. Volatile pesticides may be present in outdoor environments and thus accumulate with various materials. Pesticides are added to carpets, paints and building materials, this makes indoor environment suspected for their presence and accumulation on stuffs like toys, carpets and any other material (Rudel & Perovich 2009).

Although persistent organic compounds like OCPs were banned to be used as pesticides, but still they can be detected in environmental samples. Stockholm convention has declared so for 23 POPs and among them 13 belongs to OCPs. These include aldrin, dieldrin, endrin, chlordane, heptachlor, DDT, hexachlorobenzene, toxaphene, mirex, lindane, chlordecone, α-hexachlorocyclohexane and β-hexachlorocyclohexane (Xu et al. 2013).

Generalizing health effects of OCPs is a really difficult task because they comprise a diversity of chemicals. Pesticides are reported to have very severe neurotoxicity, effects on developing reproductive system and ability to induce various forms of cancers. They also damage the normal function of thyroid hormones in humans and animals (Brouwer et al. 1999).

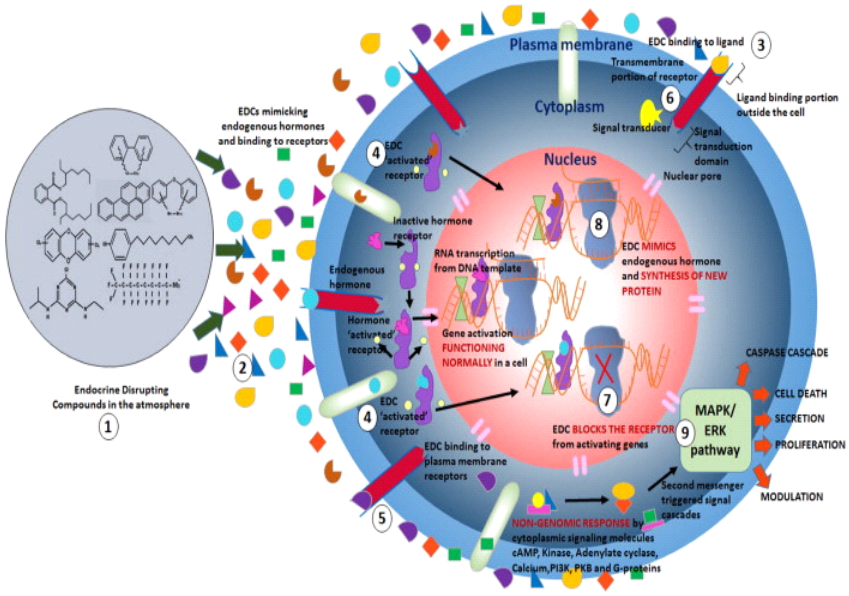

Fig. 2: Mechanisms involved by cell in response to EDCs: EDCs in the atmosphere (1) are present as aerosols or particulate phase. On inhalation, they are capable of exhibiting endocrine disruption either by genomic or non-genomic pathway in a cell. EDC (2) has certain specificity and potency in binding either to ligands (3), nuclear receptors (4) or membrane bound receptors (5). After binding to ligand, alteration in signal transduction occurs (6); in the case of EDC activated nuclear receptors either mRNA transcription is terminated (7) or new protein is synthesized (8). Certain EDCs bind to cytoplasmic signaling molecules and exhibit non-genomic pathway (9). Copied and Reprinted from (Annamalai and Namasivayam, 2015) with permission from Elsevier.

Pharmaceuticals and personal care products: Although pharmaceuticals and personal care products (PPCPs) have not been declared POPs, but they are thought to be potential endocrine disruptors and there are a lot of questions about their persistence in environment. The presence of pharmaceuticals and their bioactive metabolites in the environment has been studied very extensively in the last decade. The part of the environment which is focused in this regard is water and particularly drinking water. Most comprehensive study for emerging organic pharmaceuticals in water resources was carried out by U.S Geological Survey. A wide range of pollutants such as antibiotics, prescription and non-prescription pharmaceutical drugs were detected (Kolpin et al. 2002).

