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REVIEW

Partitioning, bioavailability and effects of oestrogens and xeno-oestrogens in the aquatic environment

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This review provides insights into the distribution and impact of oestrogens and xeno-oestrogens in the aquatic environment and highlights some significant knowledge gaps in our understanding of endocrine disrupting chemicals. Key areas of uncertainty in the assessment of risk include the role of estuarine sediments in mediating the fate and bioavailability of environmental (xeno)oestrogens (notably their transfer to benthic organisms and estuarine food chains), together with evidence for endocrine disruption in invertebrate populations.

Emphasis is placed on using published information to interpret the behaviour and effects of a small number of 'model compounds' thought to contribute to oestrogenic effects in nature; namely, the natural steroid 17β-oestradiol (E2) and the synthetic hormone 17α-ethinyloestradiol (EE2), together with the alkylphenols octyl- and nonyl-phenol (OP, NP) as oestrogen mimics. Individual sections of the review are devoted to sources and concentrations of (xeno)oestrogens in waterways, sediment partitioning and persistence, bioaccumulation rates and routes, assays and biomarkers of oestrogenicity, and, finally, a synopsis of reproductive and ecological effects in aquatic species.

INTRODUCTION

Endocrine disruption and reproductive effects may be caused by elevated levels of natural and synthetic hormones and by the ability of compounds to mimic or antagonize the effects of endogenous hormones (through disruption of their synthesis and metabolism). Thus, in addition to steroid sex hormones (oestrogens and androgens) and other naturally-occurring compounds such as phyto-oestrogens (resorcylic acid, lactones, isoflavones, coumestans, lignans), endocrine disruptors (EDs) which may be affecting aquatic organisms include industrial chemicals such as alkylphenols, phthalates, polychlorinated compounds (biphenyls, dioxins and furans) and polybrominated ethers.

Anthropogenic release of oestrogens and xeno-oestrogens is a widespread concern. These chemicals may enter the aquatic environment via effluents from sewage treatment plants, industry and other forms of run-off, including from agricultural land (tentatively attributed to carriers in pesticide applications, together with natural hormones and steroid drugs used as growth promoters in livestock).

The mode of action and effects of oestrogenic chemicals has been tested, largely, using mammalian systems and, to a lesser extent fish, and results indicate there may be large differences in responses between taxonomic groups. Responses of many invertebrate groups have scarcely been studied and this represents a major area of uncertainty in our assessment of risk from these chemicals. Another significant knowledge gap addressed in this review relates to the possibility that much of the oestrogenicity in aquatic environments may reside in benthic sediments and, if bioavailable, may be transferred to benthic biota (and hence through the food chain). If this hypothesis is correct, it is important to establish information on the factors that control partitioning behaviour and bioavailability of oestrogens and xeno-oestrogens, in order to provide more accurate predictions of impact.

The current review centres on available knowledge related to these issues and is not intended to be comprehensive. We have, however, tried to set the review in the context of the wider understanding of endocrine disruption, particularly the existing evidence for oestrogenicity in the marine environment. Because many of the sources of environmental (xeno)oestrogens are land-based, and enter the hydrosphere via rivers, previous research on endocrine disruption in aquatic organisms has focused mainly on freshwater systems, a rationale based partly on the assumption that endocrine disrupting chemicals would be diluted to below harmful levels upon reaching saline waters. This argument is now believed to contain a number of uncertainties, notably as a result of biotic and abiotic concentration processes, which we shall address here. In order to explore the potential significance of environmental (xeno)oestrogens in the estuarine and marine environment, however, it is essential to include information on inputs to catchments as a whole, and to draw on observations of bioaccumulation and effects in freshwater counter-parts. Our review begins with an overview of the distributions and concentrations along waterways, and a synthesis of partitioning behaviour into sediments. Subsequent sections examine biological processes such as bioaccumulation and metabolism, together with the *in vivo* and *in vitro* manifestations of

oestrogenicity, and the implications for natural populations.

Throughout this review, concentrations of (xeno)oestrogens in sediments and organisms are expressed in terms of dry weight unless otherwise stated. For clarification of definitions and abbreviations used, a glossary is included in the Appendix.

SOURCES AND LEVELS OF (XENO)OESTROGENS IN WATERWAYS

Chemicals in sewage effluent responsible for oestrogenic effects in male fish include the natural steroids 17β-oestradiol (E2), oestrone (E1), the synthetic hormone 17α-ethinyloestradiol (EE2), together with components attributable to less well characterized EDs (Desbrow et al., 1996, 1998; Routledge et al., 1998). The list of candidate EDs, other than hormones, is extensive and includes alkylphenols and phenols, phthalates, chlorotriazines, dioxins, cadmium, mercury, lead, PAHs, PCBs, PCDDs, PCDFs, and various pesticides (Lintelmann et al., 2003). A comprehensive examination of all these compounds is impractical, therefore the emphasis is on the behaviour of selected oestrogenic compounds E2, EE2, and established 'model' oestrogen mimics, octyl- and nonyl-phenol (OP and NP) which, the weight of evidence (from fresh water (FW) fish studies) suggests, are among the dominating threats to aquatic environments. The basic structures of these compounds are shown in Figures 1 and 2. Characteristic concentration ranges in effluent from sewage treatment works (STWs) are shown in Table 1.

Natural and synthetic oestrogens (17β-oestradiol and 17α-ethinyloestradiol)

Naturally occurring oestrogens 17β-oestradiol (E2), oestrone (E1) and oestriol (E3) are mainly derived from female hormones where they play an important role in maintaining reproductive tissues. A comparison of the relative potency of this group of steroid hormones has shown that the activity of 17β-oestradiol (E2, Figure 1) may be 5–1000 times greater than that of oestrone and oestriol (Lee et al., 2003 and references therein). However, synthetic oestrogens such as 17α-ethinyloestradiol (EE2, Figure 1), a major component of the contraceptive pill, may have even higher oestrogenic activity than their natural counterparts. Both natural and synthetic hormones are excreted and released into the environment (rivers and estuaries) via wastewater treatment plants, and also via run-off from agricultural land (from livestock excreta).

Effluent samples from STWs in the UK have been found to contain 1–50 ng l^{-1} E2 and 0.2–7.1 ng l^{-1} EE2-concentrations which appear to be sufficient to induce oestrogenic activity in an *in vitro* yeast-based oestrogen screen (Desbrow et al., 1998). Comparable concentration ranges have been reported from Japan (Nasu et al., 2000), The Netherlands (Belfroid et al., 1999), Germany (Kuch & Ballschmiter, 2001) and Canada (Ternes et al., 1999b). Somewhat lower levels of E2 (1.1 ng l^{-1}) and EE2 (4.5 ng l^{-1}) were measured in effluent from a Swedish STW, mostly present in unconjugated form, suggesting deconjugation may occur in the sewage system (Larsson et al., 1999). A similar study of STW influent and effluents in the Paris region reported EE2 and E2 concentrations up to 3.21 ng l^{-1} and 17.61 ng l^{-1}, respectively (1–3 ng l^{-1} in surface waters of the Seine). Despite the low proportion of EE2 (10–27%) in these French STW samples, the authors re-affirm that the synthetic hormone may account for a disproportionately high percencentage (up to 50%) of the total oestrogenic activity, based on an *in vitro*-reporter gene assay (Cargouet et al., 2004).

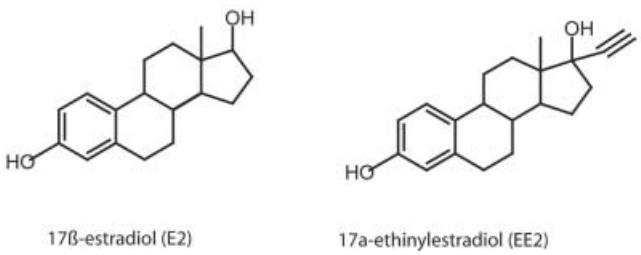
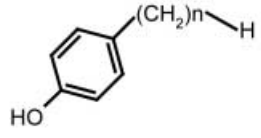


Figure 1. Natural steroid 17β-oestradiol (E2) and the synthetic hormone 17α-ethinyloestradiol (EE2).

Effects-levels for E2 and EE2 in sensitive species are reported to be in the range 0.1–10 ng l^{-1} (e.g. Purdom et al., 1994) and therefore concentrations of these natural and synthetic hormones are thought responsible, at least in part, for *in vivo* oestrogenic effects observed downstream of STWs. Occasionally, higher values have been reported (e.g. up to μg l^{-1} levels of EE2) near outfalls, though these may reflect conjugated (less biologically active) as well as unconjugated, forms. Potentially, adsorption to sediments and subsequent food chain transfer could add to the risk from water-borne exposures, at least locally; this threat has yet to be evaluated rigorously.

Hormones are removed during sewage treatment, though the rate and efficiency of removal appears to be highly variable, depending on factors such as residence times, temperatures, plant design and loadings. There are indications from UK studies that STWs remove 38–83% of the E2 from the influent (Desbrow et al., 1998) whilst Stumpf et al. (1996) reported 58–91% removal of steroids in German STWs. Estimates for STWs serving the Paris region suggest removal rates of 43–60% for E2 and 38–45% for EE2 (Cargouet et al., 2004). A significant proportion of the oestrogen loading in STW effluent samples in the Thames area appears to be as oestrone which may be partly attributable to the oxidation of oestradiol while in contact with activated sludge (Xiao et al., 2001; Ternes et al., 1999b).

Livestock wastes may include significant oestrogen concentrations, both as solids (14–533 ng E2 g^{-1} dry wt in poultry waste) or in urine (13 ng l^{-1} in cattle). Run-off from land manured with poultry litter may contain up to 3500 ng l^{-1} E2. Steroid drugs are used to control the oestrous cycle and treat reproductive disorders in various



n=8 octylphenol n=9 nonylphenol

Figure 2. Basic structure of alkylphenols.

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Table 1. *Examples of concentration ranges of oestrogenic compounds in STW effluent.*

Compound	Concentration range
17β-oestradiol (E2)	1–50 ng l^{-1} [a,b] up to 64 ng l^{-1} [c]
17α-ethinyloestradiol (EE2)	0.2–7 ng l^{-1} [a,b] up to 42 ng l^{-1} [c]
Alkylphenol polyethoxylates (APEO)	8.8–77.8 ng l^{-1} [d] up to 45 μg l^{-1} [e]
Nonylphenol (NP)	<0.2–5.4 μg l^{-1} [f] up to 3 μg l^{-1} [e]
Octylphenol (OP)	0.4–1.82 μg l^{-1} [g] 0.12–1.7 μg l^{-1} [h]

References: [a], Desbrow et al. (1996); [b], Routledge et al. (1998); [c], Ternes et al. 1999b; [d], Naylor et al. (1992); [e], Lye et al. (1999); [f], Blackburn & Waldock (1995); [g], Isobe et al. (2001); [h], Lee & Peart (1995). STW, sewage treatment works.

livestock and could increase the loadings to surface and ground waters from agriculture (Ying et al., 2002a and references therein).

Although oestrogen levels are biologically significant near to sources, there are few data on concentrations in more open natural waters, due to analytical difficulties. Published values generally indicate concentrations in the ng l^{-1} range or less (Figure 3A). For example, in the River Thames between Reading and Richmond E2 ranged from 0.5–7.1 ng l^{-1}, and at Crossness, in the middle estuary, from <0.03–7 ng l^{-1}; oestrone (El) tended to be present at slightly higher concentrations close to sewage discharges (up to 17 ng l^{-1}) , whilst EE2 was undetectable throughout (Xiao et al., 2001). Concentrations of E2 and EE2 were also predominantly below the limits of detection (~0.2 ng l^{-1}) in the Elbe Estuary (Stachel et al., 2003) and ranged from <0.1 – ~5 ng l^{-1} in Dutch estuarine waters (Belfroid et al., 1999).

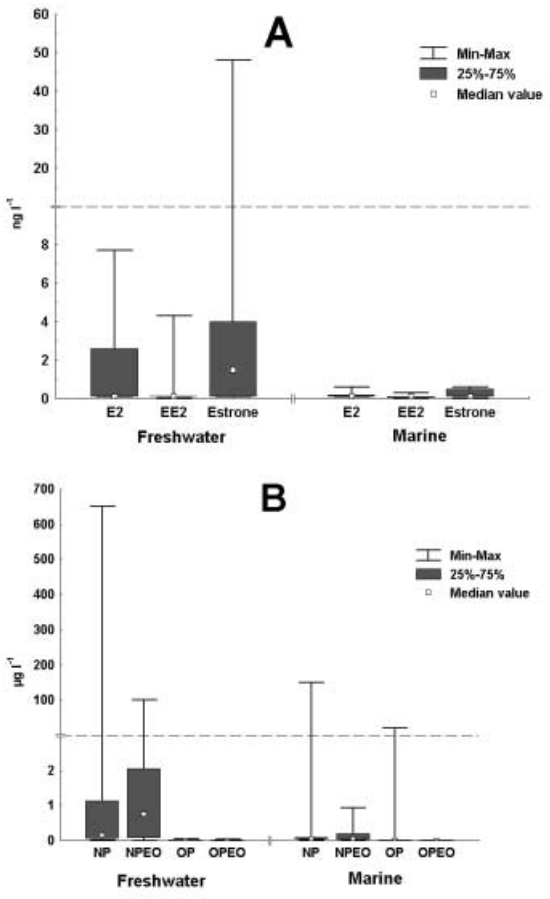

Figure 3. Concentration ranges, upper and lower quartiles, and median values for natural and artificial oestrogenic hormones (A), and alkylphenols (B), in fresh and saline waters. NB; note break in scales. (From concentrations reported by Baronti et al. (2000); Belfroid et al. (1999); Furuichi et al. (2004); Stachel et al. (2003); Rodriguez-Mozaz et al. (2004); Ahel et al. (1991); Blackburn & Waldock (1995); Jonkers et al. (2003); Li et al. (2004b); Solé et al. (2000); Lintelmann et al. (2003); Lye et al. (1999)).

Alkylphenols (octyl-and nonyl-phenol)

Alkylphenol polyethoxylates (APEOs), based mainly on nonyl- and to a lesser extent, octyl-phenols (Figure 2), are non-ionic surfactants which have been used for more than half a century for domestic, industrial and agricultural purposes (review by Ying et al., 2002b). Over 300,000 tons are produced worldwide of which an estimated 18,000 tons of nonylphenol polyethoxylate (NPEO) and 3600 tons of octylphenol polyethoxylate (OPEO) are manufactured in the UK (Lye et al., 1999). It has been calculated that some 6000 tons of APEOs enter UK rivers and estuaries annually from various sources including sewage outfalls, industrial discharge and agricultural run-off (Blackburn et al., 1999).

Industrial applications comprise about 55% APEOs global usage; currently this broad group of compounds are utilized in paper and pulp mills, textile processing, resin and paint manufacture and other chemical applications. Up until 1996 they were also a component of waste discharged into the sea from off-shore oil/gas production platforms, where they were used in lubricant and surfactant formulations (Olsgard & Gray, 1995). About 33% of APEOs are (or were, until voluntary EU restrictions) employed as ingredients in household and industrial detergents which are mainly disposed of into the sewer system and are biodegraded in the environment or in sewage treatment works, via stepwise loss of ethoxy groups to more hydrophobic, recalcitrant metabolites including nonylphenol (NP), octylphenol (OP), as well as alkylphenol mono- di- and tri-ethoxylates. Thus, alkylphenols (AP) based on NP and OP accumulate in the aquatic environment as complex mixtures of isomers containing branched alkyl groups with a formula of C_9H_{19} and C_8H_{17}, respectively (Figure 2). Despite recent EU restrictions APEOs are used legally in large amounts elsewhere e.g. in many Asian countries.

Alkylphenols are common contaminants in STWs and may be present in μg l^{-1} concentrations in effluent samples and mg g^{-1} quantities in sludges and sediment (Tables 1 & 3). For example, concentrations up to 45 μg l^{-1} NPEO (3 μg l^{-1} NP) have been measured in an outfall on the Tyne Estuary (Lye et al., 1999). In untreated septage samples APs may even reach mg l^{-1} concentrations (Ying et al., 2002b).

