Review

Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review

Chris G. Campbell [a,*], Sharon E. Borglin [b], F. Bailey Green [b], Allen Grayson [a], Eleanor Wozei [b], William T. Stringfellow [b]

[a] Water Guidance and Monitoring Group, Environmental Protection Department, Lawrence Livermore National Laboratory, P.O. Box 808, L-627, Livermore, CA 94551, United States

[b] Earth Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Rd., Berkeley, CA 94706, United States

Abstract

Endocrine disrupting compounds (EDCs) are contaminants that may be hormonally active at low concentrations and are emerging as a major concern for water quality. Estrogenic EDCs (e-EDCs) are a subclass of EDCs that, when organisms are exposed to them, func-tion as estrogens. Given that there are numerous e-EDCs that can negatively affect humans and wildlife, general screening techniques like biologically based assays (BBAs) may provide major advantages by estimating the total estrogenic effects of many e-EDCs in the envi-ronment. These techniques may potentially be adapted for field portable biologically directed sampling and analyses. This article sum-marizes available BBAs used to measure estrogenic e-EDCs in the environmental samples and also presents results relating to fate and transport of e-EDCs. Estrogenic EDCs appear to be almost ubiquitous in the environment, despite low solubility and high affinity of organic matter. Potential transport mechanisms may include: (1) transport of more soluble precursors, (2) colloid facilitated transport,
(3) enhanced solubility through elevated pH, and (4) the formation of micelles by longer-chain ethoxylates. Due to their persistent and ubiquitous nature, source control strategies for e-EDCs may reduce influent concentration to wastewater treatment plants so that the post treatment effluent will decrease concentrations to estrogenically inactive levels. Alternatively if source reduction is not possible, then more testing is needed on tertiary treatment technologies and treatment efficiencies for e-EDCs. There is still a need for research on reme-
diation and restoration approaches for habitats disturbed by elevated e-EDC concentrations.
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Keywords: Estrogenic endocrine disrupting compounds; Fate and transport; Measurement methods

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1. Introduction to endocrine disrupting compounds

Endocrine disrupting compounds (EDCs) are chemicals with the potential to elicit negative effects on endocrine sys-tems of humans and wildlife. Various natural and synthetic chemical compounds have been identified that educe estro-gen-like responses including pharmaceuticals, pesticides, industrial chemicals, and heavy metals (Giesy et al., 2002).

The US Environmental Protection Agency (EPA) defines an EDC as:

> An exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, develop-ment, and/or behavior. (USEPA, 1997, p. 1)

This paper focuses on estrogenic EDCs, we will desig-nate as e-EDCs, that are either hormonal estrogens or chemicals which mimic or induce estrogen-like response in an organism. These compounds have varying degrees of potency, some being strongly active compounds, some having weak estrogenic activity. These compounds number in the hundreds, if not thousands in the environment, and many may be yet undiscovered. Therefore, activity assays that can measure overall estrogenic potential including concentration, cumulative effects, and potency of the chem-ical would be necessary to assess total environmental estro-genic potential.

This broad class of chemicals includes both natural and synthetic estrogens (e.g. xenoestrogens and pseudoestro-gens). Specific examples of e-EDCs include: pesticides like atrazine, dieldrin, and toxaphene (Arnold et al., 1996a; Ramamoorthy et al., 1997; Hayes et al., 2002), surfactants such a alkyphenol-ethoxalates (Folmar et al., 2002; Legler et al., 2002a; Ying et al., 2002), natural hormones and pharmaceutical estrogens 17b-estradiol and 17a-ethynylest-radiol (Folmar et al., 2000; Legler et al., 2002a), phytoes-trogens including isoflavonoides and coumestrol (Bacaloni et al., 2005; Stopper et al., 2005), as well as other industrial compounds like bisphenol A (Mocarelli et al., 1996; Ramamoorthy et al., 1997; Howdeshell et al., 1999).

Given that many of the e-EDCs identified have the potential to cause an estrogenic response at very low con-centrations (parts per billion to parts per trillion) it is cause for concern that measurable concentrations of many of the chemicals mentioned above have been found in wastewater, surface waters, sediments, groundwater, and even drinking water (Benfenati et al., 2003; Petrovic´ et al., 2003; Snyder et al., 2003; Petrovic´ et al., 2004). Wastewater treatment plants have been studied as a major source for e-EDCs (Kolpin et al., 2002; Legler et al., 2002a; Snyder et al., 2003).

Various e-EDCs have been concluded to be the cause of reproductive disturbance in humans and wildlife (Colborn et al., 1993). Human exposure to these chemicals in food, water and the environment is a critical concern with unknown long-term impacts. Measurable concentrations of the e-EDC, nonylphenol (NP) were found in all of the 60 different common food products sampled in a study in Germany (Guenther et al., 2002). Exposure to the e-EDCs (i.e. diethylstilbestrol) has been implicated to cause decreased sperm counts in human males (Sharpe and Skakkebaek, 1993). While additional studies have not con-firmed decreasing sperm counts in males in Sapporo, Japan (Itoh et al., 2001): Dallinga et al. (2002) did find a correla-tion between lower sperm counts and elevated polychlori-nated biphenyl concentrations in blood serum in subjects from The Netherlands. In addition, a reanalysis of the glo-bal trend data for male sperm count found a decline in sperm density in the Unites States and Europe (Swan et al., 1997). A number of human tissues show estrogen receptor expression including the brain, immune system, cardiovascular system, lungs, mammary glands, liver, kid-neys, reproductive tract (ovaries, testes, uterus, prostrate), adipose tissue, and bone (Mu¨ller, 2004). As human toxicol-ogy is beyond the scope of this review, additional discus-sions on many aspects of human heath and exposure to e-EDCs may be found in Nicolopoulou-Stamati et al. (2001).

The US EPA has set an Ambient Water Quality Criteria for nonylphenol of 28 lg/l acute exposure (maximum 1 h concentration) and 6.6 lg/l for chronic exposure (4 d expo-sure occurring more than once over 3 yr) in freshwater environments (USEPA, 2006). In saline waters the acute criteria lower at is 7.0 lg/l and the chronic is 1.7 lg/l. Ambient Water Quality Criteria are not regulatory limits, but suggested water quality benchmarks to protect aquatic life based on studies performed by the agency.

There are mounting problems with monitoring and managing this form of environmental pollution (Petrovic´ et al., 2004). Environmental management of e-EDCs will rely on source reduction, limiting exposure of vulnerable populations, and treatment or remediation of waste streams or contaminated sites. Successful management of e-EDCs will require large scale monitoring networks, a bet-ter understanding of transport mechanisms in the environ-ment (soil, water and air), innovative treatment processes, and analysis of the potential costs and benefits of source

mitigation (e.g. removal of nonylphenols from household chemicals).

Recent literature on e-EDCs has focused on methods of detection (e.g. Huang and Sedlak, 2001; Legler et al., 2002b; Heisterkamp et al., 2004; Zhang et al., 2004; Fan et al., 2005), distributions of EDC in some specific loca-tions (e.g. Allen et al., 1999; Isobe et al., 2001; Cargoue¨t et al., 2004), and e-EDC treatment in conventional waste-water systems (e.g. Ko¨rner et al., 2000; Johnson and Sump-ter, 2001; Wozei, 2004; Huber et al., 2005). This article will review methods for e-EDC detection and quantification from the perspective of developing an environmental mon-itoring network. In addition, some results relating to fate and transport of e-EDCs will be discussed including sources, potential transport mechanisms, and some strate-gies for large sale characterization. Both the monitoring and fate and transport of e-EDCs will be related to envi-ronmental management, as possible.