It has been reported by many researchers that many bacterial strains have developed resistance against existing antibiotic drugs and there is a need to find new antibacterial agents. Keeping in view this aspect of resistance, natural synthetic chemists are searching and preparing new drugs to overcome resistance offered by bacterial strains (Levy 1998). Their number is increasing and through wastewater or sewage discharges, they absorb into soil or sediments and remain persistent. Persistence depends on photo stability, degradation capacity and leaching into water. Concentrations higher than estrogenic or proestrogenic end point are found in environment samples. Most of veterinary drugs go with manure, which is spread in agricultural fields and enter into food and water chain. Some reports indicated their improper removal from wastewater that leads to contaminate other water resources (Ternes et al. 1999).

Personal care products (PCPs) are being used by all communities in the world as disinfectants, conservation agents, fragrances and sunscreens. Fate of PPCPs in any environment is governed by chemical, environmental, retention and transport, and accumulation factors (Tijani et al. 2013). Chemical compounds which are added to sunscreen cosmetics to save the consumers from the harmful effects of solar UV radiation are known as Organic UV filters. But these compounds are not only being added to sunscreen cosmetics but all types of cosmetic products. Although they are applied on the outer skin but they have ability to absorb through skin and take part in metabolism resulting in bioaccumulation or excretion. This absorption may also trigger estrogenic (Ma et al. 2003) and carcinogenic activity (Schlumpf et al. 2001). Biotransformation pattern decides about the fate of an exogenous compound in the body, and it has been evaluated for only a few compounds only in serum and plasma, and hence there is a pressing need to investigate biotransformation patterns of all other compounds in variety of tissues (liver, brain, heart, kidney, intestine, skin) (Chisvert et al. 2012). Parabens are also widely used in cosmetics, pharmaceutical and preservatives in food. They have been widely adopted because

of their low toxicity, inertness, broad activity, low cost and long history of safe use. However, they have now been reported to show weak estrogenicity which encourages to develop analytical methods for their quantification at low level concentrations (Sajid et al. 2015a).

Phthalates and food contact materials (FCMs): Phthalates have very wide spread applications in cosmetics, plasticizers, insecticide carriers, chloride resins, adhesives and cellulose film coatings. The worldwide production of these compounds is more than 2.7 million metric tons per year and they are released to environment via direct or indirect corridors. Direct emissions take place during the production of plastics, while indirect emissions take place through leaching and volatilization from plastic products and bottles, particularly when they are subjected to disposal and incineration. Large number of phthalates are included in the "priority pollutants". Although these substances have low acute and chronic toxicity, but they are suspected EDCs (Jobling et al. 1995).

Food contact materials (FCMs) are the chemicals which leach from the packing of food and contaminate it. They are underestimated source of chemical but humans are exposed to these EDCs on a regular basis. Population is exposed to low level concentrations of FCMs throughout their lives (Sajid et al. 2016). Exposure of the general public to contaminants by FCMs is quantified based on the amount of food consumed and FCMs leached into food. Food simulants are used for this purpose, but they don't predict leaching with desired precision. A famous FCM substance, Bisphenol A (BPA), its leaching is always misjudged based on conventional methods. Exposures of these compounds are normally analysed from mutagenicity and geno-toxicity. In this approach all other toxicological effects are neglected including endocrine disruption, development toxicology and toxicity due to the combined effects of several FCM chemicals. Keeping in view these new toxicology parameters, the more sensitive population group comprises children, women of children bearing age and women with pregnancy. Any innovative method for assessment of toxicology due to FCM should incorporate all toxicological parameters and routine analysis of EDCs, with special reference to sensitive age groups (Muncke 2009).

Plastics are the most famous materials used for packaging of food and thus their polymeric layers are always in contact with food materials. Plastics, coatings and polymeric layers are combination of complex chemical substances. Understanding the nature of all these chemicals would be helpful to calculate risk assessment related to FCMs. Chemistry involved in formation of polymeric plastics is very complex and some unknown chemicals are added to the final products which can migrate into food. It is difficult to identify all the substances in the plastics even if starting chemicals and additives are well known, particularly due to non-intentionally added substances. Monomers and additives are considered while assessing the toxicity effects, while rest of the leachate is ignored. It is pertinent to mention that in final packaging of food, adhesives, printing inks and labels may introduce some supplementary compounds into the food (Blumberg et al. 2011). This department of food contamination should be focused more, because all the population is unceasingly exposed to these products.

