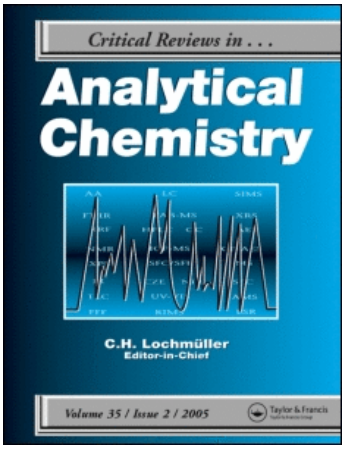

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Environmental Fate of Endocrine Disrupting Compounds—Analytical Problems and Challenges

Błażej Kudłak[a]; Jacek Namieśnik[a]
[a] Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, Gdańsk, Poland

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Environmental Fate of Endocrine Disrupting Compounds—Analytical Problems and Challenges

Błażej Kudłak and Jacek Namieśnik
Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, Gdańsk, Poland

Exposure to substances possessing sex steroid activities can adversely effect endocrine and reproductive systems in humans and wildlife. Studies have found significant increases in the incidence of breast, prostate and testicular cancer. The others have reported decreasing sperm counts and semen volume and longer times to conception. These findings are complemented by field study data indicating that wild and domesticated organisms are also experiencing compromised reproductive and developmental abruptions.

The paper presents the review of recent works conducted in the field of specific environmental pollution posed by endocrine disrupting compounds (EDCs). The modes of toxic action, division of chemicals belonging to EDCs, *in vivo* and *in vitro* assays serving endocrine potency determination, as well as results of concentration levels determinations in air, water and solid samples are given in a comprehensive way.

Keywords Endocrine disrupting compounds (EDCs), environmental pollution, hormones, xenobiotics

INTRODUCTION

Development of new technologies, progressive urbanization, increasing consumerism and industrial boom in developing countries has lead to elevated pollution of the environment. The spectrum of pollutants produced and released to the environment has increased in the last few decades including the agricultural, industrial, pharmaceutical and plastic industries.

The occurrence of some specific micro-pollutants has become more and more concerning in the last decade. These pollutants are endocrine disrupting compounds (EDCs)—chemicals mimicking the action of desired hormones or acting on the proper endocrine systems. According to U.S. Environmental Protection Agency (EPA) the EDCs are exogenous agents that interface with synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction or behavior (1). Since the early 1990s some compounds released to the environment began to be recognized as pollutants of a new kind of mode of action. The first evidences of endocrine disruption in nature were observed in fishes and amphibians exposed to paper mills' sewages (2). All xenobiotics present in the environment can be divided into two groups:

- regulated pollutants
- non-regulated pollutants.

Among these non-Regulated xenobiotics, one can distinguish:

- non-identified pollutants
- new-emerging pollutants, as can be seen in Fig. 1.

The negative symptoms observed firstly in fishes have made authorities issue guidelines and start projects aiming at detection and determination of EDCs (1, 2).

There are three main target systems in living organisms that should be selected for tests: estrogen, androgen and thyroid hormones. Affecting the receptor is a key mechanism by which xenobiotics disrupt the estrogen- and androgen-hormone systems; however, that is not the case for the thyroid system.

Presence of such substances as EDCs in particular environmental compartments can have such detrimental effects as:

- partial or total mimicking of steroidal hormones by interacting with hormonal receptors or influencing intercellular signalling,
- blocking or preventing binding between desired signalling compounds and their receptors as well as

Address correspondence to Błazej Kudłak, Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, 11/12 Naturowicza, Gdansk 80–952, Poland. E-mail: blaizek@gmail.com

