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Topic 2.5

Endocrine active industrial chemicals: Release and occurrence in the environment*

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Abstract: Of the xenobiotic endocrine active substances (EASs), tributyltin (TBT) has had the clearest link to an impact on aquatic ecology. Its release from marine antifouling paints had a drastic impact on dogwhelk populations in polluted harbors due to a masculization effect. 4-*tert*-Nonylphenol is seen as the most significant of the industrial xenobiotic estrogen mimics, being implicated as the dominant endocrine disruptor in certain industrialized river reaches. Apart from hot spots associated with particular industries, the estrogenic alkylphenols, phthalates, and bisphenol A are present in effluent and receiving water at concentrations below that which would give cause for concern. Other more bioaccumulative compounds such as polybrominated flame retardants, dioxins, and furans may possess some endocrine active properties. The possibility of additivity effects may yet mean that low concentrations of xenobiotic EASs will need careful consideration. It is noted that considerable quantities of many of these compounds are often found in sewage sludge and sediments.

INTRODUCTION

Assessing whether any of the xenobiotic endocrine active substances (EASs) pose a threat to the natural environment requires balancing information on its potency against observed environmental concentrations. At least for fish, the overwhelming form of endocrine disruption (ED) observed in the aquatic environment has been estrogenic. Much effort has gone into screening xenobiotic compounds for estrogenic potential using in vitro tests. Thus, while we can report on the xenobiotic EASs of current concern, it is important to be aware of the following complicating factors that prevent an accurate assessment from being made with confidence:

- Exposure of fish or other animals to xenobiotic EASs may have a greater impact than predicted from in vitro screening tests, because the liver system may be able to clear estradiol (the reference compound in many in vitro tests) much quicker than a xenobiotic estrogen, which although it has less affinity for the estrogen receptor, may remain in the body for longer [1]. Possibly, they are responsible for the pathogenicities such as malformed sperm ducts which are of greatest consequence for fish populations [2].
- Additivity may mean that mixtures of low concentrations of xenobiotic EASs could yield overall significant ED to animals. Such summation effects have been shown in vitro [3] and in vivo [4]
- The impact may be greatest on species of aquatic fauna other than fish which we have yet to study. TBT, for example, had little effect on fish, but had a disastrous impact on the female mollusk population due to a masculization effect [5].

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- Perhaps we have yet to discover the most important xenobiotic EAS! This may be a breakdown product of an innocuous parent molecule formed in sediment, or transit down the river.

Similarly, care must be taken in the interpretation of water or soil concentrations of EASs. The concentration will in the first case largely depend on the local dilution available in the receiving water, and in the second case on the tons per hectare of sewage sludge spread to land, and its soil incorporation [6,7]. Sediment concentrations will be influenced initially by the octanol–water partition coefficient (K_{ow}) and recalcitrance of the compound, but sample location, such as distance from a discharge, will have an enormous impact on the concentrations reported.

CHEMICALS OR GROUPS OF INTEREST

The list of known or suspected EASs gets longer almost every day. For example, a working list produced for the European Union contains 564 chemicals or groups of which 66 were chosen as priority substances [8]. This chapter attempts to give an overview of some important EASs of industrial origin—for endocrine active natural compounds, pharmaceuticals, or pesticides please refer to Topics 2.2–2.4 and 2.6.

Tributyltin (TBT)

Sources and potencies

Tributyltin acts as a biocide and is mainly used as a slow-release antifouling agent incorporated in paints for ships. TBT has been linked with the breakdown of commercial oyster breeding in some areas and masculinization (imposex or intersex) of various marine invertebrates often to the point where they were unable to reproduce. This has led to the local extinction of the dogwhelk Nucella in many areas [5]. Because of these effects, which can be observed at concentrations as low as 1 ng/l [9], its use on pleasure boats is now banned in most countries and the International Maritime Organization (IMO) has adopted a convention, which prohibits the application of TBT-containing paints on all ships by 2003 and requires old paints to be removed or covered by 2008. Even though the use of TBT is now restricted, large amounts can still be released when old paint is stripped off prior to repainting a ship [10]. Polluted sediments may also release organotin compounds into the water column, especially during harbor dredging or storms. The use of organotins in stabilizers for plastics [5] and in wood preservatives [10] could lead to potentially more diffuse contamination of the environment including soil.