METHODS FOR ANALYSIS OF POPS AND EDCS AND PROBLEMS ASSOCIATED WITH THEM

Extraction procedures: Efficient extraction and sample preparation methods are required to extract extremely low level concentrations of POPs and other potential emerging pollutants present in complex environmental and biological matrices. Classical extraction techniques are time consuming and labour extensive because several clean-up cycles are needed after extraction which may take several hours to few days (Tang 2013).

Solid phase extraction (SPE) and liquid-liquid extraction (LLE) are among most commonly employed extraction procedures for POPs in complex environmental and biological samples. SPE uses a solid sorbent which captures a particular analyte through adsorption when liquid or gas sample is passed through it. A washing solution is used to remove any unwanted constituents captured with target analyte and finally target analyte is eluted by using a suitable organic solvent or mixture of organic solvents. Selection of suitable combination of sorbent material and solvents for extraction of particular class of target compounds is critical. The sorbent is normally packed into small tubes or cartridges that are available in different shapes and prepared by many companies with specific housing for suction or pressure generation to make the elution through the column a faster process.

Conventional SPE and LLE require large volume of organic solvents and thought to be environmentally unfriendly. Moreover, requirement of large volumes of samples, huge time consumption and multiple clean-up procedures make them rather difficult extraction techniques. In current years, there remained a trend in the development of miniaturized extraction procedures which can be easily automated and configured according to analysis requirement and more importantly they are environment friendly. The main idea behind miniaturized extraction techniques is development of methods which can easily extract target compounds at trace level concentration in different matrices by employing mini-

mum amount of sorbents, samples and solvents. But still they are accurate, precise, and reproducible.

Solid phase microextraction (SPME) uses fibres coated with different organic materials and these fibres are either dipped in the aqueous samples or placed in headspace in case of a complex matrix. Target compounds are adsorbed or absorbed on the fibre which is then injected into the GC column where analytes are desorbed by providing high temperature for a certain period of time. Very high enrichment factors are achieved by using SPME. SPME can be fully automated with GC systems. SPME was used for extraction of polybro-minated diphenyl ethers (PBDEs) in sediments (Jia et al. 2012). Another recent publication describes the use of headspace SPME (HS-SPME) for extraction of POPs in serum samples followed by GC-MS analysis. As this HS-SPME was coupled with an instrument, so the steps like sample handling, removal and transfer of solvents, separate extraction steps and sample transferring to instrument station were reduced. High enrichment factors are obtainable because of analyte transfers from a relatively large volume of liquid sample to solid fibre. SPME has some limitations which include expensive and fragile fibres, lesser affinity and selectivity of commercial fibres towards target compounds (Flores-Ramírez et al. 2014).

Liquid phase microextraction (LPME) has been attempted in various ways. Earlier in 1997, static and dynamic LPME was developed using a single drop of organic liquid at the tip of conventional micro syringe. In static mode, tip of syringe is immersed in aqueous sample and then a drop of organic liquid is formed at the tip and exposed for certain time to the sample and retract back into syringe and injected to the instrument for analysis. In dynamic mode, organic solvent is taken in a micro syringe and syringe is immersed in the sample solution. Few microlitres of samples are withdrawn into syringe and then re-injected into a sample vial. Procedure is repeated for several times and finally an organic drop from the syringe is injected into the instrument (He & Lee 1997). Single drop LPME (SD-LPME) has also been tried as headspace technique, but the stability of the drop is the main drawback, because most of the organic solvents have high vapour pressures and they are easily evaporated. So in such case, selection of suitable solvent is crucial. Another modification in LPME procedure was done by putting a hollow fibre at the tip of the syringe. Fibre is filled with organic solvent which activates the pores of the fibre. Then this is exposed to liquid samples. Small pore size of the fibre does not allow large sized molecules and hence this can be an ideal method for extraction of organic pollutants from complex biological matrix, because it can easily reject any unwanted fatty stuff (Shen & Lee 2002). Hollow fibre protected LPME (HF-LPME) can easily be used for headspace because it provides a support for the organic solvent to be held for longer time (Jiang et al. 2005).

