Endocrine-Disrupting Compounds: A Review of Their Challenge to Sustainable and Safe Water Supply and Water Reuse

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ABSTRACT: The relevance of endocrine-disrupting compounds as potential contaminants of drinking water is reviewed, particularly in the reuse of wastewater. Growing populations and increasing intensification of land and water use for industry and agriculture have increased the need to reclaim wastewater for reuse, including to supplement the drinking water supply. The variety of anthropogenic chemicals that have been identified as potential endocrine disruptors in the environment and the problems arising from their use as human and livestock pharmaceuticals, as agricultural chemicals and in industry are discussed. The potentially adverse impact of these chemicals on human health and the ecology of the natural environment are reviewed. Data for the removal of estrogenic compounds from wastewater treatment are presented, together with the comparative potencies of estrogenic compounds. The relative exposure to estrogens of women on oral contraceptives, hormone replacement therapy, and through food consumption is estimated. A brief overview of some methods available or under development for the assessment of estrogenic activity in environmental samples is provided. The review concludes with a discussion of the directions for further investigation, which include human epidemiology, methodology development, and wastewater monitoring. © 2006 Wiley Periodicals, Inc. Environ Toxicol 21: 181–191, 2006.

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INTRODUCTION

In an era focused on sustainability, the water industry is faced with the challenge of ensuring a sustained and safe supply of drinking water from sources of varying quality, including the reuse of wastewater. Population growth, urbanization, industrial development, and associated changes in agricultural and other land-use practices have contributed significantly to reducing water quality through naturally occurring and anthropogenic contamination (Guillette and Crain, 2000; Sumpter, 2005). During the last few decades, the focus on chemical pollution has been largely directed toward the well-known ''priority'' pollutants, especially those displaying persistence in the environment. This is likely to be only one piece of a larger puzzle (Daughton and Ternes, 1999). Other surface water contaminants include a variety of metals, carcinogenic organic compounds, synthetic chemicals, pharmaceutical, veterinary, and illicit drugs, ingredients in cosmetics and other personal care products, and food supplements, together with their respective metabolites and transformation products (Weyer and Riley, 2001; Kolpin et al., 2002). These are of international concern because of their potential to adversely affect human health through consumption of drinking water (WHO/IPCS, 2002).

Reclaiming wastewater will help to supplement the existing drinking water supplies but has a range of potentially adverse health outcomes, including hormonal effects. In considering wastewater reuse, emphasis will need to be placed on endocrine-disrupting compounds (EDCs) because of the current public concern coupled with the lack of scientific knowledge. Improved understanding of the action of EDCs is required to assess and minimize the risks associated with human exposure to harmful substances in reclaimed water.

This article provides an overview of EDCs (including their adverse effects on health and techniques for monitoring them) that brings together the issues faced by the drinking water industry in trying to balance the need for a sustained water supply while maintaining the quality required to ensure the public's health. Endocrine disruption needs to be assessed as part of the consideration of all potentially toxic effects of a range of emerging and as yet poorly understood contaminants in surface water and wastewater.

The aim of this review is to alert those concerned with safe drinking water to the issues and problems surrounding EDCs, especially in the context of contaminated water sources and potable reuse of wastewater.

Endocrine-Disrupting Compounds

Researchers were first alerted to aquatic contamination by endocrine-disrupting compounds through observation of a variety of reproductive changes in different species of fish and mollusks sampled immediately downstream of sewage treatment plant outfalls (Howell et al., 1980; Bortone and Davis, 1994; Jobling et al., 1998, 2004; Batty and Lim, 1999; Folmar et al., 2001; Gagne et al., 2002). EDCs, defined as ''exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)-populations'' (WHO/IPCS, 2002), can enter waterways by many routes including by direct discharge into water; use of pharmaceuticals and chemicals by householders and in agriculture and industry; accidental spills and releases of compounds; and indirectly through diffuse sources such as storm water runoff. The main sources of EDCs in the rivers and lakes of Europe and North America are sewage effluent and agricultural chemicals from runoff. In less developed countries uncontrolled domestic and industrial discharge to waterways contributes to EDCs (Barnhoorn et al., 2004).