As with natural and synthetic oestrogens, rates of removal of alkylphenols by STW plants are variable. Studies in Japan have estimated losses of NP and OP during secondary sewage treatment at 79–99% and

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47–98%, respectively (Isobe et al., 1999). Removal rates of NPEOs as high 93–99% have also been reported for US systems (Naylor, 1995) whilst Ahel et al. (1994) calculated that 35–40% NPEOs were removed during treatment in STWs along the Glatt river system in Switzerland (with 85% of the released load present as metabolic products). Alkylphenol polyethoxylate concentrations in influent samples from Dutch STWs ranged between 2 and 2300 $\mu g\,l^{-1}$, compared with 0–15 $\mu g\,l^{-1}$ measured in outfalls, where substantial removal to particulates is confirmed by the relatively high levels (up to 2400 $\mu g\,g^{-1}$ dry weight) found in sediments (de Voogt et al., 1999). Other reported concentration ranges for NP in sewage sludges include 137–470 $\mu g\,g^{-1}$ in Canada (Lee & Peart, 1995) and 100–500 $\mu g\,g^{-1}$ in Spain (Solé et al., 2000). The behaviour of APs in such systems will therefore be influenced strongly by physiochemical properties (partitioning). Since NP and OP have an estimated water solubility of 1.57 mg l^{-1} and 12.6 mg l^{-1}, respectively, and corresponding octanol-water partition coefficients (log K_{ow}) of 5.76 and 4.12 (Ahel & Giger, 1993; Ying et al., 2003) moderate accumulation in the sediment phase would be anticipated.

Ranges of APEO concentrations and degradation products OP and NP in fresh and saline waters are depicted in Figure 3B. Many surface water samples from national surveys report relatively low-levels, though in the vicinity of discharges (particularly in rivers) concentrations may reach several hundred $\mu g\ l^{-1}$—levels sufficient to threaten native organisms (Blackburn & Waldock, 1995; Solé et al., 2000; Ying et al., 2002b).

In a limited survey of UK waters, which included seven estuaries (Wear, Tees, Tyne, Blythe, Mersey, Southampton, Poole), APs in the form of NP were measured in the Tees and Mersey Estuaries at concentrations of 5.2 $\mu g\,l^{-1}$ and 0.32 $\mu g\,l^{-1}$ (and up to 180 $\mu g\,l^{-1}$ in some rivers subjected to known inputs, notably the Aire). Octylphenol was only detected in the Tees Estuary—at 13 $\mu g\,l^{-1}$ (Blackburn & Waldock, 1995). In the Tyne, 0.13 $\mu g\,l^{-1}$ NP and 0.94 $\mu g\,l^{-1}$ NP1EO were detected near to outfalls (Lye et al., 1999). Further afield, in the Elbe Estuary, concentration ranges of dissolved NP and OP were relatively low—0.013–0.029 $\mu g\,l^{-1}$ and 0.001–0.002 $\mu g\,l^{-1}$, respectively—and decreased towards the open sea. Nevertheless, the Elbe was identified as a significant pollution source for these compounds (Stachel et al., 2003). Elevated levels of AP metabolites (0.2–1.0 $\mu g\,l^{-1}$ NPEO; 0.007–0.04 $\mu g\,l^{-1}$OPEO) have been measured in waters from the sewage-impacted Jamaica Bay area, New York (Ferguson et al., 2001) and in waters of the Venice Lagoon (NPEO 1–39 $\mu g\,l^{-1}$; Marcomini et al., 2000).

At contaminated sites such as the lower Tees Estuary, APEO metabolites (intermittently $>80\ \mu g\,l^{-1}$) are implicated as contributing to poor water quality in acute toxicity bioassays with marine copepods *Tisbe battagliai* and oyster embryo *Crassostrea gigas* (Thomas et al., 1999, 2001). At the sublethal level, though having less strong oestrogenic properties than natural hormones, APs have been shown to induce vitellogenin (VTG) synthesis and testicular abnormalities in male eelpout (Christiansen et al., 1998) and inhibit the settlement of barnacle larvae (Billinghurst et al., 1998), together with other effects discussed in the 'Impact of (xeno)oestrogens' section of this paper. These sublethal effects occur at levels of $\sim 1\ \mu g\,l^{-1}$ i.e. at concentrations sometimes observed in the field, such as the lower Tees.

Oestrogenic effects in some major estuaries may therefore be due, in part, to alkylphenolic compounds from industrial and sewage discharges (and, perhaps, previous sewage dumping). Though concentrations have been reported to be decreasing recently in some European studies (due to voluntary restrictions on usage in domestic detergents), and were not detectable in >80% of the UK estuarine waters sampled by Blackburn & Waldock (1995), the AP ethoxylate transformation products, NP and OP, tend to be stable, hydrophobic, and hence probably have greater affinity for sediment sinks than the parent APEO compounds. Alkylphenol ethoxylates themselves are considered less significant, environmentally, than their corresponding transformation products due to their lower persistence, toxicity and oestrogenic activity (although there are few comprehensive data sets on the oestrogenic activity of each isomer; Düring et al., 2002).

Time-series from freshwater habitats in Switzerland, confirm that environmental APEO levels are gradually being reduced. Concentrations of nonylphenol polyethoxylates (NPnEO, n=3–20) and their major metabolic products (4-nonylphenol (NP), 4-nonylphenol monoethoxylate (NP1EO), 4-nonylphenol diethoxylate (NP2EO), nonylphenoxy acetic acid (NP1EC) and nonylphenoxy(ethoxy) acetic acid (NP2EC)) have all decreased significantly in recent years (Ahel et al., 1999). In 1997, after legal and voluntary restrictions, primary sewage effluents contained NPnEO levels at around 0.15 mg l^{-1} compared with 0.7–1.0 mg l^{-1} in 1983. Metabolites were also significantly reduced (from 0.2–1.2 $\mu g\,l^{-1}$ NP compared with 3.5 $\mu g\,l^{-1}$). Nonylphenol, NP1EO and NP2EO in rivers also dropped (0.1–0.3 $\mu g\,l^{-1}$ in 1997/1998 compared with 0.5–21 $\mu g\,l^{-1}$ in 1983/1984). This reduction in environmental inputs of nonylphenolic compounds was reflected in sediment core profiles from Swiss lakes. Peak concentrations of NP and NP1EO of 1.0–1.3 mg g^{-1} dry wt were found in sediment layers deposited between 1970 and 1985, whilst the most recent sediments contained concentrations in the range 0.1–0.2 mg g^{-1} (Ahel et al., 1999).

The threat from APs may therefore be gradually declining, though there is continuing uncertainty as to the relative contribution of APs to endocrine disruption in the field. Toxicity identification evaluation (TIE) analysis of sewage effluents, using assays such as yeast oestrogen receptor binding (YES) as the endpoint, suggest that oestrogenic activity arising from treatment plants is associated primarily with the fractions containing natural (E1, E2) and synthetic oestrogens (EE2) rather than alkylphenols (Servos et al., 1999; Desbrow et al., 1998). However, caution is needed in extrapolating from these *in vitro* assays of oestrogenicity to *in vivo* consequences, since processes such as bioaccumulation are not taken into consideration. Given the higher levels of AP in the environment, relative to natural and synthetic oestrogens, magnification of residues in tissues of benthic organisms could increase their biological impact and relevance. This is discussed further in 'Bioaccumulation rates and routes' and 'Impact of (xeno)oestrogens' (below).

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(XENO)OESTROGENS IN SEDIMENTS

The initial observations of endocrine disruption in freshwater fish populations suggested that effects decreased rapidly within relatively short distances from sources (usually STWs). It was therefore anticipated that dilution in estuaries and the open sea would render the threat harmless in these environments. Since then, the discovery that some benthic estuarine fish, notably flounder (which have close contact with sediment and feed on benthic infauna), also exhibit symptoms of impact from oestrogens, has led to the recognition that adsorption to particulates may be responsible for impeding dispersal and dilution and that sediments could be acting as a secondary source (Allen et al., 1999a,b). Here we examine evidence for the role of freshwater, marine and estuarine sediments as sinks for (xeno)oestrogens, together with the current level of understanding on factors controlling partitioning between particulate and aqueous phases.

Natural and synthetic oestrogens (17β-oestradiol and 17α-ethinyloestradiol)

Published values on levels of natural and synthetic steroids in marine, estuarine and freshwater sediments are summarized in Table 2. Data are scarce due to analytical difficulties—techniques to measure low environmental concentrations are still under development.

Microwave extraction in the presence of organic solvents, followed by gas chromatography/mass spectrometry (GC/MS) analysis, has been applied to determine the levels of steroids in bed-sediments from above and below sewage treatment outfalls entering the River Uck and two stretches along the River Ouse, UK (Liu et al., 2004). The compounds 17β-oestradiol (E2) and 17α-ethinyloestradiol (EE2) were present at concentrations of <0.5–4 ng g^{-1} and <0.5–12 ng g^{-1}, respectively, in these freshwater sediments. However, the highest concentrations of these compounds did not always coincide with outfall or downstream sample sites.

The presence of oestrogens in nine freshly deposited sediment samples from the River Elbe and the mouths of its tributaries has recently been described by Stachel et al. (2003), though in every sample the concentrations of E2 and EE2 were beneath the limit of quantification of 2 ng g^{-1} dry wt sediment. Higher environmental levels of EE2 (28.8 ng g^{-1}) have been determined in a river bed-sediment from Catalonia using solid phase extraction and quantification by liquid chromatography-electro-spray ionization–MS (López de Alda & Barceló, 2001).

Because of analytical difficulties 'concentrations' of (xeno)oestrogens in sediments are sometimes expressed as oestrogen equivalents, from bioassays. Based on this methodology, very low concentrations of E2 ranging from 3.27 to 10.6 pg g^{-1} sediment (calculated as equivalent concentrations—EEQ—using E-screen assay) have been described in the downstream sediment samples of four rivers in South Korea (Oh et al., 2000). The oestrogenic potency of 12 marine and coastal sediments in The Netherlands has also been determined using *in vitro* reporter gene assays (Legler et al., 2002a): sediments collected from highly industrialized localities such as the Port of Rotterdam exhibited E2 EEQ of up to 40 pmol g^{-1}. In contrast, sediments sampled along the Dutch coast showed less oestrogenic activity (6.4–15 pmol EEQ g^{-1}) with the lowest values of 5–7 pmol EEQ g^{-1} reported for unpolluted reference areas of Lake Ijessel and Eastern Scheldt (Legler et al., 2002a).

Recent measurement of E2 in sediments in the Manko tidal flat, Okinawa Island, ranged from the equivalent of <2 to 23.2 ng g^{-1} (mean $\sim$8 ng g^{-1}). The highest values of E2 were reported in sediments from the confluence of Kokuba and Noha rivers, which probably transport the oestrogenic compounds from upstream areas of intensive agriculture (Tashiro et al., 2003). This illustrates that, although hormone steroids are derived from human sources, via sewage effluent outfall, livestock waste is another potentially important input. Poultry waste may contain, for example, an average of 44 ng g^{-1} E2 (Ying et al., 2002a and references therein).

Alkylphenols (octyl-and nonyl-phenol)

A summary of alkylphenol polyethoxylate metabolite loadings in freshwater, estuarine and marine sediments is presented in Table 3. Nonylphenol concentrations measured in 27 UK river sediments varied from <100 up to 15,000 ng g^{-1} dry wt (Blackburn et al., 1999), however, of the waterways sampled, detectable levels of NP were only observed in sediments from the River Aire and River Mersey: in the majority of riverine sediments NP was below the limits of detection. This may be attributed, in part, to the fact that many of the samples were sands and gravels of low organic matter content, and have lower surface area (fewer sites) to absorb contaminants, as compared with muddy and/or silty counterparts.

The occurrence and spatial distribution of alkylphenols and alkylphenol polyethoxylates has been studied in sediments sampled from heavily industrialized sites on the Great Lakes and the upper St Lawrence River in Canada

Table 2. *Concentrations of 17β-oestradiol 17α-ethinyloestradiol in sediments (ng g^{-1} dry wt).*

Location	17β-oestradiol	17α-ethinyloestradiol	Reference
Rivers Uck and Ouse	<0.5–4	<0.5–12	Liu et al., 2004
River Elbe	<2	<2	Stachel et al., 2003
Rivers in Catalonia, northern Spain		22.8	López de Alda & Barceló, 2001
FW sediment, Germany	8.5		Wenzel et al., 1998
Manko tidal flat, South Korea	<2 to 23.2		Tashiro et al., 2003

FW, fresh water.

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(Bennie et al., 1997) where NP concentrations ranged from 170 to 72,000 ng g^{-1} dry wt (mean 10,600 ng g^{-1} dry wt). In addition, OP was detected at concentrations varying from <5 to 1800 ng g^{-1} dry wt. A complementary follow-up study of sediments from Hamilton Harbour (Lake Ontario), the location of highest NP and OP concentrations, confirmed that amounts diminished rapidly with distance from sewage treatment plant outflows (Bennett & Metcalfe, 2000).

In the USA, monitoring of 30 river sediments located downstream of effluent discharges from wastewater treatment plants and industries using NPEOs, by liquid chromatography-fluoresence (LC-FL), indicated NP concentrations in the range <2.9–2960 ng g^{-1} dry wt (mean 162 ng g^{-1}). Five of the sites were characterized as highly contaminated, 12 had detectable levels of at least one or more NP species and 13 locations had concentrations beneath the limits of detection (Naylor et al., 1992). Recent GC/MS analysis of river sediments collected along the Han River in South Korea determined NP values of ~50 ng g^{-1} dry wt upstream of Seoul, increasing to a maximum of 250 ng g^{-1} dry wt in the city and decreasing to ~140 ng g^{-1} dry wt downstream (August profile). Although accumulations of NP in water and suspended particle matrices (SPM) were higher in summer, as compared with winter, no seasonal change was observed in the river sediments. The NP concentrations were generally higher in SPM than in benthic muds, presumably due to differences in granulometry (Li et al., 2004a, Table 3). The effect of urban and industrial pollution from two rivers in northern Italy has similarly been traced by measurement of NP and NP ethoxylate concentrations in sediments upstream and downstream of the River Po, at the confluence with the Lambro (Valsecchi et al., 2001). The lowest concentrations of NP (450 ng g^{-1} dry wt) were reported for upstream Po sediments, whereas the Lambro River and downstream Po sediment had NP values of 4700 ng g^{-1} dry wt and 2900 ng g^{-1} dry wt, respectively (Table 3).

Less-industrialized catchments would thus be expected to exhibit significantly lower particulate AP loadings, as confirmed in a number of studies. For example, endocrine disrupting compounds, including NP and OP, were examined in sediments from the relatively unindustrialized Rivers Uck and Ouse in south-east England, using microwave-assisted extraction and GC/MS analysis for identification and quantification (Liu et al., 2004). Quantifiable accumulations of NP and OP ranged from 2 to 5 ng g^{-1} dry wt and 4 to 12 ng g^{-1} dry wt, respectively, in these Sussex rivers, though often both NP and OP were present at very low levels—below the 1.7 ng g^{-1} dry wt limit of quantification (Liu et al., 2004).

An investigation into the occurrence of APEOs and their degradation products at 33 estuarine sites in the UK, using GC/MS, showed that concentrations of NP ranged from <100 ng g^{-1} to 1700 dry wt with most samples below the limit of detection (median value of <100 ng g^{-1} dry wt; Blackburn et al., 1999). As in river samples, these low values were perhaps due to a preponderance of coarser particles of low organic content and limited binding sites. Highest detectable levels of NP were generally those in siltier sediments from the Tees Estuary.

