OECD Studies on Water

Endocrine Disrupting Chemicals in Freshwater

MONITORING AND REGULATING WATER QUALITY

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BETTER POLICIES FOR BETTER LIVES

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Foreword

Water quality concerns are making the headlines - from pesticides in tap water to per and polyfluoroalkyl substances (PFAS, or 'forever chemicals') in lakes and estuaries. While chemicals have contributed to important socio-economic progress for human life, animal health and economic welfare in general, a variety of substances are steadily discharged into the natural environment. Endocrine disrupting chemicals are an example of contaminants of emerging concern that affect the healthy functioning of the endocrine system in humans and wildlife. Traces of these chemicals can be found almost everywhere in our freshwater environment, including rivers, lakes, groundwater, estuaries, sediments, wastewater and drinking water.

The impacts of endocrine disrupting chemicals are deeply concerning for the environment and human wellbeing. Scientists observed the complete collapse of a fish population in a Canadian experimental lake, after the introduction of very low concentration levels of estrogen commonly used in the birth control pill. The economic costs of endocrine disruptors are equally worrying. The disease burden incurred by endocrine disrupting chemicals is estimated to amount to USD 340 billion in the United States alone.

There are many uncertainties around the precise impact of chemicals on human and environmental health, and therefore the effective policy responses. This report provides an overview of the state of knowledge on the environmental, human health and economic impacts of endocrine disrupting chemicals in freshwater. It also considers the crucial role of science in this context, as OECD countries increasingly use novel water monitoring methods to detect these substances. This publication documents case studies of such new monitoring methods, and explores how they can benefit water quality regulation. The analysis focuses on the negative effects of endocrine disrupting chemicals on human health and wildlife, rather than mere detection of the individual substances in water. In line with previous OECD publications on contaminants of emerging concern in water, our policy recommendations seek to prevent and remedy the problem throughout the chemical lifecycle, from source to the end of pipe.

Building on the OECD Council Recommendation on Water, which explicitly refers to endocrine disruption and recommends that resources are allocated to "improve standards for water quality target setting, building on the latest scientific knowledge and the most effective technologies", this report acts as a concrete contribution. The OECD analyses and policy recommendations cut across multiple policy domains, including water and environment, chemicals and health. I trust this unique capacity creates value for our member countries and beyond. It is my hope that the findings will serve as a key reference for policy makers in the roll-out of next-generation water quality monitoring programmes and policy responses to curb the risk of endocrine disrupting chemicals in water.

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Director
Environment Directorate
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Abbreviations and acronyms

Abbreviations and acronyms	Definition
AhR	Aryl hydrocarbon receptor
AOP	Adverse outcome pathway
AR	Androgen receptor
BEQ	Biological equivalent
BOD	Biological oxygen demand
BPA	Bisphenol A
CECs	Contaminants of emerging concern
DBP	Dibutyl phthalate
DDT	Dichloro-diphenyl-trichloroethane
DEET	N,N-Diethyl-meta-toluamide or diethyltoluamide
E1	estrone
E2	17B-estradiol
E3	estriol
EAS	Endocrine active substance
EATS	Estrogen, Androgen, Thyroid, Steroidogenic
EBM	Effect-based methods or effect-based monitoring
EBT	Effect-based trigger (value)
ED	Endocrine disruptor
EDA	Effect-directed analysis
EDC	Endocrine disrupting chemical
eDNA	Environmental deoxyribonucleic acid
EDSP	Endocrine Disruptor Screening Program (United States EPA)
EE2	17a-ethinylestradiol
EEM	Environmental Effect Monitoring (Programme)
EPA	Environmental Protection Agency
EQN	Environmental quality norm
EQS	Environmental Quality Standard
ER	Estrogen Receptor
eRNA	Environmental ribonucleic acid
EU	European Union
EXTEND	Extended Tasks on Endocrine Disruption
GC-MS	Gas chromatography coupled with mass spectrometry
GDP	Gross domestic product
GR	Glucocorticoid Receptor
GWRC	Global Water Research Coalition
HRMS	High-resolution mass spectrometry
ICEDA	Intersectoral Centre for Endocrine Disruptors Analysis
INERIS	French National Institute for Industrial Environment and Risks
ISO	International Organization for Standardization
LAGDA	Larval Amphibian Growth and Development Assay
LOD	Limit of detection

LOQ	Limit of quantification
MAD	Mutual Acceptance of Data
MEOGRT	Medaka Extended One Generation Reproduction Test
MIE	Molecular initiating event
MoA	Mechanism of Action
MoE	Ministry of Environment
NAM	New approach method
NTA	Non-targeted analysis
PARC	European Partnership for the Assessment of Risk from Chemicals
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyl
PFAS	Per- and polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PNEC	Predicted no-effect concentration
POP	Persistent organic pollutant
PPP	Purchasing Power Parity
PR	Progesterone receptor
QSAR	Quantitative structure–activity relationship models
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (regulation under the European Union)
SIMONI	Smart integrated monitoring
SOP	Standard operating procedure
SSA	Suspect screening analyses
SWACHE	Surveys of willingness-to-pay to avoid chemicals-related health effects
TBT	Tributyltin
TCDD	2,3,7,8-Tetrachlorodibenzo-P-dioxin
TG	Test Guideline
TR	Thyroid hormone Receptor
UN	United Nations
VTG	Vitellogenin
WWTP	Wastewater treatment plant
YES	Yeast Estrogen Screen

Executive Summary

Endocrine disrupting chemicals in water may affect human health and wildlife

Exposure to endocrine disrupting chemicals (EDCs) can lead to negative health outcomes in humans and wildlife as EDCs interfere with the endocrine system of hormones and glands. EDCs can be found in household products, consumer products, agriculture, aquaculture, industrial production, and transportation. Humans are exposed through food, consumer products, air, or water, amongst others. Similarly, EDCs are released into the environment through excretion, landfills, runoff from agriculture and urban areas, industry and leaching of wastes. They are not fully captured by wastewater treatment plants, leading to detected contamination of rivers, lakes, groundwater, estuaries, sediments, wastewater, and drinking water.

Human exposure to EDCs could lead to birth defects, can affect neurodevelopment conditions and reproductive health, and is also linked to obesity and metabolic diseases. In wildlife, EDCs affect physiology, behaviour or health, notably through alterations of the hormonal system, reproductive dysfunctions, and the feminisation of male fish. Aquatic organisms are continuously exposed to EDCs when living in a contaminated habitat. Contamination can cascade onto other organisms, populations and communities, through food chain for instance.

Parts of the costs of exposure to EDCs can be monetised. Estimated health care costs amount to CAD 24.6 billion in Canada; EUR 163 billion in the European Union; and USD 340 billion in the United States. The health-related costs from the exposure to per- and polyfluoroalkyl substances (PFAS), a group of persistent chemicals associated with environmental and human health concerns including endocrine disruption, have been estimated to range from EUR 2.8-4.6 billion, and the estimated environment-related costs ranged from EUR 46 million – EUR 11 billion over 20 years.

Managing and monitoring EDCs in water is a challenge and effect-based tools, such as bioassays, have distinctive advantages

EDCs are distinctively challenging to monitor and manage, which affects the effectiveness of regulation in OECD and non-OECD countries. Endocrine disruption is characterised by uncertainty. EDCs originate from a diverse group of uses, products and processes. They can circumvent traditional ways of monitoring as they trigger adverse effects at concentrations lower than detection limits of traditional chemical analysis. Moreover, only a fraction of all EDCs is regularly monitored.

Prevailing methods of substance-by-substance analysis of chemical concentrations in water have reached their limits. Additional monitoring tools better capture the effects of EDCs and their mixtures and are increasingly applied across OECD countries:

- *Targeted chemical analysis* (substance-by-substance monitoring), testing on concentrations of a given substance in a water sample, is a common practice to keep.

- *Bioassays* (effect-based monitoring) are recommended as additional method. Bioassays are *in vitro* (cells or cell-free) or *in vivo* (whole organism) methods that detect and quantify the effects of chemicals on toxicological endpoints, e.g., Estrogen, Androgen, Thyroid and Steroidogenesis.
- *In situ* wildlife monitoring methods are valuable supplements. They survey species in the wild for physical, molecular or behavioural changes, potentially caused by exposure to EDCs.
- *Non-targeted analysis*, such as high-resolution mass spectrometry or eDNA, is encouraged to obtain a snapshot picture of water quality, species richness and to identify culprit chemicals.

Effective policies focus on adverse effects on humans and wildlife, address the full lifecycle of EDCs and are strengthened by multilateral action

Policies that tackle the effects of EDCs, without initial knowledge of the culprit chemical, can effectively reduce and respond to the risk of EDCs in water, at the least cost, while mitigating monitoring challenges and knowledge. Effect-centred policy approaches can be clustered as below:

- Response plans or protocols can quickly mobilise action following observed abnormalities in bioassays or wildlife. Such plans reduce the lag time between a suspected risk and action.
- National action plans on EDCs can build bridges across sectors, strengthen monitoring, assessment, and information, reduce uncertainties, and send a policy signal.
- Regulatory environmental quality standards based on effects or endpoints, instead of single chemical concentrations, capture the risk of EDCs. This involves setting effect-based trigger values or threshold values that determine the acceptable level of risk.
- Policy interventions that consider the impact of EDCs on vulnerable populations (including risk assessments specific to vulnerable groups, information campaigns, and assessment of biodiversity changes specific to endangered species and cultural keystone species) can reduce vulnerability.

Effective responses consider a broad range of entry points, as EDC emissions are influenced by several stages of the lifecycle of chemical product design and production. They combine:

- *Source-directed approaches*, such as chemicals assessments (e.g., groupwise assessments), substance bans, maintaining public EDC lists (confirmed and suspected), and product design.
- *Use-oriented approaches*, such as labelling consumer products, public environmental campaigns, substance restrictions, and best environmental practices for sectors.
- *End-of-pipe approaches*, such as improved wastewater treatment, setting standards for wastewater reuse and sewage sludge recovery, and discharge permits.

EDCs are transported across international basins or trade, and their impacts are global. What is more, water quality monitoring tools are standardised at international level. International actions could focus on:

- Upscaling the standardisation and verification of test methods that are appropriate for water quality testing, based on the mutual acceptance of data principle.
- Stimulating the demand for and development of new bioassays, based on non-animal methods, relevant to water quality testing.
- International research partnerships are essential for sharing knowledge and data, reducing uncertainties, supporting the transition to new technologies and supporting regulatory processes.
- Mainstreaming the issue of endocrine disruption in international science-policy agendas, such as the One Health agenda, to address pollution by endocrine disrupting chemicals at the global scale.

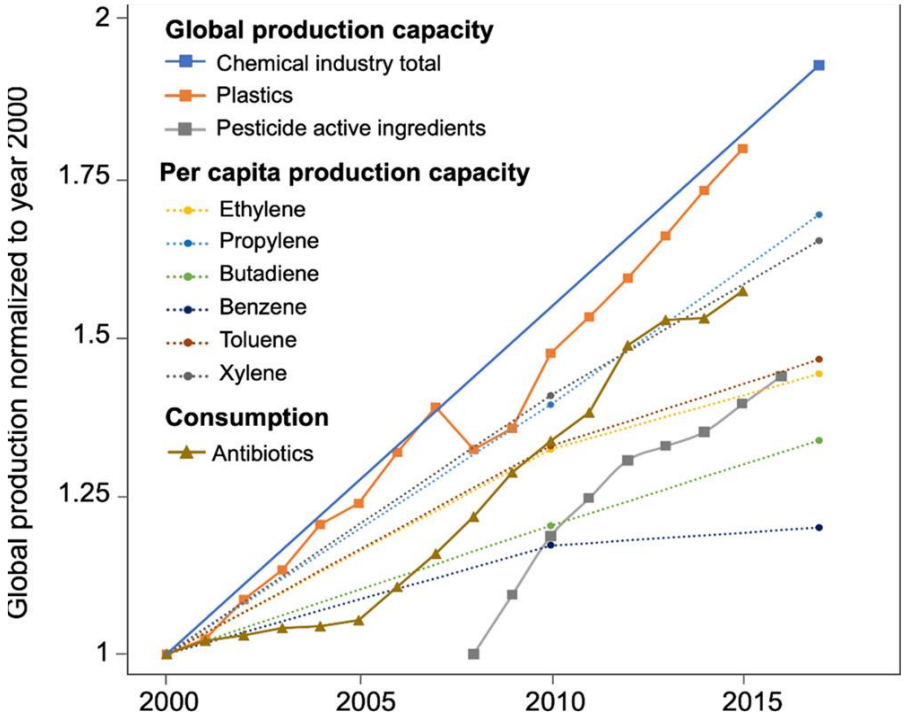
1 The challenge of endocrine disruptors in freshwater

This publication on endocrine disruption is part of a series on policy responses to contaminants of emerging concern (CECs) in freshwater. Previous work focused on pharmaceutical residues and microplastics. Building on these earlier publications, this publication focuses on endocrine disrupting chemicals (EDCs) in freshwater. This publication takes a different approach to water quality regulation: it explores the opportunity to complement a substance-by-substance approach of chemicals management with an effect-based approach, centred around the negative effects of EDCs on humans and wildlife. This chapter characterises the challenge of endocrine disruption in freshwater. It provides a typology of EDCs and their effects on human health, ecology and the economy. It also examines the sources, pathways and sinks of endocrine disrupting chemicals in freshwater. Lastly, this chapter provides an outlook of driversthat increase the future release of endocrine disrupting chemicals in freshwater.

