

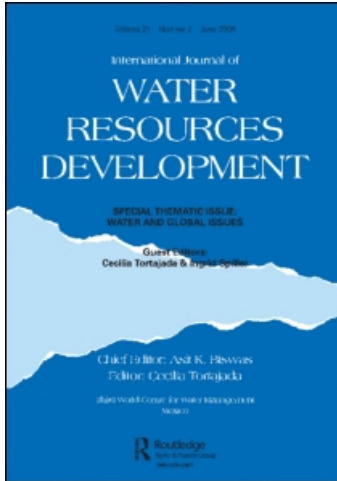
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Endocrine Disruptors and Water Quality: A State-of-the-Art Review

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ABSTRACT *Endocrine disruptors (EDs) are compounds which interfere with the hormone system and may adversely affect the health and reproduction of man and animals. These compounds comprise heavy metals and organic chemicals, such as polychlorinated biphenyls (PCBs), organochlorine pesticides, plasticizers, surfactants, pharmaceuticals, natural and synthetic estrogens as well as phyto- and mycoestrogens. In this review, an overview of most frequently asked questions is presented. These questions are: What are the sources of endocrine disruptors we find in the environment? What concentrations do we have in our wastewater? What happens in the wastewater treatment plant? Do we have EDs in other water bodies also? Is drinking water at risk? Why is it so difficult to prove whether EDs are harmful? Could sperm decline and fertility disturbance in man be due to EDs? Could breast cancer be due to exposure to EDs? What are alterations in wildlife and fish? What can be done?*

Introduction

We can assume that everybody is exposed to chemicals with endocrine effects in everyday life; such compounds are found in low doses in literally thousands of products. However, little is known about the implications for health of man and environment, especially with respect to chronic toxicity from continuous exposure and from exposure to multiple compounds. Accordingly, there is growing concern, especially with regard to the development and management of water resources. In order to reflect the current state of knowledge, in this review it is explained which water resources are of concern, what we know about the endocrine disruptors (especially focusing on estrogenically active compounds) and their known and suggested effects in man and on the environment.

However, since literature in this field is literally exploding, and many disciplines are involved, it is impossible to present a complete overview. Instead, a compilation of some of the major messages in this field is presented (Sumpter & Johnson, 2005) considering aspects which are important with regard to water resource management in agglomerations. For the ease of the reader's convenience, this review is structured in form of 'frequently asked questions'.

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What Are Endocrine Disruptors?

Endocrine disruptors (EDs) or endocrine disrupting compounds (EDCs), also referred to as hormonally active agents, are defined as compounds which affect the endocrine system. According to the World Health Organization (WHO), “an endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations” (WHO, 2002). However, the Environmental Protection Agency of the United States (USEPA) states more the specific biological effects: endocrine disrupting compounds are agents which interfere with the “synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behaviour”.

Surprisingly, endocrine-disrupting chemicals are structurally very diverse. They encompass a variety of chemical classes, which makes a systematic search and study difficult. In general, many EDs contain one or more aromatic rings and some are chlorinated (Byrne *et al.*, 2009). However, all these compounds share common mechanisms and biological effects, such as:

- mimicking or antagonizing the effects of hormones;
- altering the pattern of synthesis and the metabolism of hormones; and
- modifying hormone receptor levels.

By their interaction with hormone receptors and various processes in the endocrine and neuronal system they interfere with the homeostasis of the body. They might alter hormone biosynthesis, hormone storage and/or release, hormone transport and clearance, hormone receptor recognition or binding, post receptor activation or induce oxidative stress. As a consequence, these compounds have the potential to exert detrimental effects on man, plants, animals and eventually whole ecosystems.

Most of the chemicals with endocrine activity described so far are estrogenic, only a few have androgenic or anti-androgenic potency (McLachlan *et al.*, 2006) and only some are yet studied which show activity of other endocrine systems, such as thyroid, growth or stress hormones, and so on. The European Union compiled a list of estrogenic disruptors (also named environmental estrogens), of which 150 were synthetic compounds and seven were natural. Some of those chemicals are persistent organic pollutants (POPs), others are degraded rapidly in the environment or human body. Some heavy metals are also known to affect the endocrine system. Thus, endocrine disruptors comprise different groups (Byrne *et al.*, 2009; Markey *et al.*, 2003):

- natural hormones and metabolites (e.g., 17- β estradiol (E2) and their metabolites estriol (E3) and estrone (E1));
- artificial hormones (e.g., diethylstilbestrol; sex steroids in contraceptive pill);
- phyto- and mycoestrogens (e.g., isoflavones, lignans, coumestans, stilbenes; zearalenone);
- drugs with hormonal side effects (e.g., clofibrate);
- industrial and household chemicals (PCBs, flame retardants, paints, plasticizers (such as bisphenol A, phthalates), alkylphenol ethoxylate detergents, UV-screens);
- pesticides and metabolites (e.g., DDT, methoxychlor, kepone, dieldrin, lindan, endosulfan, toxaphene);

- side products of industrial and household processes (polycyclic aromatic hydrocarbons (PAH), dioxins, etc.); and
- metals (e.g., cadmium, lead).

However, with newly generated bioassays, indications for the presence of other hormonal activity, such as androgen-receptor, glucocorticoid-receptor and progesterone receptor-mediated transactivation, is found in wastewater effluents and surface water (Van der Linden *et al.*, 2008). Surprisingly high levels were measured; glucocorticoid-receptor mediated activity for example shows potency of up to 2900 ng/l cortisol equivalents in some samples—glucocorticoid hormones playing a pivotal role in the regulation of glucose metabolism and inflammation. The identity of the active compounds has to be established (Van der Linden *et al.*, 2008).

How Do They Act?

Endocrine disruptors act either organizationally, i.e., during development when they can disturb the sex determination and influence brain development, or they have an activating effect during the whole life time, i.e., interact with activating cellular signals thereby stimulating growth and activity of organs (Guillette *et al.*, 1995). The former effects are mostly irreversible, the latter are reversible. This results in different effects of ED in the embryo and in the adult. Homeostasis is maintained in adults; the capability for this in embryos and the young is uncertain (Sikka & Wang, 2008).

Endocrine disruptors act via receptor-dependent or receptor-independent effects. In the former case, an activation or blocking of the receptor-mediated effects results, for example an up-regulation of the receptor resulting in quantitative or qualitative differences in the downstream events. Receptor-independent effects, are, for example, the activation or blocking of metabolizing enzymes, leading to changes in the concentration of hormones.

Whether an interaction between ED occurs has been intensively studied in recent years. A joint action of environmental estrogens is now established, at least when the same mode of action, e.g., receptor activation, takes place. This explains that even weak estrogenic disruptors are able to act together to produce significant effects even when combined at concentrations below the threshold of effects (Silva *et al.*, 2002). Therefore, the concept of concentration addition seems to be the most appropriate to predict combined effects of substances with the same mode of action (Silva *et al.*, 2002).

What Are the Sources of Endocrine Disruptors Found in the Environment?

Estrogenic compounds enter the environment from different sources, such as:

- effluents from sewage treatment plants;
- surface water run-off from settlements, road system and agriculture;
- direct discharge into waters; and
- leakage from septic tanks and landfill sites, leading to compounds entering waterways through leachate.