Dispersive liquid-liquid microextraction was developed in 2006 for extraction of PAHs in water samples. This involves a rapid injection of proper mixture of extraction and disperser solvent in water samples which changes to cloudy and extraction solvent is dispersed throughout the sample and is recollected by centrifugation. Method has been extremely used for extraction of organic pollutants from aqueous samples (Rezaee et al. 2006). More recently, DLLME was used for extraction of organochlorine pesticides (OCPs) in honey (Zacharis et al. 2012). The major advantages of DLLME lie in less extraction time, simple operation, lesser volumes of organic solvents and high recoveries and enrichment factors (Rezaee et al. 2006). DLLME has been applied for extraction of variety of organic pollutants in variety of matrices (Rezaee et al. 2010). Area of LPME has gone through a rapid progression and new advancements have been included in the procedure. These review articles can be helpful in understanding advancement in LPME with time (Pedersen-Bjergaard & Rasmussen 2008, Rasmussen & Pedersen-Bjergaard 2004, Sarafraz-Yazdi & Amiri 2010).

Micro solid phase extraction (μ-SPE) uses small amount of sorbent compared to SPE. It works in two ways (1) solid is directly immersed in liquid samples to extract target compounds and then separated by centrifugation and desorbed in small volume of organic solvent (2) solid is packed inside a porous membrane and thrown into liquid sample and then this membrane packed solid is taken out and desorbed with small amount of organic solvent. Nature of sorbent is critical in such extractions and choice is dictated by nature of target compounds. Main advantages are requirement of lesser amount of sorbents and organic solvents, higher enrichment factors and freedom in choice of sorbents (Sajid & Basheer 2016). μ-SPE was used for extraction of persistent organic pollutants from biological tissue samples (Basheer et al. 2008). As sorbent is effectively secured inside the porous membrane in μ-SPE, it does not allow extraneous matter or fats to be adsorbed over the sorbent. Advantages and disadvantages of commonly used extraction techniques for POPs and EDCs from environmental and biological samples have been described in Table 2.

Detection procedures: POPs and EDCs are very diverse classes of chemical compounds and hence large number of instrumental methods are employed to analyse these compounds. Gas chromatography and liquid chromatography with MS and tandem MS detectors are among few best instrumental techniques because of their inherent ability to analyse certain classes of analytes present within very com-

plex matrices, providing high sensitivity and selectivity (Helmlin et al. 1996). Main problem concerned with chromatographic methods is their limitation of separating single class of compounds in one run under specified set of operating conditions. Developing a method which would be capable of detecting multi classes of POPs will be a great achievement.

Because of their tendency to induce serious health problems in humans and wild life at very low concentration and inherently low concentrations in environmental and biological samples, researchers intend to improve sensitivity of analytical methods for detection of POPs that can go down to parts per billion (ppb) or even low.

Gas chromatography coupled with electron capture detector (GC-ECD) is most commonly employed when compounds of interest are halogenated such as OCPs and PCBs. This is so for least expensive instrumental approach for chlorinated organic pollutants. GC-MS has now arrived in variety of MS detectors which perform a specific job. Problem of co-elution of similar compounds which is experienced in GC-ECD is resolved by application of MS detectors. Low resolution MS is now a days most commonly employed detector with GC systems and it can go up to PG levels detection of PCBs and OCPs when operated under selective ion monitoring (SIM) mode under electron impact ionization. High resolution MS can give more specificity for closely related chlorinated compounds and it can go up to ultra-trace levels (Xu et al. 2013).

High pressure liquid chromatography (HPLC) is also another chromatographic method used for analysis of EDCs. Hyphenated liquid chromatography with mass spectrometry has resulted in increased sensitivity and has become a unique method for analysis of organic pollutants in environmental and biological samples. Analytical methods are judged based on their ability of performing high-throughput analysis and time required to complete the analysis. Three approaches have been adopted to achieve these goals in HPLC without affecting resolution and separation efficiency. These approaches involve use of monolith-sorbent based columns (Legido-Quigley et al. 2003), performing separation at higher temperatures (Heinisch & Rocca 2009) and use of ultra-high pressures (Carr et al. 2011). Mainly for bioanalysis, monolith columns are promising and they allow very high flow rates of solvent, nearly 10 mL/min, without producing significant back pressures and compromising characteristic performance of HPLC. High temperature liquid chromatography is the one where high temperatures are used to perform the separation process in normal length columns. High temperature reduces viscosity of mobile phase, which makes analysis faster and minimizes the total run time. But the main problem with high temperature liquid chromatography is availability of column materials which would be stable under high temperature conditions. Also, any thermally sensitive compounds may degrade at high temperatures. Ultra-high pressure liquid chromatography (UHPLC) is the one in which very short columns with narrow diameters are employed. This allows using very high pressures which results in very well peak separation and reduces analysis time significantly (Sosa-ferrera et al. 2013).