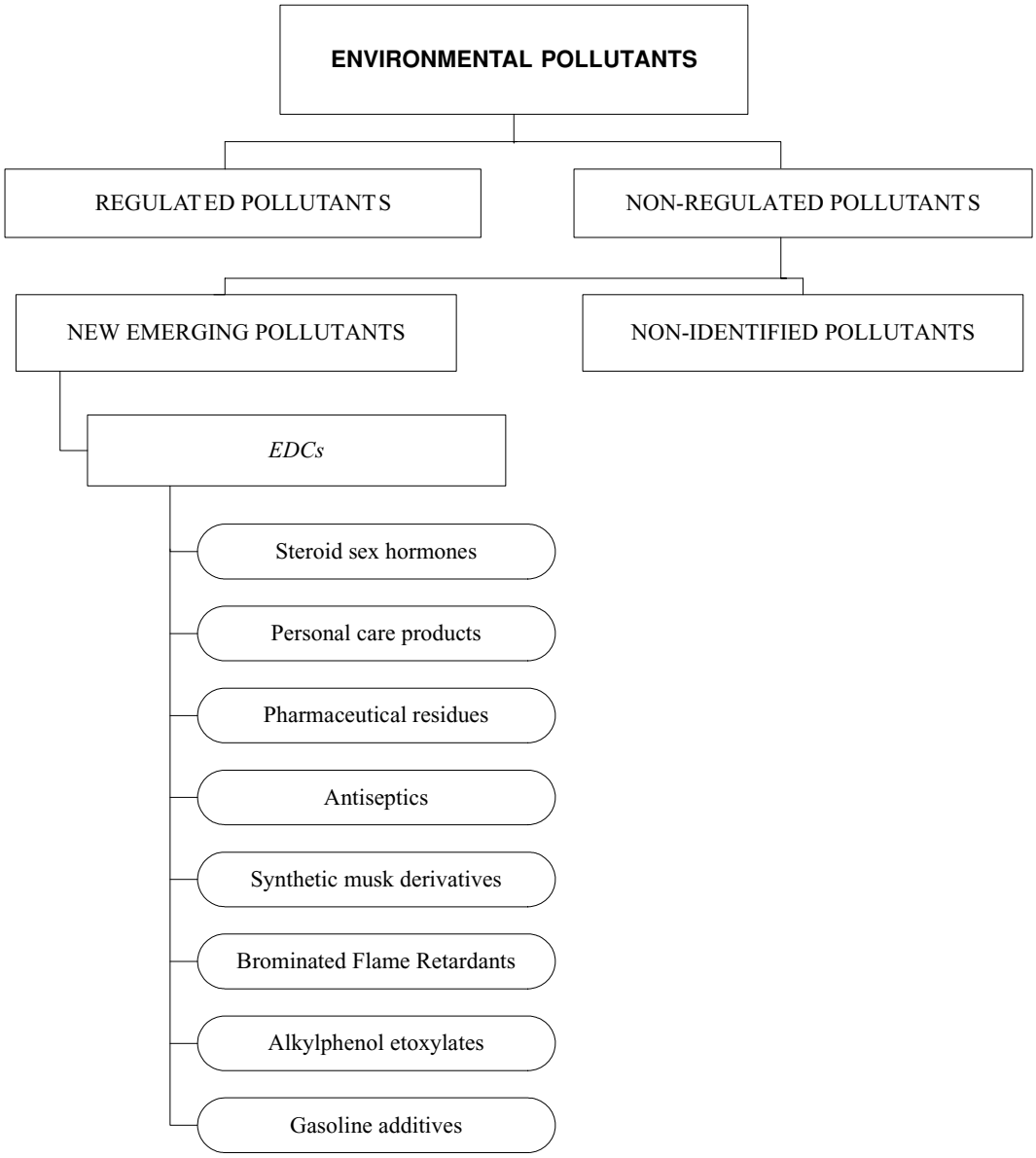

FIG. 1. Classification of environmental pollutants due to their legal regulations.

affecting these processes (anti-endrogens and anti-estrogenes),
- production and decomposition of hormones present in organisms,
- affecting creation and functioning of hormonal receptors.

Estrogen receptors (ER) belong to a superfamily of receptor proteins together with glucocorticoid, mineralocorticoid, androgen, progesterone, retinoic acid, vitamin D and thyroid receptor. Binding with ligand (antagonist or agonist) *in vivo* causes conformational changes, dimerisation and binding to a specific DNA sequence responding to characteristic receptor. There exist two subtypes of ER—α and β; the second one is found in human testis, ovary, thymus and rat prostate and ovary (3).

Paracrine, autocrine and synaptic are three types of local hormone signalling (Table 1). In paracrine signalling, hormones are released into the fluid between cells (the interstitial fluid) and diffuse to nearby target cells. Hormones that influence secretions or other processes on the same cells that released them are said to be autocrine signallers. The more specialized synaptic signalling occurs between neurons (the nerve cells that make up the nervous system) and between neurons and muscle cells, allowing nerve cells to talk to each other and to muscles (8, 34). In Fig. 2, the mode of hormonal action is schematically presented as well as possible signalling modes.

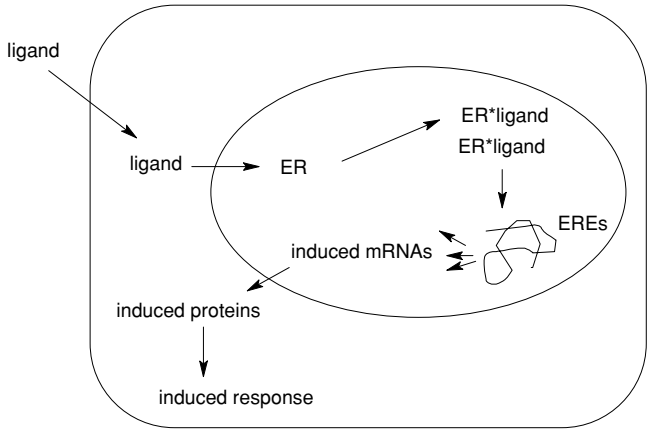

FIG. 2. Mode of hormonal action in target receptor.

The problem of endocrine disruption concerns mostly vertebrate organisms; however, over 95% of global taxonomy consists of invertebrates and commercial application of organisms like shrimps, crabs, oysters, molluscs, etc., consists which of an important part of the world economy. For this reason scientific considerations and tests on endocrine potency should not be disrespected. Numerous insecticides deliberately act on an endocrine level (13), just to mention a few :

- precocene—anti-juvenile hormone analogue,
- methoprene—mimics juvenile hormone,
- diflubenzuron—chitin synthesis inhibitor,
- tebufeno-zide—analogue of ecdysone,
- fenoxycarb—molting disruptant.

TABLE 1
Division of hormones due to structural properties

Type	Charateristics
Steroid hormones	They have lipophilic character, contain in their structure fragments similar to cholesterol, belong here mostly sex hormones like: estrogens, androgens and progesterone. Both males and females produce all these hormones however in different quantities
Aminoacids' derivatives	They are of hydrophilic character, stored in endocryne cells until the moment of need for release, they connect with specific surface receptors and activate secondary signalling factors, epinephrine is an example of such hormone
Polypeptides	Contain aminoacids varying from few to over 200 residues, these are water-soluble hormones like insulin, growth hormone, prolactine, they are stored in endocrine cells till they are needed, e.g., during metabolic regulations, lactation, growth, breeding

ENVIRONMENTAL PROBLEM

Environmental pollutants suspected to pose endocrine threat are classified most often to one of three classes (Table 2). The chemical structures of selected xenobiotics belonging to EDCs are presented in Fig. 3.

There is still not enough knowledge on endocrine effects to invertebrates; however, these organisms seem to be good intermediates in modelling hormonal potential toward higher organisms. Ease of handling, short life cycles, low cost and labor consumption are pros that promise evaluation of hormonal adverse effects both to vertebrates and invertebrates. Still, some investigations

TABLE 2
Basic classification of pollutants belonging to endocrine disrupting group

Class	Description
Industrial chemicals	• Plasticizers (e.g., phthalates applied in polymers industry, e.g., PVC) • Alkylphenols (and their derivatives functioning as detergents) • Bisphenol A (lacquers and coatings ingredients) • Polychlorinated dibenzophenols and dibenzofuranes (PCBs) • Dioxins • Brominated flame retardants (textiles and plastics) • Pharmaceuticals • Parabens (cosmetics) • Butylated hydroxyanisoles (BHAs) (food antioxidants) • Surfactants and detergents • Natural and synthetic musk
Natural hormones	• Phytoestrogens present at high concentration levels in soya • Female hormones • Mycotoxines (e.g. α- Zearalenol)
Pesticides	• e.g., DDT, lindane, vinchlozolin, carbendazim, benomyl, procymidon, chlorpyrifos, deltamethrin, dimethoate, carbofuran, amitraz, trichlorfon, atrazine, linuron

are required in this field to receive reliable and reproducible results in translating toxicological data from lower organisms to higher taxa (4–6).