Municipal sewage water was found to be estrogenic in many places, due to excretion by the human population. Most data were found for Brazil, Canada, China, Germany, Israel, Italy, Japan, Spain, Sweden, Switzerland, Netherlands, the UK and USA (Adler *et al.*, 2001;

Caliman & Gavrilesu, 2009). Modelling the input into the sewage treatment plant is helpful to make an estimation on estrogenic load in surface waters. Several parameters are needed, such as the number of people served by a sewage treatment works, the local pregnancy rate, demographic data and the amount of steroids excreted by the sub-populations. A pregnant woman, for example, excretes 259 $\mu\text{g/day}$ E2, 600 $\mu\text{g/day}$ E1 and 6,000 $\mu\text{g/day}$ E3 (Johnson *et al.*, 2000). As single contributors, industrial and hospital effluents also often show considerable amounts of endocrine activity (Van der Linden *et al.*, 2008).

However, estrogens were also detected in streams in areas with intensive agriculture (Kolpin *et al.*, 2002). The fungus *Fusarium*, infesting corn and other grains on the field or in storage, produces the potent estrogenic chemical called zearalenone which causes cessation of lactation and hyperestrogenization in pigs. Faecal and urinary deposits produced during animal husbandry contribute large amounts of steroidal hormones. The highest E1 and E2 concentrations were found in swine farrowing pits which are more than four times higher than in dairy waste (Raman *et al.*, 2004). However, the amount and species of manure-borne estrogens from livestock waste depend on the species, sex, age, hormonal status, among other traits of the animal (Hanselman *et al.*, 2003).

Even the mass spawning of salmon causes levels of steroidal hormones comparable to concentrations from sources such as municipal wastewater effluents. Although these natural hormones are not necessarily problematic to aquatic life, such natural events complicate efforts to identify sources of endocrine disruptors and to understand the various pathways through which steroids reach surface waters (Kolodziej *et al.*, 2004; Raman *et al.*, 2004).

Which Concentrations Do We Have in Our Wastewater?

The concentrations of estrogens in wastewater treatment plant influents and effluents were measured in several countries (Koh *et al.*, 2008). Comparing sewage influent and effluent data is particularly difficult, due to different sampling strategies and techniques. Of the compounds with endocrine activity, natural estrogens (E1, E2) and the synthetic 17- α estradiol (EE2) together contribute most to the estrogenic activity in wastewater, which is consistent across the various countries (Cao *et al.*, 2008; Johnson *et al.*, 2005). It has to be considered that estrone and estradiol can be interconverted by diverse animals and microorganisms.

Another consideration is the question of appropriate sampling and detection methods (Joss *et al.*, 2006). Several analytical methods have been developed to determine ED in the environment. Gas chromatography and HPLC coupled with mass spectrometry are most commonly used (Caliman & Gavrilesu, 2009), and have become recommended methods (Koh *et al.*, 2007). They provide the highest certainty of detection and the lowest detection limits, which are in the range of 0.1–0.5 for surface waters and in the range of 1–2 ng/L in sewage effluents (Young *et al.*, 2004). It has to be emphasized that the effective concentrations (see below) are often in the range of the limit of detection (Sumpter & Johnson, 2005). As always, matrix effects caused by, for example, the presence of natural organic matter or coeluting substances affect the final measurable concentration (Koh *et al.*, 2007). In every case, enrichment of the analytes is mandatory. Most commonly, solid-phase extraction is used but affinity techniques have proven successful and very selective (Ferguson, *et al.*, 2001). Sampling techniques have their pros and cons; grab sampling, for example, allows an instantaneous view but does not consider the fluctuation

of concentrations over time. Online monitoring improves the reliability of data, but is expensive and needs extensive regular maintenance. Passive sampling methods, such as polar organic chemical integrative samplers (POCIS), do not give absolute concentrations, rather time weighted average concentrations (Vermeirssen, *et al.*, 2005b). Sometimes, rather than the specific compound, the estrogenic activity, or the biologically active amount, is of importance to assess the effect on biota. For this, mostly bio-assays are used, since they are sensitive and respond only to a well-defined mode of action. Most commonly the YES and or YAS assay are used, however, reporter gene panels are developed to monitor a broad range of EDC-type activity (Routledge & Sumpter, 1996; Van der Linden *et al.*, 2008; Vermeirssen, *et al.*, 2005a).

Although the concentrations of EDs vary considerably in sewage effluents, typical ranges given by Young and co-workers correspond well with data of various countries (Adler *et al.*, 2001; Young *et al.*, 2004).

Estrone	5–20 ng/L (detected in great frequency)
17- β estradiol	1–10 ng/L
17- α estradiol	below detection limit – 10 ng/L

However, single data exceeding these ranges can be found in the literature as well, such as up to 76 ng/L estrone or 48 ng/L for 17- β estradiol in a sewage effluent in the UK (Desbrow *et al.*, 1998). In the Netherlands, up to 47 ng/L estrone and 12 ng/L E2 was detected (Belfroid *et al.*, 1999) whereas in Italy, estriol reached concentrations of up to 120 ng/L and estrone 75 ng/L (Johnson *et al.*, 2000). In southern Germany, up to 130 ng/L were measured for estrone (Adler *et al.*, 2001).

What Happens in the Wastewater Treatment Plant?

In removing the EDs in wastewater treatment, biological processes play a central role, especially biotransformation, biodegradation and adsorption. Sewage treatment plants differ widely in the treatment process technology (Koh *et al.*, 2008). The efficiency depends as well on the age of the activated sludge, the hydraulic retention time, the organic loading, redox potential, the cultivated environmental microorganisms and the size of the catchment as well as the number of inhabitants. In addition, degradation varies with the season. Implicitly, temperature and sunlight are factors positively correlated with increased biodegradation kinetics and (photo)degradation, respectively. Besides conventional treatment technologies, natural waters, such as lagoons, sometimes coupled with other technology, such as UV disinfection, are used to treat wastewater. Whereas most compounds are degraded or reduced at high rates in natural wetlands, some xenobiotics, such as carbamazepin or solatol are relatively stable (Caliman & Gavrilescu, 2009).

Steroidal estrogens are excreted in the form of glucuronide or sulphate conjugates which are considered to be biologically inactive. Deconjugation by bacteria in the sewer and the treatment plant or even in the environment leads to cleavage of these conjugates, releasing highly active free estrogens (Boxall *et al.*, 2004). Furthermore, some non-estrogenic chemicals can be metabolized into estrogenic products (Routledge & Sumpter, 1997), or compounds with higher estrogenicity. Alkylphenol polyethoxylates, as well as nonylphenol polyethoxylates are surfactants used in industrial and household detergents. Their partial degradation in wastewater treatment plants leads to shorter chain molecules

which have higher endocrine activity than their parent compounds (Koh *et al.*, 2005). This is also the case for methoxychlor, which is weakly estrogenic, but its metabolite, 2,2-bis-(p-7)-1,1,1-trichloroethane (HPTE) exhibits higher estrogenic, anti-estrogenic and anti-androgenic activity (Gaido *et al.*, 2000).

Laboratory batch studies revealed that EE₂ is highly stable and persistent in activated sludge, with no detectable degradation in the treatment process for five days, up to half-lives of 81 days (Ying & Kookana, 2005). In contrast, 17-β estradiol is biodegradable to estrone in less than one day and estrone is then biodegraded at a slightly slower half life (1–4 days) (Young *et al.*, 2004). Under anaerobic conditions, no degradation of EE₂ was observed at all during a three- year incubation period with lake water (Czajka & Londry, 2006).