1.1. Introduction

Worldwide, the production capacity for the chemical industry, plastics and pesticides has dramatically increased since 2000 (Figure 1.1: "Global production capacity"). Some of these chemicals, plastics and pesticides have properties that could have a negative effect on human and wildlife. One such property is their ability to alter function(s) of the endocrine system of organisms. These compounds are called endocrine disrupting chemicals (EDCs). A modification of function(s) of the endocrine system in the body can lead to adverse health effects, some of which may not manifest until many years after exposure. EDCs are associated with disease outcomes such as obesity, fertility loss, hormone-sensitive cancers, thyroid malfunctions and neurodevelopment impacts (Gore et al., 2015[1]). In wildlife, similar effects can occur. Moreover, in wildlife, endocrine disruptors can negatively affect populations - potentially contributing to biodiversity loss and undermining the provision of ecosystem services.

Figure 1.1. Trends of chemical industry production capacity between 2000 and 2017 (expressed as the relative growth)



Note: 1. Global production capacity; 2. Per capita production capacity, 3: Global consumption of antibiotics.
Source: (Persson et al., 2022[2])

Endocrine disruptors are ubiquitous in the environment – that is, in water, air and soil (Section 1.5). Moreover, the impacts of climate change, environmental degradation and global population growth are drivers for an even more ubiquitous presence and effect on human health and ecosystems (Section 1.8).

Endocrine disruptors are not extensively regulated in OECD countries to date. This Chapter characterises some of the challenges to manage EDCs in the freshwater environment, which can be summarised as:

1. Endocrine disruptors are not “ordinary” chemicals. EDCs can work at low doses (ng/l), in mixtures with other chemicals and the dose does not always compare to the level of toxicity (Section 1.2).

2. Regulators have limited control over the release of EDCs into the environment, as they are not completely removed by wastewater and drinking water treatment plants, and they are directly released into the environment through diffuse sources or by upstream activities in other countries and continents (Section 1.4).
3. Endocrine disruption is characterised by uncertainty. Causal relationships between exposure and adverse effects on humans and wildlife are not fully understood and many chemicals are not recognised or even suspected as endocrine disruptors (Sections 1.5 and 1.6).
4. The chemicals circumvent our traditional ways of monitoring as they can trigger adverse effects at very low doses (ng/l), below threshold values. Moreover, chemicals that interfere with the endocrine system may comprise close to 800 chemicals (WHO-UNEP, 2013[3]), most of which are not routinely monitored in water. In comparison, the European Union Water Framework Directive (WFD) currently regulates a total of 45 priority substances (Chapter 2).
5. EDCs stem from a very diverse group of uses, products and processes. The cross-sectoral, transboundary and multidisciplinary nature of this problem demands attention across multiple policy domains, such as those related to water resources management, chemical safety, public health, agriculture and food, environment and biodiversity, industry, trade, and waste management. Countries face a major challenge in attempting to holistically address the issue, and regulatory efforts to date have been fragmented (Section 1.5 and Chapter 2).

Knowledge of adverse effects of chemicals in the environment is evolving, with better-characterised health effects of better-studied pollutants (Landrigan et al., 2018[4]). With improved knowledge and advanced technologies, countries are better equipped to respond to pollution challenges. This is also the case of endocrine disruptors. This publication comes at a time where there is a technological and epistemic readiness to respond to challenges posed by endocrine disruption in freshwater.

1.2. Endocrine disruption and endocrine disruptors

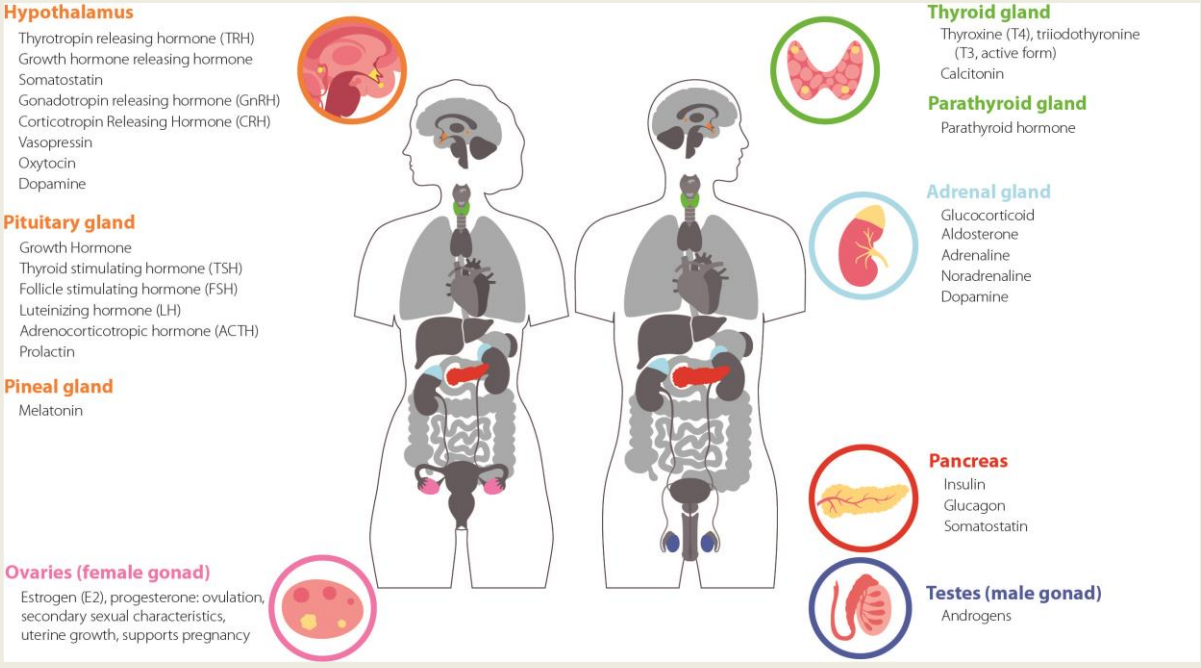
The World Health Organization's (WHO) International Programme on Chemical Safety (IPCS) defines an endocrine disruptor as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (IPCS, 2002[5]). Various actors globally have since applied the WHO's definition, such as the OECD (2018[6]) and the European Commission (EU Regulations 2017/2100 and 2018/605). The Environmental Protection Agency of the United States (US EPA) instead employs a more specific definition that notes the biological effects of EDCs, stating that they interfere with the "synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behaviour" (US EPA, 1997[7]).

While EDCs cause adverse effects, acting through an endocrine mode of action, endocrine active substances (EASs) can interfere with the endocrine system with or without an adverse effect. EASs have "the inherent ability to interact or interfere with one or more components of the endocrine system resulting in a biological effect, but need not necessarily cause adverse effects" (EFSA, 2013[8]).

Box 1.1. The endocrine system: an overview

The endocrine system allows to control various functions in the body through a complex system of messages orchestrated by the endocrine glands and their hormones. **Endocrine glands** are organs that synthesise and release hormones in the blood stream. The main endocrine glands are illustrated in Figure 1.2. They comprise the hypothalamus, the pituitary, the gonads (ovaries or testes), the thyroid, the parathyroid, the pancreas, the adrenal gland, and the pineal gland. Other organs can also secrete hormones, such as the gastrointestinal tract, the heart, the kidney, the thymus, and the adipose tissue. **Hormones** are chemical messengers that are released by an endocrine gland into the blood stream. Homes will then travel to their target organ and tissue. To deliver their message, hormones will bind to their receptor.

Figure 1.2. Scheme of the endocrine system with its main glands and organs and their respective hormones



Note: Most of the hormones presented are conserved among vertebrates and some are also conserved in invertebrates.
Source for image: Authors, with drawings adapted from Pikovit through Adobe Stock
Source for information: (Norris and Carr, 2020[9]; WHO-UNEP, 2013[3])

EATS modalities/pathways refer to estrogen (E), androgen (A), Thyroid (T) and Steroidogenesis (S). The EATS modalities are the most studied and well understood pathways for endocrine disruption and have the most developed methodologies (OECD, 2018[6]). In the revised OECD Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, the juvenile hormone (Jh) and the ecdysteroids (Ec) modalities were added to include invertebrate hormones. It should be recognized that EATSJhEc modalities, while important, are not the only ones that can be affected by endocrine disruption as many more hormones exist such as seen in Figure 1.2.

Source: (Norris and Carr, 2020[9]; OECD, 2018[6]; WHO-UNEP, 2013[3])

The endocrine system regulates and controls the release of hormones in humans and animals (Box 1.1, Figure 1.2). Well-known organs within the endocrine system are the ovaries and prostate, but also glands such as the pituitary, parathyroid, thyroid, adrenal and pancreas are part of the endocrine system (CCOHC, 2022[10])(Figure 1.2). Endocrine disruptors or endocrine active substances can work in roughly three ways: 1) they can either imitate the endocrine system ("agonist"), 2) work against the endocrine system as antagonist, 3) or interfere with the synthesis of the hormone, its transportation to the receptor, or its metabolism (CCOHC, 2022[10]; Kabir, Rahman and Rahman, 2015[11]; WHO-UNEP, 2013[3]). When the endocrine system is stimulated or inhibited by the action of EDCs on specific hormonal pathways, EDCs can act like a natural hormone and bind to a receptor or stimulate or inhibit the production or the transport of the natural hormone. It may then give the same or a more powerful signal than the "original" hormone, or give a signal at the "wrong" time, or disturb the signal at the appropriate time.

To help identify health hazards, La Merrill et al. (2020[12]) defined ten key characteristics of endocrine disrupting chemicals, summarised in Box 1.2.

Box 1.2. Ten key characteristics of endocrine disrupting chemicals

La Merrill et al. (2020[12]) made a scientific consensus statement on the ten key characteristics (KCs) of the impact of endocrine disrupting chemicals on the endocrine system. The key characteristics can help in hazard identification of EDCs to humans and animals, support in group assessments of chemicals, provide a basis for chemical risk assessments and support in prioritising knowledge and data gaps. This overview is not a checklist; EDCs may share one or a few KCs.

Ten key characteristics of endocrine disrupting chemicals

KC1.	Interacts with or activates hormone receptors
KC2.	Antagonizes hormone receptors
KC3.	Alters hormone receptor expression
KC4.	Alters signal transduction in hormone- responsive cells
KC5.	Induces epigenetic modifications in hormone- producing or hormone responsive cells
KC6.	Alters hormone synthesis
KC7.	Alters hormone transport across cell membranes
KC8.	Alters hormone distribution or circulating hormone levels
KC9.	Alters hormone metabolism or clearance
KC10.	Alters fate of hormone- producing or hormone-responsive cells

Source: (La Merrill et al., 2020[12])

Close to 800 chemicals are known or suspected to be capable of interfering with hormonal processes (WHO-UNEP, 2013[3]). EDCs can be clustered in different ways (WHO-UNEP, 2013[3]; Metcalfe et al., 2022[13]; Karthikeyan et al., 2019[14]; Kassotis et al., 2020[15]; Kabir, Rahman and Rahman, 2015[11]). Common clusters are pharmaceuticals for humans and livestock, pesticides, and additives to plastics (e.g. to make plastics fire proof, extra flexible, coloured, hardened or resistant against UV radiation). For the purpose of conducting a water policy analysis, Table 1.1 presents a typology based on product groups and their most common EDCs.