Concentrations of NP and OP have also been determined in sediments from the Tyne and Tees Estuaries by Lye et al. (1999), using GC and GC/MS. Highest amounts of both compounds were again found in the Tees, where concentrations of NP varied in the range 1600–9050 ng g^{-1} dry wt sediment and OP 30–340 ng g^{-1} dry wt (Table 3). A clear decrease in the concentrations of both target compounds was observed from upstream to downstream sample sites, such that respective

Table 3. *Concentrations of octyl- and nonyl-phenol in sediments (ng g^{-1} dry wt).*

Location	Nonylphenol	Octylphenol	Reference
River Tyne and estuary	30–80	2–20	Lye et al., 1999
River Tees and estuary	1600–9050	30–340	Lye et al., 1999
Rivers Uck and Ouse (Sussex)	2–5	4–12	Liu et al., 2004
UK estuaries	<100–1700		Blackburn et al., 1999
UK rivers	<100–15,000		Blackburn et al., 1999
Baltic Sea marinas	10–153		Bester et al., 2001
North Sea	0.1–17	<0.1–2	de Voogt et al., 1997
River Elbe and tributaries	27–428	5.5–62	Stachel et al., 2003
Rivers Lambro and Po, Italy	450–4700		Valsecchi et al., 2001
Great Lakes, St Lawrence	170–72,000	10–1800	Bennie et al., 1997
Great Lakes	up to 37,000	up to 23,000	Bennett & Metcalfe, 1998
Georgia Strait, Canada	18–29		Shang et al., 1999a
Jamaica Bay, New York	7–13,700	<5–45	Ferguson et al., 2001
USA rivers (industrial/urban)	<2.9–2960		Naylor et al., 1992
Han River, South Korea (benthic muds)	25–932		Li et al., 2004a
(suspended particulates)	1000–16,600		Li et al., 2004a
Masan Bay, South Korea	113–3890	4–179	Khim et al., 1999
Japanese Harbours	100–2600		Hosokawa et al., 2003
Tokyo Bay	120–640	6–10	Isobe et al., 2001
Tokyo rivers	30–13,000	3–50	Isobe et al., 2001

concentrations of OP and NP were about five- and ten-times lower at the mouth of the estuary, at Redcar Jetty, than at Bamlett Bight, upstream, reflecting the transition from marine sand to estuarine fines. The high concentrations of OP and NP in the Tees have been attributed to a variety of sources including treated effluent extracts from sewage treatment works and surfactant manufacture (nonylphenol at Billingham and nonylphenol ethoxylates at Wilton). The NP and OP concentrations in sediments from the Tyne were generally lower (30–80 ng g^{-1} dry wt and 2–20 ng g^{-1} dry wt respectively). No single source of NP or OP was identified, although sewage treatment works on the Tyne yield about 3 μg l^{-1} of NP and may contribute significantly to the total estuarine budget. The lower concentration of OP, relative to NP, in both the Tyne and Tees Estuaries reflects the lower commercial use of octylphenol polyethoxylate (20%) as compared with nonylphenol polyethoxylates (80%). Comparison of the concentrations of NP in Tyne and Tees sediments with waters from the same area indicates a strong affinity for suspended particulate matter and benthic sediments, attributable to the hydrophobic characteristics of NP (moderately high logK_{ow}) and the recalcitrant nature of phenolic compounds to biodegradation (Ahel & Giger, 1993).

Outside the UK, some of the highest concentrations of alkylphenol ethoxylate metabolites in maritime sediments have been observed in sewage-impacted urban estuary sediments from the Jamaica Bay area of New York (Table 3). Sensitive liquid chromatography/mass spectrometry (LC/MS) analysis revealed levels of NP ranging from 6.99–13,700 ng g^{-1} dry wt (mean 2107 ng g^{-1} dry wt). The average concentration of OP was lower—12.4 ng g^{-1} dry wt (maximum value 45 ng g^{-1} dry wt)—though it should be noted that OP is considered to be up to five times more oestrogenic than NP (Routledge & Sumpter, 1997; Ferguson et al., 2001). A strong correlation between the distribution and concentration of conventional sewage tracers and alkylphenol polyethoxylate metabolites in the Jamaica Bay sediments supports the notion that NP and OP were derived from a wastewater source (Ferguson et al., 2001).

Some high NP and OP values have been reported in Japanese inshore and estuarine sediments (Table 3). A recent nationwide survey of 39 harbours in Japan reported NP concentrations of ~100–2600 ng g^{-1} dry wt of surface sediment (Hosokawa et al., 2003) whilst an investigation of alkylphenols from a 10 km stretch of the Sumidagawa River, and bay sediments from the Tokyo metropolitan area, returned NP values ranging from 30 to 13,000 ng g^{-1} dry wt and OP concentrations of 3–670 ng g^{-1} dry wt (Isobe et al., 2001). Examination of distributions down cores in the latter study suggests that NP and OP may be persistent in sediments over decades and hence profiles could reflect the history of inputs. Nonyphenol and octylphenol were present down to a depth of 39–42 cm (estimated to be deposited in 1950) and increased up to a depth of 22–24 cm, corresponding approximately with the year 1975 when production and use were probably at a maximum.

Other surveys, such as that carried out at Masan Bay, South Korea, have illustrated the effects of industrial and municipal wastewaters from cities (Chagwon and Masan) on the AP content of coastal sediment (Khim et al., 1999). Reverse-phase high pressure liquid chromatography (HPLC) measurements of 28 surface sediments indicated NP concentrations in the range 113–3890 (mean 510) ng g^{-1} dry wt, with highest values inshore, at the head of the bay and a general 2–4 fold decrease in values in sediments towards open waters. The lower industrial usage of octylphenol polyethoxylates, as compared with NPEO, in the Masan Bay area was reflected by OP concentrations in surface sediments which were 20–10 fold less (~4–79 ng g^{-1}) than NP concentrations.

Gradual dispersion and dilution of NP in offshore particulates is confirmed in comparisons of estuarine and marine sediments in the North Sea, and Baltic Sea marinas, by Bester and co-workers, who used normal-phase HPLC with fluorescence detection for the identification and quantification of APs (see Bester et al., 2001 and references therein). The concentrations of NP ranged from 10 to 153 ng g^{-1} dry wt, with the highest values reported for marinas and the lowest values for marine sediments sampled over 100 km from the coast (Table 3). Relatively low NP and OP levels also were observed in 22 North Sea marine sediments by de Voogt et al. (1997)—values varying from below the limit of detection to 2 ng g^{-1} dry wt for OP, and from 0.1 to 17 ng g^{-1} dry wt for NP. These appear typical of values offshore, in areas remote from sources; using normal phase-liquid chromatography–electrospray–MS, Shang et al. (1999a) measured NP in sediments from the Strait of Georgia, British Columbia, at an average concentration of 18 ng g^{-1} dry wt.

Partitioning behaviour

The natural oestrogen E2 and the synthetic oestrogenic steroid EE2 have solubilities of 13 mg l^{-1} and 4.6 mg l^{-1}, respectively, and both have low vapour pressures of 2.3×10^{-10} and 4.5×10^{-11} Pa (Lai et al., 2000). These properties, combined with moderately high hydrophobicity (estimated log K_{ow} of 3.10–4.01 for E2 and 3.67–4.15 for EE2) imply that affinity for particulates is likely to be relatively high (Lai et al., 2000; Defra, 2001). There have been few realistic studies describing partition coefficients (K_d or organic-normalized K_{oc}) to verify this for estuarine/marine sediments, though from the available data, K_ds in the low hundreds might be expected for most natural and synthetic oestrogens (Petrovic et al., 2001).

Freundlich sorption coefficients (K_f—a measure of the K_d which describes a non-linear relationship between adsorbed and dissolved chemical, used when sorption is limited) of 21.8 for E2 and 24.2 for EE2 have been calculated from sorption studies on sediment and ground water from aquifers in Australia (Ying et al., 2003). These values have been substituted to derive organic-normalized K_{oc} values of 4360 and 4840 for E2 and EE2, respectively, which, when used in comparisons of partition coefficients with other endocrine disrupting compounds (including alkylphenols 4-n-NP and 4-n-OP), indicate that oestrogenic steroids display moderate sorption, at least on the limestone aquifer sediment (Ying et al., 2003).

The sorption of oestrogenic steroids on natural (undried) estuarine sediment is relatively rapid, with maximum sorption occurring after 1 h; reported log K_f values for El, E2, E3, EE2 are 1.71, 1.56, 1.33 and 1.72, respectively (Lai et al., 2000). Transformation of these K_f

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values to sorption constants (K_{oc}) by Ying and co-workers (2002a) produces values of 3300 and 4770 for E2 and EE2 and subsequent conversion to K_d (essentially the ratio between particulate and dissolved phases) results in approximated values of 40–210 l kg^{-1} for E2 and 55–350 l kg^{-1} for EE2 (Holthaus et al., 2002). These coefficients confirm the general notion that oestrogenic steroids are likely to adsorb to sediments and soils, though perhaps not as strongly as alkylphenols. The sorption of E2 and EE2 was indicated by Lai et al. (2000) to correlate with the total organic carbon (TOC) content of sediment, and increasing salinity, but less so with particle size. In addition, iron oxide was found capable of sorbing up to 40% of that of a sediment composed of only 1.1% TOC. Thus it was concluded that organic carbon may not always be required for sorption of oestrogens: other contaminant-binding sediment phases such as (Fe and Mn) oxyhydroxide coatings could also be important (Lai et al., 2000).

Further insights into partitioning of oestrogenic steroids are provided by Holthaus et al. (2002) who compared the potential for E2 and EE2 to sorb to bed- and suspended-sediments collected from rivers in urban/industrial (Aire, Calder, Tyne and Tees) or mainly rural environments (Thames at Wallingford). Using ^{14}C radio-labelled material under anaerobic conditions (to minimize biodegradation), the organic carbon-normalized distribution coefficient (K_{oc}) for E2 in bed-sediments ranged from 641 to 2975, whilst that for EE2 ranged from 1926 to 10,000. Taken over a range of seasons and locations, results for bed-sediments gave distribution coefficients (K_d) which varied from 4 to 74 l kg^{-1} for E2 and from 8 to 121 l kg^{-1} for EE2. Sorption to suspended sediments gave slightly higher K_d values, which ranged from 21 to 122 l kg^{-1} for E2 and 19 to 260 l kg^{-1} for EE2. In the majority of cases, the K_d values for EE2 were up to three times higher than those of E2.

Part of the apparent variation in partitioning behaviour among different studies can be attributed to granulometric factors and sediment conditioning. In the experiments of Holthaus et al. (2002) highest K_d values were associated with sediments rich in TOC and with small particle sizes (as evidenced by a positive correlation between K_d and silt content and negative correlation with sand). Contrary to the results of Lai et al. (2000), Holthaus and co-workers also found that the illite fraction of clay minerals was a more attractive sorbent to E2 and EE2 than kaolinite, smectite, or sediments with a high TOC content (Holthaus et al., 2002). The apparent difference in behaviour may reflect differences in clay mineralogy and possibly the use of dried sediments in the latter study. Though the authors suggested this was not a major factor influencing K_d in their samples, it is interesting to note that equilibrium times (>2 d) were significantly longer than those reported for wet sediment by Lai et al. (2000).

The effects of salinity, sediment concentration, particle size and surfactant concentrations on the sorption behaviour of oestrone (E1) and E2 have been investigated by Bowman et al. (2002). Kinetic experiments using ^{14}C radio-labelled E1 and E2 confirm a systematic, time-dependent increase in the concentration removed to sediment, coinciding with a decrease in the dissolved phase. Adsorption rates were relatively slow compared with the study by Lai et al. (2000), with 80% of E2 removal onto the sediment after 50 h and equilibrium at 170 h. The use of freeze-dried material, as opposed to natural sediments, may again be partly responsible for this difference in behaviour. Partition coefficients on sediment particles were 141 l kg^{-1} for E1 and 102 l kg^{-1} for E2, though much higher partition coefficients of 22,200 l kg^{-1} (E1) and 13,500 l kg^{-1} (E2) were determined for colloidal particles, leading Bowman and co-workers to conclude that colloids are stronger sorbents for natural oestrogens than bulk sedimentary counterparts.

Measurement of partition coefficients over a salinity range of 0–35 did not indicate any significant change in the sorption of E2 (unlike the salinity dependent increase described by Lai et al., 2000); however, E1 sorption did increase with higher salinities (Bowman et al., 2002). The increase in E1 partition coefficient with increasing salinity was attributed to its decreasing aqueous solubility, due to the presence of salts, thereby increasing its attraction to particles in the sediment. A salting out constant of 0.3 l mol^{-1} was determined for E1 (Bowman et al., 2002).

Measured partition coefficients of both E1 and E2 decreased as a function of increasing sediment loadings (over the range 0–10 g l^{-1}) in the experiments of Bowman et al. (2002). This was ascribed to the increased transfer of highly-sorbing colloids from the solid to liquid phase at the higher sediment loadings. Incomplete removal of the colloid phase during filtration of samples thus causes anomalously elevated concentrations within the 'dissolved' fraction and an apparent decrease in partition coefficients.

In contrast to the study by Lai et al. (2000), a significant effect of particle size on partition coefficients for E1 and E2 was demonstrated by Bowman et al. (2002) perhaps because their experimental sediment contained a broader spread of size fractions. Interestingly E1 had a higher affinity than E2 for small particles (63–150 μm) whilst, conversely, E2 had the higher affinity for particles >150 μm and up to 2000 μm. Somewhat unexpectedly, K_ds for both compounds were just as high, if not higher in the larger sand particles as in the finer silt fraction; this was explained in part by a strong positive correlation between K_d and both specific surface area and particulate organic carbon, which are therefore more likely to be the controlling factors than particle size *per se*. Bowman and co-workers report average K_{oc} values for E1 and E2 as 2970 and 3003, respectively—close to the carbon corrected partition coefficients described by Ying et al. (2002a).

The emerging data on partitioning are thus providing some useful insights into environmental behaviour of oestrogenic hormones and the determinands of sorption to particulates. Clearly, however, there are some significant inconsistencies which have yet to be resolved, preferably using natural sediments to rule out anomalies caused by the effects of pre-treatment on physicochemical properties and binding capacity.

The sorption of OP and NP onto particulate organic matter and minerals also appears to be a critical process in controlling the fate of alkylphenol polyethoxylate metabolites in rivers, estuaries and coastal environments, but, like hormones, is not fully resolved. Based on environmental data for NP on suspended particulates in the Han

River, South Korea, Li et al. (2004a) obtained variable estimates for K_d between 501 and 15,848 l kg^{-1}. In one of the few attempts to characterize determinands of alkylphenol partitioning, laboratory batch methods were used by Johnson et al. (1998) to examine the sorption behaviour of OP in three English rivers differing in sediment and water quality characteristics. Measurement of sorption kinetics in sediments from the Aire, Calder and Thames, using ^{14}C-labelled OP, produced variable partition coefficients (K_d) of 6–700 l kg^{-1} and organic carbon-normalized partition coefficients (K_{oc}) of 3500–18,000 l kg^{-1}, which indicates that, given sufficient time and mixing, a large proportion of OP in solution is likely to become partitioned into the bed sediment, in association with organic carbon. Octylphenol was, in fact, bound preferentially to bed-sediments with the highest organic carbon contents (% TOC) and the highest proportion of silt (2–63 μm) and clay ($<2\,\mu$m), as compared with sand (63–900 μm) particles. The high smectite content (80%) of the River Thames sediments may have also acted as a competitive sorbent to the organic matter. Highest partition coefficients were those derived from four suspended stream sediments collected from the River Calder and River Aire—attributed to the high affinity of OP for organic (algal) aggregates in these samples. Overall, the study concluded that in the rural section of the Thames much OP would remain free in solution, whereas a significant proportion of OP would sorb to the organic matter phase of suspended and bed sediments of the urban and industrial riverine systems of the Aire and Calder (Johnson et al., 1998).

The sorption/desorption of relatively hydrophobic organic compounds such as NP and OP, to and from the organic portion of sediments, is broadly analogous to partitioning between an aqueous and organic solvent. Partitioning coefficients (log K_{ow}) have been determined for *n*-octanol/water and *n*-hexane/water systems and are of the order of 4.12 for OP and 4.48 for the more hydrophobic NP. Because the organic carbon normalized partition coefficient (K_{oc}) is related to the K_{ow}, K_{oc} values of about 6000 would be anticipated for both compounds (within the range observed for OP in the three river systems studied by Johnson et al., 1998), reaffirming that OP and NP would be expected to have a relatively high affinity for organic-rich bed-sediment as compared with water.

There are a number of quantitative estimates of this affinity: for example some 60% transfer of NP from water to bed-sediment has been estimated to occur over a 35 km stretch of the River Glatt, Switzerland, through degradation and sediment sorption (Ahel et al., 1994). Observations of the distribution between aqueous and particulate phases in estuaries also hint at the importance of removal processes. Jonkers et al. (2003), for example, have investigated the behaviour of NPEO, NP and carboxylated metabolites in the Western Scheldt and Rhine Estuaries—where maximum dissolved concentrations were 2.3, 0.9 and 8.1 μg l^{-1}, respectively, and corresponding sediment maxima 242, 1080 and 239 ng g^{-1}, respectively. Axial profiles for dissolved NP and NPEO in the Scheldt (non-stratified) indicated non-conservative mixing (removal due to sorption/degradation) with concentrations decreasing strongly along the salinity gradient. This decrease was weaker for carboxylated metabolites—attributed to aerobic (oxidative) biodegradation. However, for the stratified Rhine Estuary, profiles were less easily interpreted due to a combination of complex point sources and flows. No gradient in sediment concentrations could be discerned in either estuary—interpreted as indicating *in situ* sorption. Organic carbon is suggested to be important in this process since correlation coefficients with NP were 0.71 and 0.91 for the Scheldt and Rhine, respectively. The mean log K_{oc} (NP) for suspended particulates, based on field measurements, was ~6 in both estuaries. There was little indication from this study that sorption varied systematically along the estuary or that there was a significant salting out effect (Jonkers et al., 2003).