Do We Also Have EDs in Other Water Bodies, e.g., Groundwater, Rivers, Oceans?

In general, passage through the various compartments of the water cycle leads to a reduction of estrogenic pollution. The main processes decreasing the concentrations in surface waters are dilution, adsorption and biodegradation (Young *et al.*, 2004). Nevertheless, the environmental fate of estrogens still poses some questions. Whereas laboratory studies showed a reduction or elimination of EDs within several hours or days, in the field, conditions are variable leading potentially to a higher persistence and mobility of estrogens impacting surface and groundwater quality (Hanselman *et al.*, 2003). Similar to the sewage treatment plant, 17-α estradiol is more persistent than the natural estrogens. 17-β estradiol and estrone were reported to exhibit similar half-lives of between 0.2 and 9 days (Jürgens *et al.*, 2002).

In virtually every stream and waterway tested in the US, estrogenic hormones were found (Kolpin *et al.*, 2002). However, concentrations are mostly below 1–5 ng/L estradiol equivalents, which is at or below the effect concentration in fish (Adler *et al.*, 2001; Belfroid *et al.*, 1999). One of the most frequently detected estrogens is estrone, possibly due to the breakdown of other estrogens (Hanselman *et al.*, 2003; Velicu & Suri, 2009). In addition, other organic compounds with endocrine activity were frequently measured, for example bisphenol, pesticides, nonylphenols, and many more compounds as reported by a nationwide US survey of 139 streams. However, concentrations were generally low with only 5% of the measured concentrations exceeding 1 μg/L. The maximum total concentration of EDs was 57.3 μg/L (Kolpin *et al.*, 2002).

Due to their persistence, they survive sewage treatment and can be detected up to several kilometres downstream of the sewage treatment plant. In addition to the factors responsible for the concentration in the effluent, the concentration in the river water also depends on the dilution in the receiving water.

In groundwater, < 0.05 – 0.8 ng/L for estrone, < 0.05 – 0.3 ng/L for 17- β estradiol and < 0.05 – 0.6 for EE₂ ng/L was found in southern Germany (Adler *et al.*, 2001). Although data are scarce for groundwater contamination with estrogens, studies indicate a migration from the river water to the sub-surface sediments where an accumulation may result, depending on the geology and vertical flow rate. Again, estrone was the estrogen predominantly detected (Labadie *et al.*, 2007). Further research is needed to assess the risk of estrogen transfer to groundwater during artificial recharge schemes.

In coastal areas, estrogens can leach into the marine environment from septic fields and contaminated groundwater, and enter the sea via sewage treatment plants. Concentrations

increase with decrease of distance to the agglomerations and sewage treatment plants (Atkinson *et al.*, 2003).

Is Drinking Water at Risk?

The presence of estrogenic disruptors in drinking water was reported from several researchers from various countries (Adler *et al.*, 2001). Where does the endocrine activity come from? First of all, the water could be estrogenic by itself when the underlying untreated groundwater contains compounds with hormonal activity, for example, due to a reflux of pharmaceuticals and synthetic estrogens from wastewater discharge. Secondly, in the production process, including bottling and cleaning, plasticizers and detergents or disinfectants are used. Either the compounds themselves, or their residuals can leach during the process or from the plastic bottles (Wagner & Oehlmann, 2009). Additives, some of them showing estrogenic activity, are used as plasticizers in polyethylene terephthalate (PET) bottles, and they were shown to migrate from the packaging material to the water. The consumption of bottled mineral water, up to 80% sold in PET bottles, is steadily rising all over the world. Accordingly, the risk due to estrogen activity is currently studied in various brands and under differing conditions (Pinto & Reali, 2009; Wagner & Oehlmann, 2009). 78% of all samples from PET bottles bought in Germany showed estrogenic contamination, up to concentrations equivalent to 75 ng/L 17- β estradiol (Wagner & Oehlmann, 2009). In Italy, 10% of the investigated samples showed estrogenic activity, with a maximal concentration of 23 ng estrogene equivalents/L. Storage conditions (exposure to UV and sunlight), as well as the technology and material used in production and bottling, may contribute to the contamination differently (Pinto & Reali, 2009). The production process is probably responsible if estrogenic activity is found in glass containers, as was reported in a German study where 33% of the water samples bottled in glass were positive in the yeast estrogene assay (Wagner & Oehlmann, 2009).

Occasionally, tap water contains estrogenic activity as well. The concentration depends on the origin and process of treatment. Between 0.46 ng EEQ and 17.2 ng EEQ/L were measured in Italian tap water originating from spring water, ground water and surface water (Pinto & Reali, 2009). However, a legislation specific for endocrine disruptors is not in action in the European community.

Why Is It So Difficult to Prove Whether EDs Are Harmful?

In general, concerns regarding exposure to EDs are due to three causes: (1) adverse effects observed in wildlife, especially fish; (2) increased incidence of certain endocrine-related human diseases and disorders; and (3) laboratory studies showing adverse functional changes due to exposure to endocrine disruptors. However, a consistent proof of a causal link between exposure to EDs and effect is difficult.

One problem with endocrine disruptors is not their acute toxicity but their effects due to chronic exposure at low level of concentrations. For example, the synthesis of the yolk protein precursor vitellogenin, a specific and sensitive biomarker in egg-laying vertebrates, is significantly increased at concentrations of 1 ng/L EE2 which is 1,600,000-fold lower than the concentration which is necessary to cause the death of 50% of the test fish population (LC₅₀) (Sumpter & Johnson, 2005).

A causal link between EDs and effects can be established for those compounds which operate via hormone receptor mechanisms. But how many receptors have to be occupied by EDs before an activation ensues? Of weak estrogens, for example the alkylphenols, up to 10^6 times more of the agent is required to bind to 50% of the estrogen receptor than if estradiol binds to the estrogen receptor (White *et al.*, 1994). For some hormones, such as human choriogonadotropin, less than 5% of the receptors have to be occupied for full activation response to start (Sikka & Wang, 2008).

A further characteristic of endocrine disruptors is the long delay between the time point of exposure and measurable effects. Accordingly, results of a preceding exposure may not be detected until adulthood (reproductive maturity) or even in the next generation. Such transgenerational effects due to epigenetic events are of special rising concern. We have evidence that endocrine active compounds induce altered developmental programming. When pregnant rats were treated with methoxychlor or the anti-androgenic fungicide vinclozolin, the male offspring pass the defect of subfertility through the male germline for at least four generations (Anway *et al.*, 2005; Bromer *et al.*, 2009). Correspondingly, lack of exposure data during critical periods of development are one of the biggest hindrance for determining whether observed adverse effects are linked to such an exposure (WHO, 2002).

Mixture effects have to be considered. EDs which act via the same mode of action may replace each other without loss of effectiveness. This implies that combination effects can occur even at doses well below levels at which adverse effects can be observed. When eight xenoestrogens were combined, with each at a concentration of 50% of its NOEC, 40% of a maximal estrogenic effect for the applied test system was noted (Silva *et al.*, 2002). Similar studies were performed with other mixtures and raised comparable results. Even when a dissimilar mode of action was stated, there was evidence for a mixture effect (Kortenkamp, 2008).

All these aspects make it extremely difficult to establish causal relationships between exposure to EDs and reproductive disorders. In addition, many disorders are multifactorial or even of unknown etiology.

What Are Critical Concentrations?