Table 1.1. Examples of EDCs per product group

Product group	Examples of EDCs (suspected or recognised)
Consumer products (e.g. children products, electronics, textiles)	Flame retardants, bisphenols, phthalates, perfluorooctanoic acid (PFOA)
Cosmetics, personal care products	DBP, benzophenones, parabens, triclosan, DEET, phthalates
Food contact materials (e.g. plastic food containers, food wrappers, baby bottles)	Bisphenols, perfluorooctanoic acid (PFOA)
Industrial chemicals	Bisphenol A, PCBs, triphenyl phosphate, PBDEs, TCDD
Metals	Lead, cadmium, mercury, arsenic
Pesticides (including herbicides, fungicides)	Chlorpyrifos, chlorotriazine, pyraclostrobin, DDT, PCBs, atrazine, vinclozolin
Pharmaceuticals (for humans and livestock)	Trenbolone acetate, ethinylestradiol (EE2, synthetic estrogen), dexamethasone, levonorgestrel, rosiglitazone, non-steroidal synthetic estrogen
Synthetic and naturally occurring hormones	Progesterone, testosterone, cortisol, oestrone

Notes: 1. Some of the chemicals listed above are under investigation or suspected of having endocrine active properties, such as in EDC assessment programmes or scientific journals, but they are not officially classified as EAC or EDC under national legislation; 2. Some substances are banned or restricted, but still appear in environment as legacy compounds; 3. This list is not exhaustive; 4. Glyphosate has been mentioned as endocrine active substance in sources used to produce this table (Kabir, Rahman and Rahman, 2015[11]; Kassotis et al., 2020[15]). However, further research by US-EPA and EFSA has shown that there is no indication that glyphosate is an endocrine disruptor (U.S. EPA, 2015[16]; EFSA, 2023[17]). EFSA notes that no firm conclusions can be drawn concerning the risks for biodiversity (EFSA, 2023[17]).
Source: (Gore et al., 2015[1]; Kabir, Rahman and Rahman, 2015[11]; Kassotis et al., 2020[15]; Metcalfe et al., 2022[13])

Endocrine disrupting chemicals are a subset of contaminants of emerging concern (CECs), micropollutants and persistent organic pollutants (POPs). EDC pollution may therefore be covered in action plans and strategies that do not necessarily carry the title of endocrine disruption (Table 1.2).

Table 1.2. A typology of pollutants and their relation to EDCs

Typology	Definition	Link to EDCs
Contaminants of emerging concern (CECs) *Also known as "emerging contaminants"*	A vast array of contaminants are of recent concern because they have only recently been introduced in water, or because they have only recently been detected at, or their risk to human and ecosystem health is only recently acknowledged (OECD, 2019[18]; Houtman, 2010[19]).	Many endocrine disruptors are CECs. Examples include pharmaceuticals, industrial and household chemicals, personal care products, pesticides, manufactured nanomaterials, and their transformation products (OECD, 2019[18]).
Micropollutants	Natural or synthetic chemicals that exist in the environment at very low concentrations (microgram to nanogram per litre) and that are of toxicological concern (Schwarzenbach et al., 2006[20]).	Many EDCs are micropollutants. Particularly relevant to EDCs as they can be harmful at a low dose or through mixture effects.
Persistent Organic Pollutants (POPs) *Also known as 'forever chemicals'*	Pollutants that stay in the environment for long periods of time (where the half-life of the chemical in water is greater than two months), that bio-accumulate or bio-concentrate in organisms and that have adverse effects on human or environmental health.	The majority of POPs are endocrine disruptors or endocrine active substances (WHO-UNEP, 2013[3]). The Stockholm Convention recognises some EDCs as POPs, such as aldrin, BDE, chlordane, DDT, HBCD, HCB, HCH, PCB, PCDD, PCDF, PFOA, PFOS (Metcalfe et al., 2022[13]). Other substances, such as long-chain PFCAs, are undergoing a risk management evaluation as part of the POP review process (UNEP, 2022[21]). Not all EDCs are persistent, but some are continuously released into the environment (NORMAN Network and Water Europe, 2019[22]).

Note: EDCs may fall under multiple pollutant categories.
Source: (OECD, 2019[18]; Houtman, 2010[19]; Schwarzenbach et al., 2006[20]; WHO-UNEP, 2013[3]; Metcalfe et al., 2022[13]; NORMAN Network and Water Europe, 2019[22])

1.3. The distinctive dynamics of EDCs

EDCs can catalyse adverse effects on humans and wildlife in multiple ways. Four areas of critical complexity of EDCs affect the application of traditional toxicology and risk assessment methods:

- The cocktail effect or mixture effect[1]: robust evidence has emerged over the last 15 years that shows that EDCs can work together to produce combined effects, with the result that they can produce adverse effects when combined, even when they occur at concentrations wherein no effect from the individual EDC has been observed (Kortenkamp, 2007[23]; Carvalho et al., 2014[24]) (Box 1.3). This is particularly pertinent for EDCs in water sources, as chemical mixtures are likely to occur in water bodies (Gosset, Polomé and Perrodin, 2020[25]). Mixing with other pollutants can have an additive effect, and in some cases even synergistic (Kabir, Rahman and Rahman, 2015[11]). From a policy perspective, mixture effects in water imply the need to shift from traditional approaches of targeted chemical analysis (focusing on individual chemicals) towards monitoring of endocrine effects (Ministère de la transition écologique et solidaire, 2019[26]; WHO-UNEP, 2013[3]). This challenges the regulatory practice of many countries, which currently take a substance-by-substance approach to analysis and regulation.
- The low-dose effect: EDCs are believed to have what is referred to as a low-dose effect (Welshons et al., 2003[27]; WHO-UNEP, 2013[3]). This is based on evidence that implies that there is no safe threshold of minimal exposure (i.e. the dose below which no adverse effect is expected to occur) and that monitoring conducted on this basis would be insufficient (ANSES, 2013[28]; Vandenberg et al., 2012[29]).
- The non-monotonic dose-response relationship: related to the low-dose effect, it has been appraised that some EDC dose responses are non-linear and potentially non-monotonic (Vandenberg et al., 2012[29]; WHO-UNEP, 2013[3]). It is suspected, albeit with uncertainty, that EDCs may follow “inverted curves”, i.e., can exhibit greater or even opposite effects at low doses compared to those observed at high doses. This means that traditional toxicology, which hinges on the premise that high-dose toxicity testing will proportionally inform us about low-dose exposures, sometimes does not hold (Vandenberg et al., 2012[29]).
- Continuous release into the environment causing chronic exposure: not all CECs, including endocrine disruptors, are persistent: they can be broken down. However, as some chemicals are continuously released into the environment, they are routinely found in the environment and food webs and could form a source of chronic exposure (NORMAN Network and Water Europe, 2019[22]; Windsor, Ormerod and Tyler, 2018[30])

Box 1.3. The cocktail effect: how substances mix

Endocrine disrupting compounds can co-exist and have a joint endocrine disrupting effect when combined: the cocktail-effect or mixture effect. The point is that, while individual substances may not be harmful or toxic, their combination is. This is particularly challenging from a regulatory perspective. Such mixtures are formed through different pathways, including in the environment. Mixtures are grouped in the following categories:

1. **Intentional mixtures**: manufactured formulations e.g., commercial mixtures of industrial substances; technical mixtures; product formulations.
2. **Discharge mixtures**: substance combinations that are emitted by a single industrial site e.g., effluent of a production site.
3. **Coincidental mixtures**: substances from different sources occurring in a medium e.g., combination of substances applied dermally from use of two or more product formulations.
4. **Environmental mixtures**: substance combinations in the environment e.g., substances found in soil from various exposure sources (application of product formulation, deposition from air, water run-off, etc.).

Mixtures can comprise multiple categories, e.g., a coincidental mixture that mixed in a freshwater body is also an environmental mixture.

In this context, **combined exposure** is another important concept as it relates to the exposure of humans and environment to mixtures. Combined exposure is defined by the OECD (2018[31]) as "exposure to multiple chemicals by a single route and exposure to multiple chemicals by multiple routes, from one or multiple sources of release and/or use(s)".

Source: cited from (OECD, 2018[31])

1.4. Sources, environmental pathways and sinks of EDCs in freshwater

Endocrine disruptors are ubiquitously present in water bodies; they have been observed in aquatic organisms, freshwater bodies, soil, sediments, cryosphere and the ocean. EDCs are released into the environment through point sources and diffuse sources. The environment further transports EDCs through atmospheric currents, river flows, ocean currents, groundwater-surface water exchange and fish spawning. Table 1.3 provides a summary of sources, pathways and sinks.

Table 1.3. Summary of sources, environmental pathways and sinks of EDCs in freshwater and oceans

Sources	Entry pathways into the environment	Sinks
Households and consumer uses E.g. Cleaners, Electronics, Food packaging, Personal care products, Pharmaceuticals, Plastics, Toys *Agriculture and aquaculture* E.g. Treated sewage sludge, Pesticides, Pharmaceuticals, Poultry and fish feed *Industrial production* E.g. Combustion, Disinfection by-products, Metals, Plasticizers *Transportation* E.g. Fossil fuel combustion, Ships	*Point sources* Wastewater treatment plants *Diffuse sources* Agricultural runoff Urban runoff Industrial outfalls Waste disposal Leaching (wastes, septic tanks) *Environmental migration* Atmospheric currents River flows Ocean currents Groundwater-surface water exchange Fish spawning	Aquatic organisms (biological retention) Freshwater bodies (rivers, lakes, groundwater) Soils Sediments Cryosphere Oceans

Source: Authors

1.4.1. Sources

The key sources of EDCs in the environment are (Metcalfe et al., 2022[13]; Pironti et al., 2021[32]; Karthikeyan et al., 2019[14]):

- *Households and consumer uses*. Cleaners, personal care products and (occasionally) pharmaceuticals are drained through sinks and showers; pharmaceuticals, leachates from food packaging and metabolites are excreted; electronics, packaging, toys and other consumer products end up in waste collection sites or landfills.
- *Agriculture and aquaculture*. Pesticides and remaining EDCs in recycled effluents are discharged into the freshwater system as runoff; poultry feed, pharmaceuticals and their metabolites are excreted by livestock; or directly enter the aquatic ecosystem through fish farming.
- *Industrial production*. Combustion can release EDCs into the atmosphere before deposition on land or water bodies; drinking water production can release EDCs from disinfection by-products or as a leachate from pipe systems with endocrine-active additives.
- *Transportation*. Fossil fuel combustion can release EDCs into the atmosphere before deposition on land or water bodies; ships contain anti-fouling coatings that are released into the environment (many harmful anti-fouling coatings have been phased out).

Next to current-use sources, legacy chemicals are still present in the environment and organisms, in spite of global use restrictions or bans. Legacy chemicals are still found in the environment and humans due to their ability to dissolve in fats and their persistence (Yilmaz et al., 2020[33]). Some legacy chemicals are causing “more severe and widespread damage to many wildlife species than current-use chemicals” (Matthiessen, Wheeler and Weltje, 2018[34]). For example, tributyltin (TBT) is an antifouling paint. Since its ban in 2008 the levels of TBT have declined in the marine environment, but TBT is still present in sediments and marine species although some species have recovered (Marty et al., 2017[35]; Metcalfe et al., 2022[13]).

1.4.2. Entry pathways to freshwater ecosystems

EDCs enter the environment through point sources and diffuse sources. They are also transported from one ecosystem to another through environmental migration. Figure 1.3 provides an overview of EDCs entry pathways into surface water bodies.

Figure 1.3. Pathways of endocrine disrupting compounds into surface water bodies

Source: Authors based on (Pironti et al., 2021[32])

Point sources

Direct discharge from municipal wastewater treatment plants (WWTPs) is one of the primary sources of emission of EDCs into the environment (Kasprzyk-Hordern, Dinsdale and Guwy, 2008[36]; NORMAN Network and Water Europe, 2019[22]; Luo et al., 2014[37]; WHO-UNEP, 2013[3]; Ruhí et al., 2016[38]; IPCP, 2017[39]; Wee et al., 2021[40]). WWTPs do not remove all pollutants. As a consequence, some EDCs are released into the freshwater environment (Box 1.4). The removal of micropollutants, including EDCs, depends on the properties of the pollutant (hydrophobicity, biodegradability, and volatility), the treatment process and the composition of the wastewater itself (pH and temperature) (Luo et al., 2014[37]). Removal rates also differ across countries and even within countries (Tran, Reinhard and Gin, 2018[41]). Yet the risk for human and wildlife health does not only depend on the concentration of EDCs discharged. Chemicals can be diluted through the main water system which reduces their concentration, although some EDCs remain active even at very low concentrations.

Hospitals are other point sources of pharmaceutical EDCs, although the household contribution of pharmaceutical residues is higher (OECD, 2019[18]). Industries can also release EDCs through point

sources, such as paper and pulp mills and chemical manufacturers (WHO-UNEP, 2013[3]; Ussery et al., 2021[42]).

Diffuse sources

Among diffuse sources, storm runoff from agricultural fields and livestock activity and leaching from waste disposal sites or landfills account for a significant amount of EDCs in aquatic environments (WHO-UNEP, 2013[3]; Luo et al., 2014[37]). Storm runoff from agricultural fields can contain EDCs from pesticides (Pironti et al., 2021[32]), animal excretion (oestrogens, for example) (Matthiessen et al., 2006[43]) and recycled effluents (Schapira et al., 2020[44]; Edwards et al., 2009[45]). The estrogen release into the environment from livestock is possibly twice as large as the estrogen release from humans (Adeel et al., 2017[46]). Aquaculture may also potentially release EDCs in the environment, such as through disinfectant formulations (Ahmad et al., 2022[47]).