Certainly hormones are present also in plants, not only in animals, just to mention cytokinines, auxines, giberelines, jasmonides or structurally simple ethylene.

DETERMINATION OF ENDOCRINE POTENCY

Several criteria were given when considering the exposure problem to these pollutants (7):

- health risk,
- possible high concentration in given portion of the environment,
- risk perception of the consumers,
- annual production volume,
- environmental, occupational, and exposure data,
- speciation form (e.g. tissue analysis),
- fate and transport models,
- persistence.

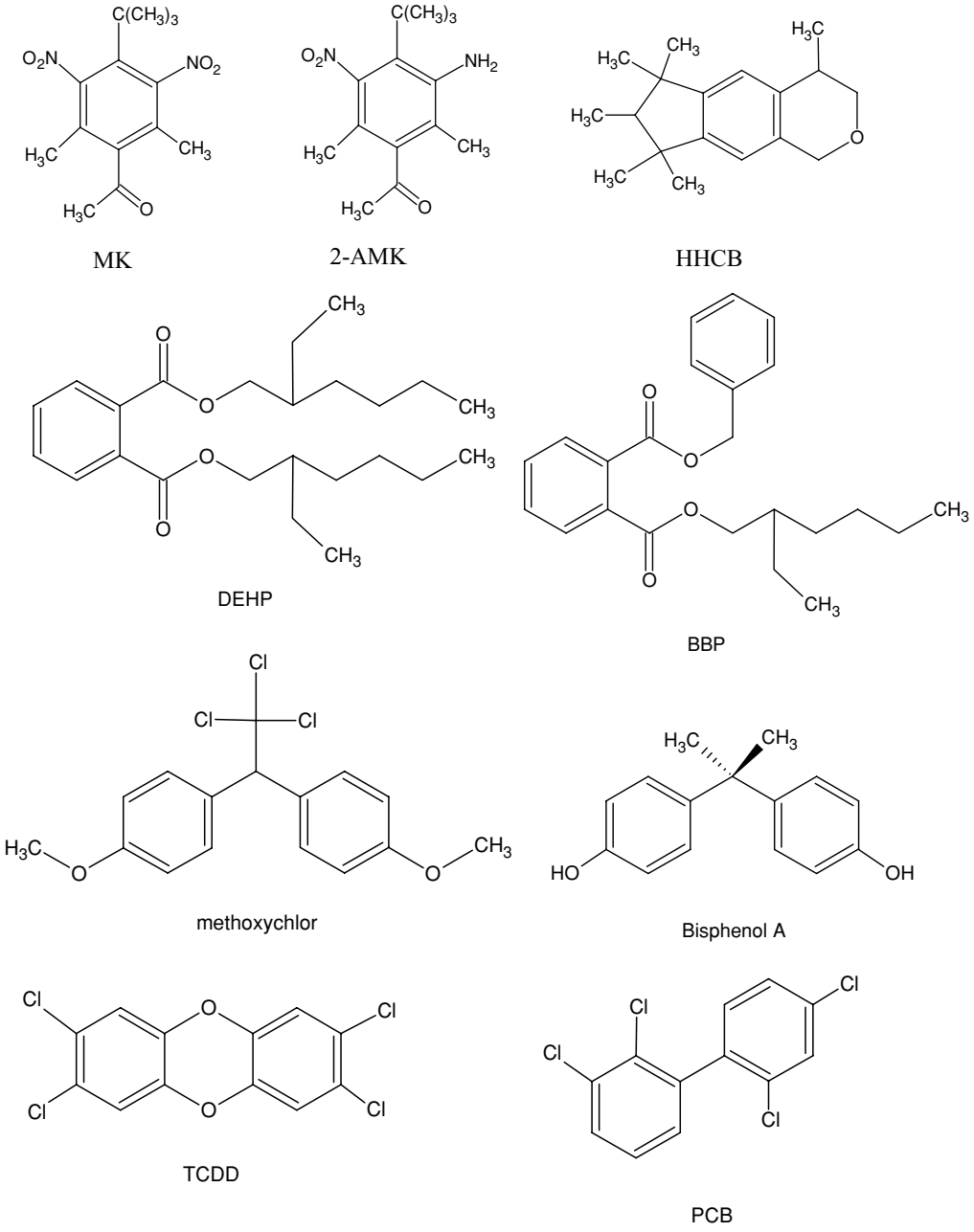

FIG. 3. Chemical structures of selected xenobiotics belonging to EDCs.