Concentrations at and above which effects occur are called effect concentration (EC). They differ between compounds and species, and, of course, experimental conditions. Regarding the various EDs, potency is a key factor; many chemicals show estrogenic activity, however, their potency is very different. Most potent is the synthetic estrogen, the 17 α -ethinylestradiol (EE2), the main estrogenic component of the combined oral contraceptive pill. The next in potency are natural estrogens (17 β -estradiol) and its metabolites, such as estrone and estriol. Accordingly, higher concentrations of other estrogenically active compounds are needed to cause a comparable effect than the natural and synthetic estrogens.

In aquatic environments, fish are considered the most sensitive taxon for adverse effects, at least as far as steroid estrogens are concerned (Young *et al.*, 2004). For the induction of vitellogenin in fish, an often used specific biomarker for estrogenic effects, the relation between the compounds are as follows: when 17- β estradiol was assigned a potency of 1, estrone has a potency of 0.3 and estriol of 0.03 (Routledge, E. J. & Sumpter, 1997), whereas ethinylestradiol has a 20-fold higher potency, namely 20. In comparison, known chemicals have the following potency: 4-tert-nonylphenol 0.0025; 4-octylphenol 0.002;

bisphenol A 0.0004 and methoxychlor 0.0025 (see Sumpter & Johnson, 2005 for literature). The phytoestrogens have activity in the range of 1/130 to 1/100 the activity of E2 (McLachlan *et al.*, 2006). In aquatic environments, only a few EDs contribute substantially to the estrogenic potential, mostly the steroid hormones and nonylphenol (Galli & Braun, 2008; Johnson *et al.*, 2005; Ternes *et al.*, 1999).

It is a matter of debate in (eco)toxicology how to propose a concentration at which no effect of biological significance occurs, the Predicted-No-Effect-Concentration (PNEC). In short, for 17- α ethinylestradiol and for 17- β ethinylestradiol, a PNEC of 0.1 ng l⁻¹ and 1 ng l⁻¹ has been proposed for fish (Young *et al.*, 2004). For estrone, 3 ng was set as PNEC (Gross-Sorokin *et al.*, 2006). Since steroids are likely to occur simultaneously in the environment and behave in a similar manner, a PNEC for total steroids would make sense. However, this has to be developed (Young *et al.*, 2004).

Establishing safe exposure levels or risk assessment for humans is difficult, and therefore no data can yet be proposed (Sikka & Wang, 2008).

Could Sperm Decline and Fertility Disturbance in Man Be Due to EDs?

Worldwide interest was raised by publications in the 1990s when it was suggested that sperm count might have declined by nearly half during the previous 50 years, and this might be related to increased estrogen exposure during embryo development (Carlsen *et al.*, 1992; Sharpe & Skakkebaek, 1993). Since then, many more studies have been carried out. According to these studies, countries with low and medium sperm counts include Denmark and Norway with 41 x 10⁶ /ml, and Germany (42- 46 x 10⁶ /ml) or Switzerland (47 x 10⁶ /ml), whereas other countries, such as Estonia and Finland, show very high values with 57 and 54 x 10⁶ /ml sperm concentrations, respectively (Jorgensen *et al.*, 2002; Paasch *et al.*, 2008; Crausaz *et al.*, 2008). According to the WHO, a value below 20 x 10⁶ /ml is considered abnormal. Data and the possible causes for differences are still controversially discussed (Sikka & Wang, 2008). Interpretation is in fact difficult since methods differ in respect to subject recruitment, season, and semen analysis (Sharpe & Skakkebaek, 2003). Nevertheless, one has to keep in mind that the human male produces relatively fewer sperm on a daily base than other animals and is, therefore, already not much above the level necessary for reproduction. Correspondingly, in many men over 30 years, a lower daily sperm production places them close to the level of sub- or infertility (Sikka & Wang, 2008). Finally, poor semen quality affects 6–8% of men and is the biggest defined cause of couple infertility (Sharpe & Skakkebaek, 2003).

Declining semen quality is only one out of several indicators that suggest a risk for male health and reproduction associated with endocrine disruption. In the male, reduced fertility, erectile dysfunction, testicular and prostate cancer, abnormal sexual development, alteration in pituitary and thyroid gland functions, immune suppression and neurobehavioural effects are further potential hazardous effects of endocrine disruption (Sikka & Wang, 2008). An increasing trend of congenital cryptorchidism is observed in Denmark and England (Boisen *et al.*, 2004). In addition, testicular cancer is continuously increasing in incidence in many countries. Also confirmed is the fact that this disease is associated with reduced fertility prior the tumour occurrence (Sharpe & Skakkebaek, 2003). Testicular cancer is the most common cancer in young men with life time risk of 0.3 to 0.8% in most countries (Sharpe & Skakkebaek, 2003).

Having a closer look at the role of endocrine disruption in male reproduction, it is obvious that such compounds may affect several potential target sites in the male reproductive tract. Most important are the testes, as the sites of spermatogenesis and androgen synthesis. As a secondary effect of disturbed spermatogenesis following hormonal alterations, sperm concentration, sperm X:Y concentration ratio, motility and morphology are affected, for example due to exposure to organic agricultural and industrial chemicals or heavy metals, such as lead, boron, cadmium, or mercury (Sikka & Wang, 2008). The most common disorders are maldescended testis (cryptorchidism) and hypospadias affecting 2–5% of boys (Sharpe & Skakkebaek, 2003). These malformations can be caused by antiandrogens (f. ex. DDT and metabolites, some antifungal agents such as procymidone and vinclozolin, the herbicide linuron, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and plasticizers such as phthalate esters) (Toppari, 2008). Millions of mothers took diethylstilbestrol (DES) in the middle of the last century to sustain pregnancy. Males exposed to DES in fetal life showed multiple adverse effects, such as undescended testis, epididymal cysts, distended seminal vesicles and impaired spermatogenesis and had an approximately double risk for testicular cancer (Toppari, 2008).

Is Breast Cancer Due to Exposure to Estrogenic Disruptors?

Breast cancer is the most frequent cancer around the world and the leading cause of cancer death worldwide. The increase in breast cancer is 1% per year (Garfinkel & Mushinski, 1994). In this context, what is the contribution of exogenous hormones? Again, the causative link is difficult to establish since EDs are ubiquitous and there is no unexposed control group. As discussed for general aspects of EDs, the long delay between exposure and effect makes it especially difficult. However, data from human epidemiological studies demonstrate positive correlations between tissue levels of estrogenic compounds and breast cancer risk. In rodents, fetal and prenatal exposure to estrogenic disruptors induce premalignant and malignant transformation of adult mammary glands. Drawing together all evidences, it has been strongly suggested that EDs may participate in the increasing incidence of breast cancer (Fenichel & Brucker-Davis, 2008).

Further, ovarian development, maturation and reproductive functions are regulated by hormones and are consequently subject to disruption by environmental estrogens. For example, disruption of the oestrus cycle, reduction in pregnancy rates and lowered number of pups were stated in animal experiments with methoxychlor (Chapin *et al.*, 1997). Bisphenol A (BPA) was found to pass through the placenta. Abnormalities, as described for methoxychlor were described, associated with reduced fertility, onset of reproductive senescence and pathological events later in life (Markey *et al.*, 2003). A striking adverse effect was found when investigating estrogenic organochlorine compounds on lactation (Gladen & Rogan, 1995). Vinclozolin alters the pattern of sexual differentiation and adult sexual function (Uzumcu & Zachow, 2007). DES was taken by some 4–6 million pregnant women in the US alone (McLachlan *et al.*, 2006). In female offspring, DES led to abnormalities of reproductive tract, such as malformations, reproductive dysfunctions and poor pregnancy outcome. Additionally, effects in the cardiovascular, neuroendocrine and immune system were stated. As well, malignant tumours developed in some cases (Uzumcu & Zachow, 2007). Even a transient exposure during development led to abnormal function in adult life. This might be a result of a mechanism called epigenetics, describing persistent,

heritable changes in gene expression without change in the DNA sequence itself. This could be due to changes in the pattern of the DNA methylation. In fact, a persistent overexpression of genes which were then hypomethylated was demonstrated in mice when treated with DES during development (McLachlan *et al.*, 2006).