Many anthropogenic chemicals, as well as naturally occurring estrogens and plant secondary metabolites, experimentally have been found to have endocrine-disrupting properties (Coalition, 2003; Naz, 2005). Anthropogenic chemicals include pesticides such as dichlorodiphenyltrichloroethane (DDT; Kelce et al., 1995), dieldrin, and lindane, chemical industry contaminants such as dioxins and dioxinlike compounds; the formerly widely used ship antifouling agent tributyltin (TBT); organotin compounds used in PVC water pipe manufacture; industrial chemicals such as polychlorinated biphenyls (PCBs) and phthalate plasticizers (Jobling et al., 1995); detergent breakdown products such as nonylphenol (U.S. EPA, 1997); and bisphenol A, a compound employed in the manufacture of polycarbonate plastics, used in baby bottles and teats (Lyons, 2000; Table 1).

The relevant issue for the water industry is determining the extent and concentration of EDCs at present and in the future in drinking water sources, including ground- and wastewaters, as well as surface water into which discharges occur. This will require extensive monitoring in order to establish the nature and quantity of the compounds. Recent studies of surface water contamination in the United States have shown that a wide variety of potential EDCs have concentrations unlikely to be of concern to the water industry (Kolpin et al., 2002). However, as discussed later, wastewater discharge can contain EDCs at levels that pose a potential risk when such wastewater is used as a drinking water source.

Contamination of the environment or of drinking water may occur from such simple routes as use of manufactured plastic articles. For example, plasticizers such as d-2-ethylhexyl phthalate (DEHP; Kolpin et al., 2002) have been frequently detected in surface water, groundwater, and treated (potable) water in the United States, and DEHP has been classified as a priority pollutant. Another plasticizer, diethyl phthalate (DEP), is used in cosmetics, insecticides, and aspirin (U.S. EPA, 2001). It too is used in PVC water pipe manufacture, creating the possibility of its leaching into drinking water. Also used in PVC water pipes are organotin compounds, which leach into treated drinking water (Sadiki and Williams, 1999).

The quantity and variety of pharmacological agents passing into sewage treatment vary considerably, depending on the upstream origin such as from hospitals and urban areas or from intensive animal production. Some pharmaceuticals are excreted in an unmetabolized form or in conjugates that release the active molecule into sewage

TABLE I. Potential endocrine-disrupting chemicals (based largely on animal studies)

Synthetic origin
Hormones
17α-Ethinylestradiol
Diethylstilbestrol
17β-Trenbolone
Herbicides
Atrazine
Simazine
Methoxychlor
2,4-D
Insecticides
DDT
Dieldrin
Endosulfan
Lindane
Industrial chemicals
Phthalates
Bisphenol A
p-Nonylphenol
PCBs
Tributyltin
Biological origin
Hormones
17β-Estradiol
Estriol
Estrone
Progesterone
Testosterone
Phytoestrogens
Sesquiterpenes
Phytosterols

being treated. Some pharmaceuticals persist in the environment for more than a year (Halling-Sorensen et al., 1998). In the highly populated Po Valley of Italy, a range of pharmaceuticals was measured in river water, sediment, and drinking water, resulting in the identification of seven human pharmaceuticals and one agricultural growth promoter. South of Milan, Italy, an additional three drugs and two agricultural growth promoters were measured in the River Lambro. Only two pharmaceuticals, clofibric acid and diazepam, were found in treated drinking water at detectable concentrations, and neither has been shown to have EDC activity (Zuccato et al., 2000).

Natural mammalian hormones, including estrogens, can be released into the environment via sewage effluent and from sources such as animal feedlots. There is potential for biologically active concentrations of these substances, along with pharmaceuticals, in surface water in areas of intensive livestock production and downstream of sewage discharges. Studies in the United Kingdom have shown that the hormones 17β-estradiol, 17α-ethinyl estradiol, and estrone, although excreted in inactive conjugates, can be degraded in sewage treatment plants to release the active steroid hormone (Desbrow et al., 1998). In Australia concentrations of 17β-estradiol from <5 (the minimum limit for reporting) to 20 ng/L and of estrone up to 78 ng/L have been detected in secondary treated effluent (Table 2). The pharmaceutical 17α-ethinyl estradiol (EE2), one of the active ingredients in birth control pills, was not detected, possibly because of the analytical method employed. At the Landsborough Water Reclamation Plant in Queensland, Australia, estrogens were not detectable at the reporting limit, 5 ng/L, after tertiary treatment (sand filtration, ozonation, and UV disinfection; Chapman 2002a; Leusch et al., 2005). However, these hormones are known to be above threshold concentrations for detection of < 1 ng/L in laboratory bioassay systems (Koerner et al., 1999). Concentrations of 17β-estradiol of up to 11 ng/L, 17α-ethinyl estradiol up to 9 ng/L, and estrone up to 17 ng/L have been reported in treated effluent from the United Kingdom (Table 3). Estrogenic effects in wild fish in the United Kingdom have been shown to be widespread in UK rivers (Jobling et al., 1998; Sumpter, 2005).