EDCs also enter the freshwater environment through the urban water cycle. Diffuse urban entry points are wet and atmospheric deposition, storm water runoff, direct discharge of untreated wastewater and sewage overflow from combined sewers (Pal et al., 2014[48]; Pironti et al., 2021[32]; König et al., 2017[49]). In a study on the occurrence of endocrine disruptors in the urban water cycle of Bogotá, Colombia, plasticisers (e.g. phthalates and bisphenol A) occurred the most in the water samples taken from aquatic media, while the pharmaceutical carbamazepine contributed with the highest concentrations (Bedoya-Ríos et al., 2018[50]).

Leaching from landfills and septic tanks is another diffuse entry pathway. EDCs used in industrial and household products can leach from landfills into surface water, sediments, and groundwater. Leaching of flame-retardants, such as PBDEs that have been banned in many jurisdictions, from e-waste dumpsites is a case in point (Alcock et al., 2003[51]; Oloruntoba et al., 2022[52]). Plastic additives, such as PBDE, phthalates, nonylphenols (NP), bisphenol A (BPA) and antioxidants, also leach into the environment during production, usage and disposal (Hermabessiere et al., 2017[53]).

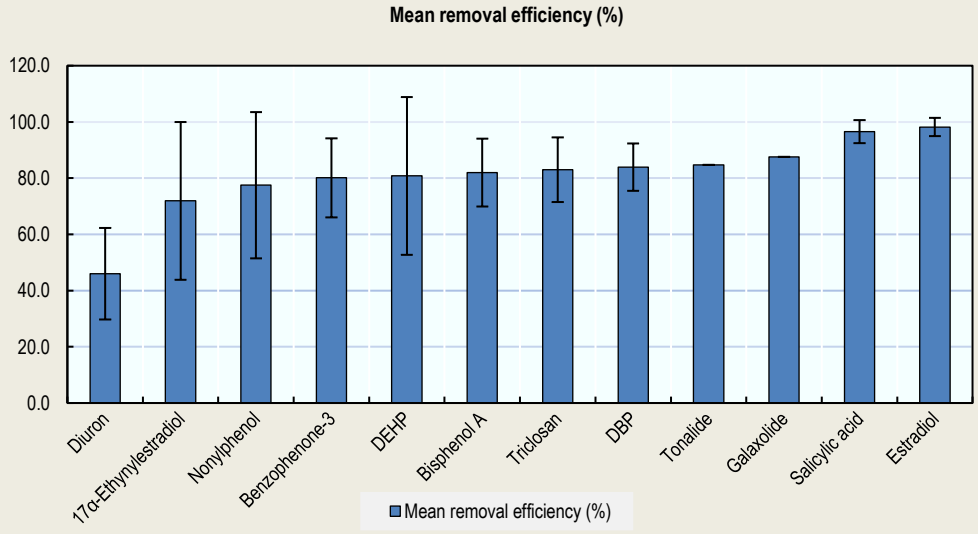
Environmental migration

EDCs migrate through the environment and across ecosystems, with transboundary pollution as a result. For example, perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are distributed globally by ocean currents (WHO-UNEP, 2013[3]). EDCs can also be transported from oceans to freshwater ecosystems by anadromous fish living in saltwater and returning to freshwater bodies to spawn (Nilsen et al., 2019[54]). Air currents also transport and deposit EDCs. This is particularly the case for highly persistent, semi-volatile compounds such as PCBs, DDTs, pesticides and predecessors of PFOS and PFCA (WHO-UNEP, 2013[3]). Certain hormones can travel long distances through rivers to seas and oceans. For example, the Jordan river carried testosterone, estrogen (and to a lesser extent ethinylestradiol and estriol) up to 100 km from the source of pollution, although concentrations dropped going downstream (Barel-Cohen et al., 2006[55]). Groundwater-surface water exchange is another inter-ecosystem pathway of groundwater pollution (Lapworth et al., 2012[56]). As EDCs are deposited in water bodies through many environmental media, water bodies provide perfect conditions for mixture effects to appear (see also Box 1.2).

Box 1.4. Figures on the removal efficiency of wastewater treatment plants

EDCs represent a wide range of compounds, some of which are better removed than others. Removal efficiency is highly context-specific. Figure 1.4 gives an impression of the discrepancies and general removal efficiency of a range of micropollutants.

Figure 1.4. Removal efficiency of selected micropollutants in WWTPs



Note: Mean removal efficiency (bars) and standard deviations (error bars). Data were taken from WWTPs in 14 countries/regions, including OECD countries. Micropollutants were selected based on their status as endocrine disruptor, endocrine active, or under evaluation as endocrine active, largely based on (edlists.org, n.d.[57]).
Source: (Luo et al., 2014[37])

Luo et al. (Luo et al., 2014[37]) developed a classification of the removal efficiency of several compounds:

Table 1.4. Simple classification of micropollutants based on removal efficiency

Degree of removal	Compounds
Poorly removed (< 40%)	Atrazine, carbamazepine, diazinon, diclofenac, erythromycin, metoprolol, mefenamic acid, tris(2-carboxyethyl)phosphine (TCEP), tris chloroisopropyl phosphate (TCPP)
Moderately removed (40–70%)	Atenolol, bezafibrate, clofibric acid, durion, ketoprofen, nonylphenol, sulfamethoxzole, tebuconazole, trimethoprim
Highly removed (> 70%)	Acetaminophen, benzophenone-3, bisphenol A, caffeine, clotrimazole, dibutyl phthalate, N,N-diethyl-meta-toluamide (DEET), Di(2-ethylhexyl) phthalate (DEHP), dimethyl phthalate (DMP), estradiol, estriol, estrone, ethinylestradiol, galaxolide, gemfibrozil, ibuprofen, naproxen, nonylphenol, octylphenol, salicylic acid, tonalide, triclosan

Note: The actual removal is highly context-specific
Source: (Luo et al., 2014[37])

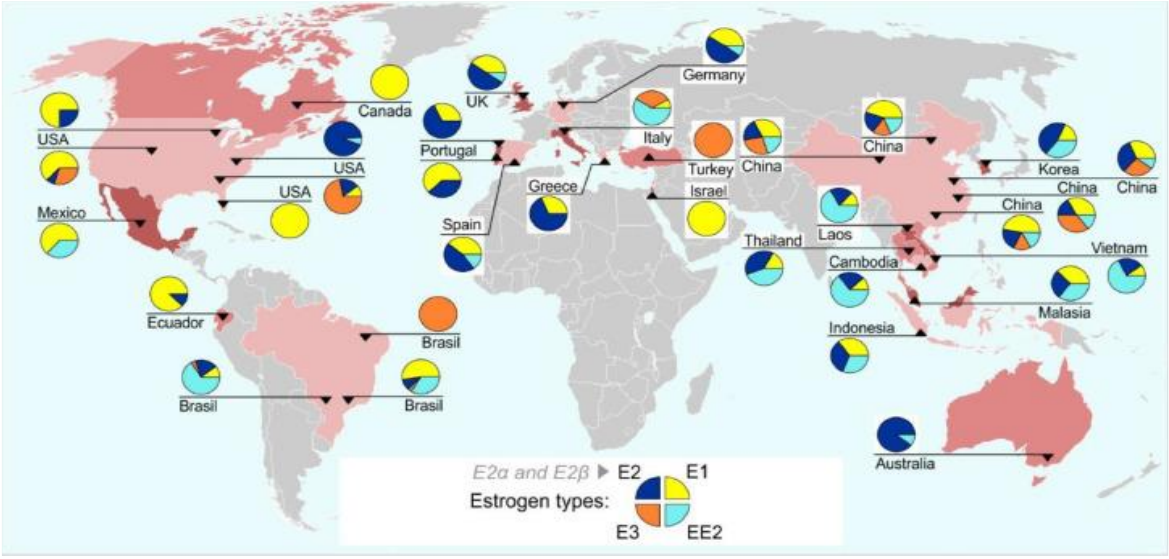
Freshwater sinks

EDCs are found in surface water bodies, groundwater bodies, drinking water and the marine environment. EDCs are also detected in aquatic organisms. Since EDCs represent a broad group of chemicals, there is

no consolidated analysis of EDC concentrations and distribution in freshwater systems. Few international comparative analyses exist for individual substances, such as estrogens (Figure 1.5) and PFOS and PFOA emissions and concentrations in Europe (Pistocchi and Loos, 2009[58]).

Many monitoring initiatives focus on WWTP effluents at the outlet. Downstream concentrations and distribution, including impacts on downstream aquatic organisms, are much less studied (Windsor, Ormerod and Tyler, 2018[30]). This forms a considerable knowledge gap.

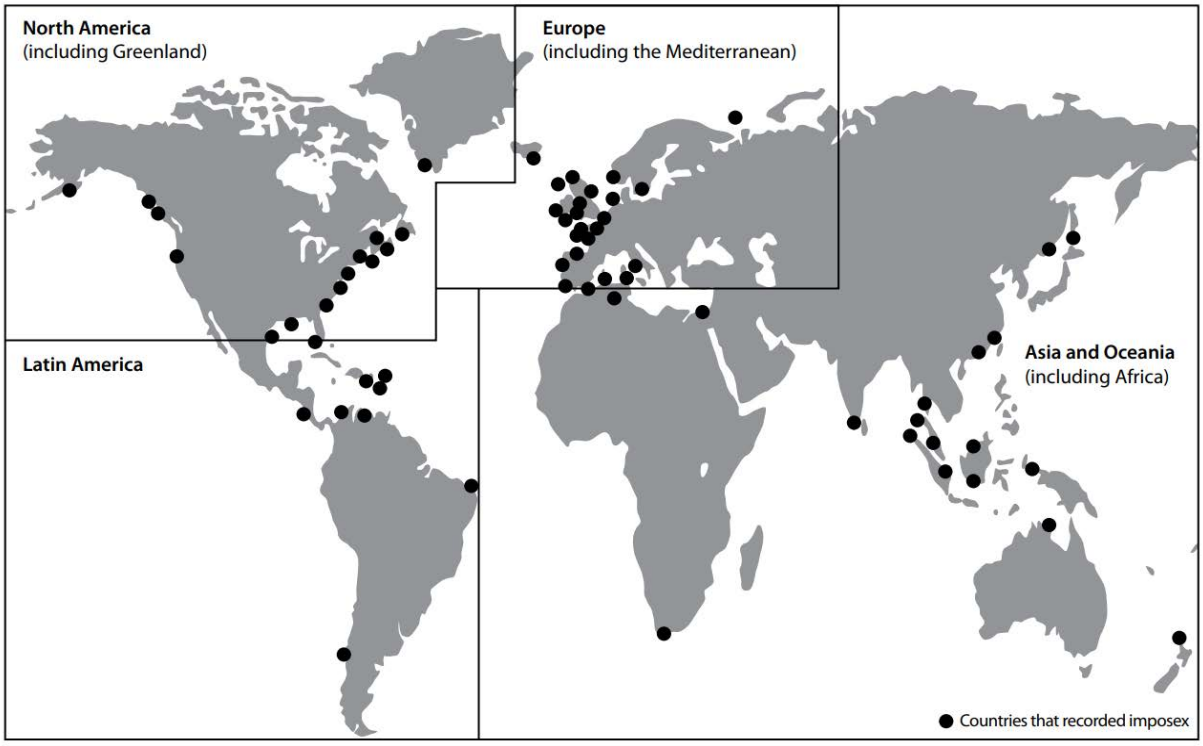
Figure 1.5. Global distribution of estrogens in river and surface water sites



Note: Each pie chart comprises the concentrations (ng/l) of E1, E2, E3 (natural estrogens) and the EE2 (synthetic estrogen applied in birth control pills) as percentages (%) of total at each site. Year of data collection is unknown.
Source: (Ciślak et al., 2023[59]) based on (Adeel et al., 2017[46])

To get an understanding of the sinks of EDCs it is meaningful to look at the global distribution of endocrine-related effects within freshwater organisms. See for example Figure 1.6, showing detected signs of masculinisation of female gastropods (snails and slugs), associated with exposure to TBT - an antifouling paint applied on ships in the 1970s-1990s (WHO-UNEP, 2013[3]). EDCs and pharmaceuticals can bioaccumulate in organisms (Ruhí et al., 2016[38]), forming an additional exposure route through diets within the food web. Knowledge gaps exist regarding the distribution of EDCs. Recent literature does not report on the regional or global distribution of EDCs in freshwaters and freshwater organisms.