What Are the Alterations in Wildlife and Fish?

The literature on exposure and effects in fish and wildlife is immense. It started with the publication of Rachel Carson's *Silent Spring* which described the vanishing of birds due to exposure to pesticides (Carson, 1962). Thirty-five years later, the issue was brought to the attention of the broad public by Theo Colborn and co-workers with *Our stolen Future* which presented copious evidence for effects of endocrine-disrupting chemicals, both in man and wildlife (Colborn *et al.*, 1996). Since then tens of thousands publications presented further evidence, asked questions, and pointed to miraculous phenomena. Excellent reviews compile the data and draw conclusions (Cheek, 2006; Porte *et al.*, 2006; Segner, 2005; Sumpter & Johnson, 2005). Evidence of hormonal derangements in wild populations of invertebrates, fish, amphibians, reptiles and birds and mammals were accumulated. Invertebrates, although comprising > 95% of the animal species, are comparatively poorly studied in respect to endocrine disruptors. This is partly due to their hormonal system which deviates from that of the better known vertebrates. Nevertheless, one of the best documented examples of endocrine disruption leading to a population decline and even to a population extinction is that of tributyltin-induced imposex formation in molluscs (Oehlmann & Schulte-Oehlmann, 2003). Tributyltin is used in boat paints for antifouling and causes the imposition of male characteristics in female gastropods (snails) thereby reducing reproductive success. In vertebrates, the endocrine system is very conservative (McLachlan *et al.*, 2006) and the specificity of the estrogen receptor shows essentially no differences across a wide range of species (Sumida *et al.*, 2003). Accordingly, we have substantial evidence from many vertebrate taxa linking fertility impairment to exposure to EDs. In wild fish, particularly in waters receiving treated (or untreated) sewage effluents, feminization of male fish was often observed. One of the best studied examples is the male roach showing up to 100% incidence of intersex (gonads composed of male and female tissue) compared to 0% intersex in male fish of the reference lake (Jobling *et al.*, 2002). The association between feminization of fish and sewage effluents is consistent across species, geographical regions, habitats and investigators. Dose-response relationships between xenoestrogens or alkylphenols and feminization of fish or increase of vitellogenin (an yolk-precursor protein) in male fish was demonstrated in many laboratory and field studies in a great variety of fish species and experimental conditions (Burki *et al.*, 2006; Cheek, 2006; Sumpter & Johnson, 2005). Effects on fish population were unequivocally demonstrated by Karen Kidd and co-workers (Kidd *et al.*, 2007) where an exposure to environmentally relevant concentration to EE2 leads to the extinction of a fish population in an experimental lake. Masculinization of fish is strongly associated with pulp and paper mill effluents and consistent across countries, species and investigators. However, it has not been demonstrated as frequently as feminization (Cheek, 2006). In amphibians, a strong temporal association between intersex in frogs and organochlorine use was demonstrated, however, a clear dose-response relationship is absent and the mechanism of the suspected causal agents for reproductive disruption is unclear (Cheek, 2006). Studies in reptiles show contradictory

results as well. Whereas a strong association was demonstrated between organochlorine contamination, sex differentiation and sex hormone levels, and abnormal penises in young alligators in Lake Apopka (Guillette *et al.*, 2000), confirmed by laboratory studies (Gunderson *et al.*, 2001), turtle populations in heavily contaminated sites seem to be unaffected (de Solla *et al.*, 2002). Fish-eating birds have suffered various health problems and poor reproductive success due to organochlorine contamination (Dawson, 2000). In aquatic mammals, high contamination burden of organochlorine contaminants were found and are associated with health impairments, such as reproductive and thyroid disturbances. However, causal links to population declines are not clear (Derocher *et al.*, 2003).

In conclusion, endocrine disruption has adverse effects on fish and wildlife. Although not shown conclusively in every case, this might be due to subtle effects, emerging with long delay. Especially in combination with other stressors, such as climate change or habitat loss, the threat to wildlife has to be taken very seriously (Burkhardt-Holm *et al.*, 2005).

What Can Be Done?

Centralization of the world's population in urban areas leads to a likely increase of water pollution; a part of those compounds are EDs. The first step is to assess potential hazardous compounds. For this, regulation is different across countries, with many countries lacking requirements at all. Some hazardous compounds are well known and have been taken out of production. DDT and PCBs have been banned in North America and Europe since the 1970s and 1980s, but persistence and global distribution in all environmental compartments present a daunting threat to health for both wildlife and man. Even more, it was calculated that only 1/3 of the produced PCBs, approximately 1 million tons, is in the environment, 2/3 will still be released over time.

Treatment of sewage or the improvement of this treatment is one option. In the sewage treatment plant, there are several options to remove or reduce EDs. Oxidative treatment of EE₂ with chlorine, bromine, ozone, hydroxyl radical chlorine dioxide and ferrine results in > 87% reduction of estrogenic activity within seconds to minutes in laboratory experiments (Lee *et al.*, 2008). This corresponds to studies showing that ozonation is efficient in removal of approximately 80% of E1, E2 and BPA. However, this treatment is not efficient in removing alkylphenols (Nakada *et al.*, 2007). In general, a combination of ozonation, sand filtration and activated sludge treatment gives the best removal result for ED (Nakada *et al.*, 2007). To remove EDs in the drinking water treatment process, granular activated carbon has been identified as the method to be used (EPA, 2001). The various methods were recently critically reviewed (Caliman & Gavrilescu, 2009; Koh *et al.*, 2008). However, one has to take in account that ozonation requires a lot of electric power. Accordingly, economic feasibility depends on the price of energy (Joss *et al.*, 2008). Apart from energy consumption, CO₂ emissions should be taken into consideration and alternative techniques, such as increased sludge ages and hydraulic retention times, should be further evaluated and developed (Jones *et al.*, 2007).

Even if elaborated treatment with ozonation is not feasible sometimes for economic reasons, it was shown that remediation measures have the potential for alleviating endocrine disruption. Additional treatment of the effluents before final release in the river Aire, UK, lead to a significant reduction of biomarker response, vitellogenin synthesis in wild fish downstream of the effluent release (Sheahan *et al.*, 2002).

What about your personal contribution? The disposal of unused or expired medicine through toilet, sink or trash is a common practice, nearly 75% of the households (n = 1005) practice these disposal methods according to a survey in Southern California (Kotchen *et al.*, 2009). Also, disposal in trash means that compounds in many places in the world end up in landfills and may eventually enter waterways through leachate. Clearly this contributes considerably to the contamination of our aquatic environment. The amount of estradiol in a vaginal ring contraceptive device at disposal was 2.4 mg—enough that one disposed ring releases enough estradiol to cause biological effects in fish in 24,000,000 l of water. The recommendation is to establish pharmaceutical disposal programmes and encourage people to participate in such programmes (Kotchen *et al.*, 2009).