Adverse Endocrine Effects

Effects on Humans

As a preliminary comment on the existing data on adverse human effects from endocrine-disrupting compounds in the natural environment, it needs to be made clear that current human epidemiological data is inadequate for drawing any definitive conclusions. Only in cases of pharmacological dosing, accidental exposure, and occupational exposure has unequivocal evidence of injury been demonstrated (see WHO/IPCS, 2002).

Organochlorine pesticides have been used to control a variety of pests since the 1940s (U.S. EPA, 1997). There is evidence of a causal link between human occupational exposure to some pesticides during pregnancy and subsequent stillbirth (Vaughan et al., 1984; McDonald et al., 1988; Taha and Gray, 1993; Pastore et al., 1997). Exposure to PCBs and dioxins, particularly by prenatal exposure, has been associated with long-term or permanently adverse effects on neurological development in children, including psychomotor developmental delays, cognitive impairment, and behavior abnormalities (Stewart et al., 2003). Exposure to organochlorines and dioxins has also been associated with endometriosis, a condition in which endometrial glands and stroma occur in locations outside the uterine lining, but the evidence for this in humans is not conclusive (WHO/IPCS, 2002).

The association between exposure to DDT and the most prevalent of its breakdown products, dichlorodiphenyldichloroethylene (DDE), and breast cancer is also not conclusive (Snedeker, 2001). Dieldrin, another organochlorine pesticide, has been studied for its association with breast cancer and endometrial cancer. These associations, together

TABLE II. Concentrations (ng/L) of estrogens from wastewater treatment (STP) and reclamation (WRP) plants in Queensland, Australia in 2001–2002[a]

Level of treatment	Location	17β-Estradio 1	17α-Ethinyl estradiol	Estrone
Influent	Landsborough WRP	18	< 5	78
Influent	Landsborough WRP	20	< 5	< 5
Secondary	Landsborough WRP	19	< 5	< 5
Secondary	Carole Park STP	< 5	< 5	46
Secondary (chlorinated)	Cooroy STP	11	< 5	46
Secondary	Cooroy STP	11	< 5	13
Secondary	Oxley STP	< 5	< 5	54
Tertiary	Landsborough WRP	< 5	< 5	< 5

Limit of detection: 5 ng/L. Data from Falconer, Moore, Chapman and Ranmuthugala (2003).

with the reports suggesting a relationship between exposure to pesticides and testicular cancer, need further investigation prior to drawing definitive conclusions (WHO/IPCS, 2002). The clearest epidemiological links between human exposure to pesticides and adverse health outcomes have been made for occupational exposure, not for general environmental contamination or ingestion of food or water. This suggests that at present drinking water supplies do not present a detectable risk to consumers from pesticides.

The association between exposure to pharmaceutical estrogens and effects on the endocrine system (either desired or manifested in adverse reproductive outcomes) is well established. The current use of pharmacological doses of estrogens (5–20 μg/day in oral contraceptives, 1000 μg/day in hormone replacement therapy) to achieve positive health outcomes is both widespread and recognized as safe. However, the earlier use of large doses of up to 100 mg/day of the synthetic estrogen diethylstilbestrol in human therapy had clearly documented adverse outcomes (Swan, 2000). For example, offspring of women exposed to diethylstilbestrol (DES) have shown higher than normal rates of cancer and of malformations of the reproductive organs (Gill et al., 1979; Nollar et al., 1990).

Exposure of women to stilbestrol during pregnancy has been linked to several reproductive abnormalities in male offspring, including cryptorchidism, hypospadias, fertility problems from abnormal sperm and low sperm counts, and higher risk of testicular cancer and testicular dysgenesis syndrome (Henderson et al., 1976; Gill et al., 1977; Stillman, 1982; Sharpe and Skakkebaek, 1993; Moller et al., 1996; Toppari et al., 1996).

The adverse effects of stilbestrol, formerly used in therapy, has raised the issue of whether all estrogens are similar. That is, do stilbestrol, natural estrogens, and plant estrogenic compounds act through the same mechanisms and hence are associated with the same risks. A recent study showed that the initial gene activation responses to all three estrogens are similar, leaving open the question of whether stilbestrol presents a unique human hazard (Moggs et al., 2004).