2. Monitoring estrogenic endocrine disruptors

The recent interest in e-EDCs has promoted the devel-opment of analytical methods, including HPLC, GC/ MS,GC–MS/MS, and LC–MS/MS (Petrovic´ and Barcelo´, 2000; Huang and Sedlak, 2001; Petrovic´ et al., 2002). These methods have been presented in detail in many references (Huang and Sedlak, 2001; Heisterkamp et al., 2004; Wozei, 2004; Zhang et al., 2004; Fan et al., 2005). These analytical techniques provide excellent sensitivity and precision for monitoring e-EDC mass. Mass measurements are neces-sary in studies of e-EDC fate and transport in the environ-ment, however, they do not provide data on estrogenic effects or synergistic or antiestrogenic influences from mul-tiple estrogenic compounds. In addition, these techniques measure specific e-EDCs individually, so the target com-pound must have been already been identified as have estrogenic properties. Such restrictions must be considered before selecting a technique for e-EDC monitoring, so that the monitoring objectives may be satisfied.

Biologically based assays (BBAs) provide alternative detection methods to traditional mass-based analyses. Detection in a BBA occurs by a number of mechanisms, including cell proliferation, ligand binding, vitellogenin induction, luciferase induction, or antigen–antibody inter-action. Cell proliferation estimates cell growth and repro-duction in different samples and ligand binding uses a specific binding site for estrogens that can be quantified (Soto et al., 1995). Vitellogenin is a yolk protein in female fish liver produced in response to estrogens that can be extracted from plasma and measured (Jimenez, 1997). The production of vitellogenin in male fish is an indication of endocrine disruption. Luciferase induction uses estro-gen receptors and response elements to produce the pro-tein luciferase that may be quantified by luminescence after cell lysing and the addition of luciferin (Legler et al., 2002a). Antigen–antibody interactions provide the basis for immunoassays based upon the non-covalent binding of antigen to antibodies (Gasco´n et al., 1997). A discussion of the details and complexity involved in many of these methods is in the following section. BBAs may provide either a qualitative or quantitative response. BBAs may use whole organisms, whole cells, or biological mate-rials like antibodies or estrogen receptors. The following discussion organized these techniques by whole organism, cellular, and non-cellular assays. A comprehensive sum-mary of compound-specific bioassay studies along with the mode of estrogenic activity is provided in Giesy et al. (2002).

Table 1
Examples of whole organism studies as indicators of estrogenic endocrine disruption

Species	Common name	EDC effect	Reference
Rana pipiens	Leopard frogs	Gonadal abnormalities	Hayes (1998), Hayes et al. (2002)
Chrysemys picta	Painted turtle	Vitellogenin induction	Irwin et al. (2001)
Oncorhynchus mykiss	Rainbow trout	Reproductive deficiencies, egg and offspring development, and vitellogenin induction	Fenner-Crisp et al. (2000), Folmar et al. (2000), Anderson et al. (1996)
Pimephales promelas	Fathead minnow	Gonad development; reproductive deficiencies, development; vitellogenin induction	Fenner-Crisp et al. (2000), Folmar et al. (2000)
Cyprinodon variegates	Sheephead minnow	Vitellogenin induction	Legler et al. (2002a), Fenner-Crisp et al. (2000), Folmar et al. (2000)
Danio rerio Brachydanio rerio	Zebrafish	Gonad development; physiological development; vitellogenin induction, luciferine (luminescence)	Maack et al. (1999), Legler et al. (2002a)
Oryzias latipes	Medaka fish	Gonadal development; reproductive success; green fluorescence protein (GFP)	Gray et al. (1999), Kurauchi et al. (2005)
Platichthys flesus	Flounder	Vitellogenin induction; gonad development; physiological development;	Allen et al. (1999)
Salmo salar	Atlantic Salmon	Zona radiata protein and vitellogenin induction	Arukwe et al. (2000)
Halineetus leucocephalus	Bald eagle	Reproductive and teratogenic effects	Bowerman et al. (2000)
Coturnix coturnix japonica Colinus virginianus	Japanese quail and bobwhite quail	sexual behavior; embryo development; egg shell thickness	Lien et al. (1985), Berg et al. (1999)
Gallus domesticus	Domestic chicken	Embryo development; egg shell thickness	Berg et al. (1999)
Daphnia magna	Water flea	Physiological and biochemical disruption	Baldwin et al. (1997)
Tisbe battagliai	Marine copepod	Fecundity, longevity, and rate of development	Bechmann (1999)

2.1. Whole organism assays

Measuring endocrine disruption in amphibians, fish, birds, and insects may be a potential approach to monitor e-EDC pollution in aquatic environments. Table 1 lists many of the species that have been studied as indicators of estrogenicity in natural waters. Frog populations have been suggested to be particularly sensitive to EDC expo-sure. Gonadal abnormalities have been observed in 10–92 percent of male wild leopard frogs (Rana pipiens) examined from throughout the United States (Hayes et al., 2002).

Many fish assays for estrogens have been developed by the US EPA and others using rainbow trout (Oncorhynchus mykiss), fathead minnow (Pimephales promelas), sheephead minnow (Cyprinodon variegates) and zebrafish (Brachyda-nio rerio) (Fenner-Crisp et al., 2000; Folmar et al., 2000; Legler et al., 2002a). There are various approaches for determining estrogenic response in these organisms includ-ing deformities, reproductive deficiencies, egg and offspring development, and serum protein production like vitello-genin. Some species have been genetically engineered to respond to e-EDCs including transgenic zebrafish (Brac-hydanio rerio) that has been bioengineered with luciferase expression coordinated to vitellogenin production (Legler et al., 2002a) and medaka fish (Oryzias latipes) bioengi-neered to express a green fluorescence protein in response to vitellogenin production (Kurauchi et al., 2005).

Whole organism monitoring of e-EDCs has the advan-tage of being an in vivo assessment of true impact of estr-ogenicity on a target species. In addition, these species inhabit a range of environments and could serve as biolog-ical indicators of areas particularly impacted within a watershed or landscape. The major disadvantage of this approach is a lack of specificity of organism response to various e-EDCs. Response in a biological indicator species may not identify cause and effect or point to a specific loca-tion as the source. However, these indicators do have the potential to provide a cumulative estrogenic response to exposure to a mixture of e-EDC in a given environment.