Environmental concentrations

Concentrations of several hundred to three thousand ng/l have been found in harbors and marinas within Germany, Switzerland, and Canada [5]. These concentrations exceed the threshold level for induction of imposex in marine neogastropods by over a hundred times. The log K_{ow} of TBT is 3–4, and therefore it should have a low to medium attraction to the organic fraction of sediments, but with its positive charge, TBT can also bind as a cation to sediments and biota, therefore partitioning to solids and bioaccumulation is higher than would be expected from the K_{ow} alone [10]. TBT levels in sediments can be up to µg/g levels especially in confined harbors and marinas [5,11].

Summary

The impact of TBT on marine and limnic organisms is very considerable. The measures restricting its use are showing some success, such as in some recently sampled North Sea sites [12], but TBT levels remain unacceptably high in many places. In contrast, a comparison of imposex parameters measured in 1995 and 2000 along the Portuguese coast found no improvements [13], and levels up to 200 ng/l were still found around Corsica in 1999 [14].

Alkylphenol polyethoxylates and their degradation products

Sources and potencies

The alkylphenol polyethoxylates (APEOs) parent compounds are commonly used nonionic surfactants which are partially degraded in sewage treatment works (STWs) to yield a wide variety of by-products [15]. These include the formation of APEOs with shorter ethoxylate (EO) chain lengths (such as the alkylphenol mono and diethoxylates). These undergo further degradation to form the alkylphenols, nonylphenol (NP) and octylphenol (OP). Log K_{ow} reported for OP and NP are 4.2–4.5 [16]. 4-*tert*-Octylphenol is the most potent of these compounds, being 1.5×10^3 less potent than 17β-oestradiol (E2), followed by 4-*tert*-nonylphenol at around 10^4 times less potent than E2 [17,18]. The NP1EO and 2EO metabolites are ten to a hundred times less potent than nonylphenol and so represent much less of a threat [19,20].

Environmental concentrations

Sewage treatment works effluent concentrations of NP have been reported at between 0.1 and 3.7 µg/l in Scotland (UK), Germany, Switzerland, and Italy [21–24]. In a recent monitoring exercise [25] of the UK rivers only in the Mersey and Aire were NP concentrations above 0.2 µg/l. High concentrations, up to 180 µg/l (total extractable NP), had been reported in the R. Aire (UK) [26] due to effluent from the local textile industries and in two rivers in Catalonia, Spain (0.15–644 µg/l) [27]. Concentrations of up to 0.4 mg/kg NP have been detected in river sediment in the UK [28] and up to 0.7 mg/kg in Canadian and 6.7 mg/kg in Italian marine sediments associated with cities [29,30]. The highest octylphenol value (13 µg/l) has been reported for the Tees estuary [26], which is heavily impacted by industry, however other values measured in the United Kingdom and Canada were <0.5 µg/l [26,31]. The hydrophobic NP and OP are readily sorbed to sludge and appear to be resistant to anaerobic digestion [32]. A study of sludge in Spain and Germany found NP concentrations ranging from 25–600 mg/kg [33]. There are some data indicating that NP does not persist in sludge-amended soil [34,35].

Summary

Potency studies would suggest that where concentrations greater than 1 µg/l of OP or NP in receiving waters occur then a danger of ED may exist. Usually, dilution in the receiving water would bring concentrations down to below 1 µg/l, but there are a few industrially impacted sites where NP concentrations of 10–100 µg/l have been measured, and ED dominated by these compounds. The other short chain EO metabolites are at least an order of magnitude less potent, but may be important if additivity is taken into account. It is not yet known whether alkylphenol binding to sediment or sludge has any ED significance.

Bisphenol A

Sources and potency

Bisphenol A (BPA, 4,4′-isopropylidenediphenol) is a widely used intermediate in the production of polycarbonate and epoxy resins. In vitro studies indicate BPA to be 10 000–30 000 times less potent than E2 [3,20,22]. A study using Japanese Medaka gave 10 µg/l as the lowest concentration where ED as induction of testis-ova could be observed [20]. One study on *Xenopus* frogs suggested an ED effect could be occurring at 23 µg/l [36], however, this was contradicted by a similar study [37]. Concentrations as low as 5 µg/l were reported as causing potentially fatal superfeminization in a species of prosobranch snails [38]. Staples [39] reported log K_{ow} values for BPA of 2.2–3.82.