Recent advancements in chromatographic systems have led to highly efficient separations. This is mainly attributed to fast GC and LC methods by using narrow bored, short columns, high mobile phase flow rates with the help of ultra-high pressures. With these advanced systems, total run time for analysis has greatly reduced from tens of minutes to few minutes. Shortening of analysis time is very promising step towards high throughput analysis which is generally required by research and analysis laboratories (Petrovic et al. 2004). These highly efficient separation systems must be accompanied by suitable sample preparation and after all a good detection system for ultra-trace level analysis of EDCs.

Advancement in detection area is coupling of GC or LC with mass spectrometry (MS) detector. This detector can determine molecular structure with high sensitivity and selectivity. Compounds are separated by chromatographic methods and then identified qualitatively and quantitatively by the MS. Use of selective ionization mode, coupling of quadruple with time of flight (TOF) enables the method to analyse and quantify target compounds from very complex matrices (Frank 2000).

Whole organism assay is also used for monitoring of EDC in some aquatic organisms and it gives an indication of total estrogenic activity. But these assays suffer from lack of specificity of organism response towards certain classes of EDCs. Cellular based assays are another attractive alternative to mass based analytical techniques, but the results are not consistent and repeatable. Non-cellular assays, which don't require whole cells, make the analysis simple and more quantitative with much better detection limits (Campbell et al. 2006).

Among non-cellular assays, enzyme linked imuunosorbent assay (ELISA) is most famous method, but it has several disadvantages including longer analysis time, washing and addition of reagents, multi-step procedures, and requirement for experienced personnel. Another Immuno-chromatographic assay combines unique detection capabilities of molecular recognition probes like antibodies, aptamers with separation advantages chromatography. Strips based on Immuno-chromatographic principle are known as lateral flow

Table 1: List of some common organic compounds which show endocrine disrupting properties

Class of compounds	Chemicals	Uses	Problems induced in human	Activity	Ref.
Pesticides	DDT, Chlordane, Methoxychlor	Insecticides, fungicides etc.	Neurotoxicity, Disorders in reproductive systems, cancers including breast cancer	Estrogenic, anti-estrogenic, anti-androgenic	(Brody et al. 2007, Cohn et al. 2007) (Rudel & Perovich 2009)
Polybrominated compounds	Polybrominated diphenyl ethers (PBDEs)	Flame Retardants	Harm to developing brain and reproductive organs	Effect on thyroid hormones	(McDonald 2005)
Plolychlorinated compounds	Polychlorinated biphenyls (PCBs), Dioxins	Electrical equipment, by product of incineration	Endometriosis	Anti-estrogenic	(Schug et al. 2011)
Phthalates	Dibutyl phthalate, Benzylbutyl phthalate	Plasticizers, detergents, resins	Infertility, effects on developing male reproductive tract	Estrogenic	(Henley & Korach 2006)
Alkylphenols	Octylphenol, nonylphenol	surfactants	Reproductive disorders in human, carcinogenic	Estrogenic	(Villar-Navarro et al. 2013)
Parabens	Para-substituted hydroxybenzoate	Preservatives and anti-microbial agents	Decreased sperm number and motile activity	Estrogenic	(Kang et al. 2002)
Natural and synthetic hormones	Estradiol, 17β-Estradiol, Estrone, EstriolEthinylestradiol		Reproductive disorders	Estrogenic	(Campbell et al. 2006)

test strips (LFTS). These strips are being used in detection of EDCs such as pesticides (Sajid et al. 2015b). But the major problem related with these strips is their selectivity for only one or two compounds at a time and cannot detect number of EDCs at a time. Also they suffer from the problems in the selection of molecular recognition probes, conjugation with label molecules and less sensitivity. From this detail, it can be concluded that gas and liquid chromatography coupled with mass spectrometric detector are best techniques to analyse the number of EDCs in single sample in a single run.

CHALLENGES IN ANALYSIS OF POPS AND EDCS IN BIOLOGICAL SAMPLES

Despite of all advancements in analytical instrumentation, pretreatment of sample to remove complex matrix effects is considered as the bottleneck in all analytical methods. In this way, sample pretreatment plays a vital role in enhancing the sample amount and removing interferences due to matrix.