Environmental estrogens have also been linked to the recent decline in the human sex ratio (WHO/IPCS, 2002), but the evidence for this association is not conclusive (Jongbloet et al., 2001, 2002). Recent research and discussion, however, support the implication that environmental pollution leads to an decreased number of male births (Hood, 2005; Mackenzie et al., 2005).

The types of naturally occurring compounds that have been demonstrated to have endocrine-disrupting activity include the so-called phytoestrogens and androgens, which are naturally occurring plant compounds related chemically to steroid hormones. This was powerfully illustrated by the occurrence of lactation in castrated male sheep and infertility in female sheep grazing on phytoestrogen-rich pasture species (Adams, 1998) and by the masculinization of female fish from exposure to phytoandrogens in effluent from paper mills (Howell et al., 1980; Durhan et al., 2002). Numerous plant extracts used in herbal remedies have endocrine activity, such as the various terpenoids found in

TABLE III. Concentrations of estrogens (ng/L) in sewage effluent in Europe, North America, and Australia

Treatment type	County of origin	17 β-Estradiol	17 α-Ethinyl estradiol	Estrone
Activated sludge system[a]	Netherlands	0.09	< lor	4.5
Activated sludge system[a]	Netherlands	< lor	< lor	0.4
Clarification and aeration[a]	Germany	< lor	1	9
Clarification and aeration[a]	Canada	6	9	3
Treated effluent[a]	Britain	11	0.73	17.3
Activated sludge[b]	Australia	< 5	NA	< 1–27

[a] Desbrow et al. (1998, Table 1): lor - limit of reporting, < 1 ng/L
[b] Leusch et al. (2005c, Table 3).

products like neem, a pharmacologically active extract from an Indian tree (*Azadirachta indica*), used medicinally in India as a contraceptive and an abortifacient (National Research Development Corporation of India, 2003). Outside India, it also is now in use as a herbal skin care product.

Recently there has been recognition that water may contain components of widely used personal care products (e.g., triclosan) and veterinary pharmaceuticals such as antibiotics, some of which have known EDC properties (Klaassen, 2001). The development and function of the immune system is also sensitive to a range of toxicants; however, the majority of immunotoxic agents do not appear to act through the endocrine system.

Several chemicals have been identified as adversely affecting neural development in the human fetus. However, this is unlikely to occur through endocrine disruption, and further research is needed to clarify both mechanisms and dose/response effects. Dioxins and polychlorinated biphenyls (PCBs) have particularly been implicated. PCBs were widely used in lubricants, coatings, and insulation material in electrical transformers (Schettler et al., 2000). As PCB use has now been discontinued, and the adverse effects of dioxins are widely appreciated, the likelihood of PCBs in water supplies has been reduced.

The evidence to date for endocrine disruption in humans from exposure to environmental chemicals is limited, and the issue remains controversial (Degen and Bolt, 2000; Safe, 2000).

Effects on Wildlife

The most consistent evidence for endocrine disruption by water contaminants comes from wildlife (Sumpter, 2005). Reduced fertility has been observed in some fish species (Bortone and Davis, 1994). The effects range from masculinization of female fish (Denton et al., 1985) and feminization of male fish (Gimeno et al., 1998a, 1998b; Batty and Lim, 1999) to problems with spawning time (Kramer et al., 1998). Alkyl phenols (e.g., nonylphenol and octylphenol), which are breakdown products of alkylphenol polyethoxylates used as industrial surfactants and bases for household products, have been linked with estrogenic effects in fish (Jobling et al., 1996).

Among agricultural chemicals in current use suspected of having endocrine-disruptive activity are atrazine and related herbicides such as simazine; endosulfan, methoxychlor, and fenitrothion (pesticides); and vinclozolin and ketoconazole (fungicides). Simazine has been demonstrated to impair reproduction and molt frequency in the water flea *Daphnia pulex* at 4–20 mg/L (Fitzmayer et al., 1982). Atrazine is a herbicide with neuroendocrine effects on reproduction and mammary tumor growth in rodents and on reproduction in fish (WHO/IPCS, 2002).