Environmental concentrations

Median effluent concentrations of 0.14, 0.15, and 0.03 µg/l were observed in Canada [40], Germany [41], and Japan [42], respectively. Industrial effluent around Toronto had a median value of 11 µg/l [40]. BPA concentrations in the effluent of paper production (mean 41 µg/l), metal/wood production (17 µg/l), and the chemical industry (18 µg/l) have been reported [43]. In a survey of river systems in

Japan [44] and Germany [45], the majority of samples were below 0.1 μg/l with only one sample above 1 μg/l. An analysis of sewage sludge extracts taken from 18 treatment works in Canada gave a median concentration of 1.1 mg/kg [40]. High concentrations of BPA of 25–146 μg/l have been found in waste dump water and compost water [45].

Summary

Reported concentrations in domestic sewage effluent are typically below 1.5 μg/l. Based on the reported potency of BPA and receiving water dilution, this should not give rise to concern. Significantly higher concentrations (often 10s of μg/l) were observed in the effluent emanating from some specific industries which could lead to locally elevated sediment concentrations. In these cases, particularly where dilution is negligible, some ED of sensitive invertebrates may occur.

Phthalates

Sources and potencies

Phthalates are widely used in the manufacture of plastics. The phthalate esters with the most evidence for estrogenic activity in vitro are butyl benzyl phthalate (BBP), dibutyl phthalate (DBP), and di(2-ethylhexyl) phthalate (DEHP) with in vitro potencies from 10^{-5}–10^{-8} compared to E2 [46]. These in vitro results however could not be reproduced in vivo. Concentrations as high as 5 mg/l DEHP had no observable effect on Japanese Medaka fish [20]. The phthalates have reported log K_{ow} values in the range of 4–5 for DBP and BBP and >7 for DEHP [47].

Environmental concentrations

In UK and German sewage effluent studies DBP was detected at <1–14 μg/l; BBP <1–2.8 μg/l; and DEHP <2.4–182 μg/l [21,45,48]. Looking across Canadian, Michigan (USA), German, and Swiss municipal sludges, phthalates were found in the following ranges; DEHP 21–230 mg/kg; BBP 0.3–10.1 mg/kg; DBP 0.2–17 mg/kg [45,49,50]. Concentrations in sludges from industrial catchments in Canada had a median 10 mg/kg for DBP, and 80 mg/kg for DEHP [51]. DEHP was also found in German waste dump waters with concentrations between 17–169 μg/l [52]. In a recent review of thousands of surface water measurements [53], most values were below 1 μg/l for DBP and BBP, but a later German survey found higher concentrations of 0.3–98 μg/l (median 2.3 μg/l) DEHP and 0.12–8.8 μg/l DBP (median 0.5 μg/l) in surface water. [45]. Although some of these values exceed predicted no effect levels for toxicity [45], there is no evidence of endocrine effects at these levels.

Summary

The in vitro estrogenic potency of the phthalate esters is similar or weaker than BPA, with little evidence of in vivo effects except at exceptionally high concentrations. Concentrations in treated sewage effluents are at levels which would be considered unlikely to pose a significant risk to aquatic life. However, data indicates several phthalate esters can have a significant presence in sewage sludge as would be predicted from their high log K_{ow} values. It is unclear whether high sludge concentrations pose any endocrine disruptor risk.

Brominated flame retardants

Sources and potencies

Polybrominated biphenyls (PBBs) and polybrominated diphenylethers (PBDEs) are widely used as flame retardants in a wide variety of products [54]. Due to their persistence and hydrophobicity, brominated flame retardants can be detected in animal tissue from all over the world [54,55]. These compounds have a high hydrophobicity with log K_{ow} 6–10 [47]. Effects on thyroid function have been found at high concentrations in rodents [56,57] and possibly estrogenic or antiestrogenic effects as suggested by in vitro studies [58]. Because the compounds accumulate, exposure via the food chain is likely. This group of chemicals is discussed in detail in Topic 3.7.

Environmental concentrations

As may be expected with their hydrophobic nature, all data available for water or sewage concentrations is focused on the amount sorbed to suspended particles. In municipal sewage effluent from the Netherlands, 0.3–0.9 mg/kg BDE 209 has been found on the suspended particles [47,59]. High concentrations of BDE 209 (up to 4.6 mg/kg) were found in suspended matter in a Dutch estuary [47], but lower levels of 10 µg/kg or less for the individual PBDE in sediments of the river Elbe in Germany [60]. The highest levels found are associated with sediments downstream of plastics manufacturing sites or sewage works, with a clear trend over time for increasing concentrations found in sediments from the late 1970s onwards [55]

Summary

The potential widespread distribution in sediments, and its persistence in sediment is a matter for some concern. Their hydrophobic character and persistence will lead to some degree of bioaccumulation. However, it is still too early to determine whether these compounds could pose a realistic ED threat.