Biological samples present various challenges prior to their analysis by using chromatographic methods. These challenges arise because of the complex nature of biological samples which sometimes contain huge protein content. This protein content can adsorb on the column in an irreversible way and thus reduce column efficiency and produce significant backpressure. This complex biological matrix when combined with trace level concentrations of EDCs emphasis to treat the sample before analysis by any instrumental technique. This pretreatment helps in enriching the analyte and removing interferences from biological matrices. But these sample pretreatment methods are most time consuming parts of analytical process. In multiresidue analysis, the biggest challenge is optimization of experimental conditions. Some compromises are to be done in this step.

Biological samples include urine, tissues, plasma or serum, breast milk, faeces, and semen. One recent study reveals that organic UV filters which are used in cosmetics (which are potential EDCs) have been mostly studied in urine (57%) and then plasma or serum (23%) and rest of matrices have not been focused much (Chisvert et al. 2012).

For the extraction of organic pollutants from urine samples, different extraction methodologies are applied such as solid phase extraction (SPE), liquid-liquid extraction (LLE), membrane assisted liquid-liquid extraction (MALLE). But the big disadvantage with these extraction methods is that they consume too much organic solvents. These organic solvents are not only toxic and expensive, but they are also con-

Table 2. Advantages and pitfalls of extraction techniques

Extraction	Advantages	Pitfalls
Solid phase extraction	• Selective sorbents have high affinity towards target POPs • Molecularly imprinted polymers are sorbents which can be designed according to the structure of target analytes. • Method is suitable for large volume of samples.	• Consumption of large volumes of organic solvents. • Huge time consumption • Multi clean-up procedures
Liquid-liquid extraction	• Simplicity of operation • Rapid analysis • Reasonable selectivity	• Large volumes of organic solvents are needed. • Environment unfriendly • Formation of emulsions which prevents proper separation of two phases. • Co-extraction of some interferences
Solid phase microextraction	• Solvent less technique • Works on adsorption equilibrium between SPME fiber and sample containing analytes. • It can be easily automated with GC and HPLC systems. • SPME can be performed in normal as well as headspace mode for more complex samples. • Due to small size of device, equally suitable for in field applications. • Rapid, simple and sensitive method for variety of target compounds. •	• Fibers are highly expensive and fragile. • Carry over effects even after longer period of thermal desorption. • Low recommended operating temperature ranges. • Fibers are highly sensitive to organic solvents and easily deshaped on contact.
Micro solid phase extraction	• Sorbent required is in range of milligrams • Small volume of organic solvents are needed for desorption. • It has variety of formats like immersing a solid directly into sample solution or packing the solid in a membrane. • Same sorbent can be used for various cycles of extraction. • Extraction can be assisted by high temperature or microwave radiation.	• In case of porous membranes packed μ-SPE, pores may get blocked by the contents of real samples. • Selection of sorbent is critical in order to increase the performance of extraction process.
Liquid phase microextraction	• Few μL of organic solvents are needed. • Huge reduction in acceptor to donor phase ratios. • It has lot of operational flexibility. • Number of well-known LPME techniques has been developed by variation in extraction procedure. • Some examples are single drop liquid micro extraction, hollow fiber liquid microextraction, dispersive liquid-liquid microextraction and many more.	• Like SPME, no preconditioning is needed. • In case of single drop LPME, solvent with low vapor pressure are employed. • Difficult to maintain a single drop. • SD LPME, not suitable for complex samples. • In case of hollow fiber membranes, extraction time is enhanced because membrane acts as a barrier between donor and acceptor phase. • Fiber pores can be blocked in case of fatty or dirty samples.

sidered as environmental pollutants. In order to overcome this problem some other extraction techniques such as solid phase micro extraction (SPME) (Felix et al. 1998), liquid phase micro extraction (LPME), hollow fibre liquid phase micro extraction (HF-LPME) (Kawaguchi et al. 2009), stir bar sportive extraction (SBSE) (Kawaguchi et al. 2008) were employed and these methods consume organic solvents in microlitres.