Similar sediment-seeking properties are probably applicable across a range of particulate types because of the hydrophobic properties of OP and NP (Ahel & Giger, 1993), though some variation due to sediment properties and physicochemical influences is to be expected. Thus, in terrestrial soils, NP has an estimated organic carbon sorption constant (log K_{oc}) of 3.97 (Düring et al., 2002); in estuaries, log K_{oc} values range from 5.22 to 5.9 for NP, and from 4.65 to 5.46 for OP (Isobe et al., 2001; Ferguson et al., 2001; Jonkers et al., 2003). However, sorption data are scarce and if the distributions and oestrogenic impact of NP and OP in estuarine systems are to be fully characterized and predicted with accuracy, a better understanding of their binding behaviour, and the factors influencing partitioning, is crucial.

Persistence and biodegradation

Metabolism and speciation of (xeno)oestrogens in sediments are influential in modifying the risk of impact to benthic organisms. For example, in experimental studies with activated sludge, the natural oestrogen E2 may be oxidized over time to oestrone (E1), thereby reducing oestrogenic potential—whilst the synthetic hormone EE2 appears to be more persistent (Cargouet et al., 2004); in some situations glucuronides of E2 and EE2 may be cleaved in activated sludge solution releasing free, biologically-active 17β-oestradiol and 17α-ethinyloestradiol into the aquatic environment (Desbrow et al., 1998; Ternes et al., 1999a,b). The relative importance of these processes will vary according to site-specific conditions.

The available information on the longevity of E2, EE2, NP and OP in the aquatic environment indicates that oxygenation is a critical factor influencing degradation. The consensus so far suggests that, in aerated surface waters, degradation of most of these compounds is likely to be relatively rapid, with half-lives from a few days to weeks—e.g. 0.2–9 d for E2 in river water (Ekelund et al., 1993; Jurgens et al., 2002; Ying & Kookana, 2003). Biodegradation experiments on aquifer materials demonstrated that, under aerobic conditions, the majority of E2 was lost in ten days, with a half-life of 2–7 d (similarly for NP), though EE2 degraded more slowly—with concentrations decreasing by 38% (wt/wt) after 70 d exposure (half-time 81 d). In contrast, under anaerobic conditions approximately 40% E2 was lost over 70 days (approximate half-life 107 d), and no appreciable loss of EE2 occurred. Octylphenol concentrations also remained virtually unchanged in these experiments (Ying et al., 2002b, 2003). A similar laboratory study on marine

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sediments collected from South Australia, found that E2 was lost rapidly under aerobic conditions (estimated half-life of 4.4 d) whereas only 50% of E2 was degraded after 70 d anaerobic incubation (Ying & Kookana, 2003).

The principal mechanisms for loss are therefore likely to involve oxidative biodegradation, although breakdown of alkylphenols may involve an abiotic component. For OP and NP in seawater, experimental half lives have been estimated at 60 and 5–58 d, respectively (Ekelund et al., 1993; Ying & Kookana, 2003). Generally, degradation of APs is temperature dependent (usually highest in summer months) reflecting optimum conditions for microbial communities.

In laboratory experiments with activated sludges, microbial degradation of oestradiol appears to be fairly rapid—of the order of a few weeks (Lintelmann et al., 2003). Again the synthetic EE2 appears to be more resistant to microbial degradation than the natural oestrogens, both in water and sediment. The half-life of EE2 has been demonstrated to be ten times that of E2 under the same incubation conditions (Williams et al., 2001; Jurgens et al., 2002). In spiked aerobic marine sediments half lives of E2 and NP have tentatively been estimated at ~5 d, and OP and EE2 at >20 d (Ying & Kookana, 2003), whilst in experimental littoral ecosystems a dissipation half-life of 28–104 d was reported for NP (Liber et al., 1999a). Crucially, in more anaerobic benthic conditions, typical of estuarine muds, it is likely that, as with E2 and EE2, little or no degradation of NP and OP occurs, and most of these compounds show much longer persistence, e.g. half lives from ~70 d for E2 to >60 y for NP (Ekelund et al., 1993; Ying & Kookana, 2003). Field studies in sheltered bays and lagoons subjected to anthropogenic inputs also indicate that OP and NP tend to be persistent in anoxic marine sediments (Shang et al., 1999b; Isobe et al., 2001).

In summary, E2, EE2, OP and NP are predicted to have moderately high potential for sorption on to particulates and to exhibit low degradation rates in anoxic conditions, explaining why benthic sediments, particularly those near treatment plants and industrial outfalls are a potential sink for these compounds (Williams et al., 2001; Young et al., 2002; and Tables 2 & 3). Sediments may subsequently act as a secondary source (particularly to filter-feeders) as a result of a combination of processes including direct ingestion, slow release by diffusion at the sediment/water interface, or through sediment resuspension and transport during high water flows. Though there are useful data to indicate trends in partitioning, the paucity of systematic studies of (xeno)oestrogen sorption (and desorption) in *natural* estuarine sediments—particularly investigations of controlling criteria—are significant gaps in our understanding and are important requirements for better predictions of environmental behaviour, transport and bioavailability.

BIOACCUMULATION

Bioaccumulation of a given chemical is the net product of uptake, elimination and metabolism. Uptake in aquatic organisms is often assumed to occur principally from water (respiration) and experiments to characterize this process focus on aqueous exposures, though diet (including sediments) and dermal contact have been shown to be important sources to some species in nature. In the absence of extensive kinetic models which adequately separate these pathways, experimentally-derived bioconcentration factors (BCF=ratio between body burden and water concentration, at steady state) are widely used as a comparative measure of bioaccumulation, along with field-based bioaccumulation factors (BAF=ratio between body burden and exposure level—though in reality this often includes a component from diet or sediment). Comparisons of burdens in organisms from different trophic levels (biomagnification factors) are used to assess the importance of food chain transfer. These terms have been adopted in the current section to evaluate available information on the bioaccumulation of (xeno)oestrogens.

Natural and synthetic oestrogens (17β-oestradiol and 17α-ethinyloestradiol)

Hormone titres in aquatic organisms are likely to be moderated significantly according to the activity of their metabolic systems. Metabolism of natural oestrogens involves the P450 enzymic pathway (phase I) along with sugar or sulphate conjugation enzymes (phase II metabolism) and, in mammals at least, these transformations occur in the liver prior to elimination in urine or faeces. Metabolic half-lives in humans are relatively rapid (approximately 20 min or 20 h for E2 and EE2, respectively) but may well be considerably longer in fish and, particularly, in invertebrates. This difference in metabolic rate will clearly be of significance in determining endogenous levels, steady-state concentrations accumulated during exposure to environmental oestrogens, and the longevity of these residues. Because oestrogens have high potency at relatively low concentrations, the implications of bioaccumulation (or induction of synthesis), even at levels only marginally above background, warrant caution and detailed investigation.

Levels of serum E2 in male fish (channel catfish *Ictalurus punctatus*) have been shown to be raised by 13–16 fold, from a basal level of 85 ng ml^{-1}, following three weeks' exposure in the outfall of municipal treatment plants. It is unclear whether this is entirely endogenous, due to induction, or whether this represents an element of accumulation (via water, as the caged fish were not fed). Laboratory experiments have indicated, however, that exposure to 1000 ng l^{-1} E2 in water can result in equivalent levels in serum (up to 1500 ng l^{-1} E2), accompanied by stimulation of vitellogenin (VTG) by up to 500% (Tilton et al., 2002). Similar studies with juvenile rainbow trout caged downstream of STWs, or exposed in the laboratory (to 50 μg l^{-1} E2 and EE2), have indicated accumulation of oestrogens to levels 10^4–10^6 times higher than water (~200–400 μg g^{-1} in bile), though these measurements of BCF/BAF in bile represent total (conjugated + unconjugated forms) rather than free oestrogen (Larsson et al., 1999).

Information on E2 and EE2 titres in invertebrates is scarce and the potential for bioaccumulation from different sources appears not to have been addressed adequately. Hormones of varying types are thought to be involved in the biochemistry of all invertebrate phyla (Depledge & Billinghurst, 1999), though this may not

always include steroid hormones. For example, whilst control of growth and reproduction in some echinoderms, cnidarians and molluscs has been shown to be under the control of vertebrate-like steroid hormones (usually synthesized in reproductive tissues), other hormones such as ecdysteroids may serve equivalent functional roles in nematodes, platyhelminthes, annelids and crustaceans.

One of the few studies to confirm the bioavailability of environmental oestrogens in an invertebrate concerns the short-term uptake of E2 by the brine shrimp *Artemia*. Dry weight bioconcentration factors ranged from 9 to 28 after 24 h exposure to concentrations of 5–20 mg l^{-1} (Martin-Robichaud et al., 1994). Longer exposures to more realistic environmental concentrations may result in higher BCFs. The same study also showed the importance of dietary transfer along food chains: the feeding of dosed *Artemia* (231–407 ng E2 mg^{-1}) to larval lumpfish resulted in 100% feminization of males.

Endogenous levels of steroid hormones in any organism will vary according to metabolic activity and other sex-dependent and tissue-specific physiological parameters and, to an extent, these will tend to be influenced by seasonal changes. This is demonstrated in the annual pattern of endogenous E2 levels in the anthozoan *Renilla koellikeri*, which is synchronized with the reproductive cycle. Levels of E2 in this sea-pen are low during autumn and winter and rise in spring at the onset of gonad development and lipid production. A second significant surge in E2 (most marked in female colonies—from 0.09 up to 6.3 ng g^{-1} dry wt, compared with an increase in males from 0.08 to 0.4 ng g^{-1} dry wt) is seen between May and June, as spawning is initiated, which may help to synchronize oocyte maturation (Pernet & Anctil, 2002). Interestingly, in spring, highest levels are present in somatic tissues, rather than eggs, suggesting a non-reproductive origin for E2 and subsequent transport to reproductive tissue. Nevertheless, an important role for E2 in the timing of reproduction is indicated. A similar role is evident in starfish *Sclerasterias mollis* (Xu & Barker, 1990) and perhaps another echinoderm, the sea urchin *Lytechinus variegatus* (Wasson et al., 2000). However, in the latter species, oestradiol metabolism varies greatly between individuals during the annual cycle and, though highest during gonadal growth, levels are comparatively low (maximum 0.01 ng g^{-1} E2 in female *L. variegatus*). Hence relationships with oogenesis and spawning are less obvious in *L. variegatus*, hinting at a paracrine, rather than endocrine, mechanism for regulating gonad function in the echinoid (Wasson et al., 2000). Perhaps because of these variables (and because levels may be close to detection limits as measured by radio-immunoassay), peak titres of E2 appear, in fact, to be higher in testes of *L. variegatus* (0.1 ng g^{-1}) than in ovaries.

In the absence of substantial bioaccumulation data on natural and synthetic steroid oestrogens, Lai et al. (2002) have attempted trophic model estimates of bioaccumulation factors and body burdens for a range of aquatic organisms in river systems and compared them with simpler estimates based on physicochemical properties (K_{ow} and molecular connectivity index). Predictions for E2 and EE2 are summarized in Figure 4. It should be recognized that, depending on the species, such estimates can be unreliable since they can be substantially affected by the ecology, biochemistry and feeding regime of each type of organism, and therefore represent only a first-order approximation. Hence, predicted bioaccumulation factors (1.8–332), particularly those based solely on K_{ow} (where the general expression log BCF$=a$log K_{ow} is assumed appropriate for a range of organisms), have sometimes proved to be orders of magnitude lower than measured values in the laboratory/field (e.g. 10^4–10^6 in trout; Larsson et al., 1999). Despite these uncertainties, some interesting simulations for E2 and EE2 were made (Figure 4) and although predicted bioaccumulation factors were generally lower than observational values from experiments or surveys, bioaccumulation was indicated to occur in all aquatic organisms. Biomagnification among higher members of the food chain (i.e. the food pathway) was not predicted to be of major importance, with the exception of EE2 in fish. Sediment-bound oestrogens were, however, considered likely to be an important contributor to accumulation in benthic invertebrates, particularly where environmental concentrations are elevated. This reinforces the requirement for realistic measurements of sediment bioavailability of oestrogens. More accurate estimates of metabolic and elimination rates are also highlighted as a means of improving predictions (Lai et al., 2002). It would seem that, although the functionality of steroid hormones in many invertebrates is often uncertain, their presence in tissues could reflect uptake from the environment (from water, sediment and food) and needs to be studied in greater detail, across a range of phyla and feeding types.

Alkylphenols (octyl-and nonyl-phenol)

Available data suggest that certain alkylphenols have moderate potential to bioaccumulate in aquatic organisms, whereas in mammals the likelihood of biomagnification is low, due to metabolism and excretion. For example, experiments indicate that OP does not bioaccumulate in rats receiving low oral doses, because of rapid elimination

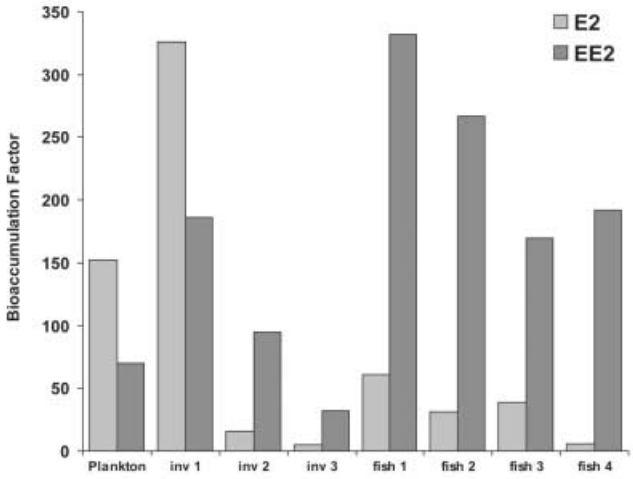

Figure 4. Predicted bioaccumulation factors for E2 and EE2 in freshwater organisms based on the mass-balance food-web model. Plankton represent all zooplankton and phytoplankton; inv 1, pelagic invertebrate; inv 2 and 3, benthic organisms; fish 1–4 represent a range of fish of ascending trophic levels (drawn from data in Lai et al., 2002).

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by the liver (via glucuronidation and sulphation) though at high doses, bioaccumulation can occur as a result of saturation of detoxification pathways (Certa et al., 1999).

The evidence from aquatic organisms points to a greater propensity for bioaccumulation of APs, even at low exposure levels. As a result, although APs may display 100-fold less oestrogenicity than the hormone E2 on a mole for mole basis, bioconcentration of APs, coupled with their relative abundance in some environments, could well result in equivalent levels of oestrogenicity in organisms. Experiments with fathead minnows *Pimephales promelas*, exposed to 0.05–3.4 μg NP l^{-1} for 42 d, confirm this degree of bioaccumulation is likely (BCF of 241; Giesy et al., 1999). Other uptake studies in whole fish show that even higher BCFs are possible—from 280 to 1300 (McLeese et al., 1981; Ekelund et al., 1990). A review of laboratory-derived BCF for alkylphenols in freshwater and marine species by Staples and co-workers cites values ranging from <1 to 1250 for fish and 1 to 3400 for invertebrates, on a fresh weight basis (Staples et al., 1998). In the same review, field-based values (BAF) for APs in a variety of organisms range from 6 to 487. Original survey data reported by Ahel et al., 1993, for the Glatt Valley, Switzerland yielded BAF for NP from 13 to 410 in fish and much higher values—up to 10,000—in macrophytic algae, *Cladophora glomerata*, though these were based on dry weights.

Figure 5 summarizes BCF/BAF data (median, maximum, minimum and upper and lower quartile values and ranges) for NP in fish, invertebrates and macroalgae, based on the review of Staples et al., 1998 and other papers listed. This illustrates the considerable variability in bioaccumulation patterns for such xeno-oestrogens, both within and between taxonomic groups, and between laboratory-derived (BCF) and field-based estimates (BAF).