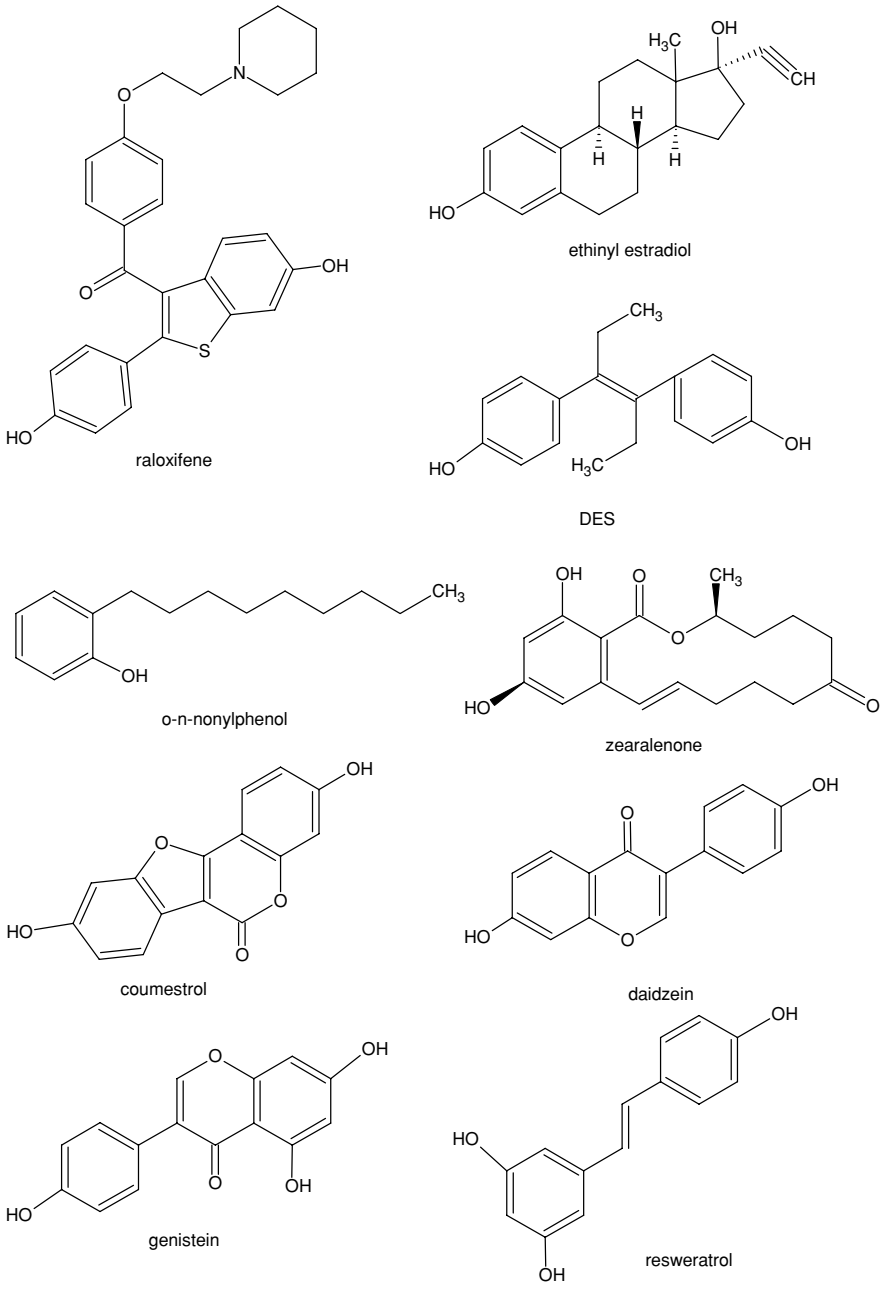

FIG. 3.

Based on previously mentioned criteria, the list of priority compounds is prepared, which should next undergo further investigations for possible endocrine properties. Approximately 90,000 chemicals are suspected to possess endocrine activity; sorting and prioritization of the list of these chemicals is indispensable. Some chemicals directly can be sorted as unlikely to belong to EDCs. Large molecules (with molecular weight greater than 1000 Da, e.g., polymers) cannot cross the membranes or be transported and as such do not pose hormonal risk. Due to degradation processes, however, the decay products of these xenobiotics may become hormonally active and further experiments on such products are required as well as a determination of the environmental fate of the chemicals .

The initial screening aims at detecting whether or not a specific chemical or a mixture of chemicals can disrupt the hormonal system. If such action is not detected, the chemical is indicated as not requiring further investigations; otherwise, it is subjected to the next level of tests. Their aim is to determine endocrine properties in quantitative way, to characterize the mode of action and to assess dose-response relationships.

If the endocrine adverse effects are not proven, the xenobiotic or their mixture is designated as not possessing a negative impact on hormonal system. However, if new improved tests or suspicious action is detected, the investigations can be restarted. Confirmed negative effects toward the endocrine system result in implementing the data in a risk assessment process and elaborating analytical methodologies to connect the biological data with environmental analysis and screening.

While preparing the prioritization list one is obliged to search through (42):

- ecotoxicological databases,
- epidemiological studies and databases,
- predictive activity or effect models (like Quantitative structure-activity relationships [QSAR]),
- high throughput screening (HTPS) assays results.

Estrogenic activity can be determined by means of numerous methods:

- enzymatic reactions,
- cell lines,
- tissue cultures,
- organs,
- whole organism.

More detailed information on possible biotests that can be applied for hormonal alteration symptoms are presented in Tables 3 and 4.

As far as the application of databases is common and widely used by scientists, the HTPS tests are recommended for chemicals with incomplete or insufficient knowledge on effects and modes of action. Such preliminary tests answer the problem of screening large numbers and volumes of chemicals for their endocrine-oriented properties. The HTPSs must be applied toward: chemicals with an annual production volume greater than 5500 kg, active ingredients of pesticides and all chemicals that were previously omitted from screening assays for any reason. The answers obtained *via* HTPS should at first increase knowledge on androgen, estrogen or thyroid-like activity of the xenobiotic, answer exposure- and dose-effect relationships to enable prioritization of substances tested and increase reliability of QSAR models. It certainly requires proper standardization and validation of assays conducted in HTPS combines.

Next to chemicals, their mixtures, mostly understood as liquids, require special attention. One should bear in mind the following:

- human breast milk,
- phytoestrogens in infant formula based on soya, naturally occurring non-steroidal estrogens
- mycotoxins,
- mixtures of chemicals deposited at hazardous waste dumping sites,
- mixtures of pesticides and fertilizers,
- byproducts of disinfectants,
- fuel and its additives.