Xenobiotics can act as endocrine disruptors without affecting hormone binding by modulating endogenous hormone levels. Fenitrothion, vinclozolin, and ketoconazole have antiandrogen effects on mammals by inhibiting key enzymes of androgen synthesis (WHO/IPCS, 2002). Methoxychlor is an organochlorine pesticide used against a range of pests, and methoxychlor and/or its metabolites are believed to have estrogenic effects on mice (U.S. EPA, 2001). In this case contamination of drinking water is regarded as a potential health risk, and the U.S. EPA advises that water containing more than 0.05 mg/L of the parent compound or 0.2 mg/L of the metabolite should not be consumed by children or adults, respectively (U.S. EPA, 2001).

The surfactants widely used in agricultural chemical formulations frequently contain nonylphenol derivatives, which are suspected of having low-level EDC activity. Human exposure to alkylphenols (AP) or alkylphenol ethoxylates (APE) may occur through drinking water that is drawn from a polluted source. However, there is no conclusive evidence that APs or APEs can adversely affect human health. There are, however, reports of alkylphenols causing production of the female-associated liver protein vitellogenin in male fish (Jobling, 1995) and delaying the onset of puberty as well as causing testicular damage in juvenile rats (Tan et al., 2003). However, at environmentally relevant concentrations, nonylphenol did not have adverse effects on Japanese medaka, compared to the highly disruptive effects of estradiol (Nimrod and Benson, 1998).

The basic mammalian toxicology of agricultural chemicals has been thoroughly investigated, so that they could be registered for use in individual countries. As a consequence, their toxic effects and the doses at which they are toxic are known for test animal species. Low-level chronic exposure leading to endocrine and early developmental effects may not be as well characterized, as this characterization is not generally a requirement for registration for use.

Occurrence of EDCs in Surface Waters

Agricultural runoff is responsible for most of the pesticides found in surface waters, with concentrations highest after the first storm following application (Chapman, 2002b).

The locations with the greatest likelihood for human and ecological impact from agricultural chemicals are irrigation areas that use chemicals in the highest quantities and with the most frequency.

The United States Geological Survey (USGS) recently examined 139 streams across 30 states in the United States. This survey was biased toward streams susceptible to contamination (i.e., downstream of intense urbanization and livestock production). Organic wastewater contaminants (OWC) were detected during this study in 80% of the streams sampled (Kolpin et al., 2002). The detection rate for steroids and hormones in these waters ranged from

TABLE IV. Estrogenic equivalents (EEQs) of EDCs compared to 17β-estradiol using E-screen cell proliferation assay

Compound	EEQ[a]	Reference
Diethyl stilbestrol	10	Soto et al. (1992)
17β-estradiol	1	Soto et al. (1992)
Genistein	0.00020	Fang et al. (2000) and Koerner et al. (2001)[b]
4-*tert*-Octylphenol	0.000065	Fang et al. (2000) and Koerner et al. (2001)[b]
Nonylphenol	0.000003	Soto et al. (1992)
o,p′-DDT	0.000001	Soto et al. (1992)

[a] Quantification of estrogenicity: EEQ (estrogen equivalent) = $EC_{50ESTRADIOL}/EC_{50\ SAMPLE}$.
[b] Mean of values reported in Fang et al. (2000) and Koerner et al. (2001).

1.4% (equilin) to 85.7% (coprostanol). Other frequently detected contaminants, in order of frequency of detection, were nonprescription drugs, insect repellents, detergent metabolites, disinfectants, plasticizers, fire retardants, antibiotics, insecticides, PAHs, reproductive hormones, prescriptions drugs, antioxidants, fragrances, and solvents (Kolpin et al., 2002).

In the United States, the highest concentration of wastewater contaminants come from detergents, followed by natural fecal steroids and plasticizers. Hormonally active compounds of natural origin— testosterone, 17β-estradiol, and progesterone—are present in somewhat smaller concentrations than the pharmaceuticals 17α-ethinyl estradiol and 19-norethisterone from oral contraceptives. The maximum detected hormone concentration is lower than 1.0 μg/L, and the median concentration is around one tenth of this (Kolpin et al., 2002).

Exposure Issues

Most potential EDCs enter the human body via ingestion as components of pharmaceutical products such as contraceptive pills and estrogen replacement therapy and through food. A minor proportion comes from drinking water. It is informative to examine the relative potencies of potentially ingested compounds, including hormonal pharmaceuticals and plant and fungal estrogens (Table 4). A 1998 estimate of the comparative intake of estrogens and their biological equivalents indicated that oral contraceptives would be the main source of exposure to estrogen equivalents in women using these pharmaceuticals (Table 5).

