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Endocrine disrupting chemicals in the marine environment

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Preface

The Department of Environmental Science and Analytical Chemistry (ACES) was commissioned by Stockholm University Baltic Sea Centre to write an overview on endocrine disrupting chemicals in the marine environment.

The report was prepared by Ellen Ingre-Khans, Marlene Ågerstrand and Christina Rudén.

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Introduction

There is growing concern that declines in wildlife populations and loss of species as well as increasing incidences of hormone-related diseases in humans are linked to chemicals interfering with the endocrine (hormone) system known as endocrine disrupting chemicals (EDCs) [1]. Disruption of the endocrine system can result in various developmental, reproductive, neurological, immune and metabolic diseases [1-3]. Over the past years, an increasing number of ubiquitous chemicals have been identified to have endocrine disrupting properties to which humans and wildlife are consequently exposed [1-3]. These include well known persistent organic pollutants that are restricted in the Western World, such as PCBs and DDT, as well as chemicals in current use, including phthalates used in plastics and personal care products, brominated flame retardants and perflouorinated compounds. Some EDCs are known to cause effects at the present levels found in biota and the environment [1, 2]. This is disconcerting since the survival of species depends on normal development and successful reproduction for which a healthy endocrine system is a prerequisite.

This report gives a brief overview of EDCs, how they differentiate from general toxicants due to their special characteristics, observed effects by EDCs on wildlife with a particular focus on marine species, as well as important data gaps in our knowledge regarding EDCs.

Endocrine disrupting chemicals – background

EDCs can generally be defined as an exogenous substance that interferes with the endocrine system causing an adverse effect in the organism, its offspring, or on the population level [1]. The endocrine system consists of several tissues interacting with each other through hormones. Hormones control a number of processes in the body from differentiating cells and forming organs during development to the normal functioning of organs and tissues in adults. The body comprise many hormones and hormone systems which regulate functions, such as reproduction, metabolism, growth and behaviour, which are all critical for organisms to function well. Hormones that function as signalling molecules are produced by endocrine glands and they are transported in blood to exert effects on target cells and tissues through specific hormone receptors [1].

Hormones can bind to receptors either on the surface of the cell (membrane receptor) or within the cell (nuclear receptor) [1]. Hormones are receptor-specific and for the majority of the hormones, receptors are present only in certain cell types and/or at specific time points, for example during early life stages. The same type of receptor can be present in different cell types but initiate different effects, and the same receptor in the same cell type can also cause different effects at different time points [1].

Due to the complexity of endocrine systems, EDCs can interfere with the hormone function in a number of ways. Substances can disrupt hormone action either by acting on the receptor or by modifying the production, transport, metabolism or secretion of hormones [1]. Endocrine disrupting chemicals that act on the receptors can either mimic the natural hormone by activating the receptor and produce a response (agonistic effect) or bind to the receptor and block the action of the natural hormone (antagonistic effect) [1, 4]. Mimicking EDCs do not necessarily trigger the same response as endogenous hormones [4]. The expected observed effects by EDCs interacting with the hormone system depend on how the chemicals interfere with the hormone action [1].

The research on endocrine disruptors has particularly focused on the interaction of EDCs with the oestrogen hormone system as well as the androgen and thyroid hormone systems [1, 3]. Oestrogens are female sex hormones which play a key role in female reproduction whereas androgens, such as testosterone, are the equivalent male sex hormone, which plays a key role in the development and function of the male reproduction system. Thyroid hormones are important in controlling

neurological development, metabolism and skeletal growth in mammals and also play an important albeit different role in fish and amphibian metamorphosis [5]. An increasing number of studies show that EDCs also can interfere with other endocrine systems that for example control the immune system and fat development [1, 2], but more research is needed to understand the full extent to which EDCs interfere with hormone action. EDCs are often described by a specific outcome or mode-of-action, such as oestrogens and anti-androgens, however, most EDCs interfere with several physiological systems simultaneously.