Conclusions

As our technological advances in instrumentation have improved in recent years, we can now measure contaminants in parts per trillion. Emerging contaminants are looming as serious potential problems for human and environmental health. The threat is greater than for other contaminants since some endocrine disruptors are able to interfere with reproduction by harming recruitment and ultimately biodiversity.

There are several open questions which have to be taken into account when trying to assess the risk of endocrine disruptors for humans and the environment.

- Due to effects caused by very low concentrations occurring with a long delay, sometimes bridging generations, proof of cause–effect relations is extremely difficult.
- This is even harder in real-world situations where mixtures of contaminants are acting, causing so far mostly unknown additive, synergistic or antagonistic effects. The fact that water is not the only pathway by which such contaminants can enter human bodies makes the work on endocrine disruptors even more challenging.
- Extrapolations from experimental studies from lower vertebrates and rodents to humans are hardly possible. The endocrine system is rather conservative, and accordingly effects in one species can often be observed in related species and other classes of vertebrates as well. Nevertheless, specific effects cannot be predicted for species not tested due to the highly complex and sensitive nature of the endocrine system. However, there is evidence that EDs may participate in the incidence of breast cancer.
- Presently, we are facing methodological and conceptual limitations on assessing the risk due to endocrine disruptors for man and environment which should be solved with strengthened research efforts.

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References

- Adler, P., Steger-Hartmann, T. & Kalbfus, W. (2001) Vorkommen natürlicher und synthetischer östrogenen Steroide in Wässern des süd- und mitteldeutschen Raumes [Distribution of natural and synthetic estrogen

- steroid hormones in water samples from southern and middle Germany], *Acta hydrochimica hydrobiologica*, 29(4), pp. 227–241.
- Anway, M. D., Cupp, A. S., Uzumcu, M. & Skinner, M. K. (2005) Epigenetic transgenerational actions of endocrine disruptors and male fertility, *Science*, 308(5727), pp. 1466–1469.
- Atkinson, S., Atkinson, M. J. & Tarrant, A. M. (2003) Estrogens from sewage in coastal marine environments, *Environmental Health Perspectives*, 111(4), pp. 531–535.
- Belfroid, A. C., Van der Horst, A., Vethaak, A. D., Schaefer, A. J., Rijs, G. B. J., Wegener, J. & Cofino, W. P. (1999) Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands, *The Science of the Total Environment*, 225(1–2), pp. 101–108.
- Boisen, K. A., Kaleva, M., Main, K. M., Virtanen, H. E., Haavisto, A. M., Schmidt, I. M., Chellakooty, M., Damgaard, I. N., Mau, C., Reunanen, M., Skakkebaek, N. E. & Toppari, J. (2004) Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries, *Lancet*, 363(9417), pp. 1264–1269.
- Boxall, A. B., Sinclair, C. J., Fenner, K., Kolpin, D. & Maund, S. J. (2004) When synthetic chemicals degrade in the environment, *Environmental Science & Technology*, 38(19), pp. 368A–375A.
- Bromer, J. G., Wu, J., Zhou, Y. P. & Taylor, H. S. (2009) Hypermethylation of homeobox A10 by in utero diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming, *Endocrinology*, 150(7), pp. 3376–3382.
- Burkhardt-Holm, P., Giger, W., Güttinger, H., Ochsenein, U., Peter, A., Scheurer, K., Segner, H., Staub, E. & Suter, M. J. F. (2005) Where have all the fish gone?, *Environmental Science & Technology*, 39(21), pp. 441a–447a.
- Burki, R., Vermeirssen, E. L. M., Körner, O., Joris, C., Burkhardt-Holm, P. & Segner, H. (2006) Assessment of estrogenic exposure in brown trout (*Salmo trutta*) in a Swiss midland river: integrated analysis of passive samplers, wild and caged fish, and vitellogenin mRNA and protein, *Environmental Toxicology and Chemistry*, 25(8), pp. 2077–2086.
- Byrne, C., Divekar, S. D., Storchan, G. B., Parodi, D. A. & Martin, M. B. (2009) Cadmium—a metalloestrogen?, *Toxicology and Applied Pharmacology*, 238(3), pp. 266–271.
- Caliman, F. A. & Gavrilescu, M. (2009) Pharmaceuticals, personal care products and endocrine disrupting agents in the environment—a review, *Clean-Soil Air Water*, 37(4–5), pp. 277–303.
- Cao, Q., Yu, Q. & Connell, D. W. (2008) Degradation rate constants of steroids in sewage treatment works and receiving water, *Environmental Technology*, 29(12), pp. 1321–1330.
- Carlsen, E., Giwercman, A., Keiding, N. & Skakkebaek, N. E. (1992) Evidence for decreasing quality of semen during past 50 years, *British Medical Journal*, 305(6854), pp. 609–613.
- Carson, R. (1962) *Silent Spring* (Boston: Houghton Mifflin).
- Chapin, R. E., Harris, M. W., Davis, B. J., Ward, S. M., Wilson, R. E., Mauney, M. A., Lockhart, A. C., Smailowicz, R. J., Moser, V. C., Burka, L. T., Collins, B. J., Haskins, E. A., Allen, J. D., Judd, L., Purdie, W. A., Harris, H. L., Lee, C. A. & Corniffe, G. M. (1997) The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function, *Fundamental and Applied Toxicology*, 40(1), pp. 138–157.
- Cheek, A. O. (2006) Subtle sabotage: endocrine disruption in wild populations, *Revista De Biologia Tropical*, 54, pp. 1–19.
- Colborn, T., Dumanoski, D. P. & Meyers, J. P. (1996) *Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival? —A Scientific Detective Story* (New York: Penguin Books).
- Crausaz, M., Vargas, J., Parapanov, R., Chollet, Y., Wisard, M., Stettler, E., Senn, A. & Germond, M. (2008) First evaluation of human sperm quality in various geographic regions of Switzerland, *Chimia*, 62(5), pp. 395–400.
- Czajka, C. P. & Londry, K. L. (2006) Anaerobic biotransformation of estrogens, *Science of the Total Environment*, 367(2–3), pp. 932–941.
- Dawson, A. (2000) Mechanisms of endocrine disruption with particular reference to occurrence in avian wildlife: a review, *Ecotoxicology*, 9(1–2), pp. 59–69.
- de Solla, S. R., Bishop, C. A. & Brooks, R. J. (2002) Sexually dimorphic morphology of hatchling snapping turtles (*Chelydra serpentina*) from contaminated and reference sites in the Great Lakes and St. Lawrence River basin, North America, *Environmental Toxicology and Chemistry*, 21(5), pp. 922–929.
- Derocher, A. E., Walkers, H., Colborn, T., Schlabach, M., Larsen, T. S. & Wiig, O. (2003) Contaminants in Svalbard polar bear samples archived since 1967 and possible population level effects, *Science of the Total Environment*, 301(1–3), pp. 163–174.