It is unlikely that compounds that are not water soluble will pose a risk to drinking water safety in conventionally treated drinking water supplies. However, natural and synthetic chemicals with very low water solubility may still be present in untreated or solely chlorinated drinking water, carried adsorbed to hydrophobic particles. Lipophilic (fat-soluble) EDCs tend to be more persistent, whereas other, more soluble compounds may only be present in the environment for short periods. However, even less persistent compounds may remain in the environment long enough to have adverse effects on a fetus at a critical stage of development (WHO/IPCS, 2002). Many lipophilic EDCs are highly persistent and accumulate in animal tissues, particularly in body fat.

As water becomes scarce because of population growth and increasing agricultural usage, reuse of wastewater, directly and indirectly, becomes an attractive mechanism for supplementing the drinking water supply. This issue is important for coastal cities currently discharging wastewater to the ocean and also for inland cities discharging wastewater for irrigation. In many countries the available good-quality water supplies are already in use. In addition to the potential problems in wastewater of pathogens, heavy metal accumulation, toxic and carcinogenic organic chemicals, and salt content, the potential for redistribution of endocrine-disrupting compounds is an issue of concern. Monitoring data for endocrine disruptors in wastewater discharge (Tables 2, 3, and 6) indicates that several potent estrogenic compounds (ethinyl estradiol and 17β-estradiol, for example) occur in wastewater in concentrations that are biologically active and bioavailable to aquatic organisms. Any adverse effect on human populations at these concentrations remains to be evaluated. Assessment of EDC removal in wastewater and drinking water treatments then becomes essential if their reuse for drinking water is to be considered. The current widespread use of solids from wastewater plants as fertilizers for garden and agricultural applications also allows the redistribution of EDCs that adsorb to wastewater sludge and may be released and taken up in crops or enter waterways via runoff.

TABLE V. Estimated daily doses of estrogen in females consuming pharmaceutical products in terms of estradiol equivalents, EEQ (Pugh and Moore, 1998)

Compound/s	EEQ[a]
Oral contraceptives	16,675
Hormone replacement therapy	3350
Plants and food	102
17β-estradiol (endogenous)	1
Organochlorines	0.0000025

[a] Quantification of estrogenicity: estimated EEQ (estrogen equivalent) = $EC_{50\ ESTRADIOL}/EC_{50\ SAMPLE}$.

TABLE VI. Results of biomarker assays indicating estrogenic activity (EEQ) in reclaimed water[a,e]

	E-screen[b]	ERBA[b]
Treatment	ER activation expressed as EEQ[c]	ER binding assay expressed as EEQ
Influent	20 ± 2.4	54 ± 26
Biological P removal	5.7 ± 0.7	79 ± 11
Bioreactor	20 ± 0.1	4.6 ± 1.8
Clarifier	2.2 ± 0.2	< 0.75 ng/L
Sand filter	2.4 ± 0.2	< 0.75 ng/L
Ozone	Cytotoxic[d]	< 0.75 ng/L
UV	< 0.03	< 0.75 ng/L

[a] Landsborough Water Reclamation Plant, Queensland.
[b] E-Screen—cell culture assay (MCF-7 cells), ERBA—estrogen receptor binding assay.
[c] EEQ (estrogen equivalent) = $EC_{50\ ESTRADIOL}/EC_{50\ SAMPLE}$.
[d] Cells died during assay.
[e] From Leusch et al. (2005a).

Detection and Monitoring of EDCs

Measurement of EDC concentrations (and their rates of natural degradation and biological activity) in surface waters and wastewater effluents are required to assess associated risks. Established analytical methods are available for many of the compounds in water and wastewater including those implicated as endocrine-disrupting compounds (EDCs). Most developed countries have established regulatory authorities with requirements for chemical analysis and methods for testing food or the environment (WHO/IPCS, 2002). These methods are well developed for chemicals such as pesticides, metals, industrial chemicals, and PCBs. However, for some of the other EDCs such as hormones, drugs, and personal care products, the methods for analyzing endocrine activity are less well developed. Some of these substances (e.g., pharmaceuticals) are designed to be biologically active at trace concentrations (in parts per trillion) and may exert an effect on the environment below the routine limit of reporting using standardized chemical analytical methods (Geisy et al., 2002). There are potentially significant classes of compounds that are not studied in detail because of a lack of suitable instrumental techniques or analytical standards.