In case of blood, it is treated to get plasma or serum. Plasma is obtained by removing cellular components of the blood and retaining proteins that are responsible for the coagulation by centrifuging the blood sample in heparinized tubes, while serum is obtained by removing both cellular components and proteins and this is attained by doing centrifugation in non-heparinized tubes (Okereke et al. 1994). Once the serum or plasma sample is obtained, hydrolysis is carried out to determine the bonded or conjugated target analyte. When anlayte is attached to proteins, acidic hydrolysis helps to determine it. On the other hand, proteins are the materials which are thought to be the biggest hurdle in analytical detection of organic compounds as they represent a very complex class of matrix. These proteins are precipitated and then removed from the samples by the use of organic solvents to avoid interferences. Further purification could be achieved by LLE or SPE.

In case of semen, breast milk and tissues hydrolysis as well, removal of proteins is a mandatory step before detection of target analyte in the sample through any instrumental technique. Selection of suitable solvent is crucial. Tissues are normally homogenized with water or acetonitrile and LLE is done to remove any protein content. Soxhlet extraction or LLE is used for pretreatment of faeces (Abdel-Nabi et al. 1992, Okereke et al. 1994).

HEALTH EFFECTS OF EDCS AND POPS

EDCs are associated with changed endocrine functions both in animals and humans. They create long lasting adverse effects on the metabolism, neurological function, reproduction, and other physiologically important processes. Health concerns are being raised with every passing day and more and more abnormalities are being associated with the use of these toxic compounds. Some recent reports show that EDCs have resulted in high incidences of thyroid and testicular cancers in males (Adami et al. 1994, Davies & Welch 2006). EDCs disturb hormonal signalling. Those which block or interrupt the function of sex hormones have been paid much attention but it is a recognized fact that EDCs have very high impact on insulin signalling, functioning of thyroid and bone growth. Some PCBs and pesticide atrazine have been reported to have effect over proteomes of the breast cancer cells. Literature reveals that there is a great debate on the classification and effects of EDCs on humans. EDCs are present in environment as mixture and they can have synergistic or additive effects. So determining the effects from individual EDCs will be really difficult without considering all other affecting parameters (Cottingham 2009).

Human exposure to EDCs have gained too plentiful consideration in recent years due to experimental results indicating endocrine-related effects on reproduction, development, cancer, and metabolism, and observations for increasing tendencies (as well as geographic trends) in endocrine-related ailments among populations (Soto & Sonnenschein 2010). There are thousands of persistent organic pollutants for which toxicity information either does not exist or insufficient (Judson et al. 2009). The main targets of EDC effects are homeostasis of sex steroids and the thyroid; hence, reproductive health, is also concerned with endocrine disruption (Mantovani 2002). However, studies carried out to find the impact of environmental EDCs in relation to endocrine associated cancers and outcomes in human are limited in number.

A significant increase in breast cancers in last 50 years has developed a perception that hormonally active industrial chemicals which are result of industrial revolution could be possible reason for these cancers. Same half century witnessed an increased incidence of testicular cancers and disorders in quantity and quality of human sperm (Davis et al. 1993, Sharpe & Skakkebaek 1993). EDCs are involved in disruption of reproductive system in human and other animals. They are thought to be responsible for incidences of cancers and transgenerational effects (Brouwers et al. 2006, Yi et al. 2010). Glyphosate, which is used in pesticides was thought to be safe but a new study states that it has endocrine disrupting properties and promotes breast cancer on human cells through estrogen receptors at very low concentrations of 10^{-12} to 10^{-16} M (Thongprakaisang et al. 2013). When human peripheral blood cells were exposed to micro molar concentrations of EDCs, it resulted in change of their gene expressions. This change in gene expression was independent of gender (Wens et al. 2013). Luteinizing hormone receptor (Lhcgr) of zebrafish follicle cells showed very high response to estrogenic EDCs (Liu et al. 2013). Human MCF-7 cells were exposed to PCB-153 and atrazine concentrations for a period of 36 hours and proteins isolated from cytosol and membrane were tested for presence of biomarker as a result of this exposure. It resulted in an altered protein expression which is an indication that these compounds can have potential impacts on human health (Lasserre et al. 2012). A study was carried out to see the effects of mixture of five pesticides when exposed to rats, developmental changes in

gestation length and increased number of nipples and genital disfigurements were observed in male offsprings (Hass et al. 2012). It is an indication that how these compounds can effect on sexual developments in human and other animals. A comprehensive review was done which included 91 studies dealing with BPA and human health and it showed that BPA exposure has adverse effects on human of all age groups and also early exposure in children leads to asthma and changed behaviour (Rochester 2013). A study which was designed to determine endocrine disrupting activity of some compounds in bottled flavoured and mineral drinking water showed hormonal activity in 78 % of samples (Plotan et al. 2013). EDCs have great impact on reproductive system particularly disorders in female fertility (Petro et al. 2012). Estrogenic activity of fruits and vegetables was determined and it showed positive results. This activity was attributed to the presence of pesticide residues in fruits and vegetables (Schilirò et al. 2013).