Currently, roughly 800 chemicals are known or suspected to have endocrine disrupting properties, which include a wide variety of man-made and natural substances [1]. Some chemicals are persistent and bioaccumulative and consequently can accumulate to toxic levels in top predators and stay in the environment long after the chemical has ceased to be actively used. This include many legacy pollutants, such as polychlorinated biphenyls (PCBs) and the insecticide DDT, but also recently restricted compounds, including the flame retardant polybrominated diphenyl ethers (PBDE) and the perfluorinated surfactants perfluorooctane sulfonate (PFOS) and perflurooctanoate (PFOA) [1]. Other substances are rapidly degraded but are still of concern due to their widespread use and continuous release to the environment, known as pseudo persistent, or due to exposure at critical time points during development [1]. Some metals and organometallic compounds, for example cadmium, lead, mercury and tributyl tin (TBT), have also been identified as EDCs [1, 3].

Chemicals known or suspected to have endocrine disrupting chemicals, such as flame retardants, plasticisers, fragrances, preservatives and metals, are used in a wide range of industrial and consumer products, including personal care products, furniture, electronics, building materials and clothing [1, 3]. Pharmaceuticals like contraceptives and anti-depressants also contain EDCs [1, 3].

How does risk assessment of EDCs differ from general toxicants?
The improved understanding that EDCs can interfere with the hormone system challenges fundamental principles in toxicology on which today's chemical risk assessments and subsequent policy regulation of chemicals are based [1, 3, 4, 6]. Certain features of EDCs differ from that of general toxicants and include the ability to act at low doses, non-monotonic dose responses, varying/differing effects over an organism's life cycle, delayed effects that are not evident until later in life or in generations to come, and potential combination effects when exposed to multiple chemicals [1, 3, 4, 6].

Low dose effects and non-monotonic responses
Natural hormones are known to act at very low concentrations within and below the picomolar range [4]. EDCs that mimic oestrogenic hormones typically produce effects in the range of nano- to micromolar, although some can be active at concentrations lower than that. Low dose effects are typically defined as effects that occur at concentrations to which humans are exposed or at doses that are lower than those commonly used in standard toxicological tests [1, 4, 7]. Traditional chemical risk assessment with the exception for genotoxic substances, seek to set a safe threshold below which no adverse effects are expected to occur [1]. Setting safe thresholds may consequently not be possible for EDCs for which effects are often seen even at the lowest dose tested.

Just like hormones exhibit non-linear dose-response relationships, similar non-monotonic responses have also been shown for EDCs [1, 3, 4]. Some EDCs exert effects at lower doses but not at higher which contrasts with the traditional view in toxicology of linear dose-response curves, i.e. an increasing dose resulting in greater effects. Non-linear dose-response curves can have a U-shape, i.e. maximum response at low and high doses, or an inverted U-shape in which intermediate doses produce the greatest response [1, 3, 4]. However, the existence and significance of non-monotonic dose responses is a subject of much debate among risk assessors since it can be difficult to tell a true threshold apart from a threshold that appears due to limitations of the test design [6].

Critical windows of exposure

EDCs can, just like hormones, cause different effects depending on when exposure occurs [1, 3]. Exposure to EDCs during critical time periods when hormones act to differentiate cells and develop tissues can alter the development of tissues and lead to permanent effects [1, 3]. The effects may not be expressed until later in life and may also lead to increased susceptibility to diseases. In adults, exposure to the same EDC may have a different but nevertheless adverse effect which recedes when no longer exposed [1]. Thus, the time point of exposure to some EDCs may be of greater importance than the dose. This is not accounted for in general acute toxicity tests.

Delayed and transgenerational effects

The effects of EDCs interfering with the hormone system during critical time points of development may not be evident until much later in life expressed as infertility, cancer or other types of diseases [1, 8]. Since the effect can be delayed for a considerable time after exposure has taken place, it can be difficult to establish a causal relationship, particularly for non-persistent chemicals that are rapidly degraded. It has also been shown that effects may not only be manifested on an individual level but can also be transferred to next generations by heritable changes in the gene expression [1, 8]. A heritable alteration in gene expression due to environmental factors without changing the underlying DNA sequence is known as epigenetic effects. Consequently, studies that do not consider exposure during critical time points during development and screen for effects over the lifetime as well as heritable effects on the offspring may miss important adverse effects caused by EDCs. There are currently no regulatory test requirements that systematically screen for endocrine disrupting effects [6, 9].