Figure 1.6. Geographic regions where female gastropods were reported as affected by imposex, intersex and ovo-testis



Note: Figures are from 1990-2009
Source: (WHO-UNEP, 2013[3])

The understanding of endocrine disruptors in groundwater is limited compared to other freshwater bodies, even though groundwater is an important drinking water source for many regions in the world. Leaching from septic tanks, wastes and landfills, wastewater effluents, livestock activities, and groundwater-surface water exchange are common pathways of groundwater pollution (Lapworth et al., 2012[56]). Whether a compound can be transferred to groundwater depends on its physiochemical properties (Luo et al., 2014[37]). In a meta-analysis of EDCs in groundwater in several OECD countries, estrone, E2, NP and bisphenol A were the most frequently reported EDCs (Lapworth et al., 2012[56]). Personal care products, pesticides, plastic additives, fragrances and pharmaceuticals have also been reported in groundwater (Lapworth et al., 2012[56]; Jurado et al., 2012[60]). A study in Spain found that contaminant concentrations including EDCs were sometimes higher in aquifers than in their respective rivers, although generally groundwater was significantly less polluted than other water bodies (Jurado et al., 2012[60]). This may suggest that some contaminants can be persistent in groundwater.

The marine environment is important to mention in this context, as it receives EDCs from land-based activities via rivers. Endocrine disrupting chemicals from land-based activities have been found in estuaries, such as industrial xenoestrogens and natural and synthetic estrogens (Rocha et al., 2019[61]). Killer whales carry high levels of PCBs in their tissues originating from river runoff and atmospheric deposition, posing a potential risk to future killer whale populations (Desforges et al., 2018[62]). Similarly, endocrine disrupting POPs stemming from industrial and agricultural activities have reached polar bear populations through air and ocean currents (Routti et al., 2019[63]). Other EDCs found in the marine environment originate from marine-based activities, such as EDCs stemming from antifouling coatings on ships (Birch, Scammell and Besley, 2014[64]).

1.5. Human health impacts

This is a brief overview of the impacts of endocrine disrupting chemicals on the human body and public health. With the purpose of providing policy guidance to the environmental sector, this section simplifies exposure routes and disease impacts on humans. To provide adequate policy guidance for the health sector, a dedicated review is appropriate.

Humans may be exposed to endocrine disrupting chemicals through consumption of food and water, skin contact, inhalation, intravenous routes or biological transfer to the human foetus or newborn from the placenta and breast milk (Kabir, Rahman and Rahman, 2015[11]). At present, EDCs have been identified in human urine, blood, sweat and breast milk (Azzouz, Rascón and Ballesteros, 2016[65]; Shekhar et al., 2017[66]).

Endocrine disruption is a mode of action, i.e. it catalyses a change within an organism resulting from chemical exposure, that could lead to different health outcomes. In other words, endocrine disruption is not a health effect in itself. There are still knowledge gaps about the impacts of EDC exposure on human health, owing to the difficulty of separating their specific contribution from other potential causes (i.e. the heavy toll of establishing causality) in tandem with a dearth of epidemiological and experimental toxicology studies. Nonetheless, research over the last decade has made significant steps ahead in deepening our understanding and identifying an increasing number of potential new exposure-outcome associations.

The diseases induced by exposure to endocrine disrupting chemicals may comprise (based on a grouping by Kahn et al. (2020[67])):

- **Birth defects**. They include disrupted foetal development and growth (Kahn et al., 2020[67]), reduced birthweight (Steenland, Barry and Savitz, 2018[68]), preterm birth (Gao et al., 2019[69]; Ferguson, McElrath and Meeker, 2014[70]; Latini et al., 2003[71]), and reduced anogenital distance in males (Bornehag et al., 2015[72]; Swan et al., 2015[73]).
- **Neurodevelopment conditions**, such as attention-deficit hyperactivity disorder, autism, and cognitive and behavioural changes and dysfunction (Ghassabian and Trasande, 2018[74]).
- **Male and female reproductive health**. The literature documents reproductive system disorders such as infertility (Kahn et al., 2020[67]), congenital malformations of the male reproductive system (Goodyer et al., 2017[75]), endometriosis (Kim et al., 2015[76]) (Kim et al., 2015), polycystic ovarian syndrome, breast cancer (Cohn et al., 2020[77]; Mancini et al., 2020[78]; Bonefeld-Jørgensen et al., 2014[79]), testicular cancer (Soto and Sonnenschein, 2010[80]), prostate cancer (Soto and Sonnenschein, 2010[80]; Kachuri et al., 2017[81]; Meyer et al., 2007[82]), and poor sperm quality and function (Li et al., 2011[83]; Omran et al., 2018[84]).
- **Obesity and metabolic diseases**. Increased incidence of metabolic syndromes, such as obesity, insulin resistance, type 2 diabetes and cardiovascular diseases (Casals-Casas and Desvergne, 2011[85]; Giulivo et al., 2016[86]). Diabetes has been associated with PFAS exposure in Swedish and American cohorts (Lind et al., 2014[87]; Sun et al., 2018[88]; Cardenas et al., 2019[89]), whereas the strongest associations have been found with bisphenols BPA (Li et al., 2018[90]; Duan et al., 2019[91]; Murphy et al., 2019[92]; Rancière et al., 2019[93]; Sun et al., 2014[94]). However, increased PFAS exposure does not always cause increased diabetic outcomes (Karnes, Winquist and Steenland, 2014[95]).
- Other endocrine disruptors including BPA, pesticides and flame retardants (e.g. PCBs, PBBs) have consistently shown **thyroid disrupting properties** (Boas, Feldt-Rasmussen and Main, 2012[96]; Murk et al., 2013[97]).

The most sensitive window of exposure to EDCs pertains to the critical periods of development, such as embryonic development, perinatal development, puberty, pregnancy and lactation periods, and menopause, i.e. periods during which organisms are more sensitive to hormonal disruption (Woodruff

et al., 2008[98]; WHO-UNEP, 2013[3]). This implies a greater degree of risk for foetuses, infants, adolescents, pregnant women and the elderly (Leung et al., 2013[99]). Importantly, concerns for infants and young children have increased dramatically as they were found to be subject to much higher EDC exposure compared to adults through *inter alia* dust and particulates (Lunder et al., 2010[100]; Wormuth et al., 2006[101]). Moreover, early (especially prenatal) exposure can have health impacts at a later life stage (WHO-UNEP, 2013[3]).

Freshwater bodies potentially serve as a vehicle for transmitting EDC exposure from the environment to humans, mainly through contaminated drinking water, although causal linkages have not been established with certainty. Some of the interlinkages between human exposure to EDCs from freshwater are:

- **Consumption of untreated or contaminated drinking water collected from polluted freshwater sources**. It is established that EDCs are not completely removed from drinking water treatment processes (Wee and Aris, 2017[102]; Kuch and Ballschmiter, 2001[103]; Benotti et al., 2009[104]). However, the health risk of consuming the very low levels of EDCs present in treated drinking water is likely to be low (Pironti et al., 2021[32]). For example, Wee et al. (2021[40]) found no risk for different age groups via tap water consumption in Malaysia, in spite of the presence of several endocrine disrupting chemicals. Similarly, endocrine-disrupting chemicals may be present in drinking water as by-products resulting from the process of water disinfection with chlorine, so-called chlorinated by-products (Gonsioroski, Mourikes and Flaws, 2020[105]; Liu, Dang and Liu, 2021[106]). The spray-on-lining of aged piping systems, specifically those with epoxy coating which contains bisphenol A (BPA), can leach from the pipes into to the drinking water supply (Rajasärkkä et al., 2016[107]).
- **Food consumption,** for instance when food products are cultivated using recycled effluent or when EDCs bioaccumulate in crops, fish and seafood. Recycling of wastewater for irrigation could have an impact on human health. A study in Israel detected an association between vegetable consumption and relatively high concentrations of the carbamazepine drug (although carbamazepine is a CEC, it is not with certainty associated with endocrine disruption) in urine of people living in areas with extensive recycled effluent irrigation (Schapira et al., 2020[44]).
- **Bathing water**, such as pools, ponds, lakes or seas, have not been identified as a vehicle for transmitting EDCs from the environment to humans. A possible explanation is that bathing water is not a source of chronic exposure. However, phenols, oestrogens, caffeine and progestogens have been detected in swimming pool water in China (Zhou et al., 2020[108]). A risk assessment done as part of the same study suggests that swim water skin contact is a more dominant exposure route than ingestion.

1.6. Ecological impacts

There are concerns about EDCs as drivers of biodiversity loss and ecosystem degradation, terrestrial, freshwater and marine species included (Harrison, 2022[109]). The European Environment Agency concludes that “on average 20 % of aquatic species are lost due to exposure to chemical mixtures” (European Environment Agency, 2020[110]). However, there is still a limited understanding of the effects of EDCs on biodiversity.

Pollution of water, even at low concentrations, is an important source of EDC exposure for wildlife. Effects of exposure to EDCs have been observed in a breadth of aquatic species: alligators, fish, frogs, minks/otters, mussels, polar bears and snails (Hotchkiss et al., 2008[111]; Orton et al., 2018[112]; Rodil et al., 2019[113]). Fish take up endocrine disruptors through their gills, while birds and mammals are exposed mainly through drinking water (WHO-UNEP, 2013[3]). Diet (trophic transfer through the food web) is another exposure route for aquatic organisms, as EDCs can bioaccumulate in organisms (Ruhí et al., 2016[38]).

Aquatic plant species, such as algae, duckweed and wetland macrophytes can accumulate estrogens, thereby removing them from water (Shi et al., 2010[114]; Adeel et al., 2017[46]).

Ecosystems respond in different ways to contamination by endocrine disruptors (Table 1.5). Understanding the degree of impact of EDCs on wildlife and broader ecosystems is crucial yet quite challenging. Effects do not limit themselves to an individual organism, instead they can affect all levels of biological organisation. Endocrine contamination may not only affect the physiology of an organism, but can also change behaviour, fitness and evolution. Moreover, direct impacts on one species could cascade onto other species, so-called "indirect effects" (Saaristo et al., 2018[115]). Effects can be lethal or sublethal to some organisms, while other species can adapt or be(come) resistant. The effects of endocrine disrupting chemicals on wildlife and ecology are explained briefly in the following sections.

Table 1.5. Typology of effects of EDCs on wildlife and ecology

Lethality	Effects	Level of biological organisation	Temporal aspects	Coping mechanisms
Lethal Sublethal	*Direct* Physiology Behaviour Fitness Evolution *Indirect*	Molecules Cells Tissues Organs Organisms Population Community Ecosystem	Delayed effects Generational effects	Resistance Adaptation Recovery

Source: Based on: (Saaristo et al., 2018[115]; Windsor, Ormerod and Tyler, 2018[30]; Nilsen et al., 2019[54]; Parrott et al., 2017[116]; Marty et al., 2017[35])

1.6.1. Lethality

While chemical pollution can be lethal to wildlife, many species survive toxic exposure and experience more subtle effects that can still be harmful. Such sublethal toxic effects can change survival, growth and reproductive capabilities of organisms, ultimately affecting individual organisms, populations and communities (Beiras, 2018[117]; Saaristo et al., 2018[115]). Exposure to low doses of chemicals, sometimes over longer periods, can trigger sublethal effects (Nilsen et al., 2019[54]).

1.6.2. Direct and indirect effects

Endocrine disrupting chemicals can directly affect the physiology, behaviour or fitness of organisms:

- *Physiology*: Among the adverse physiological effects in aquatic organisms (alligators, fish, frogs, minks/otters, mussels, polar bears and snails) are, *inter alia*: immune system damage, alterations of the hormonal system, disruption of homeostasis, reproductive dysfunctions (embryo malformation, hatchability, sex ratio alteration, sperm alteration), feminisation of male fish (Marty et al., 2017[35]; Zhou, Cai and Zhu, 2010[118]).
- *Behaviour*: Chemical contaminants can affect the behaviour of individual organisms, which can, in turn, affect populations, communities and ecosystems (Ford et al., 2021[119]; Windsor, Ormerod and Tyler, 2018[30]). There are many uncertainties regarding EDC-induced behavioural changes. Zala and Penn (2004[120]) recorded several cognitive and neurological effects in aquatic organisms (Table 1.6). Changing feeding behaviour, avoidance of contaminated areas and changed migration routes have also been reported (Saaristo et al., 2018[115])
- *Fitness:* Fitness-related traits of species are body size, growth and locomotor skills which affect the ability to move from one place to another, such as swimming performance of fish (Arendt,

2003[121]). Fitness of aquatic species can be affected by pollution and can have consequential effects on the population growth rates of species (Egea-Serrano and Tejedo, 2014[122]; Hamilton et al., 2017[123]). The fitness parameters that are affected by endocrine disruption are largely unknown.