2.2. Cellular bioassays

Cellular bioassays are an alternative to mass-based ana-lytical techniques. While offering good sensitivity, these bioassays may not consistently provide a repeatable quan-titative response for a specific e-EDC in complex environ-mental samples. The rapid response and lower equipment requirements make cellular bioassays an attractive alterna-tive to conventional analytical technique for environmental monitoring, particularly when measuring relative increases in total estrogenic activity is the monitoring objective. Cel-lular assays often use yeast or human cells (e.g. breast can-cer or kidney), that have been use as is, or bioengineered so that an estrogen binding to the estrogen receptors produces a dimer able to bind to and stimulate an estrogen response element that promotes the expression of a measurable pro-tein. Yeast have no indigenous estrogen receptor (ER) so the receptor gene has to be added to its genome from human, fish frogs, fish or other species. This is advanta-geous as it eliminates the multiple pathways by which cells and tissues normally respond to estrogen in organisms that have existing ERs. Two examples of the response proteins include luciferase and b-galactosidase which can be quanti-fied using a luminometer (after cell lysing) and a spectro-photometer (back-calculating from the amount of colored product measured after the enzyme-catalyzed reaction has stopped), respectively.

Specific cellular bioassays include E-SCREEN (cell pro-liferation response), YES (colometric response), and ER-CALUX (luminescent response) (Table 2). Among these, only the ER-CALUX is commercially available as a license to perform the analysis and the biological reagents (BioDe-tection Systems, Amsterdam, The Netherlands). Other methods that have been developed include luminescent bio-assays using E. coli and the HEK 293 (human embryonic kidney) cell, as well as, an infrared bio-amplification approach using mammalian cells (Table 2).

In the E-SCREEN cell-proliferation bioassay, more cells are generated in the presence of estrogen (Soto et al., 1995).

Table 2
Examples of single cell bioassays for detection of e-EDCs

Common name	Cell type	e-EDC effect	Reference
E-SCREEN	MCF-7 breast cancer cells	Cell proliferation response	Soto et al. (1995)
Yeast Estrogen Screen (YES) – including LYES and BLYES variations as well	Various (Saccharomyces spp., Cryptococcus spp., and Candida spp.)	Colometric & luminescent response	Arnold et al. (1996b), Routledge and Sumpter (1996), Fang et al. (2000), Silva et al. (2002), Legler et al. (2002b), Schultis and Metzger (2004); Sanseverino et al. (2005)]
ER-luciferase assay with HEK 293 cells	Human embryonic kidney (HEC)	Luminescent response	Pawlowski et al. (2003)
NA	E. coli	Luminescent response	Gu et al. (1999)
Estrogen responsive chemically activated luciferase expression (ER-CALUX)	T47D human breast adenocarcinoma cell	Luminescent response	Legler et al. (2002b); BioDetection Systems, Amsterdam, The Netherlands[a]
IR-bio-amplification	Mammalian cells	Cellular function	Holman et al. (2000, 2003)

[a] Commercially available product.

MCF-7 breast cancer cells are exposed to both positive (17b-estradiol) and negative (no estrogens) controls, as well as, to samples potentially containing estrogenic com-pounds. The comparison of the total cell proliferation to the positive control provides the basis for demonstrating estrogenic response.

In general, the Yeast Estrogen Screen, or YES, refers to the assay developed by Routledge and Sumpter (1996), however, other yeast-based assays for environmental screening include the RCBA of Coldham et al. (1997), the yeast-based assay of Gaido et al. (1997) and a yeast two-hybrid assay of Nishikawa et al. (1999). A modified version called LYES (and a bioluminescent version [BLYES] by Sanseverino et al. (2005)) has also been devel-oped that is faster (7 h to perform) and more sensitive than other BBAs (Schultis and Metzger, 2004). These modified versions utilize the bacterial (lux) luciferase approach which differs from the traditional use of the firefly (luc) luciferase (Sanseverino et al., 2005). The approach of using the lux cassette has the advantage over the luc system the luminescent response may be produced without the addi-tion of an exogeneous substrate or excitation.

The traditional YES cells are engineered with a human estrogen receptor gene, which binds to an estrogen response element regulated-expression plasmid (lac-Z) coded to express b-galactosidase (Arnold et al., 1996b; Routledge and Sumpter, 1996). The process is as follows: (1) estrogen enters the cell, (2) the cell responds by generating more estrogen receptors, (3) estrogen binds to receptors, (4) two of the estrogen–estrogen receptor molecules bind to form a dimer, (5) the dimer binds to the estrogen response ele-ment, (6) that binding initiates transcription of lac-Z mRNA, (7) then b-galactosidase enzyme is produced, and finally (8) the enzyme catalyzes the substrate causing a product reaction. This enzyme reacts with a substrate in the culture media to release CPRG (chlorophenol red b-D-galactopyranoside) and intensity of the colometric response can be quantified using a spectrometer at specific light absorbance wavelength peaks (Legler et al., 2002b). A correction for cell density to growth ratio is measured as turbidity at OD = 600–630 nm and a red product absor-bance measured at OD = 540–550 nm. Other yeast assays like the RCBA use a colorless substrate (ONPG) and form a yellow product (ONP) measured at OD = 420 nm. Ini-tially, the YES assay did not have a correction for cell den-sity or cell growth like the RCBA, but it now does although the equations used by the different researchers to correct for this using the YES assay vary. In an application of the YES assay, investigators observed combined additive estrogenic-ity with the presence of multiple estrogenic compounds, demonstrating the need for total screening tools that are not compound specific (Silva et al., 2002).

The estrogen responsive chemically activated luciferase expression (ER-CALUX) assay is a commercially avail-able method that uses the T47D human breast adenocarci-noma cell engineered to express the enzyme luciferase (BioDetection Systems, Amsterdam, The Netherlands). The luciferase will luminesce when exposed to an estrogenic chemical by lysing the cells and adding the substrate lucif-erin (Legler et al., 2002b).

IR-bio-amplification is a technique developed at the Lawrence Berkeley National Laboratory (LBNL) that is based on synchrotron radiation (SR)-based Fourier trans-form infrared (FTIR) spectromicroscopy (Holman et al., 2000; Holman et al., 2003). It has been demonstrated that changes in light diffraction can be related to changes in molecules within living cells. Mid-infrared light is low in energy, so it is non-destructive to biological materials, allowing the detection of subtle intracellular changes in live cells as they are exposed to environmental stimuli like e-EDCs (Holman et al., 2000). The diffraction of the light is detected at 128 individual sensors and the response is cal-ibrated to measurement of a normal functioning cell (or control cell). Cell response must be documented for various life stages of an e-EDC sensitive cell to define the ''back-ground'' light diffraction pattern. Once this background is defined, a change in cell response due to exposure to e-EDCs may be tested.

Gu et al. (1999) developed a biosensor using recombi-nant E. coli containing the luxCDABE luminescent gene from Vibrio fischeri to assess both estrogenicity and toxicity of many e-EDCs. Many of the estrogenic compounds, including NP, BPA, and pesticides, were demonstrated to cause toxic response including decreased biological activity and mortality. This study demonstrated the necessity for establishing both estrogenic, antiestrogenic, and toxic biosensor responses to e-EDCs. This observation can be generalized to many of the cellular bioassays for estroge-nicity. Antiestrogenicity and toxicity inhibit the lumines-cent or colometric response in bioassay, producing an inappropriate result. Some chemicals may also be agonis-tic, promoting a synergistic estrogenic response, and antag-onistic, promoting an antiestrogenic response in a bioassay. For example other emerging contaminants, like dioxins and polycyclic aromatic hydrocarbons, have been shown to induce both limited agonistic and antagonistic responses related to binding with the arylhydrocarbon receptor (AhR) (Oh et al., 2006). Given agonism, antagonism, and toxicity the collective response induced by a complex environmental media can be difficult to characterize and differentiate from the estrogenic effect of an individual e-EDC.