The majority of bioaccumulation studies on APs are based on an assumption that water represents the sole pathway, and resultant BCF values are mainly derived for whole body residues. Whilst these endpoints establish useful comparative data on bioaccumulation potential, little information emerges about routes of uptake or mechanisms of accumulation in nature. However, a small number of more detailed studies provide insights into the bioaccumulation process, including the importance of tissue localization. Thus, studies with juvenile rainbow trout *Onchorhyncus mykiss* exposed to 4 μg l^{-1} radiolabelled (^{14}C) 4-*tert*-octylphenol demonstrate that accumulation approaches steady-state relatively quickly (4 d) and is highest in bile (BCF 68,000) > faeces (13,000) > pyloric caeca (1255) > liver (1020) > intestine (798), with most activity present as the glucuronide conjugates of *t*-OP and *t*-octylcatechol. These results indicate a relatively rapidly metabolized pool which is eliminated via the liver/bile route. In contrast, the parent compound *t*-OP is accumulated in fat (BCF 1190), with lesser amounts in brain, muscle, skin, bone, gills and eye (BCFs 110–260); dermal, as well as branchial uptake appears to be a possible route of entry. The BCF for OP in whole trout is estimated to be 470 (Ferreira-Leach & Hill, 2001). For NP, BCF in muscle of the same species (*O. mykiss*) are in the range 90–125, following exposure for three weeks to a nominal concentration of 65 μg l^{-1}, i.e. slightly lower than for OP, though it is unclear whether these represent steady-state concentration factors for NP (Blackburn et al., 1999). As with OP, a pathway involving glucoronide conjugation and biliary excretion is also postulated for 4-*n*-NP in fish, analogous to the catabolism of oestrogens (Thibaut et al., 1988).

The ability to accumulate and excrete APs may change during development as demonstrated in roach *Rutilus rutilus* by Ferreira-Leach & Hill (2000). Seven day post-hatch larvae achieved a steady state BCF for OP of 1061–1134 between 12 and 19 d, and metabolism was relatively slow during the first 5 d of exposure (57% remaining as the parent compound). In contrast, in 26 d post-hatch fry exposed for 5 d (to 5 μg l^{-1} *t*-OP), significant metabolism took place, with only 26% of the activity represented by the original *t*-OP—the remainder being conjugated to more polar metabolites (mainly as glucoronide) suggesting that activity of metabolizing enzymes was greater in the older fry. This difference in behaviour may be important in relation to the window of sensitivity to (xeno)oestrogens, when developmental effects are most pronounced (usually coinciding with the period in eggs/fry of fish when differentiation of the gonad and reproductive tract are occurring).

Attempts have been made to measure residues of AP in fish populations from UK rivers and coastal sites. Adult roach sampled from an AP-contaminated site in the River Aire contained concentrations of NP, OP and $NP_{(1+2)}EO$ of 600 ng g^{-1}, <100 ng g^{-1} and 1400 ng g^{-1}, respectively, compared with undetectable values (<100, <100, <500 ng g^{-1}) at a control site; marine fish samples from the open sea around the UK also generally contained AP below these detection limits (Blackburn et al., 1999). In contrast, measurable residues have been found in a small number of estuarine fish samples. Up to 180 ng g^{-1} NP (wet wt) and 17 ng g^{-1} OP were detected in juvenile flounder *Platichthys flesus* from the Tees (Lye et al., 1999)—thought to be sufficient to raise plasma VTG concentrations and induce ovotestis; lower levels were

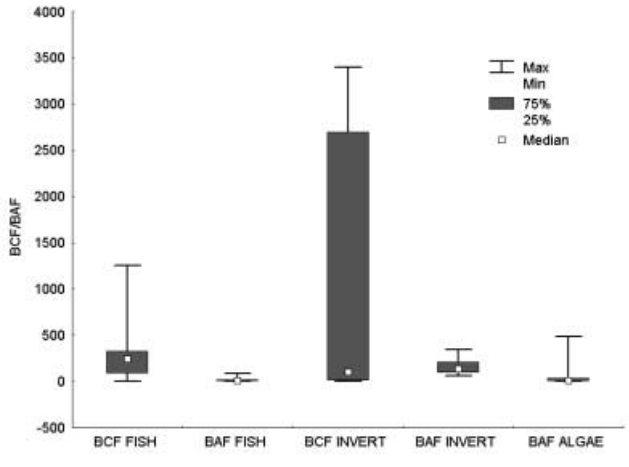

Figure 5. Box–Whisker plot of bioconcentration factors (BCF–laboratory) and bioaccumulation factors (BAF—field) for nonylphenol in fish, invertebrates and macroalgae (using fresh weight estimates reviewed in Staples et al. (1998); Ahel et al. (1993); Brooke (1993); Chemical Inspection and Testing Institute (1992); Ekelund et al. (1990); Lewis & Lech (1996); Liber et al. (1999b); McLeese et al. (1980, 1981); Ward & Boeri (1991); Granmo et al. (1991); Wahlberg et al. (1990)).

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present in flounder from the Tyne (up to 60 ng g^{-1} NP, OP not detected). Surfactant manufacture on the Tees may account for the higher levels in tissues, water and sediment. Other than at expected hotspots such as the Tees (and Mersey), however, evidence of significant AP contamination has not been forthcoming (Blackburn et al., 1999).

Sediments and sediment-dwelling organisms are postulated as possible critical (direct or indirect) sources and pathways of (xeno)oestrogens to flounder (and bottom-feeding equivalents in fresh water such as gudgeon; Blackburn et al., 1999). Bioaccumulation and biomagnification of these compounds, via infaunal organisms, could contribute to the oestrogenic responses (raised VTG and ovotestis) seen in Tees flatfish (Lye et al., 1999; Matthiessen et al., 2002). The importance of the sediment pathway for fish appears to be backed up by results from mesocosm experiments in which premature vitellogenisis was seen in female flounder exposed to harbour muds. However, as there are likely to be complex mixtures of other endocrine disrupting chemicals in sediments, including E2 and EE2, the causative agent(s) may well be acting in combination and the relative contributions of each remains uncertain (Lye et al., 1999). The importance of dietary uptake of (xeno)oestrogens is nevertheless confirmed by experiments in which flounder were fed OP (at 10, 50 and 100 mg OP kg^{-1} body weight) and E2 (0.05 mg kg^{-1} body weight); both compounds were found to be strongly oestrogenic (as measured by VTG induction) when assimilated via the diet. The VTG response was directly proportional to the amount of OP accumulated in both liver and muscle tissue, though the OP content of the liver of exposed fish (up to ~40 μg g^{-1} wet wt) was some 4–5 fold higher than in the muscle (Madsen et al., 2002).

Bioaccumulation of alkylphenols has been studied in relatively few invertebrates. Transplanted freshwater mussels *Elliptio complanata* were shown to accumulate NP and OP to ng g^{-1} (wet wt) quantities in the field (BAF= 340) whilst some NPEOs may even reach μg g^{-1} concentrations—a similar level to that sequestered by sediments downstream of STWs (Bennett & Metcalfe, 1998; Sabik et al., 2003). Most of the data for marine invertebrates concern either *Mytilus edulis* (where estimates of nonylphenol BCF range over three orders of magnitude, from 1.4–3400 on a fresh weight basis) or *Crangon crangon* (BCF 90–110) and were obtained principally from a single study by Ekelund et al. (1990). The higher BCF estimates for *M. edulis* were, however, based on extrapolated, rather than measured, concentrations since, unlike *Crangon* (and the stickleback *Gasterosteus aculeatus* also tested in the same study), steady-state in mussels was not achieved during 16 days' exposure to ^{14}C-radiolabelled NP (Ekelund et al., 1990). Bioaccumulation of NP in mussels was higher in these experiments than predicted from octanol/water partitioning, and it is possible that, as a fraction (25%) of the NP in exposure experiments was particle bound, this route could represent an important pathway; i.e. bioavailability may be increased in some filter feeders as a result of adsorption to particles. Furthermore, whilst elimination of NP from shrimp (and stickleback) was fairly rapid, and complete after a few days, a substantial residue remained in mussels—even after 30 days—and more than 80% of this residue co-chromatographed with the administered ^{14}C-NP, implying low levels of metabolism (Ekelund et al., 1990).

The transformation products NP and OP would be expected to have a higher bioaccumulation potential than the parent alkylphenol ethoxylates, by virtue of their higher log K_{ow}. This appears to be supported by field observations of APs in commercial catches of molluscs (cephalopods, clams and mussels) from 15 coastal sites in the northern Adriatic (Ferrara et al., 2001). Concentrations of NP were consistently higher than other APs (up to 696 ng g^{-1} wet wt in squid); body burdens of OP were generally some 30 times lower (up to 18.6 ng g^{-1} in squid). Ethoxylates were mostly below detection limits in all sample types (Ferrara et al., 2001). The fact that carnivorous species (squid and cuttlefish) contained the highest levels of AP hints at the potential for biomagnification of these compounds along marine food chains, though wider verification is needed.

Long-term field observations of NP body burdens appear to corroborate trends for environmental loadings, in that residues in biota may be declining in response to reduced inputs. A recent retrospective investigation of APs in *Mytilus edulis*, collected from the German North Sea coast and Baltic between 1985 and 2001, indicates that NP levels in mussels have tended to decline at most sites since 1996/1997 (Wenzel et al., 2004). This coincides with the introduction of voluntary measures intended to achieve removal of NPEO from detergent applications by 2000. (In contrast, OP showed relatively little change, suggesting only minor shifts in consumption.) Comparable trends for APs were observed in freshwater mussels (*Dreissena polymorpha*) and fish (bream *Abramis brama*) during the same period, though levels were higher than in marine counterparts. Maximum reported concentrations were 112 ng g^{-1} NP (5.5 ng g^{-1} OP) in bream in 1994. Concentrations up to 41 ng g^{-1} NP (~0.4 ng g^{-1} OP) were recorded in freshwater mussels (River Elbe) compared with up to 9.7 ng g^{-1} NP (~0.2 ng g^{-1} OP) in marine mussels. These field comparisons of residues in different sample types also led Wenzel et al. (2004) to speculate that particulates may be an important pathway for AP uptake in filter feeders.

Sediments as sources

Bioaccumulation of (xeno)oestrogens in sediment-dwelling invertebrates has yet to be characterized, although the ability of the latter (particularly deposit-feeders) to accumulate contaminants from sediments is to be anticipated. The estuarine clam *Scrobicularia plana*, for example, accumulates the majority of its tributyltin (TBT) burden from particulate sources (Langston & Burt, 1991). Significant variations in bioaccumulation factors for (xeno)oestrogens may partly reflect differing contributions from sediment (dietary) sources to different feeding types—contributing to the uncertainty surrounding existing food chain models and predictions. Since widespread and abundant sediment-dwelling clams such as *S. plana* form an important part of the diet of wading birds and benthic fish (such as flounder), their role in the transfer of endocrine disruptions (EDs) from sediments to higher trophic levels would seem an appropriate area for study in the near future.

The lack of information on ED chemicals in sediments and infauna has been highlighted by several workshops

and working groups[1]. In particular, the importance of sediments as a source of EDs to benthic organisms, and the influence of binding characteristics on bioavailability, need to be quantified more thoroughly. Until more detailed knowledge of assimilation routes and rates are obtained for EDs such as E2, EE2 and APs, across a range of taxonomic groups and feeding types, there remains a great deal of uncertainty in using BCFs to predict and assess risks to the environment. Because sediments may concentrate these compounds perhaps hundreds to thousands of times higher than in the water column, biological availability of only a small proportion of the particulate loading could result in significant uptake (and effects)—especially in filter-feeders and other benthic invertebrate organisms which are in direct contact with sediments (Langston et al., 1998). The process and rates of transfer of EDs from sediments to biota are outstanding scientific issues to be addressed in order to predict long-term consequences.

IMPACT OF (XENO)OESTROGENS

Hormones, together with industrial chemicals which mimic their actions, can occur in aquatic environments at significant physiological levels. In the UK, the main chemicals responsible for oestrogenic effects in male fish appear to be the natural steroids 17β-oestradiol (E2), estrone (E1) and to a limited extent 17α-ethinyloestradiol (EE2), together with components attributable to less well characterized EDs, including alkylphenols (Desbrow et al., 1998; Routledge et al., 1998; Blackburn et al., 1999). Treated sewage discharges and some industrial sources containing these compounds have been found to be a widespread cause of oestrogenic effects in freshwater fish. For example, male roach sampled near such sources may display severe evidence of ED, including abnormal testicular growth, intersex conditions such as ovotestis and vitellogenin (VTG) production (Harries et al., 1996, 1999; Jobling et al., 1998a,b, 2002; Matthiessen & Sumpter, 1998). Marine species, including flounder *Platichthys flesus*, exhibit similar feminization effects in industrialized estuaries (e.g. Lye et al., 1997; Matthiessen et al., 2002; Lintelmann et al., 2003).

An indication of the scope and scale of effects in fish is thus beginning to emerge. Matthiessen et al. (1998), for example, found that male flounder from the Mersey Estuary contained the highest recorded VTG concentrations of all UK marine sites sampled, many also displaying advanced ovotestis (though ecological significance is difficult to assess on these observations alone). Habitat and feeding preferences make flounder vulnerable to the type of sediment-associated pollution which occurs in estuaries (potentially, assimilation may occur by dermal uptake from the sediment in which flounder bury, as well as during ingestion of infaunal prey species). However, for the majority of benthic species (particularly sedentary invertebrates) there is considerable uncertainty over the relative bioavailability of this form of contamination, and the resulting susceptibility to ED. In the following sections the assays and biomarker techniques which may be used to assess oestrogenicity, and the broader evidence for reproductive and ecotoxological effects in aquatic species, are described.

In vitro *and* in vivo *assays and biomarkers of oestrogenicity*

Female sexual maturation in many (higher) organisms proceeds by hepatic biosynthetic processes under the control of oestrogens. In fish, oogenesis involves both zonagenesis and vitellogenesis, forming the bulk of the egg shell (zona) and yolk, respectively (Celius & Walther, 1998a). Oestradiol (and some xeno-oestrogens such as NP and DDT) induces mRNAs for zona radiata proteins (ZRP) and for vitellogenin (VTG), by interacting with the hepatic oestrogen receptor, followed by protein synthesis (Celius & Walther, 1998a,b; Arukwe, 1998; Oppen-Berntsen et al., 1999). Such inductions can be demonstrated in primary cultures of hepatocytes e.g. from juvenile Atlantic salmon, which secrete ZRP and VTG into the culture medium; *in vivo*, ZRP and VTG are transported in the blood from their point of synthesis (predominantly the liver) for specific uptake by the ovaries (Oppen-Berntsen et al., 1992, 1999; Arukwe et al., 1998).

The synthesis of VTG appears to be affected by both natural hormones (e.g. oestradiol) and xeno-oestrogens, and has therefore been widely used as a sensitive marker of exposure to oestrogenic compounds. A pronounced increase in plasma VTG levels in male fish has been observed in many laboratory studies (e.g. Arukwe et al., 1998, 2000; Hemmer et al., 2002) and also in field experiments which highlight STWs and industrial effluents (such as pulp and paper mills, detergent manufacture) as sources of oestrogenic compounds (e.g. Purdom et al., 1994; Lye et al., 1997, 1998; Larsson et al., 1999; Mellanen et al., 1999; Solé et al., 2000). Induction of ZRP has also been proposed as a potential biomarker for oestrogenic effects in male fish (Hyllner et al., 1991). Indeed, studies with juvenile salmon have indicated that the ZRP response may be more sensitive than VTG to a range of pollutants (Arukwe et al., 2000), providing a further powerful means of detecting exposure to environmental oestrogens.

Because the phenomenon of oestrogenicity is highly linked to transcription activation, some of the most promising biomarkers for oestrogenic effects involve genes expressed under the control of hormone-activated transcription factors. These include the detection and quantification of oestrogen receptor mRNA or vitellogenin mRNA (see for example Ackermann & Fent, 1999). The regulatory need for cheap and effective screening of potentially harmful chemicals has been an effective driver, stimulating the development of such technology and its application in a number of sensitive *in vitro* and *in vivo* tests for oestrogenicity (and androgenicity) in environmental samples. These techniques have confirmed, for example, that much of the oestrogenic activity in STW effluents is often due to natural and synthetic hormones and, to a lesser extent to xeno-oestrogens such as alkylphenols (Desbrow et al., 1998; Snyder et al., 2001; Cargouet et al., 2004).