- Desbrow, C., Routledge, E. J., Brighty, G. C., Sumpter, J. P. & Waldock, M. (1998) Identification of estrogenic chemicals in STW effluent. 1. chemical fractionation and in vitro biological screening, *Environmental Science & Technology*, 32(11), pp. 1549–1558.
- EPA (2001) *Removal of Endocrine Disruptor Chemicals Using Drinking Water Treatment Processes*, R & D Technology Transfer EPA/625/R-00/015 (Washington DC: EPA).
- Fenichel, P. & Brucker-Davis, F. (2008) Breast risk cancer and environmental endocrine disruptors, *Gynecologie Obstetrique & Fertilité*, 36(10), pp. 969–977.
- Ferguson, P. L., Iden, C. R., McElroy, A. E. & Brownawell, B. J. (2001) Determination of steroid estrogens in wastewater by immunoaffinity extraction coupled with HPLC-MS, *Analytical Chemistry*, 73(16), pp. 3890–3895.
- Gälli, R. & Braun, C. (2008) Integrative risk assessment of endocrine disruptors in Switzerland, *Chimia*, 62(5), pp. 417–423.
- Gaido, K. W., Maness, S. C., McDonnell, D. P., Dehal, S. S., Kupfer, D. & Safe, S. (2000) Interaction of methoxychlor and related compounds with estrogen receptor alpha and beta, and androgen receptor: structure-activity studies, *Molecular Pharmacology*, 58(4), pp. 852–858.
- Garfinkel, L. & Mushinski, M. (1994) Cancer incidence, mortality and survival: trends in four leading sites, *Statistical Bulletin (Metropolitan Life Insurance Company)*, 75(3), pp. 19–27.
- Gladen, B. C. & Rogan, W. J. (1995) DDE and shortened duration of lactation in a northern Mexican town, *American Journal of Public Health*, 85(4), pp. 504–508.
- Gross-Sorokin, M. Y., Roast, S. D. & Brighty, G. C. (2006) Assessment of feminization of male fish in English rivers by the environment agency of England and Wales, *Environmental Health Perspectives*, 114, pp. 147–151.
- Guillette, L. J., Jr., Crain, D. A., Gunderson, M. P., Kools, S. A. E., Milnes, M. R., Orlando, E. F., Rooney, A. A. & Woodward, A. R. (2000) Alligators and endocrine disrupting contaminants: a current perspective, *American Zoology*, 40, pp. 438–452.
- Guillette, L. J., Jr., Crain, D. A., Rooney, A. A. & Pickford, D. B. (1995) Organization versus activation: the role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife, *Environmental Health Perspectives*, 103(Supplement 7), pp. 157–164.
- Gunderson, M. P., LeBlanc, G. A. & Guillette, L. J. (2001) Alterations in sexually dimorphic biotransformation of testosterone in juvenile American alligators (*Alligator mississippiensis*) from contaminated lakes, *Environmental Health Perspectives*, 109(12), pp. 1257–1264.
- Hanselman, T. A., Graetz, D. A. & Wilkie, A. C. (2003) Manure-borne estrogens as potential environmental contaminants: a review, *Environmental Science & Technology*, 37(24), pp. 5471–5478.
- Jobling, S., Coey, S., Whitmore, J. G., Kime, D. E., Van Look, K. J. W., McAllister, B. G., Beresford, N., Henshaw, A. C., Brighty, G., Tyler, C. R. & Sumpter, J. P. (2002) Wild intersex roach (*Rutilus rutilus*) have reduced fertility, *Biology of Reproduction*, 67, pp. 515–524.
- Johnson, A. C., Aerni, H. R., Gerritsen, A., Gibert, M., Giger, W., Hylland, K., Jurgens, M., Nakari, T., Pickering, A., Suter, M. J. F., Svenson, A. & Wettstein, F. E. (2005) Comparing steroid estrogen, and nonylphenol content across a range of European sewage plants with different treatment and management practices, *Water Research*, 39(1), pp. 47–58.
- Johnson, A. C., Belfroid, A. & Di Corcia, A. (2000) Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent, *Science of the Total Environment*, 256(2–3), pp. 163–173.
- Jones, O. A. H., Green, P. G., Voulvoulis, N. & Lester, J. N. (2007) Questioning the excessive use of advanced treatment to remove organic micropollutants from wastewater, *Environmental Science & Technology*, 41(14), pp. 5085–5089.
- Jorgensen, N., Carlsen, E., Nermoen, I., Punab, M., Suominen, J., Andersen, A. G., Andersson, A. M., Haugen, T. B., Horte, A., Jensen, T. K., Magnus, O., Petersen, J. H., Vierula, M., Toppari, J. & Skakkebaek, N. E. (2002) East–West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland, *Human Reproduction*, 17(8), pp. 2199–2208.
- Joss, A., Carballa, M., Kreuzinger, N., Siegrist, H. & Zabczynski, S. (2006) Wastewater, in: T. A. Ternes & A. Joss (Eds) *Human Pharmaceuticals, Hormones and Fragrances: The Challenge of Micropollutants in Urban Water Management*, pp. 243–295 (London: IWA Publishing).
- Joss, A., Siegrist, H. & Ternes, T. A. (2008) Are we about to upgrade wastewater treatment for removing organic micropollutants? *Water, Science and Technology*, 57(2), pp. 251–255.

- Jürgens, M. D., Holthaus, K. I. E., Johnson, A. C., Smith, J. J. L., Hetheridge, M. & Williams, R. J. (2002) The potential for estradiol and ethinylestradiol degradation in English rivers, *Environmental Toxicology and Chemistry*, 21(3), pp. 480–488.
- Kidd, K. A., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M. & Flick, R. W. (2007) Collapse of a fish population after exposure to a synthetic estrogen, *Proceedings of the National Academy of Sciences of the United States of America*, 104(21), pp. 8897–8901.
- Koh, Y. K. K., Chiu, T. Y., Boobis, A., Cartmell, E., Lester, J. N. & Scrimshaw, M. D. (2007) Determination of steroid estrogens in wastewater by high performance liquid chromatography-tandem mass spectrometry, *Journal of Chromatography A*, 1173, pp. 81–87.
- Koh, Y. K. K., Chiu, T. Y., Boobis, A., Cartmell, E., Scrimshaw, M. D. & Lester, J. N. (2008) Treatment and removal strategies for estrogens from wastewater, *Environmental Technology*, 29(3), pp. 245–267.
- Koh, Y. K. K., Lester, J. N. & Scrimshaw, M. D. (2005) Fate and behaviour of alkylphenols and their polyethoxylates in an activated sludge plant, *Bulletin of Environmental Contamination and Toxicology*, 75(6), pp. 1098–1106.
- Kolodziej, E. P., Harter, T. & Sedlak, D. L. (2004) Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment, *Environmental Science & Technology*, 38(23), pp. 6377–6384.
- Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S., Barber, L. B. & Buxton, H. T. (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance, *Environmental Science and Technology*, 36(6), pp. 1202–1211.
- Kortenkamp, A. (2008) Low dose mixture effects of endocrine disruptors: implications for risk assessment and epidemiology, *International Journal of Andrology*, 31(2), pp. 233–237.
- Kotchen, M., Kallaos, J., Wheeler, K., Wong, C. & Zahller, M. (2009) Pharmaceuticals in wastewater: behaviour, preferences, and willingness to pay for a disposal program, *Journal of Environmental Management*, 90(3), pp. 1476–1482.
- Labadie, P., Cundy, A. B., Stone, K., Andrews, M., Valbonesi, S. & Hill, E. M. (2007) Evidence for the migration of steroidal estrogens through river bed sediments, *Environmental Science & Technology*, 41(12), pp. 4299–4304.
- Lee, Y., Escher, B. I. & Von Gunten, U. (2008) Efficient removal of estrogenic activity during oxidative treatment of waters containing steroid estrogens, *Environmental Science & Technology*, 42(17), pp. 6333–6339.
- Markey, C. M., Rubin, B. S., Soto, A. M. & Sonnenschein, C. (2003) Endocrine disruptors: from Wingspread to environmental developmental biology, *Journal of Steroid Biochemistry and Molecular Biology*, 83(1–5), pp. 235–244.
- McLachlan, J. A., Simpson, E. & Martin, M. (2006) Endocrine disruptors and female reproductive health, *Best Practice & Research Clinical Endocrinology & Metabolism*, 20(1), pp. 63–75.
- Nakada, N., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N. & Takada, H. (2007) Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant, *Water Research*, 41(19), pp. 4373–4382.
- Oehlmann, J. & Schulte-Oehlmann, U. (2003) Endocrine disruption in invertebrates, *Pure and Applied Chemistry*, 75(11–12), pp. 2207–2218.
- Paasch, U., Salzbrunn, A., Glander, H. J., Plambeck, K., Salzbrunn, H., Grunewald, S., Stucke, J., Vierula, M., Skakkebaek, N. E. & Jorgensen, N. (2008) Semen quality in sub-fertile range for a significant proportion of young men from the general German population: a co-ordinated, controlled study of 791 men from Hamburg and Leipzig, *International Journal of Andrology*, 31(3), pp. 371–371.
- Pinto, B. & Reali, D. (2009) Screening of estrogen-like activity of mineral water stored in PET bottles, *International Journal of Hygiene and Environmental Health*, 212(2), pp. 228–232.
- Porte, C., Janer, G., Lorusso, L. C., Ortiz-Zarragoitia, M., Cajaraville, M. P., Fossi, M. C. & Canesi, L. (2006) Endocrine disruptors in marine organisms: approaches and perspectives, *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 143(3), pp. 303–315.
- Raman, D. R., Williams, E. L., Layton, A. C., Burns, R. T., Easter, J. P., Daugherty, A. S., Mullen, M. D. & Saylor, G. S. (2004) Estrogen content of dairy and swine wastes, *Environmental Science & Technology*, 38(13), pp. 3567–3573.
- Routledge, E. J. & Sumpter, J. P. (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen, *Environmental Toxicology and Chemistry*, 15(3), pp. 241–248.
- Routledge, E. J. & Sumpter, J. P. (1997) Structural features of alkylphenolic chemicals associated with estrogenic activity, *Journal of Biological Chemistry*, 272(6), pp. 3280–3288.