2.3. Non-cellular assays

Assays that do not require whole cells can avoid some of the difficulties related to membrane permeability, cell func-tion, organism life stages, and toxicity responses to a given sample (Table 3). Many of these assays are quantitative and provide reasonable detection limits for measurement of e-EDCs. Some quantitative assays like the enzyme-linked immunosorbent assays (ELISA) and the enzyme-linked receptor assay (ELRA) require laboratory systems for quantification, but provide a measurement of e-EDC

Table 3
Examples of noncellular assays and biosensors for detection of e-EDCs

Assay name	e-EDC response	Quantification	Reference
Enzyme-linked immunosorbent assays (ELISA)	Colormetric	Luminometer, spectrophotometer	Gasco´n et al. (1997), Sun et al. (2001), Oubin˜a et al. (1997), Huang and Sedlak (2001), Bretcht et al. (1998)[a]
Enzyme-linked receptor assay (ELRA)	Luminescent, colormetric	Luminometer, spectrophotometer	Garrett et al. (1999), Seifert et al. (1999), Seifert (2004)
Endotect™	Fluorescence	Evanescence fluorometer	Erb et al. (2001); ThreeFold Sensors, Ann Arbor, MI[a]
River analysis (RIANA)	Fluorescence	Fluorometer	Rodriguez-Mozaz et al. (2004b)
Biacore™	Surface plasmon resonance	Laser diffraction	Usami et al. (2002), Seifert et al. (1999), Hock et al. (2002), Biacore Company Piscataway, NJ, USA[a]
Electrochemical biosensors	Piezoelectric sandwich-type assay/histidine-tag fusion system/daunomycin labeling	Multimeter	Zhihong et al. (1999), Murata et al. (2001), Kuramitz et al. (2002)
Single cell coactivator recruitment (SCCoR)	Fluorescent indicator	Fluorometer	Awais et al. (2004)
Microarray relative binding assay (RBA)	Fluorescent fluorophore	Fluorometer	Kim et al. (2004)

[a] Commercially available product.

concentration (Seifert, 2004). ELISA kits are currently available for many of the environmentally relevant surfac-tants and estrogen compounds, as well as pesticides, antibi-otics, and other personal care products (Gasco´n et al., 1997; Neogen Corp Lexington, KY; ALPCO Diagnostics Salem, NH; Assay Designs Inc. Ann Arbor, MI; Bio-Quant Inc., San Diego, CA; BioSource Internacional, Cama-rillo,CA; Cayman Chemical Company, Ann Arbor, MI; Immuno-Biological Laboratories, Inc., Minneapolis, MN; Envirologix. Portland, Me: York Nutritional Laboratory, Osbaldwick, York, UK). Both ELISA and ELRA have been successfully applied to environmental samples and also developed into biosensors using a Biacore™ system, a surface plasmon resonance device sold by Biacore Com-pany (Piscataway, NJ, USA) (Seifert et al., 1999; Hock et al., 2002; Usami et al., 2002). We will define a biosensor as a BBA that has a self-contained method of quantitation (e.g. luminometer or voltmeter). More details on these assays may be found in the references provided in Table 3.

Other examples of non-cellular assays include biosen-sors like the Endotect™ and the RIver ANAlyser (RIANA) systems that have the potential to be made field portable. The Endotect™ biosensor receptor-binding assay uses a human estrogen receptor (hER) connected to a fluorescent molecule that is quenched until binding with the estrogen and the fluorescence is then measured in an evanescence-type detector (Erb et al., 2001). It is commercially available as a field portable, hand-held device with refills for the reagent and evanescent optical fibers from ThreeFold Sen-sors (Ann Arbor, MI). While this biosensor is still under development, it has been successfully field tested (Erb et al., 2001). A comparison of the Endotect™ to other tech-niques has not yet been published.

The RIANA is a multi-analyte immunosensor that uses total internal reflection fluorescence to determine the atra-zine, isoproturon, and estrone levels in water (Rodriguez-Mozaz et al., 2004b). An immunosensor uses antibodies rather than hormone receptors, so in this case the ''recep-tor'' is the region of the antibody which shows recognition of an antigen. The estrogen binds to chemical-specific anti-bodies with a fluorescence tag, which are excited by that binding. Initial testing of the RIANA is very promising with clear determination of the three target analytes, low variability, and a demonstrated ability to measure the ana-lytes in various water sources including river water, groundwater, and wastewater (Rodriguez-Mozaz et al., 2004b).

In addition to these non-cellular assays, there have been some recent advances in electrochemical sensors, fluores-cent indicators, and microarray relative binding assays that have the potential to improve monitoring capabilities. Zhi-hong et al. (1999) examined a piezoelectric sandwich-type assay using an estrogen response element (ERE) immobi-lized in the biosensor. The 17b-estradiol forms a complex with an estrogen receptor which is detected by binding to the immobilized ERE with a lower detection limit of 2.2 lg/l. Another potential biosensor uses a histidine-tag fusion system, where the histidine-tag interacts with a Ni(II) chelate adsorbant, the author found an estrogen concentration dependent voltammetric response (Murata et al., 2001).

More recently a fluorescent indicator approach that allows for discrimination between estrogen agonists and antagonists (Awais et al., 2004). This was achieved through a specialized ligand binding domain approach that creates a coactivator recruitment surface which allows natural and synthetic estrogen screening in living cells using a fluores-cence resonance energy-transfer technique (Awais et al., 2004). It was demonstrated that the fluorescent indicator could be applied to living cells and the dose-dependent fluo-rescent response measured to determine estrogenic activity in cells. This indicator approach, called the single cell coac-tivator recruitment (SCCoR), has the potential to make tar-get cells of many different species into biosensors.

Table 4
Reported limits of detection and limits of quantification for different methods to detect the various e-EDCs mentioned in this review. Ranges include different e-EDCs and water media including drinking and wastewater

Method	Detection limit (ng/l)
E-Screen	0.27
ER-CALUX	0.14
YES	0.3–30
ELISA	20–40
LC–MS/MS	0.08–33
GC–MS	0.2–2
GC–MS/MS	0.05–2.4
SPME–HPLC	0.064–1.2
HPLC/ESI-MS/MS	0.2–1
MEKC	44–89

ER-CALUX: Estrogen responsive chemically activated luciferase expression.
YES: Yeast estrogen screen.
ELISA: Enzyme-linked immunosorbent assay.
LC–MS/MS: Liquid chromatography tandem mass spectrometer.
GC–MS: Gas chromatography mass spectrometer.
GC–MS/MS: Gas chromatography tandem mass spectrometer. SPME–HPLC: Solid-phase microextraction high performance liquid chromatography.

HPLC/ESI-MS/MS: High-performance liquid chromatography with positive electrospray ionization and tandem mass spectrometry. MEKC: Micellar electrokinetic chromatography.
Sources: Wozei (2004), Fan et al. (2005), Huang and Sedlak (2001), Heisterkamp et al. (2004), Zhang et al. (2004), Voulvoulis (2003, Table 3.5).

Note: See Petrovic´ and Barcelo´ (2004, Table 1) for analytical methods and detection limits for sediment and sludge.