Table 1.6. Cognitive and behavioural effects of EDCs present in the environment on aquatic species

Species	Behaviour	Changes	EDC
Mosquitofish	Reproductive behaviour and sex characters	Females masculinized Precocious and aggressive (males)	Paper mill effluent*
	Dominance	Increased	
Brown pelican	Reproductive behaviour	Aberrant	Chlorinated hydrocarbon*
Common tern	Reproductive behaviour	Aberrant	DDT metabolites*
Gulls	Mate choice	Homosexual in females	DDT
Guppies	Sexual behaviours	Decreased (males)	4-t-octylphenol, vinclozolin
Three-spined sticklebacks	Aggression	Decreased (males)	Ethinyl oestradiol*
	Courtship and nesting	Abnormal (males)	
Atlantic salmon	Mating (response to females' pheromones)	Inhibited (males)	Cypermethrin (low doses)
Mallard ducks	Response to maternal calls	Decreased	Methyl-mercury exposure in utero or as adult
	Response to fright stimulus	Increased	
	Oviposition	Laid more eggs outside nest	
		Laid fewer eggs	
Common tern	Behaviour	Altered	Lead
Herring gulls	Begging	Decreased	Lead
	Balance	Decreased	
	Righting responses	Decreased	
	Individual recognition	Decreased	

Note: *EED chemical tested at levels found in the environment; Only aquatic wildlife species are presented in this table; Experimental and correlational evidence are presented.
Source: (Zala and Penn, 2004[120])

Contamination can cascade onto other organisms, populations and communities, too. Such indirect effects can, for example, arise when contaminants trigger changes of behaviour or populations, and subsequently changes competition or predator-prey relationships in the foodweb (Windsor, Ormerod and Tyler, 2018[30]; Saaristo et al., 2018[115]). Indirect effects can thus also affect endocrine-resistant species and ecosystems at large.

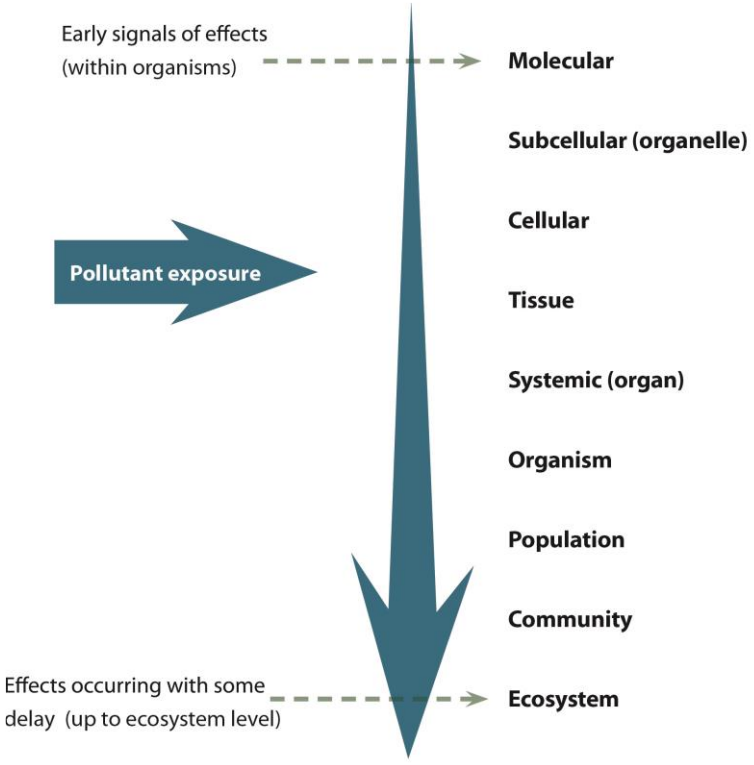
The indirect effects of estrogen 17a-ethinylestradiol (EE2) on an aquatic foodweb have been demonstrated in a whole-lake experiment by Kidd et al. (2014[124]). EE2 normally enters the environment via wastewater treatment plants, as a residue of the birth control pill. The introduction of small concentrations of EE2 (5–6 ng/L^{-1}) to the lake led to the collapse of the fathead minnow, a freshwater fish often preyed upon by larger fish species such as trout. The biomass of trout subsequently declined as they lost their prey species. Moreover, moving down the foodweb, the zooplankton population increased as their predator - the fathead minnow - had disappeared.

Our current understanding of the indirect impacts of endocrine disrupting contamination on populations, communities and ecosystems is limited. Scientists therefore advocate for a better assessment of ecological risks, taking into account all levels of biological organisation (Kidd et al., 2014[124]; Saaristo et al., 2018[115]; Nilsen et al., 2019[54]; Windsor, Ormerod and Tyler, 2018[30]).

1.6.3. Levels of biological organisation

The effects of endocrine disruption on aquatic organisms can cascade from cell, molecular and individual effects, to populations, to communities up to the entire ecosystem and the food web (Figure 1.7). This raises concerns for ecosystem balances and biodiversity. The effects of pollutants are visible earlier and detected more easily on the lower levels of biological organisation. Adverse effects on higher levels of biological organisation are more difficult to detect. Sometimes there is a lag time between exposure and effect. A timely understanding of effects at lower levels of the biological organisation can prevent significant negative effects on populations, which are much harder to recover (Wernersson et al., 2015[125]).

Figure 1.7. Effects of exposure to pollutants on different levels of biological organisation



Note: The effects of pollutants are visible earlier and detected more easily on the lower levels of biological organisation before adverse effects are observed in higher levels of biological organisation.
Source: Authors, based on (Van der Oost, Beyer and Vermeulen, 2003[126])

1.6.4. Temporal aspects

The effects of EDC exposure are not always acute, but can be delayed or deferred to future generations. Effects of exposure to endocrine disrupting chemicals can be delayed when exposure happens in a period when an organism is vulnerable to contamination, but the effects will only manifest in another development stage, even if the exposure has already stopped (Parrott et al., 2017[116]; Matthiessen et al., 2017[127]). This is particularly true for endocrine disrupting chemicals, as they tend to affect the reproductive system during critical development stages.

Multigenerational effects become visible in the subsequent generation or generations (Parrott et al., 2017[116]). The type of impact may be different for the parental generation and the successive generations (Windsor, Ormerod and Tyler, 2018[30]). In a study on transgenerational effects of EE2 and BPA exposure on medaka fish, no irregularities were observed with the parental and subsequent generation (Bhandari,

Vom Saal and Tillitt, 2015[128]). However, fertility dropped, and embryo survival dropped two and three generations later. The causes of such multigenerational effects vary. Factors include the duration of exposure, the timing of exposure (during a critical development window when the species are vulnerable to chemical exposure), or effects are carried over from one generation to the next, for example as a result of embryo exposure (Parrott et al., 2017[116]).

However, understanding of delayed and intergenerational effects is still limited (Parrott et al., 2017[116]; Saaristo et al., 2018[115]; Nilsen et al., 2019[54]; Windsor, Ormerod and Tyler, 2018[30]). The extent to which populations and evolution will be threatened is uncertain, as species may adapt, become resistant or recover from exposure (Windsor, Ormerod and Tyler, 2018[30]).

1.6.5. Coping mechanisms and recovery

Endocrine disruption does not always lead to permanent adverse effects. Species can be resistant or become resistant to endocrine disruptors, effects can be reversed, or species can adapt (Windsor, Ormerod and Tyler, 2018[30]). Species can recover from endocrine disruption, for example when the contaminant is removed from a water body (Marty et al., 2017[35]; Blanchfield et al., 2015[129]). The oyster population in the Sydney estuary recovered in a period of 10 years, following a partial ban of tributyltin as an antifouling coating (Birch, Scammell and Besley, 2014[64]). A study on impacts of pulp mill pollution in Jackfish Bay, in the Great Lakes of Canada, showed the ability of species to improve or recover from adverse effects on the endocrine system (Ussery et al., 2021[42]). In this study, three interventions over a period of 30 years had positive impacts on impairment and population size: introduction of secondary effluent treatment (1989), change in production processes (1990s) and a series of temporary closures of the mill (2000s). At the onset, adverse effects were observed in the white sucker fish species, such as smaller gonads, delayed sexual maturation, and changed production of sex steroids. After introduction of the measures, changes in “body size, liver size, gonad size and condition” reduced, but persisted (Ussery et al., 2021[42]). Some effects, such as enlarged liver sizes, bounced back to reference levels. Reproductive effects, however, persisted and can only be further reduced with mill closure. Nevertheless it was estimated that with the current measures, population levels could restore to over 93% of the lake’s carrying capacity, and improvements in population levels were observed.

Effects of EDCs differ across species, as some species are more resistant to contamination than others. The same study by Ussery et al. notes that some fish species are more sensitive to pulp and paper effluent exposure than others (Ussery et al., 2021[42]). Physical changes can also be observed following contamination, but without changing apical endpoints, i.e., without leading to any state of disease. Other species adapt to the contaminated environment and become resistant to EDCs, although these mechanisms have not been well documented (Windsor, Ormerod and Tyler, 2018[30]). Lastly, developing resistance to mixtures of chemicals with a broad range of modes of action is a much slower process than for similar-working chemicals (Saaristo et al., 2018[115]).

Uncertainty remains as to what extent the species can bounce back from the impacts of endocrine disruptive contamination. For example, Marty et al. (2017[35]) point out that after a global drop of TBT usage, some irreversible effects have been observed in female sea snails (*nucella lapillus*), while global snail populations have recovered.

1.7. Economic impacts

Environmental pollution from chemicals has substantial economic effects and costs (Landrigan et al., 2018[4]; Fuller et al., 2022[130]), in spite of the benefits that chemicals can offer. A handful of studies have assessed the economic costs of endocrine disruption and EDCs. These economic evaluations strive to

estimate the disease costs of EDCs by hinging on a range of health expenditures and health outcomes (see Box 1.5).

Box 1.5. The costs of pollution-related disease

The Lancet Commission on Pollution and Health (Landrigan et al., 2018[4]) calculate the costs of pollution-related disease as a factor of:

1. "Direct medical expenditures, including hospital, physician, and medication costs, long-term rehabilitation or home care, and non-clinical services such as management, support services, and health insurance costs;
2. Indirect health-related expenditures, such as time lost from school or work, costs of special education, and the cost of investments in the health system (including health infrastructure, research and development, and medical training);
3. Diminished economic productivity in persons whose brains, lungs, and other organ systems are permanently damaged by pollution;
4. Losses in output resulting from premature death."

Source: (Landrigan et al., 2018[4])

Trasande et al. (2016[131]) estimate a median annual cost of EUR 163 billion in the European Union stemming from exposure to EDCs, which amounts to 1.28% of EU GDP. In the same analysis, the largest burden per capita is found to be borne by Luxembourg (€791 per capita), Ireland (€583 per capita), and the Netherlands (€411 per capita). The study also estimated different probability-scenarios. Looking at the lower EDC exposure cost scenario, Trasande et al. estimate a 5% probability that costs are less than €22.5 billion/year. There is a 10% probability of the higher annual cost scenario of €215 billion/year.

For the US, Attina et al. (2016[132]) compute a cost of USD 340 billion/year (equal to 2.33% of US GDP). The lower cost scenario estimates, with 5% probability, that the cost of exposure to EDCs are less than $43.3 billion/year. The higher cost scenario calculates a 10% probability of costs amounting to $512 billion/year. The cost of exposure to EDCs are estimated to amount CAD 24.6 billion in Canada, or 1.25% of the Canadian GDP (Malits, Naidu and Trasande, 2022[133]).

Economic and social cost estimations have also been made for specific EDCs, such as PFAS and bisphenol A (BPA). The Nordic Council of Ministers (Goldenman et al., 2019[134]) estimated the socioeconomic costs from the use of PFAS in Denmark, Finland, Iceland, Norway and Sweden, covering health-related costs and environment-related costs to mitigate contamination. The annual health-related costs of exposure to PFAS have been estimated to range from EUR 2.8 – EUR 4.6 billion in the Nordic Countries, and EUR 52 - EUR 84 billion for all EEA countries. The estimated environment-related costs ranged from EUR 46 million – EUR 11 billion per country over a period of 20 years. Soil remediation measures constituted to be the highest expense, followed by upgraded treatment works and maintenance. Trasande (2014[135]) estimates the social costs of childhood obesity and adult coronary heart disease as a consequence of BPA exposure in the US at USD 2.98 billion in 2008.

Yet, the economic burden appraised via these approaches is deemed to be underestimated as only a limited subset of potential chemical exposure-outcome routes are taken into account (Malits, Naidu and Trasande, 2022[133]; Fuller et al., 2022[130]). Moreover, only the routes for which sufficient evidence of causation exists are considered. Lastly, the economic impact may be larger than the calculated direct and indirect costs, such as impacts on quality of life (Kassotis et al., 2020[15]; Malits, Naidu and Trasande, 2022[133]) or loss of property value near contaminated sites (Cordner et al., 2021[136]; Goldenman et al., 2019[134]).

In comparison, the economic costs of antimicrobial resistance (AMR), another One Health issue associated with the freshwater environment, amount to $55 billion every year in the US (CDC, 2013[137]), €1.5 billion in the EU (ECDC, 2009[138]) and an annual GDP decline of between CAD 13-21 billion in 2050 in Canada (CAC, 2019[139]). It should be noted that these figures cannot be directly compared to the figures on endocrine disruption due to differences in methodology, underlying assumptions, uncertainties and disease pathways considered in each model. Still, the figures tell us that economic costs of endocrine disruption are likely to be at par or higher than the economic costs associated with AMR.