TABLE 3
Possible bioassays for endocrine potency determination

In vitro	*In vivo*
Estrogen receptor binding/reporter gene assay	*Rodent 3-day uterotrophic assay*
Androgen receptor binding/reporter gene assay	*Rodent 20-day pubertal female with thyroid*
Steroidogenesis assay with minced testis	*Rodent 5–7 day Hershberger assay*
	Frog metamorphosis assay
	Fish gonadal recrudescence assay
Placental aromatase assay	Modified rodent 3-day uterotrophic assay (intraperitoneal dosing)
	Rodent 14-day intact adult male assay with thyroid
	Rodent 20-day thyroid/pubertal male assay

⇓

Evaluating the assays' results:
- Weight-of-evidence approach
- Balance of positive and negative test results
- Nature and range of adverse effects observed
- Dose-response relationships
- Strength of effect induced by xenobiotic
- Presence or absence of answer in different taxonomic tests

TABLE 4
In vivo assays and their description for EDCs determination

Assay	Effect	Administration	Organisms	Time	Xenobiotic detected	Notes	Reference
Two-generation mammalian reproductive toxicity study	Gonadal function, estrous cycle, mating behavior, fertilization, implantation, pregnancy, parturition, lactation, weaning, offspring reaching adult age, neonatal survival, growth and development	Oral (feed, water, gavage), inhalation	At least 20 males rat and females to produce 20 pregnant females/dose	10 weeks	Phytoestrogens, coumesterol, cyproterone acetate	Hormonally-induced effects such as abortion, resorption, or premature delivery as well as abnormalities and anomalies such as masculinization of the female offspring or feminization of male offspring can be detected, subchronic	38, 45
Avian reproduction test Japanese quail	Sex ratio at hatching, size of cloacal protuberance, brain and body weight, wing and bone length, thyroid weight, skeletal x-ray, nest attentiveness, cold test stress	Feed, spraying	30 eggs/ generation/ immersed in specific dose	~ 20days	Pesticides, fertilizers, oils, air pollutants	Widely available, excellent background information on the endocrinology of Japanese quail	17
Fish life cycle test fathead minnow (*Pimephales promelas*), sheepshead minnow (*Cyprinodon variegates*)	Growth, maturation, reproduction, egg fertility, survival of embryos, time required to hatch, hatching success, survival, body color, pads' presence,	Feed, water	10 females, 5 males	Embryo 4–5 days, larval juvenile 4–8 weeks adult juvenile 32–40 weeks	Numerous toxicants mostly water soluble	Natural spawning is possible, solution water should be sterilized with ultra-violet irradiation and tested for pesticides, heavy metals, and other possible contaminants, possible false (+) or (−)	14
Developmental uterotrophic assay	Exposure of uteri to EDC, number of uterine glands, height of luminal and glandular epithelia	Treatment on postnatal days (PND)	Rats, mice	10–22 days	Phytoestrogens, coumestrol, toremifene, numerous xenobiotics	Large weight—agonistic action, low weight—partial agonistic/antagonistic effect	15
Uterine weight bioassay in juvenile or adult ovariectomized female rats	Exposure of uteri to xenobiotics	Oral, injection	Rat females	1–3 days juvenile, 1–4 weeks adults	Numerous toxicants	Possible metabolic products, "gold" test	36
Vaginal smears (mucification and cornification)	Microscopic examination of vaginal lavages, lordosis behavior, necropsy	Oral, injection	Rats	1–40 weeks	Pesticides, PCBs, Numerous toxicants	possible false (+) and (−)	37

Puberty, age at vaginal opening	First estrus, onset of cyclicity	Oral, injection	Rats, mice	2–21 days	TCDD, metoxychlor, octylphenol, nonylphenol, pesticides, numerous xenobiotics	Estrogens or antiestrogens discrimination, possible false (+) or (−)	
Induction of female sex behavior	Perceptive and receptive behavior, lordosis	Oral, injection	Non-human mammalians	3 days	metoxychlor, octylphenol, nonylphenol, bisphenol A, o,p'-DDT,	Quantitative screen for estrogenicity, very sensitive	
Estrous cyclicity	Alterations of vaginal smears	Oral, injection	Rats, mice	> 10 days (even 9 months)	Numerous chemicals tested	Difficult data analysis, possible false (+) or (−)	
Super apical developmental toxicity test	Exposure of pregnant/lactating females and examining hormones levels	Oral, feed	Non-human mammalians	2–3 months	AhR agonists, phthalates, antithyroidal toxins, e.g., PCBs and PTU, antiandrogens	Standardization required, no false (−) detected	40
Feeding behavior	Food consumption and growth rate	Oral	Male rats	-	Metoxychlor, octylphenol, nonylphenol, bisphenol A	Very non-specific, possible mis-interpretation of data	
AR equilibrium Binding assay	Toxicants' affinity for rAR	Solutions	Rats	24 h	Numerous xenoantiadrogens	Sensitive, no metabolic transformations, few possible false (+) or (−), good for screening, requires radiolabeling	
Temperature-dependant sex determination assay	Sex determination in reptiles in function of temperature	Immersing eggs	Reptiles - turtles	4 months	Xenobiotics, e.g., dioxins and temperature	Antagonistic or agonistic androgen/estrogen action	19
Endocrine challenge test	Repeated determination of testosterone, LH and other hormones	Oral, feed, injection	Non-human mammalians	1–10 weeks	Numerous xenobiotics	Stress reduces serum T, increases prolactin and corticosterone, excellent method, requires specialized personnel and equipment	39
Hershberger Assay	Antiandrogenic/ androgenic effects in eripubertal/adult males	Oral, injection	Rats	1 week	Antiadrogenic pesticides	Validated, one of the best tests, possible false (+) or (−)	41