The selection of an appropriate technique for environmental monitoring depends upon the monitoring objective and the resources available. Table 4 lists reported limits of detection and quantification for e-EDCs achieved by vari-ous methods including mass-based and biologically based approaches. Again the mass-based analytical techniques provide a quantitative result, but usually require significant capital investment in equipment like a tandem mass spectrometer. On the other hand many biologically based sen-sors require less expensive microplate luminometers or spectrophotometers and provide a total estrogenic response, but it may be more qualitative. One proposed solution has been to use the two types of approached together in a bioassay-directed chemical analysis (BDCA) approach in which sample screening is performed using a YES assay for total estrogenic activity in combination with analyses for specific chemical species using LC-MS/MS (Heisterkamp et al., 2004). Bioassay-directed analysis is discussed further in Section 3.4.

3. e-EDC fate and transport

The ultimate goal for a monitoring system is to provide information at the temporal and spatial resolution in order to characterize source, transport, and fate of the target compounds. A representation of the transfer and partition-ing of e-EDCs into different compartments is shown in Fig. 1. Many e-EDCs are potentially released into the envi-ronment through wastewater treatment discharges, surface non-point source runoff, and atmospheric deposition of particulates and aerosols. While there are many different potential e-EDCs, there are some general similarities in chemical properties. These similarities will allow for gen-eral conclusions on the fate and transport of e-EDCs in the environment. In addition, areas requiring continued research will be identified.

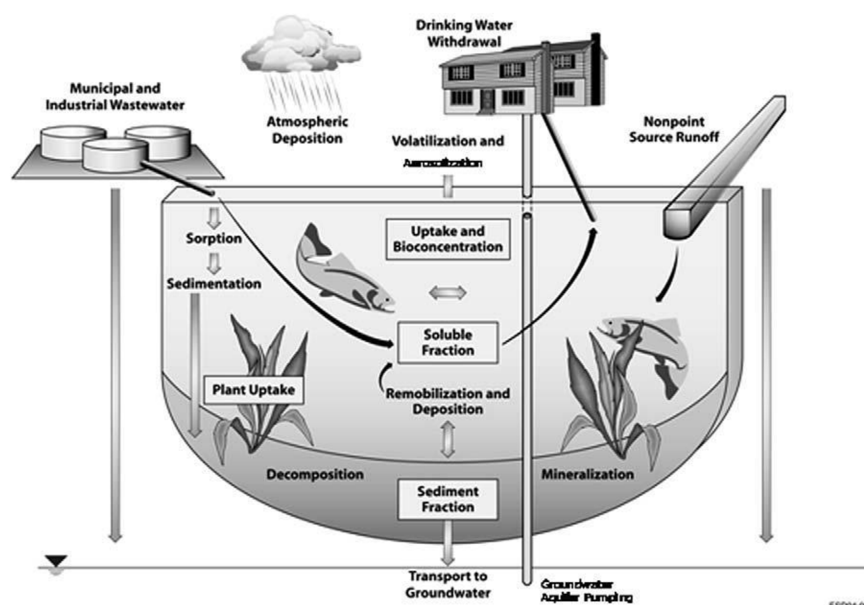

Fig. 1. Schematic representation of the different processes and compartments that need to be monitored to characterize the fate and transport of e-EDCs in the environment.

3.1. Sources of e-EDCs

While wastewater treatment facilities have been implicated as the major sources for e-EDCs (Sumpter, 1995; Kolpin et al., 2002; Legler et al., 2002a), the actual sources are upstream discharges to the treatment facilities. A few of these upstream sources include natural hormones and pharmaceutical estrogens flushed down home toilets, household cleaners containing NP, industrial processes that use cleaners containing NP and plastics containing BPA, or agrochemicals containing alkylphenol and nonyl-phenol ethoxylate surfactants (Staples et al., 1998; Ying et al., 2002; Snyder et al., 2003). Wastewater treatment facilities serve as a focal point where treatment is possible if source mitigation is impractical (e.g. removal of e-EDCs from product formulations or reducing pharmaceutical estrogens in household waste). Discharges from wastewater treatment facilities are also the likely point sources for reg-ulation under the Clean Water Act in the United States.

If source control is attempted, sources upstream of wastewater treatment facilities like industries using plasticizers, medical industries, and household discharges, would require further characterization. In addition, the costs and benefits of substituting alternative industrial chemicals for e-EDC need to be examined. Alternative surfactants to replace nonylphenol ethoxylates have been proposed and tested by Fernandez et al. (2005). Of course if source con-trol is not feasible, then options for implementing tertiary treatment at the wastewater facilities must be considered. Studies have examined fate and transport of e-EDCs through wastewater treatment facilities, finding removal of estrogens and alkyphenol-ethoxalates (e.g. NP, NPOE, OPOE) (Körner et al., 2000; La Guardia et al., 2001; Braga et al., 2005a; Johnson et al., 2005). At the same time many of these studies still found measurable, and potentially estrogenically active, e-EDC concentrations in the final effluent discharges.

Agricultural land uses have also been identified as non-point sources for e-EDCs including wastewaters from dair-ies and aquaculture (Kolodziej et al., 2004). Spawning fish may locally increase the estrogen concentrations of in river water (Kolodziej et al., 2004). Livestock feed lots have also been demonstrated to be potential sources of estrogenic compounds from excretion of hormones in manure and urine (Hanselman et al., 2003; Tashiro et al., 2003; Soto et al., 2004). In addition, the potential exists for agricul-tural runoff containing pesticides and fertilizers to contain the estrogenic surfactants (e.g. nonylphenol ethoxylates) that make up the chemical formulation (Staples et al., 1998; Ying et al., 2002). These potential agricultural sources, livestock excretion of hormones and chemicals in pesticide and fertilizer formulations, could contribute to the non-point source runoff component of e-EDCs identi-fied in Fig. 1.

3.2. Potential transport mechanisms

The partitioning coefficients of e-EDCs between the aqueous and solid phases (K_{OC} values) in relation to the local concentration of organic carbon are listed as log K_{OC} in Table 5. Many e-EDCs have moderate to high log K_{OC} values, so the mass that does not remain soluble often ends up in organic complexes in, or sorbed to, sediments or sus-pended organic material. In the sediments there is the potential for biological uptake, degradation and transfor-mation to less mobile or more mobile forms. If mobilized, the e-EDC complexes may move back into the water col-umn or downward toward groundwater. Therefore expo-

Table 5
Properties of selected e-EDCs from the literature

EDC	Log K_{OC} (l/kg)	Solubility (mg/l)	EEF[A]	CMC[B] (mg/l)	pKa
Estradiol[C]	2.55–4.01	13.0–32.0	1.0a	NA[D]	10.5–10.71
17b-Estradiol (E2)	3.10–4.01	13.0	1.0b	NA	10.71
Estrone (E1)	2.45–3.34	6.0–13.0	0.1–1.0a, 0.01–0.1b	NA	10.3–10.8
Ethinylestradiol (EE2)	2.91–3.04	4.8	0.8–1.9b	NA	NA
Estriol (E3)	2.13–2.62[E]	32	0.01–0.08b	NA	10.4
Bisphenol A	2.50–6.60	120–300	$5.0 \cdot 10^{5}$–$6.0 \cdot 10^{5}$b	NA	9.6–11.3
Nonylphenol (NP)	3.56–5.67	4.9–7.0	$2.3 \cdot 10^{5}$–$9.0 \cdot 10^{4}$a	5–13	10.28
			$7.2 \cdot 10^{7}$–$1.9 \cdot 10^{2}$b		
Nonylphenol ethoxylates (NP1EO-NPnEO)	3.91–5.64	3.02–7.65	$2.0 \cdot 10^{7}$–$1.3 \cdot 10^{5}$b	$4.25 \cdot 10^{5}$	NA
Octylphenol	3.54–5.18	12.6	$1.0 \cdot 10^{5}$–$4.9 \cdot 10^{4}$b	150 (Triton X-100)	NA