Combination/Mixture effects

It has been shown that EDCs present at levels too low to induce observable effects can produce significant effects when combined if they act on a common endpoint [1, 3, 10]. However, current chemical risk assessment practices generally only consider the risk of being exposed to one chemical at the time [1, 9]. Since humans and wildlife are exposed to a mixture of chemicals, important combination additive effects may consequently be overlooked when assessing the risk of EDCs. In order to fully assess the risk of EDCs, knowledge about the full exposure to EDCs that act through the same pathway and their potency is needed [1].

Complex/Multiple endpoints

Many of the possible adverse effects of EDCs are not covered by traditional toxicological tests which focus on histopathology and organ and body weights [1]. Moreover, it will be hard to capture effects by a subset of endpoints and extrapolate to other endpoints due to the many ways that EDCs can interfere with hormone action [1]. This means that current legislation is insufficient in identifying EDCs.

EDCs in the marine environment

EDCs can reach the aquatic environment through a number of point sources along the coast or in the catchment area on land as well as human activities at sea, land-based diffuse sources and atmospheric deposition [1-3]. Effluents from waste water treatment plants are known to contain EDCs from a variety of sources such as personal care products, pharmaceuticals, and consumer goods treated with chemicals. Other sources of EDCs include discharges from industrial processes, runoff from agriculture containing pesticides, fertilisers and pharmaceuticals, urban runoff and storm water flows. Atmospheric deposition, which originates from waste incineration, industrial and transportation emissions as well as natural combustion, can be a major route for some heavy metals and dioxin [1, 11]. Anti-fouling paints used on hulls, intentional as well as unintentional spills of oil or other substances and contaminated sediments are other types of sources of EDCs [1].

Considering the numerous sources of EDCs to the marine environment, aquatic organisms and top predators in the marine food web are exposed to a mixture/combination of chemicals [1-3]. Depending on the chemical's physical-chemical properties, the chemical will be taken up from the diet, by inhalation and/or by absorption through the skin or gills. Persistent and fat-soluble chemicals are taken up from the diet where they can bioaccumulate and transfer through the marine food chain resulting in higher levels in top predators. Water-soluble chemicals are taken up through the skin or gills in fish or through drinking-water for marine top predators. Sediment living organisms are exposed to EDCs that bind to the sediment and diffuse to the pore water. Aquatic organisms that lay their eggs in water are particularly vulnerable to EDCs since the eggs are directly exposed during the developmental stage [2]. Organisms that live in or close to urban areas or various point sources are often exposed to the highest concentrations before dilution occurs and are also continuously exposed to pseudo persistent chemicals [1].

Environmentally relevant concentrations of known or suspected EDCs have been measured in the marine environment [1, 2]. Particularly old persistent organic compounds, such as PCBs and DDT, but also new emerging persistent pollutants, such as brominated flame retardants and perfluorinated compounds, are found in top marine predators exceeding concentrations known to cause adverse effects [1]. Although, persistent and bioaccumulative substances that are banned show a corresponding decline in the environment, they are still of concern since they stay in the environment for a long time and accumulate in the food chain [1].

For example, high concentrations of the sum of PCBs and DDTs have been found in skipjack tuna and dolphin blubber in various locations worldwide [1]. In dolphin blubber, measured levels have sometimes exceeded 1000 µg/g fat [1]. Although concentrations of PCBs and DDE (the main metabolite of DDT) show decreasing trends in the Baltic Sea since the end of 1970's as a result of their restricted use, threshold levels in fish and mussels are occasionally exceeded indicating a cause for concern [11].

Brominated flame retardants, such as PBDEs and hexabromocyclododecane (HBCDD) have been increasingly measured in the environment in the last decade [1]. In the Baltic Sea, BDE concentrations four times higher than levels causing adverse effects in American Kestrels have been measured in white-tailed sea eagles and high levels have also been reported in roach in the Archipelago Sea (Åland) [11]. Measured concentrations of HBCDD in herring exceeded the threshold level in all stations measured along the Swedish coast. Although no significant trend of HBCDD in herring can be seen over time, an increasing trend can be seen in eggs from common guillemot from Stora Karlsö, Gotland.