TABLE 5
In vitro assays and their characterisation for EDCs determination

Assay	Method	Form of EDC chemicals	Assay time	Reporting form/endpoint	Notes	Reference
Whole cell ER binding assay with MCF-7 (ESCREEN)	Competition of analyte with estradiol to bind to ER	Bioavailable, metabolically activated	2–5 days	Radio-labeling fluorescence	No characterization on agonistic or antagonistic character	20
Non-human or avian ER binding assays	In vitro affinity of xenobiotics for recombinant ER	Soluble toxicants,	18 h	Radio-labeling	Both agonists and antagonists can be detected, degradation products can be problematic, false (+) and (−)	
Transiently transfected mammalian cell with hER	Estrogen-regulated transcription	organochlorines, PCBs, PAHs, phytoestrogens, alkylphenols, phthalate esters, environmental matrices, urban air PM	3 days	Luciferase CAT	Distinguishing agonist-antagonist with high sensitivity	43
AR whole cell binding assay, monkey kidney COS cells	Whole-cell binding to AR of cultured COS cells	Proantiandrogenic fungicides	4 days	Radio-labeled ligands – scintillation counts	Relative affinity of xenobiotics to compete with endogenus ligands, cells transfections, tissue cultures are expensive, possible false (+) or (−)	
MCF-7 proliferation assay	Endocrine-induced cell proliferation	Numerous chemicals and mixtures	6 days	Cell proliferation	Indirect answer, detects mostly estrogen antagonists, sensitive, reproducible, possible false positive (cell mitogens) and false negative (cytotoxicants, not specific growth inhibitors) hits	
Yeast estrogen receptor assay (YES)	Mammalian steroid (estrogen) receptors cloned into *Saccharomyces cerevisae* strain	Dioxins, PCBs, alkyl phenols, bisphenol A	4 h–6 days	Most often J-galactosidase reporter (lacZ) gene	Easy to run, lack of standardization, large differences in cell wall and membrane transport compared to mammals, numerous false negatives of many xenobiotics to ER	21

MVLN Assay (stably transfected reporter gene assay in mammalian cells)	Mammalian MCF-7 cell line transfected with ER specific reporter gene (Vit-Luc)	Numerous toxicants, limited metabolic changes	2–3 days	Vit-Luc reporter	No false (+) or (-) determined, stable lines, high specificity, can be automated, easy to run	22
Yeast-based androgen receptor assay	Human or other AR cloned into *Saccharomyces cerevisae* strain	Dioxins, PCBs, alkyl phenols, bisphenol A, hydroxyflutamide	4 h–6 days	Most often J-galactosidase reporter (lacZ) gene	Agonistic and antagonistic endocrine disruptors, simple to run, possible false negatives	
Leydig cell culture	Determination of testosterone production in purified, isolated Leydig cells	Numerous toxicants	3–9 days	Testosterone production	Difficult to run, requires specific instrumentation, possible false (+) or (-)	
hAR transactivation assays using stable cell lines	Transcriptional activation using cells (e.g. CV-1) expressing relevant reporter	Antiandrogenic fungicides,	24 h	MMTV-luciferase	Sensitive, possible false (+) or (-), cells stability must be controlled	46
Testis/ovaries cultures	Determination of testosterone production in vertebrates	Estrogens, antiadrogens, numerous toxicants	1–several days	Testosterone levels	Easy to run, sensitive, possible false (+) or (-), rapid screening	
Vitellogenin assay	Amount of an egg yolk protein precursor in males as an indicator of estrogenic activity	Good for estrogen activity, but thyroid hormones may also be involved	> 72 h	amount of an egg yolk protein precursor	Requires some advanced equipment and trained personnel, good sensitivity	16, 18
TR binding assay	Competition for T3 binding in isolated nuclei of livers of any species	Possible for all toxicants	4 h	T3 level	High specificity, sensitivity, rarely possible false (+) or (-)	

Testing of mixtures for endocrine potential is difficult due to the necessity of testing numerous content ratios between their ingredients. Different levels of specific compounds in mixture may result in elevated or not-detected hormonal activity (9, 10).

Naturally occurring non-steroidal estrogens like phytoestrogenes and mycotoxins are plant- and fungi-derived chemicals which can be widely found in human food products. They are known for potent additives synergistic or antagonistic effects and can additionally make the screening and prioritization list more difficult and more screening assays may be required to obtain reliable answers to the problem of estrogenicity (11).

When the first level of assays is considered, one must apply all measures to:

- maximize sensitivity to reduce false negative results (false positives should also be avoided, although it is much safer to receive false positives (+) as the next level of tests should clarify eventual mistakes and errors),
- set a battery of tests of a wide range of organisms to represent the full spectrum of metabolism and different taxonomic groups,
- detect and predict all possible endpoints of endocrine disrupting activity.

All these factors can be answered by applying both *in vivo* and *in vitro* assays, as presented in Table 3.

The battery of tests indicated with italics is believed to detect any possible and known endocrine disruptors present in the environment alone, as well as part of a more complex mixture of xenobiotics. Additionally, other assays are proposed to detect some prenatal/pre-hatch exposure concerns (non-italics); they can be used instead of a few first-choice tests if it is justified from scientific or economic point of view.

All chemicals and mixtures that are suspected as endocrine disruptors should follow further investigations, however with more complex assays to characterize, identify and quantify eventual adverse hormonal action. Some criteria that must be fulfilled by tests are:

- the necessity of including the most sensitive lifestage of organism development process,
- the necessity of specifying the hazard caused by the chemical and plotting dose response relationship,
- the necessity of including an extensive range of taxa.