Sources: Petrović et al. (2004), Hanselman et al. (2003), Lee et al. (2003), Folmar et al. (2002), Düring et al. (2002), Legler et al. (2002a), Ying et al. (2002), Brix et al. (2001), Ferguson et al. (2001), Müller and Schlatter (1998), Ahel and Giger (1993), Staples et al. (1998), Sylvestre et al., 1998, Kurauchi et al. (2005), Cargouët et al. (2004), Lewis and Archer (1979), Körner et al. (2000), Heisterkamp et al. (2004) and Sánchez-Camazano et al. (2003).

[A] Estrogen equivalent factor effect relative to estradiol (a) and relative to 17b-estradiol (b) – ranges include various difference bioassays and estrogen receptors including ER-CALUX, YES, E-Screen transgenic zebrafish, and sheepshead minnows, as well as, both hEH-a and hEH-b receptors.

[B] Critical micelle concentration.

[C] Estradiol here is presented separate from 17b-Estradiol as it may include a larger class of compounds including 17b-Estradiol and 17a-Estradiol, and the specific compound used was not clarified in all sources.

[D] Not available or not found in the literature.

[E] Estimated from K_{OW}.

sure pathways exist for humans and wildlife consuming either water or biomass.

Other chemical and physical properties of some com-mon e-EDCs are listed in Table 5. The solubility values would suggest that most e-EDCs would generally not remain in solution. However, the e-EDCs in this table have been identified in water samples collected throughout the world (Thurman et al., 1992; Ying et al., 2002; Fergu-son et al., 2001; Rice et al., 2003; Stachel et al., 2003; Pet-rovic´ et al., 2004; Pere´-Trepat et al., 2004). In some cases e-EDCs have been found in groundwater and drinking water samples suggesting some type of soluble transport (Petrovic´ et al., 2003; Lopez-Roldan et al., 2004). Possible hypotheses for these observations include (1) more soluble precursors or metabolites experienced transport (e.g. nonylphenol carboxylics), (2) colloid facilitated transport,
(3) enhanced solubility through elevated pH (many e-EDCs have a pK_a around 10), and (4) the formation of micelles. Longer chain nonylphenol ethoxylates can have
critical micelle formation concentrations (CMC) of $4.25 \cdot 10^{5}$ mg/l (Brix et al., 2001). The formation of micelles can greatly enhance the stability of a compound, as well as facilitate the stability of other low solubility e-EDCs in solution.

The metabolites or conjugates of many of the e-EDCs mentioned have been suggested to be important in the transport process. As mentioned, akylphenols may have long chain ethoxylate tails (APxEO), where the x denotes the length of the ethoxylate chain. More commonly exam-ined APxEO include NP1EO, NP2EO, NP3EO, OP1EO, OP2EO, and OP3EO, where OP denotes octylphenol (Fer-guson et al., 2001). Brix et al. (2001) examined the CMC resulting solubility of NPxEO, up to a tail lengths of NP12EO. Halogenated forms of NP and OP have also been reported to be produced in chlorinated wastewater effluent, but at concentrations much lower (>1% of total NPEO) than other alkylphenol metabolites (Ferguson et al., 2001). Bisphenol A (BPA) metabolites have been suggested to mainly form through oxidative rearrangement by aero-bic bacteria and many of those metabolites were observed to have similar estrogenicity to BPA (Suzuki et al., 2004). The degradation products of estradiol, ethylylestradiol, and estrone where not found to be significantly estrogenic in studies of river sediments in the UK (Jurgens et al., 2002).

Colloid particle (particles 0.001–1 lm) formation in river water has been demonstrated for e-EDCs including; estrone, 17b-estradiol, 17a-ethynylestradiol OP, NP, and BSA (Liu et al., 2005). This study estimated partitioning coefficients for each of these e-EDCs into the colloidal phase. The authors also found poor correlation between
the colloidial partitioning coefficients and the water–octa-nol partitioning coefficients (K_{ow}) (Liu et al., 2005). This result indicates that the dominant mechanisms for binding of e-EDCs to colloidal particles may not be controlled by its physiochemical properties, which is expected to domi-nate e-EDC sorption in sediments.

3.3. Fate and transport studies

The partitioning of e-EDCs in the environment will ulti-mately determine the conditions under which transport occurs and thus the fate of these compounds. As seen sche-matically in Fig. 1, e-EDCs have been found in surface water, wastewater, sediment, groundwater, aquatic life, and even in the atmosphere. Various reported concentra-tions for selected e-EDC in these different environmental media are listed in Table 6. While it is clear that the highest concentrations of e-EDCs have been observed in sediments and wastewaters, there are smaller quantities present in air and drinking water that may still be estrogenically active.

NP and NPOEs have been detected in ng/m^3 concentra-tions in air near an industrialized area in Italy (Cincinelli et al., 2003). It was determined that the concentrations of NP and NPOE were correlated to winds from the direction of a wastewater treatment plant, suggesting aerosolization from the plant. Similar NP and OP concentrations were also found in air samples collected near the lower Hudson River Estuary in the United States (van Ry et al., 2000). Xie et al. (2004) determined Henry's law constants for NP and vari-ous NPOEs and used the results from van Ry et al. (2000) to estimate net deposition of the e-EDCs from discharges onshore to the bay. These examinations demonstrate that atmospheric release from wastewater treatment plants and subsequent deposition by way of rain water have the poten-tial to be a significant component in e-EDC partitioning, transport, and fate, in the environment.

While e-EDCs comprise a number of different com-pounds, degradation does appear to occur to most of the common xenoestrogens rendering them inactive. Chang et al. (2005) observed anaerobic degradation rates for NPOEs of 0.029 l/day in a wastewater solids digester. Removal of pharmaceutical estrogens (17b-estradiol, 17b-ethylylestradiol, and estrone) by ozonation has also been observed by Huber et al. (2005). Photochemical degrada-tion of NP and NPOEs has been observed by Ahel et al. (1994), with 10–15 h half-lives. Laboratory studies of sorp-tion and degradation in aquifer materials performed by Ying et al. (2003), found half-lives for the 17b-estradiol and 4-n-nonylphenols were 2–7 days under aerobic condi-tions. This study also found that the half-life for 17a-ethy-nylestradiol of 81 days, with little change in BPA or OP. No degradation of these e-EDCs was observed under anaerobic conditions (Ying et al., 2003).