Many potential endocrine disruptors exist in the environment as mixtures, possibly acting synergistically. The individual components may not have been chemically characterized, in which case, environmental samples are best assessed for endocrine potency with a bioassay, rather than by chemical analysis.

Screening and Testing Programs

In August 1998 the U.S. EPA announced establishment of the Endocrine Disruptor Screening Program (EDSP; U.S. EPA, 2000), which uses a tiered approach (1) to identify substances that have the potential to interfere with the endocrine system and (2) to confirm the potential and characterize the effects of these substances (U.S. EPA, 2000). The EDSP scope includes effects on humans and wildlife, effects on estrogen-, androgen-, and thyroid (EAT) hormone–related processes, and evaluation of chemical substances. Tier 1 screening includes a uterotrophic screen, a Hersberger screen (male rodent-based tests for androgenic activity), a rodent pubertal female screen, a rodent pubertal male screen, estrogen and androgen receptor reporter gene screens, a fish reproduction screen, and a frog metamorphosis screen. Tier 2 includes a two-generation mammalian reproduction and development test and a mysid shrimp reproduction test (U.S. EPA, 2000). U.S. EPA validation work is being conducted in close liaison with the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM), established by the National Toxicology Program under the auspices of the National Institute of Environmental Health Sciences (NIEHS).

Alternate Test Method Development

New and revised toxicological testing methods being developed around the world that incorporate molecular and cellular biology hold promise for reducing whole animal testing. Biological methods can be used as screens to determine if EDC-active compounds are present in a given environmental sample (WHO/ICPS, 2002). These can be carried out together with chemical methods to establish cause and effect and to quantify the EDCs present (Cech et al., 1998). The majority of tests developed so far are either *in vitro* bioassays for assessing estrogenic and antiestrogenic substances and *in vivo* methods using fish or invertebrates. Ideally a battery of screens should be used to address a range of mechanisms of endocrine disruption. Tests are also being developed to detect androgens and antiandrogens, thyroid-active chemicals, and compounds that interfere with steroid biosynthesis and metabolism (WHO/ICPS, 2002).

Bioassays

In vitro and *in vivo* bioassays are useful techniques for determining receptor-mediated activities in environmental samples containing complex mixtures of contaminants. The bioassays determine contamination by pollutants with specific modes of action (Geisy et al., 2002) and also integrate possible interactions between compounds. Extracts from various matrices can be tested to evaluate their biological activity and to identify those samples that require further investigation using resource-intensive analytical techniques. *In vitro* and *in vivo* bioassays offer a rapid, sensitive, and relatively inexpensive solution to some of the limitations of instrumental analysis. Methods that rely on biological activity are finding increasing utility as screening tools because the chemical nature of the endocrine-disrupting sample may be unknown and the biological method may be the best (or only) indicator of EDC activity.

Competitive Estrogen Receptor Binding Assays

Receptor-binding assays measure binding of agonists or antagonists to a specific cellular receptor. They assess endocrine-disrupting activity at the molecular level of biological organization. This is the first level at which signal transduction of these hormones modulates the expression of specific genes. The potency of compounds is dictated by their relative affinity to these receptors. Receptor binding affinity measurements can then be used to indicate the potential of specific compounds or mixtures of compounds to act as EDCs. An estrogen-receptor binding assay (ERBA) using sheep uterus estrogen receptors has been used to derive estradiol equivalents (EEQ) from sewage effluent (Table 6; Leusch et al., 2005a).

Estrogen Receptor Activation and Cellular Proliferation Assays

Estrogen receptor activation depends on the ability of estrogens to induce cellular responses in target organs such as fish liver cells (hepatocyte bioassay; Stephensen et al., 1998) or human breast cancer cell lines such as MCF-7 cells (Koerner et al., 1999). When primary cell cultures are used, a particular protein such as vitellogenin, a protein unique to egg development, can be measured as the end point. One advantage of using whole-cell bioassays is the ability to differentiate a hormone agonist from a hormone antagonist (both of which may show receptor affinity). Cell proliferation can also be induced at very low concentrations of estrogenic substances. The E-screen, using MCF-7 human breast cancer cells, has been used to assess comparative removal of EDCs by wastewater treatments (Table 6; Leusch et al., 2005a). Some of these tests can detect concentrations in the picograms per liter (pg/L) range (Soto et al., 1995).