It must be stated that all false positive results would be either proved or negated at this stage of screening. Including all tests from the battery, one should receive a more accurate and comprehensive picture of endocrine disruption potential, mode of action and dose/response function. Still,

TABLE 6
Problems connected with analytics of EDCs, their implications and possible solving methods

Problem	Implications for analytical process	Possible solution
Low levels of concentration of EDC analytes in samples characterized by complex and fluctuating matrix content	– Too low detection limits	– Analytes' pre– concentration prior to final determination step
Possibility of presence (in sample) of other chemicals possessing similar physicochemical properties like analyte of interest	– False (+) or (–) hits	– Increase of analytical method specificity – Removal of interferences
Unknown metabolic and transformation pathways of endocrine disruptors	– No single source information contains all chemicals – Lack of validated procedures – Unknown bio– transformation products	– Fate and transport tests and researches – Combining metabolic and analytical data
Lack of reference materials and standards	– Lack of information on method selectivity and specificity	– Preparation of Standard Reference Material (SRM)
Limited number of compounds monitored	– Incomplete information on real adverse potency	– Determination of real endocrine potent with bioassays
Biological half–life, metabolism and tissue distribution variation from substance to substance	– Studying too many or too few chemicals – studying different tissues	– Preparing databases on metabolic and environmental pathways and distribution of xenobiotics

TABLE 7
Concentrations of selected EDCs determined in environmental matrices

Element of environment	Analyte(s)	Sample treatment prior to analysis	Detection technique	Results of quantitative analysis	Reference
Air samples (Germany)	Perfluorinated alkyls	Collection on PUF Extraction	GC/PCI-MS	64–546 pg/m^3	53
Air samples (sea-route from Germany to South Africa)	Perfluorinated alkyls	Collection on PUF Extraction	GC/PCI-MS	0.3–14 pg/m^3	52
River water	17β-estradiol	Stir bar sorptive extraction, in situ acylation, thermal desorption, quartz wool assisted silylation	GC-MS	0.5–2 pg/mL	22
Surface and drinking waters (Germany)	Perfluorinated surfactants	SPE clean-up and pre-concentration	HPLC-MS/MS	2–4385 ng/L	31
Surface water	Estradiol	Speedisk^{TM} extraction	GC-MS/MS	0.25—0.27 ng/L	34
	Estrone			0.37–10 ng/L	
	Ethinylestradiol			n.d.	
River water samples (Jordan River)	Testosterone	Centrifugation	YES	0.8–35.5 ng/L	27
	Estrone+estradiol	Acidication to pH=5	Radioimmunoassay	3.2–4.3 ng/L	
	Estriol	C-18 columns extraction	ELISA	0.7–3.4 ng/L	
	Ethinylestradiol			1.4–19.4 ng/L	
Sewage treatment plants (UK)	Bisphenol A	SPE	GC-MS/MS and HPLC/FTMS	1209 ng/L	23
	Estrone			30.10 ng/L	
	17β-estradiol			52.54 ng/L	
	16α-hydroxyestrone			63.7 ng/L	
	4-tert-octylphenol			188.5 ng/L	
	4-nonylphenol			39.9 ng/L	
River waters (Llobregat River, Spain)	alkylphenols	Filtration SPE Drying Elution and drying in N_2 stream	LC-ESI-MS	0.06–37.3 mg/L	47
Wastewaters (Lisbon, Portugal)	Alkyphenol	Centrifugation	ELISA	0.724–78.15 μg/L	25
	Etoxylates	Sonication	LC-MS/MS		
	Bisphenol A	Pre-concentration under N_2 stream		0.08–1.55 μg/L	
	17β-estradiol			0.57–1.73 μg/L	

(Continued on next page)

TABLE 7
Concentrations of selected EDCs determined in environmental matrices *(Continued)*

Element of environment	Analyte(s)	Sample treatment prior to analysis	Detection technique	Results of quantitative analysis	Reference
Wastewaters from swine farm (Tsukuba, Japan)	Estrone	SPE (N-vinylacetamide)	LC-MS	5200–5400 ng/L	26
	17β-estradiol	pH=3 regulation	LC-MS/MS	1000–1500 ng/L	
	17α-estradiol	Centrifugation		650–680 ng/L	
	Estriol	Pre-concentration under N_2 stream		2200–3000 ng/L	
	Bisphenol A			1100–1200 ng/L	
	Equol			940–1100 ng/L	
Lake waters (China)	Bisphenol A	In-tube SPME monolithic capillary	HPLC	0.00797 mg/L	49
	17α-ethynylestradiol			<LOD	
Waste waters (Beijing, China)	17β-estradiol	Filtration SPE	Yeat assay	0.03–13.27 EEQ/L	55
Surface, drinking and waste waters (South Korea)	Estriol	pH = 2 regulation	LC-ESI-MS/MS	1.0–36 ng/L	35
	17α-ethynyloestradiol	Storage in 4°C			
	Estrone	SPE			
	17β-estradiol				
	Testosterone				
	Androstenedione				
Waste waters (China)	Prednisone	Filtration	LC-ESI-MS/MS	2.6 ng/L	51
	Prednisolone	SPE		3.0 ng/L	
	Cortisone			30 ng/L	
	Cortisol			39 ng/L	
	Dexamethasone			1.2 ng/L	
	6α-methylprednisolone			0.62 ng/L	
Textile waste waters (Belgium, Italy)	Alkylphenols	SPE	LC-MS/MS	0.006–11.2 μg/L	48
	Akylphenol Ethoxylates				
	Ethoxycarboxylate Metabolites				
	Bisphenol A				
Sewage effluents (Hamilton, New Zealand)	Estrone	C-18 SPE	MCF-7	70 ng/L	32
	17β-estradiol		GC-MS	64 ng/L	
	Triclosan			3.2 μg/L	
	Eugenol			2.0 μg/L	
	Chloroxylenol			4.5 μg/L	
	Phthalates			7–17 μg/L	
	γ-sitosterol			2.2 μg/L	