It should be noted that the cost estimates in the previous paragraphs cannot be fully attributed to environmental causes. Other exposure routes significantly contribute to the burden of disease, such as through food contact materials, working in occupations with high chemical exposure, or breathing in contaminated air.

Economic costs are not always easily defined when it comes to the loss of biodiversity and ecosystem services caused by endocrine disruption. There have been no attempts to quantify such costs.

1.8. Drivers for future endocrine disruption in the environment

1.8.1. Climate change

Climate change-related stressors can have important implications with respect to EDCs' impacts on wildlife and ecosystems. Higher average temperatures may increase the rate of volatilisation (evaporation) and dilution of endocrine-disrupting chemicals in water (Godfray et al., 2019[140]). It has also been shown that higher water temperatures can affect organisms, as it may lead to intensified feminising effects of zebrafish following exposure to the synthetic oestrogen EE2 (Luzio et al., 2016[141]); increased vitellogenin production of fish exposed to EDC mixtures (Brian et al., 2008[142]); induced female biased sex ratio (Dang and Kienzler, 2019[143]); and, more broadly, negative impacts on fish survival, development and reproduction where there is concurrent exposure to EDCs, eventually leading to population declines (Brown et al., 2015[144]). Nonetheless, the interactive effects of co-exposure to EDCs and warming and/or acidification are not clear-cut. What is certain is that an alteration takes place, which may lead to either the enhancement or inhibition of responses to EDCs (Maulvault et al., 2019[145]).

According to Godfray et al. (2019[140]), climate change has the potential to lead to reduced precipitation in certain regions and at certain times, driving reduced flows in water bodies and less dilution of wastewater, in turn enhancing EDC concentrations in water. Or, conversely, climate change may elicit changes in extreme rainfall events that in turn increase agricultural runoff and sewer overflows into river water. Furthermore, endocrine disrupting chemicals trapped in glacial ice may be released upon melting (Godfray et al., 2019[140]). Soil erosion due to rainwater and certain types of land use has also been found to cause greater EDC pollution of nearby water bodies (Issaka and Ashraf, 2017[146]).

Climate change can also have an indirect effect on pollution. In regions where climate change causes intensified or more frequent droughts, countries may resort to alternative water resources such as wastewater recycling (California EPA, 2018[147]). Recycled effluents, however, can discharge EDCs into the environment through agricultural runoff (Schapira et al., 2020[44]; Edwards et al., 2009[45]). Moreover, an increased demand of biofuels to accommodate the energy transition, combined with increased food production, could lead to a doubling of pesticide and fertiliser use by 2050 (Harrison, 2022[109])

1.8.2. Societal changes

Urbanisation and increased population density can reduce water quality by generating a higher concentration of EDCs in water bodies, especially in those with modest dilution capacities (Miller and Hutchins, 2017[148]; Gabor et al., 2018[149]; Godfray et al., 2019[140]; Li, Zhang and Shan, 2019[150]).

Among other major global trends, various demographic and economic changes are affecting EDC releases into water sources and the broader environment. Growing populations will trigger a higher release of EDCs, even if per capita consumption remains constant, as increasing wealth is associated with greater consumption. Trends such as population ageing and the non-communicable diseases epidemic are also expected to increase the discharge of pharmaceuticals into waterways (Godfray et al., 2019[140]).

In this context of societal changes, it should also be noted that substances are being phased out in several regions of the world (Metcalfe et al., 2022[13]). This potentially has a positive effect on human health and ecosystem recovery, although legacy compounds are still found in the environment. Partial bans (e.g. based on geography or usage) cannot eradicate the transboundary movement of substances, and substitutes can still be harmful (Matthiessen, Wheeler and Weltje, 2017[151]; Barton-Maclaren et al., 2022[152]).

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Notes

[1] The cocktail effect is not specific to EASs and EDCs. Mixtures of other, non-EDC related, groups of chemicals are possible.

2 Water quality monitoring for endocrine disrupting chemicals: from traditional chemical analysis to effect-based monitoring

To manage endocrine disrupting chemicals (EDCs) in freshwater, there is a need to prioritise actions that identify hotspots and sources of emission. This can be done via robust monitoring. This chapter explores available monitoring methods, such as traditional chemical analysis, non-targeted analysis, effect-based methods (EBM) and *in situ* wildlife monitoring. The chapter also illustrates ways to build cases for action by for example identifying the culprit chemical via effect-directed analysis (EDA). Some of the enabling factors for a robust monitoring system are also discussed. They include thresholds or trigger values, budgets, laboratory capacity, avoiding animal testing, and sampling strategies. Recent developments will be discussed.

2.1. Introduction

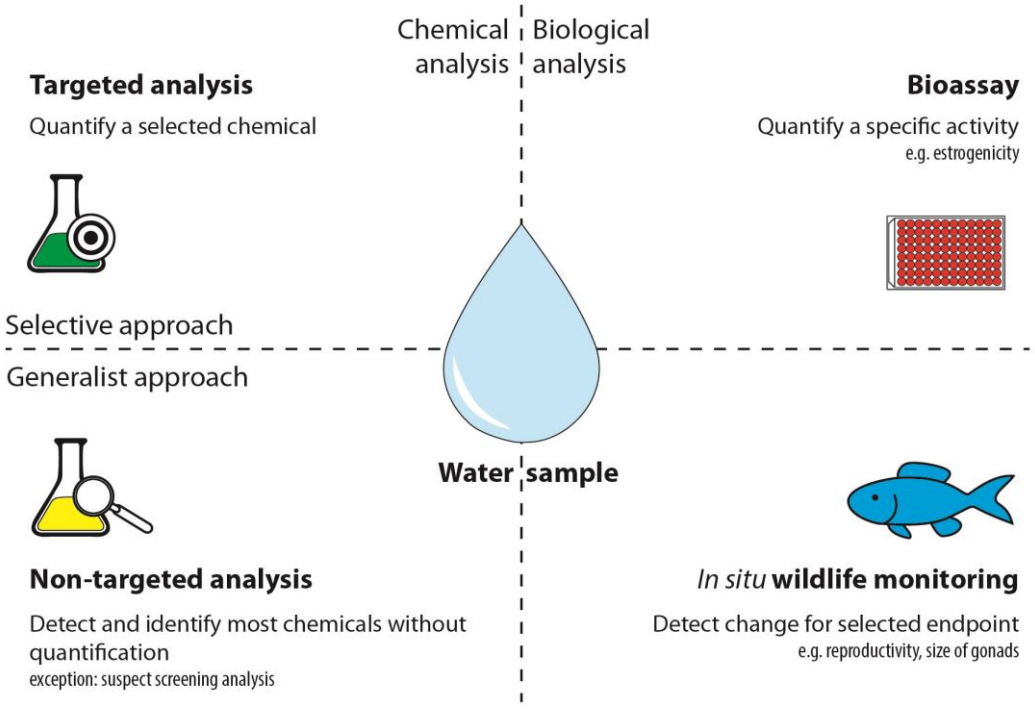
To manage endocrine disruptors in water, well-designed monitoring programmes can support policy action. While water quality monitoring usually focusses on detecting a shortlist of substances (substance-by-substance monitoring), this approach is insufficient to address endocrine disruption. As described in Chapter 1, endocrine disrupting chemicals (EDCs) are found in various classes of chemicals (e.g., pesticides, pharmaceuticals, packaging, steroids) and it is impossible to monitor each and every potential EDC. Moreover, only few chemicals are currently identified or suspected as EDCs, even though effects may be observed in freshwater organisms, posing a challenge for the selection of chemicals to monitor on a substance-by-substance basis. The problematics of EDCs call for additional monitoring approaches.

One emerging solution are effect-based methods (EBM). EBMs are increasingly applied for water monitoring in research since the 2000s (Escher, Neale and Leusch, 2021[1]; Di Paolo et al., 2016[2]; Fairbrother et al., 2019[3]; Robitaille et al., 2022[4]; Wernersson et al., 2015[5]). EBM is achieved through bioanalytical assays or bioassays that detect the activity – or effect – of water samples in organisms, embryos, tissues, or cells. If a change occurs in the bioassay, it indicates the presence of chemical(s) which can generate that change. Bioassays exist to detect various types of endocrine activity.

In 2018, California has formalised the use of cell bioassays as a water quality policy tool, including bioassays which test for estrogenicity (California State Water Board, 2018[6]). Moreover, bioassays are used by utilities, water authorities and industries across the world, usually as a screening tool to detect endocrine disruptive effects. The European Commission is also considering extending the Water Framework Directive (EU, 2000[7]) to include the regulation of endocrine effects (European Commission, 2022[8]).

This chapter inventories prevailing and promising techniques to monitor and assess endocrine activity and endocrine disruption through chemical and biological analysis. An overview of these methods is given in Figure 2.1. The chapter also addresses ways to validate the results obtained in monitoring, either to confirm the hazard, to identify the culprit substance, or to identify pollution hotspots. The chapter also highlights the enabling factors, including threshold values, funding, laboratory access and sampling, to advance effect-based monitoring. The last section of this chapter gives a brief overview of the barriers and uncertainties that may challenge the wide use of effect-based monitoring for policy development. It also argues that decision-makers must accept a certain level of uncertainty when developing policy responses.

Figure 2.1. Comparison of monitoring methods for endocrine disruptors



Source: Authors

2.2. Chemical analysis

This section defines and assesses targeted and non-targeted chemical analyses to monitor endocrine-disrupting compounds in water.

2.2.1. Targeted chemistry

Water quality management is typically done by the development of threshold values for concentrations of single chemicals found in freshwater, such as environmental quality standards, water quality criteria, or environmental norms. Targeted chemistry gives a direct conclusion on compliance with regulation: the chemical concentration is either below or above the threshold level. Countries develop threshold levels based on the available knowledge of the toxicity and the exposure levels of a chemical with the objective of protecting human and/or ecosystem health. The enforcement of those standards is done via classical single-chemical monitoring. In this type of monitoring, targeted chemical analysis is used to determine the concentration of an individual chemical of interest in a water sample. The concentration is then compared to the associated standard.

Targeted analysis can also be useful to monitor known highly active EDCs for which no quality standard exists. For example, EPA Victoria in Australia has conducted two monitoring campaigns on emerging contaminants in wastewater using targeted chemistry (Box 2.1). Data of such targeted analysis can be instrumental in linking the effects observed in bioassays to the culprit compounds (Section 2.3.1).

Box 2.1. Monitoring EDCs in wastewater and waterways in EPA Victoria in Australia

Despite a small number of incidents (e.g., spills due to factory fire), prevalence of endocrine disruptors or disruption has not been assessed in waterways in metropolitan Melbourne and regional Victoria, Australia. This lack of research was the driver for Victoria's Environment Protection Agency's (EPA) monitoring programme, which aimed to set a baseline and improve understanding of presence/absence of EDCs in wastewater and waterways. To fill this knowledge gap, EPA Victoria conducted two monitoring campaigns on Victorian waterways (2020) and influent and effluent waters (2021).

Wastewater treatment plant monitoring

Sewage influent and effluent waters from 30 wastewater treatment plants (WWTPs) were sampled across Victoria in 2021. Sites were selected based on the VicWater 2019 risk assessment on emerging contaminants in wastewater (O'Connor and Stevens, 2019[9]). For influent waters, 24-hour composite samples were collected. For effluent waters, grab samples and passive samples were deployed. Samples were analysed for EDCs as well as personal care products (PPCPs), pesticides, herbicides, PFAS and disinfection by-products (DBPs). Of the 21 EDCs measured, 13 EDCs were detected in influent waters, while 11 and 9 EDCs were detected in effluent from grab samples and passive[1] samples, respectively. The maximum predicted estradiol equivalent based on chemical concentration (EEQ_{chem}) was 83 ng/L and 13 ng/L for influent and effluent waters respectively. The mean percent of reduction rates across wastewater treatment trains for androsterone, BPA, estriol, estrone, etiocholanolone and nonylphenol were >66%.

Despite the detection of EDCs, there are only limited ecological guideline values available for EDCs in Australia (ANZG, 2021[10]). For example, concentrations of nonylphenol measured in samples exceeded low-reliability ecological guidelines for nonylphenol in freshwaters (0.1 µg/L, (ANZECC and ARMCANZ, 2000[11])). All concentrations were below the moderate-reliability international guideline for freshwaters (1 µg/L) and marine ecosystem protection (1 µg/, (ANZG, 2021[10]) and 0.7 µg/L, (CCME, 2021[12])). In this study, no exceedances were detected for drinking water guidelines (NHMRC and NRMMC, 2011[13]).