Canadian researchers applied the YES bioassay was applied, along with other methods, to examine the persis-tence and degradation of estrogenic hormones in soils (Col-ucci et al., 2001; Colucci and Topp, 2001). The YES assay results of estrogenicity over time agreed reasonably well with degradation rates monitored using radioactive carbon labeled 17b-estradiol. These authors found rapid degrada-tion of estrogenic hormones (17b-estradiol, estrone, and 17a-ethynylestradiol), decreasing estrogenic response and immobilization of these compounds close to background levels within 60 days (Colucci et al., 2001; Colucci and

Table 6
Selected examples of e-EDC concentrations measured in various environmental media

EDC	Surface water (ng/l)	Sediments (lg/g)	Groundwater (contaminated) (ng/l)	Drinking water (ng/l)	Wastewater effluent (ng/l)	Sewage sludge (lg/g)	Air (ng/m^3)
17b-Estradiol (E2)	1.9–6.0 [2] 0.15–3.6 [6] 1.4–3.2 [8] <0.1–0.7 [11]	220–2480 [25] 50–530 [26] 0.9–2.1[a]	13–80 [1]	0.20–2.1 [6]	650 [3] 0.15–5.2 [6] <0.1 [7] 4.5–8.6 [8] 1–5.6 [13] <0.4–4.3 [15]	0.00057 [7] Dewatered	
Estrone (E1)	0.10–4.1 [6] 1.1–3.0 [8] <0.1–17 [11] <0.4–2.12 [15]	<0.04–0.39 [15] 160–1170 [25] 70–2520 [26] 0.4–0.6[a]		0.20–0.60 [6]	0.35–18 [6] <0.1 [7] 4.3–7.2 [8] 1.2–19 [13] <0.4–12.2 [15]	0.00143 [7] Dewatered	
Ethynylestradiol (EE2)	0.1–5.1 [6] 1.1–2.9 [8]	<50–500 [25]		0.15–0.50 [6]	0.1–8.9 [6] 2.7–4.5 [8] <1–1.5 [13] <0.4–3.4 [15]	0.00061 [7] Dewatered	
Estriol (E3)	1.0–2.5 [8]	0.5–1.5[a]			5.0–7.3 [8]		
Bisphenol A	0.5–14 [6] 85–250 [9] <3–230 [14]		3–1410 [19] 20–44 [19] Drinking water well	0.50–2.0 [6] 20–44 [19] Groundwater well	4.8–47 [6] 18–40 [9] 15–258 [12]		
Nonylphenol (NP)	<100–15000 [5] <10–920 [5] <110–640 [5] <20–1200 [5] <77–420 [5] 6.7–134 [6] <33–225 [9] 100–7300 [22] 290–370 [23]	0.022–0.645 [5] <0.05–0.26 [5] <0.003–2.96 [5] 2.35–4.61 [5] <0.01–1.05 [5] 0.03–9.05 [5] 6.4–154 [10] 0.130–0.190 [23] 0.012–21 [24]	200–760 [18]	2.50–16 [6] 10–2700 [22]	25–770 [6] 18–185 [9]	5.4–887 [16] Dry weight	0.01 to 81 [17] <0.002–81 [20] <0.001–10 [21]
Nonylphenol ethoxylates (NP1EO–NPnEO)	<220–1050 [4] <100–31000 [5] <20–10000 [5] <60–600 [5] <40–520 [5] <20–11000 [5] 1000–97600 [22]	0.05–30 [4] <0.015–38 [5] <0.003–0.17 [5] 0.16–3.97 [5] 0.04–0.25 [5] 0.05–30 [5]	<10–8400 [18] 14 000–38 000 [19] 2900–22 400 [18] Nonylphenol carboxylic	100–300 [22]	320–1570 [12]	<0.5–254 [16] Dry weight	<0.001–14 [21]
Octylphenol (OP) and Octylphenol ethoxylates (OPEO)	7–40 [4] <10–190 [5] <5–84 [5] <20–90 [5] <100–13000 [5] 0.8–54 [6] 61–66 [23]	<0.005–0.090 [4] <0.01–1.08 [5] 0.05–0.18 [5] 0.002–0.34 [5] 1.8–8.8 [10] 0.027–0.049 [23]		0.20–4.9 [6]	2.2–73 [6] 281–358 [12]	<0.5–12.6 [16] Dry weight	0.01–2.5 [20]

[1]Wicks et al. (2004); [2] Dorabawila and Gupta (2005); [3] Kolodziej et al. (2004); [4] Ferguson et al. (2001); [5] Petrovic´ et al. (2004); [6] Kuch and Ballschmiter (2001); [7] Braga et al. (2005a,b); [8] Cargoue¨t et al. (2004); [9] Heisterkamp et al. (2004); [10] Hilscherova et al. (2002); [11] Kolodziej et al. (2004); [12] Ko¨rner et al. (2000); [13] Pawlowski et al. (2003); [14] Suzuki et al. (2004); [15] Williams et al. (2003); [16] La Guardia et al. (2001); [17] Ying et al. (2002); [18] Ahel et al. (1996); [19] Rudel et al. (1998); [20] van Ry et al. (2000); [21] Cincinelli et al. (2003); [22] Shao et al. (2005); [23] Cheng et al. (in press); [24] Mibu et al. (2004); [25] Braga et al. (2005b), Reddy and Brownawell (2005).

[a] Unpublished data from authors of this review analyzed using ELISA in samples upstream and downstream from a wastewater treatment plant on the Sacramento River, near Redding, California, USA.

Topp, 2001). Other studies of transport through soils have been performed in lysimeters, with sewage sludge and e-EDC mixtures applied at the surface (Dizer et al., 2002). This investigation found measurable estrogenic response in soils from 30 and 90 cm depth and suggested that a fast mobilization may have occurred due to the soluble fraction and colloid facilitated transport. Addition transport studies of estrone and 17b-estradiol found log K_{OC} values similar to those in Table 5 (Das et al., 2004). In addition, the authors conclude that the sorption, degradation and transport of these e-EDCs could be represented by first order kinetics, but that an accurate description of degradation would require higher order kinetic models (Das et al., 2004).

Bioaccumulation of NP and NPOEs has been observed in fish and algae, with bioconcentration factors on the order of 1–300 for fish and up to 10 000 for algae (Ahel et al., 1993). However, despite the elevated concentrations in the primary producers (algae) in the food web, no bio-magnification (concentration in consumers) was observed in the consumers (the fish) (Ahel et al., 1993). This finding was confirmed by Hu et al., 2005 who found no evidence of biomagnification for 4-NP or NPOEs. Marine organisms (oysters and snails) off the coast of Taiwan studied by Cheng et al. (in press) were found to bioaccumulate alkyl-phenols. This study estimated biomagnification factors that varied seasonally with higher values during August ranging from 1.4 to 4.3 for the alkylphenols (Cheng et al., in press). Bioaccumulation of 17a-ethinylestradiol has also been observed in freshwater endobenthic organisms, with dry weight bioconcentration factors of 254, which if extended to a steady-state condition could be up to 646. (Liebig et al., 2005). Observations suggesting bioaccumulation in fathead minnow have also been reported (La¨nge et al., 2001). Therefore, while there is no direct evidence for bio-magnification of e-EDCs concentrating in higher trophic levels of food web levels, there have been observations sug-gesting the possibility for biomagnification (Cheng et al., in press). Moreover there is convincing evidence that some e-EDCs bioaccumulate in specific aquatic species (Ahel et al., 1993; Hu et al., 2005; Liebig et al., 2005; Cheng et al., in press).