Manure treated soils (Denmark)	17β-estradiol	pH regulation = 3	GC-MS/MS	2.5 ng/L	29
	Estrone	Storage in –18°C SPE PLE Centrifugation		68.1 ng/l	
Ringed seals (*Phoca hispida*) (Canadian Arctic)	Perfluoroalkyls	Homogenization LLE Clean up	Negative electrospray LC-MS/MS	0.1–13.0 ng/g w.w.	33
Marine organisms (lugworm, clam, crustaceans, fishes, mammals, birds) from Ariake Sea (Japan)	HHCB	LLE	GC-MS SIM	0.55–51 ng/g w.w.	30
	AHTN	Lipid removal by GPC Activated silica gel column cleaning		0.4–5.9 ng/g w.w.	
Chinese sturgeon (*Acipenser sinensis*) (China)	Synthetic musk fragrances	Soxhlet extraction	GC-MS	33.7–62.1 ng/g lipid ww	28
	DDT HCB p,p'-DDE	Drying-heating at 60°C Roto-evaporation LLE Moisture removing		0.27 ng/g ww 0.61 ng/g ww 68.4–449 ng/g ww	
Breast milk/ house dust	PBDEs	Samples freezing at −20°C	GC-MS	30.2 ng/g lipid 1.91 μg/g dust	21
Freshwater crocodile (*Crocodylus Johnstoni*) (Australia)	p,p'-DDE	ASE	GC-MS	6–80 μg/g lipid	50
	Toxaphene	GPC			
Gammarus wilkitzkii	Per- and polyfluorinated alkyls	Homogenization	HPLC-TOF/MS	0.48–7.35 ng/g w. w.	54
Boreogadus saida *Cepphus grille* *Larus hyperboreus* (Barents Sea)		Extraction		0.04–31.4 ng/g w. w. 0.17–49.5 ng/g w. w. 0.26–1680 ng/g w. w.	
Lobster, shrimp, anchovy, mullet, sole, hake, angler (Adriatic Sea, Italy)	Nonylphenol	Homohenisation	SIM GC-MS	9.5–1431 ng/g fish	24
		Freeze-drying		118–339 ng/g crust.	
	Octylphenol	Fat extraction		0.3–3.8 ng/g crust. 2.7–4.7 ng/g fish	
	Polyethoxylates			0.2–21.1 ng/g crust. 1.2–16.8 ng/g fish	

before applying tests to environmental matrices other model assays are requested to answer problems of NOAEL and EC_0 determination .

Following assays should be conducted when final conclusions on estrogenicity are to be obtained (see Tables 4 and 5):

- two-generation mammalian reproductive toxicity study or an alternative one,
- avian reproduction test,
- fish life cycle test,
- mysid life cycle test,
- amphibian development and reproduction test.

As in all analytical methodology, all assays require standardization, development, research and validation to be applied world-wide in EDC screening tests.

PROBLEMS CONNECTED WITH EDCS

Natural and synthetic hormones, their analogues and derivatives can be found at very low concentration intervals; however, their potency for harmful effect is enormous. What is more, the interactions between these chemicals are not commonly studied and any divagations in this subject area are still a white page in an "environmental analytics" book.

Problems and challenges connected with EDCs analytical determination are presented in Table 6 as well as possible ways of solving them.

Fate and transport data interpretation is a very challenging task to perform. Although the amount of information is sufficient, it is crucial to identify critical processes and transport pathways for prioritization and screening purposes.

There are three environmental processes that affect environmental fate of EDCs (as well as other pollutants). They are defined as (1, 44):

- Persistence – the tendency of a chemical substance or its degradation products to survive in the environment without being transformed into other form, (measure: hydrolysis half-life, aerobic and anaerobic soil metabolism and photolysis).
- Mobility – the tendency of a chemical substance to move within environmental media or between media (measure: volatility, Henry's law constant, K_d, K_{oc}, ground water ubiquitous score, aged soil column leaching and terrestrial field dissipation studies).
- Bioaccumulation – the capacity of a chemical to accumulate (be stored in tissue) in an organism as a result of uptake from all environmental sources (measure: octanol water partition coefficient, BCF and animal metabolism).

RESULTS OF ANALYSIS OF ENVIRONMENTAL SAMPLES

Environmental material containing EDCs may vary greatly. Starting from aqueous matrices, effluents, runoff waters, biosolids, animal wastes, sediments, pharmaceutical and their residues and finishing at polymers of everyday use.

Chemical structures of selected endocrine disruptors are presented in Fig. 3. while information on determined concentration levels of particular xenobiotics belonging to EDCs in environmental samples are presented in Table 7.

CONCLUSIONS

Screening and testing for endocrine potency will always require enormous numbers of animals (both vertebrates and invertebrates) to run assays. Animals play an essential role in the determination of hormonal properties of xenobiotics; however, one should consider some ethical problems in running tests on living creatures in such amounts. Next to the problem of the amount of organisms required, the exposure route should also be standardized for better relevancy of repeatability and reproducibility. The list of alleged exoestrogens continues to grow and in order to comply with legislative amendments requiring the testing of estrogenicity, means of prioritizing substances through the use of *in vitro* assays appears to be the inevitable solution, followed by analytical procedures and determinations to assess environmental concentration levels of the EDC xenobiotics.

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ABBREVIATIONS

16aOHE	16-αhydroxyestrone
2-AMK	2-amine musk ketone
2-OHE	2-hydroxyestrone
$\beta-$HCH	β-hexachlorcyclohexane
APCI	atmospheric pressure chemical ionization
APE	Alkylphenol etoxylate
AR	Androgen receptor
ASE	Accelerated Solvent Extraction
BBP	Buthylbenzyl phthalate
BFR	Brominated flame retardants
BP-A	Bisphenol A
DEHP	di(2-ethylhexyl)-phthalate
DES	Diethylsthilbestrol
DNA	Deoxyribonucleic acid
EC	Effective concentration
EDC	Endocrine disrupting compounds
EEQ	Estradiol equivalents
ESI	Electrospray ionization
ER	Estrogen receptor
GPC	Gel permeation chromatography
HHCB	Galaxolide
HTPS	High throughput screening
LC-MS/MS	Liquid chromatograph with doubled mass spectrometry detection system
LC	Lethal concentration

LOD	Limit of detection
MK	Musk ketone
PBDE	Polybrominated diphenylethers
TBBP-A	Tetrabrominated bisphenol A
PCB's	Polychlorinated biphenyls
PCI	Positive chemical ionization
PM	Particulate matter
PUF	Polyurethane foam
PVC	Polyvinyl chloride
QSAR	Quantitative structure-activity relationships
RBA	Relative binding affinity
TBT	Tributhyltin cation
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TOF	Time of flight
TPT	Triphenyltin cation
U.S. EPA	United States Environmental Protection Agency
WHO	World Health Organization
w.w.	Wet weight

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