Polychlorinated biphenyls (PCBs), dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and other industrial chemicals

Sources and potencies

PCBs dioxins and furans

PCBs were widely used in lubrication and in isolating, cooling, and hydraulic fluids. However, when their toxicity and potential to accumulate in the environment became known their use was phased out in many countries [61,62]. PCBs have been implicated in impaired reproduction and immune function in seals (reviewed in [63]). Various dioxins and furans, which can be generated for example during waste incineration, especially the Seveso poison 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD) are arylhydrocarbon receptor (AhR) agonists and have been shown in vitro and in vivo to act as antiestrogens. However, the doses needed were very close to the lethal dose, so the acute and chronic toxic effects for this group of chemicals would probably be of greater concern than any suspected endocrine effects. Some coplanar and mono-orthocoplanar PCBs also have a weak dioxin-like effect but always less than 2,3,7,8-TCDD. Depending on the exact structure, PCBs can also have estrogenic effects (reviewed in [61]).

Styrene

Styrene is a monomer used for the production of polystyrene, synthetic rubber, etc. Monomers or small polymers such as dimers and trimers can leak from plastic containers. The highest estrogenic potential in vitro was found for some styrene trimers. The relative potency of these chemicals was 1.5×10^{-5}–2×10^{-6} [64]. Some endocrine related impacts on in female styrene-exposed workers have been reported [8], but so far it is not considered to possess a significant environmental threat.

Other polycyclic aromatic hydrocarbons

Many polycyclic hydrocarbons have been tested in vitro for estrogenic or dioxin-like activity. Generally small molecules with an unhindered phenolic moiety similar to the A-ring of estradiol and moderate hydrophobicity are likely to bind to the estrogen receptor and can act as weak estrogens [65], other hydrocarbons such as benzo(a)pyrene, benzo(a)anthrazene, and related compounds show dioxin-like activity with potencies generally at least a factor of a 1000 less than 2,3,7,8-TCDD [66].

Environmental concentrations

Due to their persistence and hydrophobicity (log P_{ow} 4.5–10 [61]), PCBs are widely distributed in sediments, soils, and animal tissue, but the measures replacing these chemicals are showing effects: for example, dated sediments from the Gulf of Finland show highest concentrations of PCBs (up to 57 μg/kg), polychlorinated dioxins and furans (PCDD/F up to 101 μg/kg) in sediments deposited in the 1960s and 1970s. This contamination was caused by a chemical plant producing a chlorophenol-based wood preservative until 1984 [67]. Surface sediments in the same area have lower concentrations (0.4–52 μg/kg PCDD/F and 1.85–39 μg/kg PCB). PCB concentrations in surface sediments from river and coastal regions of the United States were also in the low μg/kg range. [68]. However, recent studies have shown elevated PCB concentrations in marine top predators in the Mediterranean, illustrating the continuing hazard that these chemicals can pose due to bioaccumulation [69]. Low, to very low water concentrations, in the sub μg/l range are usually reported for compounds in the PAH family such as benzo(a)pyrene and naphthalene [28,70], but as observed previously, much higher concentrations, up to mg/kg levels can be found in river bed sediments [28].

Summary

While water concentrations of these compounds are rarely a matter of concern due to their poor solubility, the potential for accumulation along the food chain may be of importance especially for relatively long-lived animals. Whereas many of these compounds have toxic properties, they may also represent an ED hazard for top predators in water bounded by industries with, for example, historic PCB use.

CONCLUSIONS

In terms of the presence in freshwater receiving STW effluents, only NP occasionally reaches concentrations that might give rise to serious ED concern. The problem of TBT in certain marine and freshwater locations affecting dogwhelk populations seems set to continue due to the lag in implementing the new restrictions and its persistence in sediments. Concentrations of BPA in water and sediment may be an issue associated with a few industrial discharges. Industrial-derived endocrine disruptors can be found in high concentrations in sewage sludge. For example, a German survey found that alkylphenols and BPA contributed maximal 10 % to the estrogenicity of sewage effluents or surface water compared to 65 % for sewage sludge extracts [71], but there is no evidence yet that this will have an ED impact. The polybrominated flame retardants may become an increasingly undesirable constituent of some sediments and animal tissue, but its significance is still difficult to evaluate. The ED impact of such chemicals as dioxins, furans, and PCBs relative to their already known toxicity should not be ignored. There is still insufficient information on whether xenobiotic EASs have the potential to harm other forms of aquatic fauna, particularly invertebrates at concentrations below the no effect level in fish. Overall, the possibility of additivity effects of mixtures, which is discussed in Topic 3.11, may mean the concern of ED from these xenobiotic compounds may ratchet up in future years.