3.4. Biologically directed analyses

While a majority of the e-EDC source and distribution studies have use mass-based analytical techniques like HPLC, GC/MS, and LC/MS/MS, screening the large num-ber of samples required for fate and transport characteriza-tion could be more efficient using BBAs. Using results from these screening methods to evaluate the presence of e-EDCs, more targeted investigations may be used to identify the compounds involved and their degradation, fate and transport in that environment. However, successful appli-cation of biosensors in the field can be a complicated engi-neering problem and research is still needed to transform laboratory bioassays into portable field biosensors (Rodri-guez-Mozaz et al., 2004a). Moreover, apart from toxicity issues at high e-EDC concentrations, as well as, agonism and antagonism in complex environmental mixtures in samples, biosensors may be more versatile for screening raw samples than analytical techniques.

Screening for xenoestrogens will often express estrogenic potency in relation to an estrogen-like estradiol. Cal-culation of the estrogen equivalent concentration (EEQ) of a chemically determined mixture is based on all mea-sured estrogens with a known estradiol equivalency factor (EEF) according to:

$$EEQ_i = C_i \times EEF_i; \quad \text{and} \quad EEQ_t = \sum EEQ_i \qquad (1)$$

where i refers to compound i in the mixture with concentra-tion C, and EEQ_t is the total EEQ. The EEFs are usually expressed on a molar basis because this is toxicologically more relevant than expressing concentrations on a weight basis (de Voogt and van Hattum, 2003). Some examples of EEFs may be seen in Table 5. An assessment of estro-genicity in sediments collected from marine locations throughout The Netherlands using the ER-CALUX assay found EEQs ranging from 4.5 to 38.4 (Legler et al., 2002b). Given the affinity of e-EDC to sorb to sediments, these EEQ demonstrate the potential for accumulative estrogenic potential in sediments.

A synthesis of a large data set (including 32 different geographic locations) on e-EDCs available for coastal and harbor waters and sediment in Spain was attempted in Pere´-Trepat et al. (2004). Statistical analyses including principal components analysis and a multivariate curve res-olution using alternative least squares method were applied the data set to identify relationships between measured e-EDCs and sources. The study found that the geographic location of the e-EDC source could be reasonably identi-fied using three principle components for water samples and four for sediment samples. Interestingly the study con-cluded that, although ‘‘hot spots’’, sources of e-EDCs, could be generally identified using these techniques, the over all distribution of e-EDCs suggested ubiquitous sources (Pere´-Trepat et al., 2004). This study demonstrates the potential for non-point sources of e-EDC and that con-trolling point source discharges from wastewater treatment plants or industrial sources could be insufficient to reduce e-EDCs to below active levels in water and sediment.

4. Summary and conclusions

We have summarized many of the biologically based assays (BBAs) available for detection and quantification of estrogenic endocrine disrupting compounds (e-EDCs) from the perspective of selecting an environmental monitor-ing approach. Results relating to fate and transport of e-EDCs were discussed including sources, potential transport mechanisms, and strategies for large scale characterization. Areas in need of continuing research include the adaptation of BBAs into field portable biosensors, source control strat-egies to reduce the mass of e-EDCs introduced into the

waste stream, tertiary treatment strategies for wastewater treatment plants, continued large scale characterization of e-EDC contamination, and finally approaches to environ-mental remediation of e-EDC contaminated sites.

A comparison of the various BBAs reveals that while the most commonly applied approaches are the ELISA and YES assays, there are many promising technologies avail-able including ER-CALUX, ELRA, Endotect^{TM}, RIANA, and IR-bioamplification. There are comparability prob-lems for estrogenic activity measurements made using dif-ferent bioassays, however, these issues can be taken into consideration in designing environmental monitoring regimes. A field portable assay is needed for the environ-mental monitoring and management should be the means to achieve the goal of limiting exposure of humans and wildlife to e-EDCs. The two promising field portable bio-sensors the Endotect^{TM} and RIANA both use biological detection strategies without whole cell bioassays. Other approaches that appear to have future potential as field portable assays are the IR-bioamplification and electro-chemical biosensors.

The major advantage provided by biosensors for estro-genic activity is the capacity to estimate the cumulative e-EDC effects of a variety of chemicals in an environmental sample. Biosensors may not perform well in all the neces-sary media including wastewater, sediments, or biological materials, however, the spatial and temporal resolution from a reliable biosensor could focus investigations on a compartment where e-EDC mass has partitioned. Then the more sensitive laboratory techniques may be performed on fewer samples of similar media. Biosensors could greatly improve e-EDC monitoring schemes and aid in the devel-opment of environmental management solutions. The direct relationship between in vitro bioassays and in vivo effects on aquatic organisms and wildlife is a continuing area of research. However, the advantage of using a bioas-say a screening tool in a bioassay-directed chemical analy-sis (BDCA) or toxicity, identification, and evaluation (TIE) approach is great (Routledge, 2003; Petrovic´ et al., 2004). The impossibility of analyzing samples for all the possible known e-EDCs, even neglecting unknown e-EDCs, necessi-tates the BDCA or TIE approach. In addition to screening for sources and directing more detailed analyses, these bio-assays can be applied to numerous monitoring questions including; (1) time-repeated measurements for variability and concentration patterns (over months, seasons, years),
(2) transport through the vadose zone, and (3) partitioning between water, sediment, air at a single location. The abil-ity to address these sorts of issues would greatly enhance our understanding of e-EDC transport, fate, and impacts allowing for better environmental management.

Source control strategies may include discouraging over-prescribing pharmaceutical estrogens, the testing and use of alternative surfactants to replace nonylphenol ethoxylates as proposed by Fernandez et al. (2005), or other means to reduce the mass of estrogens, surfactants, and industrial chemicals in wastewater discharges. Reducing the e-EDC sources to wastewater treatment plants could decrease dis-charge e-EDC concentrations from those facilities to estro-genically inactive levels.

Alternatively if source reduction is not possible, then more testing is needed on tertiary treatment technologies and treatment efficiencies for e-EDCs. Various treatment options have been discussed (Ko¨rner et al., 2000; La Guar-dia et al., 2001; Braga et al., 2005a; Johnson et al., 2005), and research into the optimum way to achieve adequate treatment at wastewater facilities is needed.

While biologically directed sampling and analysis may greatly aide large scale characterization of e-EDC contam-ination, approaches are still needed for environmental remediation or restoration of degraded habitat. Most of the e-EDCs discussed have been found to have relatively short half-lives on the order of weeks to months in soils and sediments (Ying et al., 2003). Therefore, monitored natural attenuation in combination with reducing sources of e-EDCs is a viable option. Although the evidence sug-gests that e-EDCs do not biomagnify, they do bioaccumu-late in specific species (Ahel et al., 1993). Therefore the main impacts of e-EDCs are likely to be at the species level which would require additional research into reintroduc-tion and wildlife management of affected species after e-EDC exposure.

It is clear that environmental management of e-EDC contamination in surface and ground water remains a major challenge for the scientific and engineering commu-nities. However, with more research on source reduction and control, treatment technologies, environmental resto-ration, and field monitoring using BBAs, science will help to address this pressing environmental problem.

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