- Segner, H. (2005) Developmental, reproductive, and demographic alterations in aquatic wildlife: Establishing causality between exposure to endocrine-active compounds (EACs) and effects, *Acta Hydrochimica et Hydrobiologica*, 33(1), pp. 17–26.
- Sharpe, R. M. & Skakkebaek, N. E. (1993) Are estrogens involved in falling sperm counts and disorders of the male reproductive-tract, *Lancet*, 341(8857), pp. 1392–1395.
- Sharpe, R. M. & Skakkebaek, N. E. (2003) Male reproductive disorders and the role of endocrine disruption: advances in understanding and identification of areas for future research, *Pure and Applied Chemistry*, 75(11–12), pp. 2023–2038.
- Sheahan, D. A., Brighty, G. C., Daniel, M., Jobling, S., Harries, J. E., Hurst, M. R., Kennedy, J., Kirby, S. J., Morris, S., Routledge, E. J., Sumpter, J. P. & Waldock, M. J. (2002) Reduction in the estrogenic activity of a treated sewage effluent discharge to an English river as a result of a decrease in the concentration of industrially derived surfactants, *Environmental Toxicology and Chemistry*, 21(3), pp. 515–519.
- Sikka, S. C. & Wang, R. (2008) Endocrine disruptors and estrogenic effects on male reproductive axis, *Asian Journal of Andrology*, 10(1), pp. 134–145.
- Silva, E., Rajapakse, N. & Kortenkamp, A. (2002) Something from “nothing”—eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects, *Environmental Science and Technology*, 36, pp. 1751–1756.
- Sumida, K., Ooe, N., Saito, K. & Kaneko, H. (2003) Limited species differences in estrogen receptor alpha-mediated reporter gene transactivation by xenestrogens, *Journal of Steroid Biochemistry and Molecular Biology*, 84, pp. 33–40.
- Sumpter, J. P. & Johnson, A. C. (2005) Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment, *Environmental Science & Technology*, 39(12), pp. 4321–4332.
- Ternes, T. A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R. D. & Servos, M. (1999) Behavior and occurrence of estrogens in municipal sewage treatment plants—I. Investigations in Germany, Canada and Brazil, *Science of the Total Environment*, 225(1–2), pp. 81–90.
- Toppiari, J. (2008) Environmental Endocrine Disrupters, *Sexual Development*, 2(4–5), pp. 260–267.
- Uzumcu, M. & Zachow, R. (2007) Developmental exposure to environmental endocrine disruptors: consequences within the ovary and on female reproductive function, *Reproductive Toxicology*, 23(3), pp. 337–352.
- Van der Linden, S. C., Heringa, M. B., Man, H. Y., Sonneveld, E., Puijker, L. M., Brouwer, A. & Van der Burg, B. (2008) Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays, *Environmental Science & Technology*, 42(15), pp. 5814–5820.
- Velicu, M. & Suri, R. (2009) Presence of steroid hormones and antibiotics in surface water of agricultural, suburban and mixed-use areas, *Environmental Monitoring and Assessment*, 154(1–4), pp. 349–359.
- Vermeirssen, E. L. M., Burki, R., Joris, C., Peter, A., Segner, H., Suter, M. J. F. & Burkhardt-Holm, P. (2005a) Characterisation of the estrogenicity of Swiss midland rivers using a recombinant yeast bioassay and plasma vitellogenin concentration in feral male brown trout, *Environmental Toxicology and Chemistry*, 24(9), pp. 2226–2233.
- Vermeirssen, E. L. M., Koerner, O., Schoenenberger, R., Suter, M. J. F. & Burkhardt-Holm, P. (2005b) Characterization of environmental estrogens in river water using a three pronged approach: active and passive water sampling and the analysis of accumulated estrogens in the bile of caged fish, *Environmental Science & Technology*, 39(21), pp. 8191–8198.
- Wagner, M. & Oehlmann, J. (2009) Endocrine disruptors in bottled mineral water: total estrogenic burden and migration from plastic bottles, *Environmental Science and Pollution Research International*, 16, pp. 278–286.
- White, R., Jobling, S., Hoare, S. A., Sumpter, J. P. & Parker, M. G. (1994) Environmentally persistent alkylphenolic compounds are estrogenic, *Endocrinology*, 135(1), pp. 175–182.
- WHO (2002) *Global Assessment of the State-of-the-Science of Endocrine Disruptors* (Geneva: World Health Organization).
- Ying, G. G. & Kookana, R. S. (2005) Sorption and degradation of estrogen-like-endocrine disrupting chemicals in soil, *Environmental Toxicology and Chemistry*, 24(10), pp. 2640–2645.
- Young, W. F., Whitehouse, P., Johnson, I. & Sorokin, N. (2004) *Proposed Predicted-No-Effect-Concentrations (Pnecs) for Natural and Synthetic Steroid Oestrogens in Surface Waters*, R&D Technical Report P2-T04/1 (Swindon, UK: Environment Agency).