[1], For example, EDIETA (Endocrine Disruption in Invertebrates: Endocrinology, Testing and Assessment); EMWAT (Endocrine Modulators in Wildlife: Assessment and Testing); EDMAR (Endocrine Disruptors in the Marine Environment Programme).

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In trials with chemically-characterized water and particulate samples, Murk et al. (2002) recently compared three of the most widely used *in vitro* assays in—an oestrogen receptor (ER) binding assay and two reporter gene assays (YES, the yeast oestrogen screen developed by Routledge & Sumpter (1996), and ER-CALUX the ER-mediated chemical activated luciferase reporter gene expression assay). The responses with ER-CALUX and YES were more comparable with each other than with the ER binding data, probably explained by the different principles of these assays. Comparisons with chemical data revealed similar trends, although the accurate prediction of biological responses, based on concentrations and oestrogenic potencies of compounds, has yet to be achieved widely. A complex variety of mechanisms (including the presence of unquantified compounds) may confound straightforward relationships between measured concentrations of known oestrogens and *in vitro* responses (Cargouet et al., 2004).

Nevertheless, these assays are useful management tools and provide useful comparative insights into the relative sensitivities of (xeno)oestrogens and their modes of action. Using the ER-CALUX technique, for example, Legler et al. (2002a) determined an EC_{50} for oestradiol of 0.006 nM (0.00163 μg l^{-1}), compared with 260 nM (57.3 μg l^{-1}) for nonylphenol. The same authors were also able to compare oestrogenicity of a number of other ED chemicals and to study the influence of metabolism on their activity; thus, exposure of E2 to microsomal degradation clearly reduced potency, whilst decreasing the side chain length of APEO resulted in increased oestrogenic activity (the degradation products OP and NP showing highest potency). Using the same assay, Murk et al. (2002) were able to estimate the efficacies of various treatment plants in removing oestrogenic potency: for some domestic STW this was in the range 90–95%, compared with approximately 50% in an industrial treatment plant. Variable efficiencies for the removal of oestrogencity by STWs, ranging from 62 to 97%, were indicated in a similar study by Cargouet et al. (2004).

Provided that suitable extraction schemes are developed, it is feasible to adopt similar techniques to screen sediment samples. Recently, characterization of oestrogenic activity of riverine sediments from an industrialized area in the Czech Republic has been performed using ER-mediated luciferase activity (Hilscherova et al., 2002). In this study, florisil fractionation of sediment extracts indicated that certain PAHs or their metabolites were the most likely compounds contributing to oestrogenicity. An ER-CALUX technique has also been used to compare oestrogenic activity in extracts from marine sediments in The Netherlands, where maximum activity (up to 40 pmol (10.9 ng) g^{-1} sediment, as oestradiol equivalents) was recorded in industrialized areas such as the Port of Rotterdam, compared with 5 pmol (1.4 ng) EEQ g^{-1} in 'reference' sediment from the Eastern Scheldt (Legler et al., 2002a). In the latter survey, polar fractions (acetone soluble) appeared to exhibit more activity than non-polar (hexane) equivalents implying that oestrogenic compounds of a more hydrophilic nature were the most active. However, other studies have indicated that oestrogenicity may also be associated with intermediate- and relatively non-polar compounds (Hilscherova et al., 2002; Thomas et al., 2001).

Until there is more widespread understanding and confirmation of links with chemistry and whole organism response, caution is warranted when interpreting and applying results of these assays. Also, there may be substantial differences in responses to oestrogens (and anti-oestrogens) between, say, the ER-CALUX and YES assay, which are related to the influence of factors such as exogenous and endogenous binding and membrane transport—i.e. bioavailability (Legler et al., 2002b). The ability of endocrine disrupting chemicals to elicit significant taxonomic differences in ligand preferences, binding affinities and inducibility of gene expression, is demonstrated markedly by comparison of the literature for human and fish ERs. Finally, there may be oestrogenic effects exhibited in some organisms that are based on mechanisms different from ER binding. Since bioaccumulation and biological responses to EDs can vary greatly among phylogenetic groups, the use of a single surrogate model is unlikely to be suitable for the prediction of oestrogenic responses in all species (Matthews et al., 1999).

The importance of processes such as bioavailability and metabolism (not usually taken into account by *in vitro* tests), and their consequences when extrapolating to responses in multicellular organisms, is illustrated in a study with *tert*-APs by Jobling et al. (1996) in which oestrogenic potency determined by *in vivo* tests in fish was up to 100 times higher than predicted *in vitro*. The recent trialling of rapid *in vivo* assays for (xeno)oestrogenic compounds—e.g. using transgenic zebrafish modified with the same recombinant luciferase gene as used in the ER-CALUX assay (Legler et al., 2002b)—have further highlighted the potential inconsistencies between techniques. Ethinyloestradiol (EE2) appeared to be 100 times more potent than E2 using the *in vivo* method with transgenic fish, though both were of equal potency using the *in vitro* ER CALUX assay. Results from the latter assay also suggested NP was a full oestrogen agonist, whereas this was not evident *in vivo* (Legler et al., 2002b).

Consequently, although there are many advantages of *in vitro* assays, including faster throughputs and lower cost, ability to predict whole organism response may not always be relied upon. Particular care needs to be taken when extrapolating to reproductive and ecological impacts in nature (see next section). *In vitro* testing of oestrogenicity (e.g. based on ER binding or ER mediated gene expression) needs to be scaled in tandem with relevant *in vivo* tests to confirm their validity when used as a means of assessment of oestrogenicity in field situations, or when making regulatory decisions regarding the endocrine activity of chemicals. Indeed, Europe's Scientific Committee for Toxicity, Ecotoxicity and the Environment (CSTEE) has indicated that the major emphasis should be put on *in vivo* assays when screening chemicals, whilst in the United States, the Environmental Protection Agency has accepted the recommendations by its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to adopt a screening battery of three *in vitro* and five *in vivo* assays.

Reproductive and ecological effects in aquatic species

It is now well established that various natural and man-made compounds found in domestic and industrial effluents possess endocrine modulating activity. In some cases binding of xeno-oestrogens (e.g. diethylstilboestrol, 4-hydroxytamoxifen, ethinyloestradiol, genistein, zearalenone, 4-t-octylphenol, 4-n-nonylphenol, and o,p′-DDT) to the oestrogen receptor may cause equivalent oestrogenic effects which are indistinguishable from those caused by hormones (Tollefsen et al., 2002). Superficially, it may be argued that there is little difference between the inhibitory reproductive consequences of a number of different classes of pollutants including heavy metals, organophosphorous pesticides, organochlorines, APs and polyaromatic compounds (see for example Kime, 1995; Kime et al., 1999) and in some locations such effects may be ecologically relevant. For example, fish surveys in the US indicate that pulp-mill effluents, water-soluble pesticides and herbicides may be affecting endocrine systems by changing steroidal pathways (determined as the median ratio of 17β-oestradiol to 11-ketotestosterone), though precise cause–effect relationships have not yet been established (Sepulveda et al., 2002; Goodbred et al., 1997). Taylor & Harrison (1999), describing instances of endocrine disruption in wildlife, also concluded that in most cases a causal link between the observed abnormalities and chemical exposure have, so far, not been established. Below, we present an overview of research that shows how these environmental chemicals can disrupt the normal functioning and development of aquatic species, both in laboratory and field exposures. Emphasis is placed on reviewing the evidence for impacts in benthic invertebrates, because this is currently where the major uncertainties and inconsistencies lie; however, it would seem appropriate to begin by summarizing some of the more established and consistent effects in fish.

Fish

Much of the initial work on endocrine disruption focused on oestrogenic effects in male fish (mainly freshwater), manifested as the induction of the female yolk precursor protein vitellogenin (Purdom et al., 1994; Harries et al., 1996, 1997). As indicated in the previous section, the induction of VTG production in juvenile or male fish in the field has now become one of the most notable and convincing biological responses of fish linked to oestrogenic compounds exposure (Tyler & Routledge, 1998; Kime et al., 1999). Currently, there are numerous reported cases of VTG induction in fish in a variety of water bodies across Europe, North America, and further afield. Examples include: induction of plasma VTG in chub (*Leuciscus cephalus*) in the River Moselle (France) downstream of urban areas (Flammarion et al., 2000); in wild carp from the Llobregat River, Spain, notably in the vicinity of treatment plants and, overall, correlated with NP levels in water (Solé et al., 2000); in male carp and fathead minnows exposed to municipal wastewaters in the USA (Bevans et al., 1996; Folmar et al., 1996; Goodbred et al., 1997; Nichols et al., 1999); and in Sweden, plasma VTG levels were raised (to 1.5 mg ml^{-1}) in juvenile rainbow trout *Oncorhynchus mykiss* caged in STW discharge—coinciding with the appearance of E2, El, and the synthetic oestrogen EE2, in bile (Larsson et al., 1999). Among the most exceptional reported increases in VTG levels, however, are those described in fish exposed to STW discharges at sites in the UK. Levels of up to 147 mg ml^{-1} were measured in plasma of male rainbow trout caged in STW discharges, which exceeded those found in mature females. Vitellogenin levels in immature carp (*Cyprinus carpio*) caged in STW discharges also increased, but to a lesser degree (Purdom et al., 1994). In most cases, induction of vitellogenesis is generally believed to decline sharply with distance from sources of pollution, corresponding to dilution of the active compounds (Harries et al., 1999).

Additional effects that often occur concomitantly with VTG induction in fish exposed to discharges include enlarged livers and abnormal gonadal development; again, much of the information is the result of UK studies. Harries et al. (1997) found inhibition of testicular growth in adult rainbow trout held at sites in the River Aire, which is contaminated with alkylphenols, whilst trout exposed to environmentally realistic concentrations of alkylphenols in laboratory studies display similar effects (Jobling et al., 1996). The appearance of oocytes in the testes of male fish (ovotestis—an 'intersex' condition), has been observed in wild male roach *Rutilus rutilus* and gudgeon *Gobio gobio* collected downstream of STW discharges in several rivers in the United Kingdom, including the Aire and Nene (Jobling et al., 1998a, 2002; Tyler & Routledge, 1998; Nolan et al., 2001). Ovotestis is presumed to be due to oestrogenic exposure during gonadogenesis (Jobling et al., 1998b). The severity of ovotestis ranges from the occasional oocyte in otherwise normal testicular tissue, to large regions of mature ovarian tissue interspersed with abnormal testicular tissue. Feminized or absent vas deferens and impaired milt production are also reported in some of the fish exposed to STW discharges (Jobling et al., 1998b).

These effects are currently causing concern for the long-term future of fisheries. In July 2004 the Environment Agency reported new evidence to confirm that the sex change phenomenon in fish is widespread in English rivers[2]. In total, 1615 roach (654 'male' fish and 923 female fish) were sampled from five of the EA's eight designated Regions and a third of all 'males' were found to be intersex. Affected roach were present at 44 of the 51 sites, where prevalence of intersex varied from 0% to 100%, and there was a large variation in the severity of the condition at these sites. Approximately half of the intersex fish had abnormal reproductive ducts, and most of these had only a single sperm duct together with an oviduct in one or both of the gonads. In 23 fish, one or both gonads contained an additional sperm duct, as well as an oviduct. In 140 fish oocytes were found in the testes (ovotestis) and 39 fish had both abnormal reproductive ducts and ovotestis. Both the proportion of 'male' fish with ovotestis and the severity of the condition increased with age.

As indicated in the introduction to this section, endocrine disrupting effects are not confined to freshwater fish. Male flounder *Platichthys flesus* exposed to 10 ng l^{-1} of the

[2], www.enviroment-agency.gov.uk/news/821453; www.comprendo-project.org; www.credocluster.info; Taylor et al., 2004.

synthetic oestrogen EE2 in the laboratory have been shown to produce VTG within three weeks (Allen et al., 1999a). Among the earliest manifestations in the field were observations of increased VTG induction and testicular abnormalities in male *P. flesus* caught near a STW discharge in the Tyne Estuary, UK (Lye et al., 1997, 1998). Subsequently, these *in situ* effects were partly attributed to several oestrogenic alkylphenols (Lye et al., 1999). High levels of VTG (up to 20 mg ml^{-1} plasma) have also been measured in male flounder from other heavily industrialized British estuaries. In contrast, levels of VTG were low or undetectable in male flounder from estuaries draining rural areas, or from urban areas with relatively little heavy industrial activity (Matthiessen et al., 1998; Allen et al., 1999a,b).

The EDMAR (Endocrine Disruption in the Marine Environment) programme was initiated in 1998 to investigate further the extent of endocrine disrupting effects (specifically oestrogenic effects) occurring in the UK marine environment, and the possible causes. Studies with marine fish (and crustacea) included field surveys of 14 estuaries and confirmed that several—particularly the Tyne, Tees and Mersey—were contaminated with (xeno)oestrogens (Matthiessen et al., 1998; Allen et al., 1999a,b). Sentinel fish species, flounder *P. flesus*, sand goby *Pomatoschistus minutes* and viviparous blenny *Zoarces viviparus*, were found to be experiencing feminization in these industrialized estuaries. In males, symptoms included raised vitellogenin (VTG) synthesis, ovotestis induction and/or feminized sexual characteristics. However, although reproductive success may be impaired as a result of these changes, implications for these fish populations are still unclear (EDMAR, 2002).

Similar effects to those described in flounder from the UK have also been observed in Japanese flounder *Pleuronectes yokohamae* from Tokyo Bay (Hashimoto et al., 2000). The causative substances have not yet been fully identified, but as with freshwaters, mixtures of potentially oestrogenic substances have been detected in estuarine waters.

Thus, endocrine disruption is a well established and widespread phenomenon occurring in wild freshwater and estuarine fish, and whilst the precise modes of action are still not fully understood, synthetic and natural compounds entering the environment via discharges are undoubtedly responsible for many of the observed effects. In view of the extensive concerns highlighted by fish studies, there are compelling reasons to assess impacts more broadly, notably in aquatic invertebrates.

Invertebrates

There are several published works which catalogue studies on contaminants and endocrine disrupting effects in marine, freshwater and terrestrial invertebrates (Depledge & Billinghurst, 1999; Matthiessen et al., 1999; Zou, 2003; Segner et al., 2003), though, in contrast to fish, unequivocal confirmation of environmentally-relevant effects in aquatic invertebrates is scarce. This lack of evidence for ED impact is partly because some invertebrate endocrine systems may have little in common with vertebrates, and have dissimilar and diverse anatomical and physical traits under endocrine control (e.g. various forms of hermaphroditism, moulting). Therefore, endocrine disruptors known to affect vertebrates may not always present the same risks to invertebrates (van der Kraak et al., 1998). Complex reproductive cycles in certain invertebrates can involve environmental stimuli such as light intensity, temperature, desiccation and diet, and environmental conditions during embryonic and larval development can influence sex determination (Stahl et al., 1999) making potential ED effects difficult to distinguish. Partly as a result of these difficulties, there has been a lack of emphasis on assessing ED in invertebrates, and hence, inevitably, limited development of validated biomarkers for detecting endocrine disruption in invertebrates, such as VTG and zona radiata proteins. To compound the problems, there are still many gaps in our knowledge of invertebrate endocrinology.

Steroid hormones are known to be involved in the control of the biochemistry, physiology and behaviour of some invertebrate species. In echinoderms they appear to play a role in the control of growth, reproduction and oocyte development, and like many contaminants, excess levels can affect normal functions and processes. For example, artificially elevated levels of E2 in starfish can increase oestrone and progesterone levels, increase the protein content of oocytes and also increase oocyte size (Shoenmakers et al., 1981; Barker & Xu, 1993). Oestrogens and androgens may also play a regulatory role in some coelenterates and molluscs (Lupo di Prisco et al., 1973; Lupo di Prisco & Fulgheri, 1975; Reis-Henriques & Coimbra, 1990; Atkinson & Atkinson, 1992; Tarrant et al., 1999).