Of the perfluorinated compounds, PFOS is the most commonly found in animals and locations worldwide [1]. Higher concentrations of PFOS have typically been found in regions that are generally more populated or industrialised than in remote marine areas, although high levels of PFOS have also been reported in polar bears in Alaska [1]. In the Baltic Sea, the highest levels of PFOS (several hundreds to one thousand µg per kg wet weight) have been measured in marine predatory birds and mammals [11]. The perfluoroalkyl substances (PFASs) considered to be of most concern in the Baltic Sea include PFOS, PFOA and PFNA. Although PFOA and PFOS and related precursors are being restricted and phased out in many parts of the world, more than 3000 PFASs are still on the global market that are structurally similar and produced in significant quantities [12]. For most of them, little is known about their fate, exposure or adverse effects but based on available evidence they may be of similar concern as PFOA and PFOS. Since only a few selected PFASs are actively monitored in the environment, the exposure to PFASs may consequently be greatly underestimated [12].

Pharmaceuticals have also been recognised as a threat to the marine environment due to their inherent design to have biological effects on living organisms [11]. More than a hundred

pharmaceuticals have been measured in effluents and surface waters in concentrations of nanograms to micrograms per litre [1]. Concentrations of pharmaceuticals in rivers in Baltic Sea countries have been measured up to µg per litre and there are indications that pharmaceutical concentrations less than 10 ng per litre can change the behaviour of aquatic organisms [11]. Active ingredients of antidepressants, human contraceptives and antiepileptic medicines have also been found in tissues of aquatic organisms close to discharges from sewage treatment works [1].

In addition to the EDCs mentioned above, many more are present in the marine environment at levels that may cause harm [1, 11]. Moreover, most studies on EDCs in wildlife have focused on persistent organic pollutants, which means that for most known or potential EDCs that are currently used today, there are no or little data on exposure [1]. In the light of the improved understanding of EDCs, it follows that exposure to even very low concentrations of EDCs during critical times in development can have potentially significant effects on the health and survival of individuals [1, 4]. Thus, it will generally be challenging to determine safe levels of EDCs as well as assess the relevance of effects seen on the individual level to population level.

Effects of EDCs on marine wildlife

It is generally difficult to establish a causal relationship between EDC exposure and long-term effects on wildlife populations. However, observed effects on population levels, such as reproductive failure and outbreaks of diseases, can in several cases be linked to EDC exposure in the marine environment [1, 2]. There are some historic cases where exposure to EDCs are strongly correlated with effects seen in wildlife including reproductive impairment in seals, eggshell thinning in predatory birds, feminisation of fish and masculinisation in marine snails as described below [1, 2].

Mammals

Several field- and semi-experimental studies show that lipophilic EDCs accumulate to high levels in fatty tissue in marine mammals at levels that may cause adverse effects. The decline of seal populations in the Baltic Sea is strongly correlated to high body burdens of organochlorines, especially PCB, DDT and their metabolites, causing reproductive effects [2, 13]. Decreasing concentrations in the environment, as a result of PCB and DDT restrictions in the 1970's, have led to a recovery of the seal populations [1, 13]. PCB was also found to cause reproductive effects in a two-year Dutch semi field study on harbour seals in Wadden Sea that were fed fish with high respectively low contaminated fish [2]. Several studies also indicate endocrine disrupting related effects in polar bears, dolphins and whales, which seem to be correlated to body burdens of persistent organic pollutants [2].

Birds

There is strong evidence that the organochlorine pesticide DDT that came into use in the 1940's caused severe population declines of several marine and terrestrial predatory birds in Europe and North America. [1, 2]. The population declines were due to eggshell thinning which caused the eggs to break during incubation [2]. The thinning of the eggshell is linked to the DDT metabolite DDE that blocks the cellular signal which causes calcium to deposit in the shell. Many of the affected bird populations recovered as a result of banning DDT [1].

Toxicological studies on birds exposed to new emerging EDCs, such as brominated flame retardants, show adverse endocrine disrupting effects, which may have consequences for populations of wild birds [2]. For example, exposure of PBDE at environmentally relevant concentrations to captive American kestrels affected among other things courtship behaviour, hatching success and eggshell thickness, which are important for successful reproduction [2].