Studies suggest a link between exposure to low doses of POPs and incidence of type 2 diabetes. This risk is boosted when exposed to POP mixtures instead of few individual POPs. But results are somehow inconsistent in this regard because of unequal distribution of POPs in environment (Lee et al. 2014). High levels of POPs concentrations were found in biological and environmental matrices around the globe. A recent report indicates frequent presence of POPs in the serum of most of Tunisian women (Artacho-Cordón et al. 2015). POPs and EDCs are linked with malfunctions in ovarian function and the women exposed to such compounds are more likely to get menopausal at earlier ages than non- exposed women (Grindler et al. 2015). Similarly, an association between concentrations of POPs in breast milk of mothers and faeces of infants was found (Chen et al. 2014). Subject of health effects of POPs and EDCs is highly diversified and it covers wide range of ailments in human and wildlife and reports covering this aspect are increasing with every coming day. Covering every single report which describes the negative impact of POPs is beyond the scope of this review. Some review articles covering health effects of POPs and EDCs can be studied to get more comprehensive overview of the subject (Corsolini et al. 2005, Gascon et al. 2013, Haffner & Schecter 2014, Li et al. 2006, Pedersen et al. 2015).

CONCLUSIVE REMARKS

It needs to develop systematic criteria in order to assess toxicity and risk assessment related to new organic pollutants being added into environment. Most scarce aspects of risk assessment studies is that researchers focus on particular aspect of their interest. Compiling such reports is very difficult and it cannot lead to straight forward conclusions about a compound or group of compounds.

Consumable foods in different localities need to be explored for presence of POPs and EDCs levels. Studies in this regard are highly scattered. For example, a review article was conducted to compile the studies carried out in Spain to find the relationship between human diet and its effect on POP concentrations in humans. Results reveal that only few compounds were consistently studied in fish and human serum but most of the foods such as cereals, vegetables and fruits have not been evaluated for POP concentrations (Gasull et al. 2011). All food stuff has to be studied in systematic pattern at all geographical locations in order to reach some conclusive outcomes.

There exists sufficient evidence that POPs have considerable concentrations in fats and other body compartments of the people in all around the globe which have been linked with serious health implications (Arrebola et al. 2012, Croes et al. 2012, Pauwels et al. 2000, Pestana et al. 2014). These POPs come from environment and contaminated food and thus accumulate in fatty tissues. There should be some minimum allowable limits of POPs in serum, adipose tissues and other body compartments, so that above levels can be treated as a health risk. POPs concentrations in body fluids can be used as a biomarkers for near or far future health implications. Although hundreds of reports have been published describing toxicity and health effects of POPs, but standard procedures and limits to be regularized in clinical applications to determine and establish a relationship between POP levels and disease stages.

CONCLUSION

In this review article, we have comprehensively looked into the diverse types of POPs and EDCs prevalent in human environment. We have also described the various methods that could be used to enrich these compounds from tissues, body fluids and environmental samples, which could enable their detections using advanced mass spectrometry based chromatographic techniques. We have very clearly highlighted the advantages and disadvantages of more commonly used extraction methods. Advancements in analytical instrumentation have made the analysis of low levels of organic pollutants a doable job within a time span of few minutes, but still there exists some challenges while dealing with complex biological samples. Accomplishments, weaknesses and future challenges in the area of analytical chromatography are critically discussed. Gene-protein-metabolite relationships play a key role in the incidence of diseases associated with exposure to toxic chemicals in the environment. We have summarized these reports associated with role of POPs and EDCs in triggering health related problems. Studies in

this regard could be extended to the development of novel sensors and biosensors that could be used to monitor the range of toxicity associated with newly identified toxic chemicals.

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