A definitive role for oestrogen has yet to be demonstrated in crustaceans; even so, perturbance of physiological and biochemical functions by environmental (xeno)oestrogens does occur, and may involve other steroid hormone systems. Ecdysteroids serve a functional role in crustaceans (and in platyhelminthes, nematodes, and annelids; Barker & Rees, 1990; Barker et al., 1990; Fingerman, 1997); 20-hydroxyecdysone, for example, controls the moulting processes in crustacea (and, perhaps, the production of vitellogenin), and although there is no direct evidence of impact of (xeno)oestrogens on synthesis of the ecdysteroid, links have been made with the inhibition of moulting and growth retardation (Charniaux-Cotton, 1985; Quackenbush, 1986; Zou & Fingerman, 1997a,b; Brown et al., 1999). Thus, several experimental studies indicate that oestrogens and oestrogen mimics such as alkylphenols could interact with crustacean developmental pathways: exposure to NP inhibits moulting, growth and the metabolic elimination of testosterone in the water flea *Daphnia magna* (Comber et al., 1993; Baldwin et al., 1995; Zou & Fingerman, 1997b); prenatal NP exposure results in a high proportion of morphological deformities in juvenile *Daphnia galeata* (Shurin & Dodson, 1997); and life history effects have been observed in the copepod *Tisbe battagliai* at NP concentrations of 20 $\mu g\ l^{-1}$ (Bechmann, 1997). In barnacles *Balanus amphitrite*, low level exposure (0.01–10.0 $\mu g\ l^{-1}$) to both NP and the natural hormone 17β-oestradiol (E2) can significantly reduce the settlement of cyprid larvae, and, in *Elminius modestus*, can disrupt the timing of larval development and reduce the size of resultant adults (Billinghurst et al., 1998, 2001). The latter studies emphasize that there are critical stages during development which are particularly vulnerable to xeno-oestrogens.

Genotoxicological research on barnacle larvae also reveals that both NP and E2 induce common DNA effects which may arise as a consequence of 'hot spot' DNA damage (e.g. DNA adducts) and/or mutations (point mutations or genomic rearrangements) (Atienzar et al., 2002). This could help to explain how xeno-oestrogens mimic the effects of natural oestrogens. Changes at the DNA level will inevitably be precursors of many of the effects reported at higher levels of biological organization (feminization of males, developmental abnormalities, infertility) and warrant further investigation, both as mechanisms of endocrine disruption and as potential biomarkers.

Field investigations of crustacean populations have been few and far between, providing somewhat tentative evidence of endocrine disruption caused by (xeno)oestrogens. Intersex was identified in copepods *Paramphiascella hyperborea* and *Leptoiaptomus minutus* (Moore & Stevenson, 1991; Sillett & Stemberger, 1998) and in lobster *Homarus americanus* (Sangalang & Jones, 1997), but although this may be connected with sewage discharge, it is unclear whether pollutants or other factors (e.g. parasites, viruses, physical environmental factors) are responsible (Moore & Stevenson, 1991; Oberdörster & Cheek, 2000). Gross et al. (2001) described differences in populations of the freshwater amphipod *Gammarus pulex* collected from an unpolluted reference site and those living below sewage treatment works, wherein samples from the 'clean' site had a higher male/female ratio and greater male/female size differentiation. There were also differences in secondary male characteristics: notably, allometric relationships between body size and gnathopod, and genital papillae, differed between populations. Additionally, abnormal oocytes were found in *G. pulex* from the polluted site. The authors considered that substances from the sewage effluent were interfering with androgenic hormone signalling, although the causative agents were not identified. In male shore crabs *Carcinus maenas*, retarded growth in the right claw (normally enlarged in comparison to the left) has been recorded at sites where strong oestrogenic contamination is present, though again the causative agents are unknown (EDMAR, 2002). Likewise, experiments with benthic amphipods *Corophium volutator* corroborate that xeno-oestrogens such as NP can induce morphological changes to male secondary sex characteristics of crustaceans (in this case the length of the second antenna), perhaps acting through the androgenic gland, but the precise mechanisms and reproductive consequences remain the subject of speculation (Brown et al., 1999).

Benthic infauna, including amphipods, might be expected to be vulnerable to the effects of (xeno)oestrogenic compounds which tend to accumulate in the sediment phase. The estuarine species *Leptocheirus plumulosus* has been used with a degree of success to assess the endocrine disrupting effects of sediment collected near a wastewater treatment plant outfall in Jamaica Bay, New York (Zulkosky et al., 2002). The concentration of total nonylphenol ethoxylate (NPEO) in the sediment from this site was 44.2 μg g^{-1} dry wt, with 95% present as the metabolite NP and 28-day exposures reduced the production of young (juveniles and embryos) in surviving females by an average 50%, suggesting that *L. plumulosus* could be a sensitive amphipod for use in bioassays, though the mode of action and involvement of endocrine activity has yet to be investigated.

There are strong arguments for including molluscs as a high priority for research into endocrine disrupting effects and this phylum provides the focus for the remainder of our synopsis. Indeed, one of the earliest and most startling indications of the potential importance of endocrine disruption in the marine environment came in the 1980s with the recognition that extremely low concentrations of tributyltin (TBT) from antifouling paints was causing masculinization of female dog-whelks *Nucella lapillus*. The condition, termed imposex—the imposition of male characteristics on females—has been shown to lead to sterilization and elimination of populations at concentrations of ~5–10 ng TBT l^{-1} (Smith, 1971; Bryan et al., 1986; Langston et al., 1990; Gibbs, 1999). Molluscs such as *N. lapillus* appear to be highly sensitive because of the known susceptibility of their steroidally-controlled reproductive systems to endocrine disrupting effects; it is the inhibition of testosterone conversion to oestradiol, perhaps catalysed by the P450-aromatase, which affects sexual development in (female) TBT-exposed neogastropods (Spooner et al., 1991). Other molluscs may also be subject to endocrine disruption at TBT-contaminated sites: female abalone *Haliotis madaka*—an orthogastropod—were found to be masculinized and displayed ovotestis, a condition whereby the ovaries of affected females contained small amounts of testicular tissue undergoing spermatogenesis (Horiguchi et al., 2000). The condition and gonad–somatic index of clams *Mya arenaria* were significantly reduced and gonad development impaired in males collected from a TBT-contaminated harbour and, since male clams were predominant at the site, the authors considered that *Mya* were subject to the masculinizing effects of TBT (Gagné et al., 2003). Masculinizing effects of TBT have also been noted in the biochemistry of clams *Ruditapes decussata* transplanted to a TBT-polluted environment: changes included a 33% increase in testosterone levels five weeks into the transplant, and a two-, three-, and five-fold decrease in oestradiol levels after one, three and five weeks, respectively (Morcillo & Porte, 2000).

These TBT studies have established that molluscs are vulnerable to endocrine disruption, and have paved the way for further investigation into possible effects in mollusc species from the various (xeno)oestrogens found in domestic and industrial effluents which possess endocrine-modulating activity. Examples of effects and threshold values for the natural hormone 17β-oestradiol (E2), synthetic 17α-ethinyloestradiol (EE2) and alkylphenolics octylphenol (OP) and nonylphenol (NP) are listed in Table 4. Below, we summarize the consequences for bivalves and, subsequently, gastropods.

It has been suggested that the endocrine systems of molluscs may depend more on neuropeptides than on steroid hormones (LeBlanc, 1999), although E2, testosterone, and progesterone, have been found in various bivalves such as the scallops *Pecten hericius*, *Pecten maximus*, and *Patinopecten yessoensis* (Boticelli et al., 1961; Saliot & Barbier, 1971; Matsumoto et al., 1997), the mussel *Mytilus edulis* (Reis-Henriques et al., 1990), and the oyster *Crassostrea gigas* (Matsumoto et al., 1997). Possible roles in the regulation of reproduction are suggested by fluctuations

in the levels of these sex steroids, correlating with the sexual maturation cycle in these animals (Reis-Henriques & Coimbra, 1990; Matsumoto et al., 1997).

There is evidence that elevated levels of sex steroids, can interfere with the normal functioning of bivalves. When exposed experimentally to 20 and 200 ng l^{-1} E2 in water, mussels *Mytilus galloprovincialis* appear able to maintain endogenous levels of oestradiol by homeostatic mechanisms[3], however, at higher concentrations (2000 ng l^{-1}), E2 titres increase significantly, and sexual maturation in females is accelerated (Janer et al., 2004). Increased levels of E2 stimulate vitellogenesis and facilitate serotonin-induced egg release in oysters and scallops (Osada et al., 1992, 1998; Li et al., 1998; Wang & Croll, 2003). There are also reports that E2 and progesterone can potentiate sperm release and spawning in male scallops (Wang, 2000; Wang & Croll, 2003). Injections of E2 (and other sex steroids, testosterone, and progesterone) have been shown to stimulate both oogenesis and spermatogenesis in adult scallops *Mizuhopecten yessoensis*, and lead to increased gonad weight and oocyte diameter (Varaksina & Varaksin, 1992; Varaksina et al., 1992; Wang & Croll, 2004). Injections of oestradiol at early stages of the seasonal sex maturation of the oyster *C. gigas* may induce sexual reversal from male to female (Mori et al., 1969).

Despite evidence for effects of sex steroids on seasonal maturation, there have been few reports of possible effects of these substances on gender determination in juvenile stages. In an effort to establish possible effects on sexual differentiation and gonadal development, Wang & Croll (2004) injected juvenile sea scallops *Placopecten magellanicus* with E2, testosterone, progesterone and dehydroepiandrosterone[4]. Interestingly, sex ratios were significantly shifted towards males by all the sex steroids, in comparison with control animals. Thus, it appears that E2 (and progesterone) can have both feminizing and masculinizing effects and although the exact mechanisms are not yet elucidated for bivalves, there are at least two possibilities for the apparent anomaly: (1) injection of oestradiol (and progesterone) may promote the synthesis of active androgens in the animal and thus favour the development of male gonad. In vertebrates, oestradiol and progesterone have been reported to stimulate the conversion of testosterone or 4-androstene-3,17-dione to 5α-dihydrotestosterone (DHT) which is a more potent androgen (Tilakaratne & Soory, 1999). Alternatively (2), oestradiol or progesterone may bind directly to an androgen receptor and elicit androgenic effects. This too has been demonstrated in vertebrates (Veldscholte et al., 1990) and is supported by recent *in vitro* results which showed that potentiation of sperm release could be partially inhibited in scallops *Placopecten magellanicus* by the antiandrogen, flutamide (Wang & Croll, 2003). Stimulation of male reproductive activities by oestrogens (El and E2) has been reported in other molluscs/invertebrates—including stimulated spermatogenesis in the gastropod *Helix pomatia* (Csaba & Bierbauer, 1979) and induced male sex differentiation in juvenile red sea urchins *Pseudocentrotus depressus* (Unuma et al., 1999). Among vertebrates there is evidence of similar effects in female reptiles and fish (e.g. Callard et al., 1991; Nagahama, 1994).

Nonylphenols alter hormonal functions in fish by binding oestrogen receptors and mimicking the action of endogenous oestrogens (Arukwe et al., 1997; Madigou et al., 2001). Resultant VTG synthesis and alterations in testicular structure have been demonstrated both *in vitro* and *in vivo* indicating that NP may contribute to the induction of 'feminization' of wild male fish (Jobling et al., 1996, 2002; Christiansen et al., 1998; Christensen et al., 1999). Effects in molluscs are less well documented, although there is a growing body of evidence indicating that NP can exert a variety of endocrine-disrupting effects and also direct toxicity (see examples Table 4). In laboratory experiments, both acute toxicity and sub-lethal effects of NP have been demonstrated in several bivalve species. Nice et al. (2000) exposed the embryos of Pacific oysters *Crassostrea gigas* to a range of 4-nonylphenol concentrations which resulted in mortality at high doses (1000 and 10,000 μg l^{-1}), whilst at lower levels (0.1–100 μg l^{-1}) normal development to D-shaped larvae was significantly delayed compared with non-exposed embryos, and the percentage of larval deformities increased with increasing concentrations. Transgenerational effects of NP in *C. gigas* have also been reported (Nice et al., 2003): exposing fertilized *C. gigas* embryos to 1 and 100 μg l^{-1} NP resulted in an altered sex ratio (greater number of females), together with a high percentage of fully functional hermaphrodites in the resultant adults (17% at 1 μg l^{-1} NP and 30% at 100 μg l^{-1} NP). Gamete viability was also affected with poor embryonic and larval development (up to 100% mortality) of the subsequent generation. As with barnacles (discussed previously), these findings suggest that there may be critical stages in the development of bivalves during which they are particularly vulnerable to the effects of NP.

In Manila clams *Tapes philippinarium* acute toxicity of NP occurred at a similar concentration (LC_{50} 1120 μg l^{-1}) to that observed in *Crassostrea gigas* (Matozzo et al., 2003, 2004; Matozzo & Marin, 2005). Sub-lethal effects included decreased respiration and clearance rates and reduced scope for growth. In males exposed to 100 and 200 μg NP l^{-1}, levels of VTG (normally found only in females) increased significantly in both haemolymph and digestive gland, whereas no changes were observed in females. Although the mechanisms by which NP exerts its *in vivo* endocrine-modulating effects in bivalve molluscs remain unclear, results demonstrate the potential of NP to induce VTG-like proteins in *T. philippinarum*, particularly males. A significant induction of VTG-like proteins has also been observed in the soft-shelled clam *Mya arenaria* following injection of various NP concentrations into the adductor muscle (Blaise et al., 1999).

Increasing exposure times to NP may lower the toxicity threshold in bivalves, as demonstrated in experiments with mussels *Mytilus edulis*: acute toxicity, expressed by an LC_{50} of 30 mg l^{-1} (96 h), was reduced to 500 μg l^{-1} at 360 h and 140 μg l^{-1} at 850 h (Granmo et al., 1989). Sublethal effects, manifested as decreased byssus strength and reduced scope for growth, were apparent at concentrations as low as 56 μg l^{-1}. However, concentrations up to 200 μg l^{-1} (the

[3], Such mechanisms may include esterification of E2, though even esterified steroids may be considered as long-acting steroids following hydrolysis (Hochberg, 1998). More work is needed to establish their role in molluscs.

[4], Dehydroepiandrosterone (DHEA), a compound which may serve as a precursor for the synthesis of other active sex steroids in molluscs (Lupo di Prisco et al., 1973; Le Guellec et al., 1987).

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Table 4. *Sub-lethal effects of (xeno)oestrogens in molluscs.*

Species	Stage	Chemical	Concentration (mg l^{-1})	Effect	Reference
			BIVALVES		
Crassostrea gigas	Embryo	NP	0.0001–10	Delayed development, larval deformities, reduced survival	Nice et al. (2000)
Crassostrea gigas	Embryonic—days 7–8 post fertilization	NP	0.001 and 0.1	Change in sex ratio towards females and increased hermaphroditism in resulting adults. Reduced gamete viability of subsequent generation	Nice et al. (2003)
Tapes philippinarium	Pre-spawning adult	NP	0.025–0.2	Vitellogenin production in males, reduced scope for growth, respiration and clearance rates	Matozzo et al. (2003, 2004) Matozzo & Marin, in press
Mytilus edulis		NP	0.056	Decreased byssus strength and altered scope for growth no observable effects on fertilization	Granmo et al. (1989)
Mytilus edulis	Embryonic	NP monoethoxylate	1–2	Development inhibited	Swedmark et al. (1971)
Mya arenaria	Adult	NP, E2	Injected	VTG induction	Blaise et al. (1999)
Placopecten magellanicus	Juvenile	E2	Injected	Altered sex ratio towards more males. Stimulated oocyte growth	Wang & Croll (2004)
			GASTROPODS		
Marisa cornuarietis	Adult and complete life cycle	BPA and OP	0.001–0.1	Induction of 'superfemales'—additional female organs, gross abnormalities of sex glands, stimulated oocyte and spawning mass production	Oehlmann et al. (2000)
Nucella lapillus	Adult	BPA and OP	0.001–0.1	Induction of 'superfemales'. Additional female organs. Male sex organs reduced	Oehlmann et al. (2000)
Potamopyrgus antipodium	Adult	OP, BPA, EE2	0.001–0.1 ($1–100\times10^{-6}$ EE2)	Stimulation/inhibition of embryo production	Jobling et al. (2003)
Lymnaea stagnalis	Adult	NP	1–100	Few effects—reduced fecundity at highest concentration	Czech et al. (2001)
Potamopyrgus antipodium	Adult	OP, BPA, NP	*In sediment 1–300 μg kg^{-1} dry wt (1–1000 μg kg^{-1} dry wt NP)	Stimulation/inhibition of embryo production	Duft et al. (2003)

highest dose level tested) did not affect fertilization and early developmental success. Swedmark et al. (1971) also looked at the effects of NP (monoethoxylate) in *Mytilus edulis* and found that development of embryos was inhibited at 1–2 mg l^{-1}.