Future research needs

An exhaustive list could be drawn up of various aquatic fauna and EASs that should be tested. It is perhaps wiser to assess current ecosystem health in a range of representative river reaches to examine whether any problems actually exist in the first place. A toxicity identification and evaluation (TIE) technique with an in vitro estrogenicity test highlighted the importance of steroid estrogens compared to xenobiotic estrogen mimics in sewage effluent [72]. Could such a TIE approach be repeated using an in vivo test, with a suitable, easy-to-handle fish, such as Japanese medaka, or zebrafish (*Danio rerio*)? Understanding the role of mixtures of low concentrations of xenobiotic EASs on fish would help regulators in assessing how seriously these compounds should be taken.

Table 1 Examples of reported concentrations (after 1985).

Compound	In vitro potency comp. to E2	In vivo potency LOEL µg/l	Domestic (or not known) effluent conc. µg/l[a]	Industrial impacted effluent conc. µg/l[a]	Sewage sludge conc. µg/g dry wt	Surface water conc. µg/l[a]	Sediment conc. µg/g dry wt	Concern?
TBT	Not applicable	0.001 (imposex in marine snail) [61]	<0.03–35 [5,10]	<0.03–62 [5]	0.02–1.5 [5,73]	<0.005–3 [5]	0.001–53 [5,11,73,74]	Yes
Nonylphenol	10^{-3}–10^{-6} [19,46,47]	0.6, 6 (vtg fish) [4,75]	<0.02–69 [31,47]	142–330 [26,27]	8–4000 [31]	<0.01–644 [26,27,31,47]	<0.0015–72 [31,47]	Yes, in certain reaches with industrial im pact
Octylphenol	10^{-3}–10^{-6} [19,22,46,61]	5 (vtg, fish) [47]	0.005–1.7 [31,47]	0.26–9 [31]	<0.01–20 [31]	<0.005–13 [31,47]	<0.002–1.8 [31,47]	No, except for rare industrial impact
Bisphenol A	10^{-4}–10^{-5} [3,20,22]	1, 10 (snail; intersex fish) [20,76]	<0.0001–4.5 [22,47,52,77–79]	<0.01–8 [47,52,80]	0.004–1.36 [45]	<0.0001–1.4 [47,52,81]	<0.001–0.19 [47,52]	Possible in some industrial impact sites
Phthalates DEHP[b], DBP[c], BBP[d]	10^{-5}–10^{-8} [46,82]	>100 [20,75]	<0.1–30.5 [52]	<0.1–80.5 [52]	0.19–154 [52]	<0.1–98 [52]	<0.01–8.4 [52]	No, except for rare industrial impact sites
Brominated flame retardants BDE-47[e], BDE-99[f], BDE-100[g]	10^{-5} –10^{-7} [58]				0.015–0.12 [55]		<0.0002–0.9 [59,83,84]	Not until in vivo effect shown
PCBs	10^{-4}–<10^{-6} [61], But some are antiestrogenic				1.3 (sum of 52 peaks) [34]	<0.0001–0.03 (PCB153[h]) [61]	0.000002–9 (sum) [61,67]	Probably not
Dioxins and furans	Antiestrogenic				0.001–0.024 (sum) [85]	0.00009–0.0003 (sum) [61]	0.0002–0.10 (sum) [61,67]	Less than for toxicity

[a]May include chemical sorbed to suspended particles.
[b]di(2-Ethylhexyl)phthalate.
[c]di-*n*-Butylphthalate.
[d]Benzylbutylphthalate.
[e]2,2′,4,4′-Tetrabromo-diphenyl-ether (2,2′,4,4′-TeBDE).
[f]2,2′,4,4′,5-Pentabromo-diphenyl-ether (2,2′,4,4′,5-PeBDE).
[g]2,2′,4,4′,6-Pentabromo-diphenyl-ether (2,2′,4,4′,6-PeBDE).
[h]2,2′,4,4′,5,5′-Hexachlorobiphenyl (estrogenic in rat uterus test [61]).

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