Fish

Frequently reported endocrine disrupting effects in vertebrates are gonadal defects and reproductive effects including feminisation of males, masculinisation of females and reduced fertility in fish [1, 2]. Feminisation in fish has been extensively investigated and is caused by natural as well as synthetic oestrogenic compounds in effluent discharges [2, 14, 15]. Feminisation was first discovered in the 1980's in roach from UK river systems that were exposed to sewage effluents, but several studies have shown adverse reproductive effects in wild fish populations that can result in reduced fertility [2]. The biomarker vitellogenin is typically used to confirm exposure to oestrogenic compounds in males since it is associated with egg laying in females [2, 14]. Although many of the field observations and studies have been made in freshwater species, similar oestrogenic effects are being increasingly observed in marine species [2].

Invertebrates

The clearest example of exposure to EDC and subsequent population changes is the masculinisation of marine snails [2, 13]. The biocide tributyltin (TBT), a constituent in marine antifouling paint, was in the 1980's linked to masculinisation in marine snails showing high incidences close to marinas. Several studies have demonstrated that masculinisation can be induced at very low concentrations of TBT (ng per litre) [2, 13]. TBT can cause two different types of masculinisation: imposex and intersex [2]. Imposex refers to the growth of an entire or partial male organ in females still having an intact female sexual organ whereas intersex is the transformation of the female sexual organ into a male organ. Both types of masculinisation result in sterile individuals which causes reproductive failure and subsequent population declines. New studies suggest that imposex is caused by TBT acting as an antagonist on the retinoid X receptor [2]. In general, endocrine disrupting effects on invertebrates have not been studied to the same degree as vertebrates which is probably related to a poor understanding of invertebrate endocrine systems [16].

Uncertainties and data gaps

The increasing incidences of endocrine related diseases observed in wildlife today and the increasing number of ubiquitous chemicals that are identified to potentially have endocrine disrupting properties is a matter of concern [1]. This concern is based on our general knowledge on how hormone systems work, in combination with available knowledge about how chemicals can interfere with these systems. This has been convincingly shown in studies using experimental animals as well as in wildlife [1-3]. Taken together, this gives a reason for concern.

However, several knowledge gaps persist to fully understand the full scale effects of EDCs in general and for the marine environment in particular since much of the research has focused on freshwater fish, reptiles and mammals and less on marine and estuarine animals [17]. Even less is known about endocrine disrupting effects on marine invertebrates although they make up 95 % of all known animal species and constitute large groups of the marine ecosystem that are ecologically relevant [16].

Many of the chemicals that are in commerce today have not been tested for potential endocrine disrupting properties and many routes and sources of EDCs are unknown which makes it difficult to assess the full extent of exposure to EDCs [1, 6]. For example, marine species living close to sewage effluents are likely exposed to a great number of not yet identified EDCs. In addition, we have insufficient knowledge of all the possible ways EDCs can interfere with the hormone system (in particular the invertebrate hormonal system) and how exposure during critical time periods of development may contribute to diseases at later life stages as well as be passed on to the offspring [1-3]. Many of the validated toxicological tests that are used today are not designed for detecting endocrine disrupting effects, although such tests are under development [6, 9]. In general, the implication of effects seen in experiments can be difficult to extrapolate to effects on population levels [1, 2].

Even if a number of related endocrine disrupting effects have been reported in wildlife, establishing a causal relationship between effects and EDC exposure, and to a single chemical in particular, can be difficult [1, 2, 6]. For example, observed changes in population can be caused by a combination of other environmental factors including selection pressure and habitat destruction. The observed effects can also be a result of the interaction of several chemicals causing additive, synergistic or antagonistic effects. In addition, we generally have little information on wildlife population status which means that effects on population changes may be overlooked. Monitoring of chemicals in the environment also focuses to a large degree on persistent organic pollutants of which many are already regulated [18]. Hence, we know little on the exposure of new emerging EDCs. We also lack knowledge on which EDC mechanisms pose the greatest threat as well as which species are the most sensitive to EDC exposure [2].

Given the current knowledge on EDCs, it is likely that more resources spent on screening of chemicals for endocrine disrupting properties, monitoring of EDCs in the environment and the development of toxicological tests designed to detect EDCs will confirm the suspicion that risks with EDCs are currently underestimated [1].

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