Thus, laboratory exposure studies suggest that (xeno)oestrogens have the potential to perturb the reproductive systems of bivalves, and evidence is beginning to emerge of such effects occurring in the field, where organisms may be exposed to oestrogenic compounds on a long-term basis. Municipal effluents release both micro-organisms and pollutants into the aquatic environment including significant amounts of polyaromatic hydrocarbons, pesticides, heavy metals and established endocrine-disrupting (xeno)oestrogens (Chambers et al., 1997; Ternes et al., 1999a,b). The oestrogenic effects of such municipal effluents have been detected in field studies with freshwater mussel *Elliptio complanata* caged downstream from an effluent outfall (primary treatment). Within three months, increased VTG levels occurred both in the gonad and haemolymph in these *in situ* exposures (Gagné et al., 2001). The increases in VTG occurred in both sexes of *E. complanata* but were higher in females. Lipid and sugar levels in male gonads also increased, and shell growth was reduced in the caged animals. Long-term exposure to the plume led to a higher proportion of females (not usual in natural populations) suggesting that feminization is artificially induced (Blaise et al., 2003). Subsequent field studies with *E. complanata*

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showed that the normal metabolism of serotonin and dopamine, both of which are involved in sexual differentiation, are altered by exposure to the effluent plume, even at distances of 4 km from the outfall (Gagné & Blaise, 2003).

Vitellin-like proteins have also been shown to be induced in freshwater zebra mussel *Dreissena polymorpha* following exposure to tertiary treated effluent from a municipal sewage treatment works (Quinn et al., 2004). The mussels were held *in situ* for 112 days during gametogenesis (December to mid-March). Again, females were found to be more sensitive, producing more vitellin-like proteins. Histological effects on the male gonad included a large increase in undifferentiated interstitial tissue between the seminiferous tubules. The increase led to a reduction in the size of seminiferous tubules, effectively reducing the sperm-producing area.

Gauthier-Clerc et al. (2002) put forward evidence of endocrine disruption in *Mya arenaria* collected from contaminant-impacted in Saguenay Fjord (Canada). Differences in physiological condition and reproductive status were compared over the course of one year at two sites within the Fjord and a reference site in the St Lawrence Estuary. Delayed gametogenesis, and accompanying higher gonad glycogen concentrations in clams from the more perturbed upper Saguenay Fjord site were attributed to a dysfunction of the vitellogenic process (initially postulated as signifying exposure to anti-oestrogenic contaminants). Subsequently, biochemical properties normally associated with vitellin (Vn), including relative levels of sugars, lipids, proteins, phosphates, and labile Group IIb metals and calcium, were examined in greater detail at marina, municipal outfall and reference sites in the Fjord to try and explain the differences in *Mya* populations and help establish links with causative agents (Gagné et al., 2002). Altered chemical composition of the gonads and Vn in *Mya* were consistent with the type of contamination present, with the best classification provided by chemical characterization of Vn. Thus, whilst total protein concentrations were significantly reduced at both marina and outfall sites, labile IIb metal (zinc, cadmium and mercury) levels were much higher in *Mya* Vn from the marina, and alkali-labile phosphate (ALP) levels and calcium were significantly higher at the municipal outfall (compared with the reference site). Levels of ALP and possibly calcium in gonad and haemolymph have been reported to increase with exposure to oestrogens (E2 and NP), acting as a proxy biomarker for Vn (Blaise et al., 1999; Gagné et al., 2002); therefore increased levels of ALP may be indicative of the presence of environmental oestrogens at the outfall sites.

Studies on the endocrine disrupting effects of (xeno)oestrogens in other molluscs have centred almost exclusively on gastropods. Striking examples of experimentally-induced ED are described for freshwater and marine gonochoristic prosobranchs following exposure to 0.1–100 μg l^{-1} bisphenol A (BPA)[5] and 1–100 μg l^{-1} octylphenol (OP), typified by a complex syndrome of sexual abnormalities referred to as 'superfemales' in female freshwater snails *Marisa cornuarietis* (Oehlmann et al., 2000, 2001). Effects included the formation of additional female organs: a second vagina with vaginal opening to the mantle cavity, and/or an enlargement of the pallial accessory sex glands. Affected females also displayed gross oviduct malformation (rupture) resulting in increased female mortality, and a massive stimulation of oocyte and spawning mass production. *Nucella lapillus* 'superfemales' have also been observed following exposure to BPA and OP, but unlike *Marisa* no gross malformations of the oviduct, female sterilization or increased female mortality occurred. Contrary to their findings for *Marisa*, male *Nucella* exposed to BPA and OP were also affected: the percentage of males with sperm stored in the *vesicula seminalis* dropped significantly faster, and the length of the male penis and prostate gland were significantly reduced, in exposed animals, compared with the control group. These effects in *Nucella* were observed at a nominal concentration of 1 μg l^{-1} BPA or OP. Oehlmann and co-workers speculated that these alterations might be expected to have serious consequences at the population level, comparable with the TBT-induced imposex phenomenon, though as far as is known, no similar effects in the field have been reported to date. Subsequent laboratory experiments with *Marisa cornuaretis* showed contrasting, concentration-dependent, oestrogenic/androgenic effects from the environmental hormone EE2 (Oehlmann et al., 2001). Thus, whilst 0.001–0.1 μg l^{-1} EE2 induced the 'superfemales' phenomenon, at concentrations >0.25 μg l^{-1}, EE2 initiated masculinization in this species (a marked imposex development and a simultaneous impairment of oogenesis). Despite the ambiguous nature of certain responses, these results suggest that prosobranch molluscs could be useful screening tools for the identification of endocrine disruptors.

Jobling et al. (2003) exposed another freshwater prosobranch *Potamopyrgus antipodium* to EE2, OP, BPA and treated sewage effluent. Reproductive responses to the (xeno)oestrogens were dose-dependent, stimulating embryo production at low doses (<25 ng l^{-1}) and causing inhibitory effects at 100 μg l^{-1}. Exposure to the sewage effluent also stimulated embryo production, and increased duration of exposure lowered the critical threshold for response. A comparison of responses of *P. antipodium* with those in various species of freshwater fish indicated that fish are more sensitive to disruption in reproductive output caused by EE2, but that *Potamopyrgus* may be more responsive to very low concentrations of some xeno-oestrogens (and/or oestrogenic effluent).

Some snail species however, appear to be less sensitive to the effects of (xeno)oestrogens: hermaphrodite gastropods *Lymnaea stagnalis* were exposed to a range of NP concentrations in water (1–100 μg l^{-1}) with little effect. Lower fecundity was only induced at the highest level after prolonged exposure (Czech et al., 2001). It is possible therefore that individual species, or sub-classes of gastropod could be at higher risk than others, although further studies are needed to confirm this.

Current environmental protection measures and gaps in risk assessment

At present, there are no statutory or guideline values for E2 or EE2 in aquatic environments, although both appear

[5], BPA is used in the production of polycarbonate epoxy resins lining food cans and water pipes, dental coatings and fillings. BPA has been shown to be oestrogenic to rainbow trout (e.g. Kloas et al., 1999; Lindholst, 2000).

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on OSPAR lists of substances of possible concern. Generally, the data for aquatic biota suggest that the 'no observed effect concentrations' (NOECs) for OP and NP are of the order of a few $\mu g\ l^{-1}$ (though general toxicity endpoints may be lower than ED effects). Interestingly, the oestrogenic potency of OP in the field is considered higher than suggested from *in vitro* tests—perhaps as a consequence of bioaccumulation (Defra, 2001). Predicted no effect concentrations (PNECs) for OP and NP are $< \mu g\ l^{-1}$ and, hence, concentrations of these compounds may be environmentally significant near discharges, meeting criteria as chemicals of concern. Both OP and NP have been identified in the OSPAR DYNAMEC[6] procedures as substances for priority action and have been included in Water Framework Directive lists of priority substances[7]. An Environmental Quality Standard (EQS) of $1\ \mu g\ l^{-1}$ (as annual average; maximum allowable concentration $2.5\ \mu g\ l^{-1}$) has been set for NP and OP to control these substances in European water[8]. In the USA a chronic criteria level of $1.4\ \mu g\ l^{-1}$ is proposed for NP in marine waters.

Potential for effects from AP-contaminated sediments has been demonstrated for crustacea (Zulkosky et al., 2002—see above) and molluscs: Duft et al. (2003) showed that embryo production in the gastropod *Potamopyrgus antipodium* was stimulated in response to long-term exposure to sediments spiked with environmentally realistic concentrations of EDs (OP, NP and BPA). The lowest observed effect concentration (LOEC) was $1\ \mu g\ kg^{-1}$ for all test compounds. The PNECs for sediments have been suggested as $39\ \mu g\ kg^{-1}$ and $6.5\ \mu g\ kg^{-1}$ for NP and OP, respectively—however, these are based on evidence from surface water data and a partition model with a high degree of uncertainty. This illustrates the need for more accurate data on the criteria determining the fate of endocrine disrupting compounds in sediments, their bioavailability and effects, particularly in estuaries and coastal embayments subjected to substantial anthropogenic perturbance.

There is remarkably little information on EDs or their effects in many of the UK's nationally and internationally important smarine sites—including special areas of conservation (SACs) and special protection areas (SPAs) designated under European Habitats and Birds Directives (Allen et al., 2000; Langston et al., 2003). Arguably, in the debate as to where to apportion resources (regarding assessment of risk from ED), such sites may be considered a priority because of their important conservation status, and the requirement to apply the precautionary principle in the absence of full scientific knowledge. Currently, any information on these sites, if it exists, relates largely to symptoms exhibited in fish, making it difficult to interpret the broader significance of EDs in terms of impact at population or community levels. There is a need for basic ED research in a wider variety of organisms, some of which may offer better opportunities as biomonitors of ED phenomena or exhibit practical attributes as 'early-warning' indicators. Of all the aquatic invertebrates, gastropod molluscs and bivalves appear to be most susceptible to environmental oestrogens (and androgens) and therefore are important candidates for investigation in this capacity.

Since much of the oestrogenic activity in estuarine and coastal environments is likely to reside in, or originate from, benthic sediments, assessment of any accompanying ecological risks is a distinct concern. Various gonochoristic benthic molluscs may be suitable organisms as biological indicators of sediment contamination because of their sessile nature, relative abundance and ability to bioaccumulate contaminants. Some, such as the clams *Mya arenaria*, *Scrobicularia plana* and *Macoma balthica* have additional advantages as indicators because of their infaunal habit or ability to deposit-feed (accumulating a number of contaminants directly from sediment), coupled with a wide tolerance to salinity (essential for estuarine survey). Extensive geographical distributions also make them ideal for large-scale screening purposes. Similar arguments may be extended to a number of benthic amphipods which are either deposit feeders or live in contact with sediment, are numerically abundant and have broad biogeographical ranges. Responses in such candidate species must be quantified in the laboratory and at estuarine sites subjected to varying degrees of anthropogenic impact, with a view to wider application in monitoring.

CONCLUSIONS

This review has summarized current understanding on partitioning, persistence, bioaccumulation and behaviour of the natural hormone 17β-oestradiol (E2), the synthetic 17α-ethinyloestradiol (EE2) and alkylphenolics octylphenol (OP) and nonylphenol (NP) in the aquatic environment. The weight of evidence suggests that these compounds are responsible for some of the most important oestrogenic impacts affecting freshwater and marine organisms.

Details of effects and the scale of the problem are beginning to emerge from studies with fish, though characterization of responses in other organisms is much less advanced. Laboratory exposure experiments have demonstrated the potential for endocrine disruption in some marine invertebrates, though with the exception of TBT-induced imposex, there are still few documented examples of such effects occurring in the field.

It is important to confirm the significance of oestrogencity in estuarine sediments. A better understanding of the major determinands of partitioning of (xeno)oestrogenic compounds between sediments and water is needed to provide accurate estimates of distribution and transport. Other priority questions are: how important are sediments in determining bioavailability of these compounds? Are infaunal species susceptible to the effects of sediment-bound (xeno)oestrogens and if so, what is the prognosis for impact at the population level? Since benthic organisms form the diet of many birds and fish, do they contribute significantly to the transfer of endocrine disrupting compounds along marine and estuarine food chains, thus threatening higher predators?

[6], Dynamic Selection and Prioritization Mechanism for Hazardous Substances.
[7], The 'WFD', 2000/60/EC; the Priority List, COM(2001)17 final, at Annex II.
[8], This only applies to AP and does not include shorter chain ethoxylates and carboxylate metabolites of APEOs.

The time has come to address these gaps through fundamental studies on: sequestration of (xeno)oestrogens; accumulation rates and routes; development of relevant invertebrate assays and reproductive markers; and by embarking upon systematic studies of vulnerable benthic populations in potentially contaminated and sensitive areas. Results will contribute towards better-informed risk assessments and ensure that the most appropriate and cost-effective regulatory responses are made.

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APPENDIX

Descriptions of specific compounds and abbreviations included in the review

Compound	Abbreviation	Description
17β-oestradiol	E2	Natural oestrogens synthesized and excreted by all vertebrates and some invertebrates
Oestrone	E1	
Oestriol	E3	
Diethylstilboestrol	DES	Synthetic oestrogens excreted by women taking the contraceptive pill and hormone replacement therapy
17α-ethinyloestradiol	EE2	
Alkylphenol polyethoxylates	APEOs	Non-ionic surfactants used in industrial detergents, some pesticide formulations, some cosmetics and in plastics manufacture.
Octylphenol, octylphenol polyethoxylate	OP OPEO	Most commonly used alkylphenols and alkylphenol ethoxylates, containing branched alkyl groups with a formula C_8H_{17} (OP) and C_9H_{19} (NP), respectively. NPEOs and OPEOs are broken down in sewage treatment plants, whilst the resulting alkylphenols (NP and OP, respectively) tend to remain more stable in the effluent, sewage sludge and the environment
Nonylphenol,nonylphenol polyethoxylate,nonylphenol mono-ethoxylate	NP NPEO NP1EO	
Bisphenol —A	BPA	Used in production of polycarbonate epoxy resins lining food cans and water pipes, dental coatings and fillings, flame retardants and PVC polymers
Progesterone		Female steroid hormone involved in the regulation of reproduction
Testosterone		Potent androgenic hormone involved in development of male secondary sex characteristics
Vitellogenin*	VTG	Female protein, precursor of several yolk proteins
Vitellin*	Vn	Chief protein constituent of egg yolk
Zona radiata protein*	ZRP	Eggshell protein—zona radiata is a radiately striated membrane adjacent to the yolk of an ovum, or separated from it by a very delicate membrane
5α-dihydrotestosterone	DHT	A potent androgen
Dehydroepiandrosterone	DHEA	May serve as a precursor for the synthesis of active sex steroids in molluscs

*, VTG, Vn and ZRP are induced by interactions with the E2-receptor, rather than through general stress or other mechanisms of toxicity.

GLOSSARY (DEFINITIONS AND ABBREVIATIONS)

Endocrine disrupting chemicals (EDs) are defined as (1) 'exogenous substances that cause adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function' (EU, 1997); or as (2) 'exogenous agents that interfere with the production, release, transport, metabolism, binding and action or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and regulation of developmental processes' (EPA, 1996).

Oestrogen: any of various natural steroids (e.g. oestradiol) that are formed from androgen precursors, that are secreted chiefly by the ovaries, adipose tissue, and testes, and that stimulate the development of female secondary sex characteristics and promote the growth and maintenance of the female reproductive system.

Anti-oestrogen: substance that mimics or blocks the natural female sex hormones.

Androgen: male sex hormone that is produced in the testes and responsible for typical male sexual characteristics.

Anti-androgen: substance that mimics or blocks the natural male sex hormones.

Xeno-oestrogen: any of various synthetic or semisynthetic steroids (such as ethinyl estradiol) that mimic the physiological effect of natural estrogens.

Phyto-oestrogen: natural chemicals found in plants, notably whole grains, fibres and soy products, (resorclic acid, lactones, isoflavones, coumestans, lignans).

Gonochoristic: pertaining to a unisexual individual; remaining as the same sex throughout the life cycle.

Ovotestis: an 'intersex' condition whereby a gonad contains both testicular tubular elements and ovarian follicles.

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