Whole Animal Assays

Whole animal assays may also be used as a biomarker of exposure and/or effect. For example, the mosquito fish *Gambusia affinis holbrooki* has been used to assess hormonal properties of effluent. The assay for estrogenicity (Batty and Lim, 1999) and also androgenicity (Leusch et al., 2005b) uses morphological changes as the end point. These end points can be monitored in natural environments as indicators of EDC-mediated effects but may not be as sensitive as some of the molecular or cellular end points.

Mollusk genitalia are sensitive to hormonal effects in contaminated waters, and these responses have been used to measure estrogenic activity in river water downstream of wastewater discharge (Blaise et al., 2002).

Economic Considerations

In looking at how to assess the direct biological effects of EDCs on humans, animals, and ecosystems, it is useful to consider economic factors. The direct economic costs of monitoring and removing EDCs from industrial plant effluents, intensive agriculture, wastewater treatment plants, and, in the case of wastewater reuse, drinking water treatment, will be considerable. This in turn will affect the cost to consumers of drinking water. These costs may be unavoidable if a variety of wastewaters are to be employed as drinking water sources.

Impoverishment of ecosystems arising from endocrine disturbance will have economic consequences on the efficiency of the food web, which ultimately controls the availability of foodstuffs for humans. The most direct effects will be on shellfish and fish production from EDC-contaminated waters.

Directions for Further Investigation

The U.S. National Research Council, commissioned by the U.S. Environmental Protection Agency (EPA), U.S. Centers for Disease Control (CDC), and the U.S. Congress, conducted an independent review of existing literature on hormonally active agents in the environment and made several recommendations (NRC, 2000). In view of the evidence from animal studies supporting reproductive and developmental abnormalities and of the inadequacy of human data, longitudinal studies were recommended in human populations exposed to endocrine disruptors in order to assess developmental milestones from conception through adulthood. The recommendation included the use of standardized criteria to assess various aspects of development. Studies on the immunological effects of endocrine disruptors were also recommended, particularly for individuals exposed prenatally (i.e., whose mothers were exposed during pregnancy). Further investigation of the association between exposure to EDCs and development of various cancers was recommended, with the conduct of appropriately designed case–control and retrospective cohort studies. Measurement of internal dose (concentration of the substance in blood or target organ or body fluid) while conducting these studies was recommended. Further long-term studies of populations exposed to EDCs were recommended to assess the effects on age and the structure of population as well as on altering population size. Because of the long-term nature of these investigations and of delays in establishing criteria for compounds of interest, exposure assessment methods and reference populations, it is likely to be a decade or more before human epidemiological studies have a major role in setting guidelines for safe levels of human exposure.

In the meantime, the water industry needs to identify how best to maintain a sustainable supply of safe and reliable drinking water, which requires the detection and removal of potentially harmful contaminants. There is increased expectation of regulatory authorities to set safety standards. The current practice of setting safe guideline values for drinking water using animal studies with a range of single compounds to extrapolate risks to humans has limitations because of differences in body mass and physiological responses between humans and experimental animals. Also, humans are rarely, if at all, exposed to single compounds in the environment, with exposure more likely to be to complex mixtures. This issue is likely to be particularly relevant to EDCs. The effects of one compound may synergistically or antagonistically affect the end result of exposure to another compound. As a result of these and other issues, the safety/uncertainty factors used in the calculation of safe human intakes are highly conservative. These may establish guideline levels of toxicants well below those with any likelihood of posing health risks.

Given the current knowledge base, it is clear that ensuring the availability of a safe and sustainable water supply will be increasingly challenging. More data will be required than are available from conventional risk assessments; in particular, data from exposures to EDC mixtures are needed. Cost-effective treatment practices for EDC removal in wastewater and drinking water treatments will require development and implementation. In addition, good integrated management practices and technologies are needed to prevent further deterioration of our water sources by stopping endocrine-disruptive pollutants from entering our waterways. So far, most research has focused on estrogenic effects; however, there is evidence of androgens in treated effluents in Australia (Leusch et al., 2005c), and they, as well as estrogens, will require consideration.

For the safe reuse of wastewater, we consider that improvements in measurement of EDCs are needed, together with extensive monitoring of intake and discharge waters at wastewater and drinking water treatment plants. Treatment technologies will need to be assessed for their capability of removing EDCs in order to ensure a safe and cost-effective drinking water supply. Making certain that drinking water is safe will require determining and implementing guidelines for maximum acceptable concentrations of known EDCs and their mixtures in drinking water.

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