Waterway monitoring

In Victoria, 18 sites located along seven waterways were sampled in 2020 using a combination of passive samples and grab samples. Sites were chosen based on their proximity to WWTP (distance upstream and downstream) and with one urban waterway without WWTP identified as hotspot in an earlier EPA study (Sardiña et al., 2019[14]). Two reference waterways were selected downstream of areas of state forest and national parks. Population varies across the sites, with catchment land-use predominantly peri-urban with small areas of commerce, agriculture, and industry.

Only BPA was detected in grab samples and in four POCIS[1] samples. In the grab samples, the highest detection of BPA was found in an urban waterway that has no WWTP discharge points. The study data did not indicate any clear EDC concentration trends downstream from this hotspot site. Further research is required for unravelling point sources of EDC contamination.

Lessons learnt

1. A research project led by a regulator has its own challenges. Duty holders[2] are anxious about what the EPA will do with the data, especially if detected concentrations exceed guideline values. Some duty holders therefore withdrew from the sampling campaign or denied access to private land. Nevertheless, the general environmental duty (Environment Protection Act, 2017 (Victorian Government, 2017[15])) does not obviate a duty holder's responsibility to minimise the

risk of their activities harming human health and the environment, so far as reasonably practicable.

2. Limits of reporting are currently too high in commercial laboratories in Australia. As an example, in the current study, over 50% of water samples came back as non-detects. Non-detect data at µg/L level are not very useful, especially when research shows that for EE2 exposure to only 1.5 ng/L is enough to cause adverse effects in non-target organisms (Rehberger et al., 2020[16]).
3. Lack of guidelines and environmental reference standards for EDCs is a limiting factor for understanding the prevalence of EDCs in wastewater and natural waterway systems. In Australia, there are only a small number of guidelines and reference standards for EDCs, although this is improving (ANZG, 2021[10]; King et al., 2017[17]).
4. Despite the general environmental duty to minimise the risk of activities harming human health and the environment, so far as reasonably practicable, duty holders may claim that it is not reasonably practical to monitor EDCs in the environment, especially when analysis costs are AUD 300-500 per sample.

Note[1]: Polar Organic Chemical Integrative Sampler (POCIS)
Note[2]: A duty holder can be a person or entity that engages in an activity that may give rise to risks of harm to human health or the environment. For the specific definition of a duty holder, please refer to the Environmental Protection Act (Victorian Government, 2017[15]).
Source: Dr Minna Saaristo, EPA Victoria, Australia

Limitations

While targeted chemistry is widely used for various chemicals for water management, it is currently a rather limited approach within the EDC context for several reasons:

1. Currently only a few EDCs are covered in regulatory monitoring programmes. For example, the European Union Water Framework Directive (EU, 2000[7]) has Environmental Quality Standards for several suspected or conformed EDCs, such as Di(2-ethylhexyl)- phthalate (DEHP), nonylphenols, octylphenols, tributyltin compounds, perfluorooctane sulfonic acid and its derivatives (PFOS), brominated diphenyl ethers and hexabromo cyclododecane (HBCDD). However, this is only a small fraction of the more than 100 EDCs listed as identified, under evaluation, or considered as EDC on the platform Endocrine Disruptors Lists (edlists.org, n.d.[18])[1]. Moreover, endocrine disruptive substances monitored may not necessarily be the highest-potency substances. The scarcity of available standards can be linked to a lack of formal identification of EDCs, to insufficient data for their development and to inadequate methods for including endocrine endpoints (Chapter 3, Section 3.3.1). Despite this limitation in the regulatory context, targeted chemical analysis is still useful in monitoring known substances for which no quality standard exists.
2. EDCs can cause effects at very low concentrations (below ng/L). However, current chemistry analyses for common EDCs have limits of detection higher than the range required to evaluate their risk (see the example in Box 2.1). Hence, the available methods are ill-suited for the required risk assessment. Efforts are made to decrease the limits of detection, increase accuracy, and streamline sample processing (Metcalfe et al., 2022[19]). One example of such progress is the development of a method to detect steroids and bisphenols at levels as low as 0.1-0.5 ng/L (Goeury et al., 2022[20]). However, it will take time for those methods to be standardised and made accessible globally.
3. Chemical monitoring is a top-down approach that only scratches the surface of the problem (WHO-UNEP, 2013[21]) as an analysis of the wide array of chemicals present in an environmental sample is expensive and fundamentally impossible. This is due to limits in our knowledge of all existing

chemicals (“unknown unknowns”) - including breakdown and transformation products (Hecker and Hollert, 2009[22]).

4. Targeted chemical analyses do not address mixture effects (Brack et al., 2019[23]). Chemistry data is compared to individual standards and overlooks the risks posed by chemical mixtures. Bioassays can capture mixtures (Wernersson et al., 2015[5]) (Section 2.3.1).

2.2.2. Non-targeted analysis

As mentioned above, only a few chemicals are assessed in routine water monitoring programmes. It is estimated that only 5% of all known chemicals are monitored using targeted analyses (McCord, Groff and Sobus, 2022[24]). To address this issue, non-targeted analyses (NTAs) are increasingly used. Like the name indicates, NTAs do not have necessarily pre-defined target chemicals. Rather they aim to identify *all* chemicals present in an environmental sample, without quantifying the concentration of each chemical detected.

NTAs can analyse “known unknowns” and “unknown unknowns”. Most NTAs analyse “known unknowns”, which are chemicals of which at least the structure is classified in databases and of which some toxicity data is available. NTAs aim to include chemicals that are not yet regulated or routinely monitored. Those analyses are often referred to as suspect screening analyses (SSA) (Paszkiewicz et al., 2022[25]). SSA can also include the quantification of selected chemicals. NTAs can also look at “unknown unknowns” for which not even the molecular structure is clearly defined or registered in databases (Paszkiewicz et al., 2022[25]). High-resolution mass spectrometry (HRMS) is the typical method of choice for any type of NTA (McCord, Groff and Sobus, 2022[24]; Paszkiewicz et al., 2022[25]).

NTA is a useful screening tool to map EDCs and other chemicals present in water (McCord, Groff and Sobus, 2022[24]; Hollender et al., 2019[26]). Such methods are useful in developing a baseline or archive of the chemical composition of a water sample, in detecting accidental spills, in capturing (synthetic) EDCs that cannot yet be detected by bioassays, and in analysing mixtures of chemicals.

NTAs could help track the impact of pollution sources by looking at their specific fingerprint instead of by surveying specific chemicals (Brack et al., 2019[27]).For example, samples were analysed with NTAs at multiple sites of River Holtemme in Germany. By clustering the acquired data, researchers were able to identify patterns of chemicals specific to their sources of contamination, such as wastewater treatment plant (WWTP) effluents. The research even identified the contribution of each WWTP to the pollution in a section of the river (Beckers et al., 2020[28]).

Furthermore, NTA can provide a good digital record of chemical pollution over time (Alygizakis et al., 2019[29]; Hollender et al., 2019[26]). This can be used for retrospective analysis even for contaminants which were not of concern as endocrine active at the time of the measurement. Keeping records of NTAs can also help evaluate the evolution of pollution through time to see for example if a contaminant is ubiquitous (i.e., present all the time), if new contaminants were introduced, or if contaminants detected in prior studies disappeared. This information could be used in the long term to prioritise action for new and ubiquitous contaminants, as well as assessing the impact of remediation action (Brack et al., 2019[27]; Hollender et al., 2019[26]). The Norman Network has kept NTA records in a Digital Sample Freezing Platform (Norman Network, n.d.[30]).

NTA technologies are evolving into automated routine monitoring systems. This is exemplified by the case study of the International Rhine Monitoring station in Switzerland (Box 2.2). The automation of the workflow enables the station to monitor water quality daily with NTA. NTA has identified accidental spills and alerted drinking water treatment stations downstream. NTA has also led to mitigation action in a manufacturing company after the detection of a continuously released hazardous compound.

Box 2.2. Daily non-targeted analysis at the International Rhine monitoring station

The International Rhine monitoring station is located close to Basel, at the border between Switzerland and Germany. This station is managed under the International Commission for the Protection of the River Rhine which aims to protect the water quality of the river on which 20 million people rely for drinking water. Since 2012, the station monitors daily the water quality using liquid chromatography coupled with HRMS (LC-HRMS) and gas chromatography coupled with mass spectrometry (GC-MS). To be able to provide results within a day, the station designed a proper workflow of sample measurement, followed by an automated data processing. For the LC-HRMS data, automated analysis is provided for 320 suspects with their respective standard to follow long-term trends. Moreover, another 1,500 suspect chemicals are followed to identify accidental spill and continuous emission patterns.

The data obtained through the screening have two main purposes. The first one is to inform quickly on accidental spills that can occur upstream of the station. Spills trigger a warning to the downstream drinking water plant treatment station. In 2014, 10 major spill events were detected and led to the shutdown of downstream water production. Secondly, the daily screening provides a rich source of data for long-term monitoring. For example, in 2014, the compound tetracarbonitrile-1-propene was identified by the station as being continuously discharged by an upstream manufacturing industry. Moreover, the break in production of the compounds was detected as the concentration observed dropped to zero. Based on the data obtained, the company was approached to implement mitigating actions. In 2015, the monitoring station picked up the positive impacts of the mitigation actions by the company. The level of the compound stayed low in the following year. The success of this station prompted the opening of others on the Rhine River and other rivers in Europe.

Source: (Hollender et al., 2017[31])

Except when standards are used for SSA, most NTAs cannot be used in risk-based regulation as quantification remains a challenge. Still, NTAs could be useful in hazard-based regulation as only the presence of the chemical is sufficient to justify action (McCord, Groff and Sobus, 2022[24]). Hence, if an authority decides to adopt a hazard-based approach to EDCs, with a zero tolerance to EDCs present in a water sample, NTAs could be applied to detect the presence of substances. However, depending on the cost per sample, targeted chemical analysis may be more cost-effective.

While NTAs might not be readily useful for regulation, NTAs can be used to prioritise EDCs and other chemicals. For example, prioritisation of site-specific contamination can be done by looking at the rarity of a chemical in water, such as demonstrated in a German study (Krauss et al., 2019[32]). Moreover, the Environmental Agency (EA) of England, United Kingdom, is investigating how to integrate NTAs in their Prioritisation and Early Warning System (PEWS) for chemicals (Sims, 2022[33]).

Limitations

While NTA, combined with other methods, has a strong potential in the future monitoring of EDCs, there are still a lot of limitations for their use for regulatory purposes around the world.

1. NTAs are still mainly qualitative (McCord, Groff and Sobus, 2022[24]; Hollender et al., 2019[26]), as the concentration of each chemical cannot be determined, with the exception of standards used for SSA. Otherwise, only relative quantification can be done. Research efforts are being done to allow quantification for the purpose of risk assessment using surrogate standards or modelling responses based on chemical structure (McCord, Groff and Sobus, 2022[24]). Until those methods are mainstreamed, non-targeted chemistry can be used for pre-screening, setting a water quality baseline of known and unknown substances present in water, and prioritisation. NTAs could also

be useful in the context of hazard-based approaches that do not tolerate any presence of certain substances, though chemical analysis might be more cost-effective for these purposes.

2. NTA is not standardised, time-consuming and requires analytical expertise (McCord, Groff and Sobus, 2022[24]; Paszkiewicz et al., 2022[25]), which makes those methods more difficult to apply on a regular basis. To use NTAs for regulatory purposes, there is first a need for standardisation and harmonisation of methods to ensure the quality of data (McCord, Groff and Sobus, 2022[24]; Luo et al., 2022[34]; Hollender et al., 2019[26]). Efforts are also made to make the technology quicker and more accessible (e.g., price and expertise requirement) (Hollender et al., 2019[26]). There is a need for automation of the data processing for high-throughput analysis (McCord, Groff and Sobus, 2022[24]).
3. There is a growing need to develop databases for sharing NTA data to enable their comparison, retrospective analysis, facilitate technical support by experts and increase international collaboration (Hollender et al., 2019[26]). Some databases already exist, such as the Global Natural Products Social Molecular Networking (GNPS) (Wang et al., 2016[35]) or the Digital Sample Freezing Platform (DSFP) from the Norman Network (Alygizakis et al., 2019[29]). Data acquisition needs to be harmonised to facilitate data submission and data comparison. Organisations such as the International Commission for the Protection of the River Rhine (ICPR) are working towards that goal (Hollender et al., 2019[26]).
4. Most NTAs concentrate on known chemicals for which the chemical structure has at least been identified. There is a need to increase spectra identification to increase the information available in databases such as the NORMAN MassBank (NORMAN Network, n.d.[36]). However, some chemicals might not be detected and efforts need to be put in place to improve the method to enable the discovery of new chemicals (Escher, Stapleton and Schymanski, 2020[37]).

2.3. Biological analysis

This section presents and discusses the advantages and disadvantages of bioassays and *in situ* wildlife monitoring, two biological approaches that can be used to monitor the adverse effects of EDCs in water.

2.3.1. Bioassays

A promising approach to solve the issues linked to chemical monitoring for EDC risk assessment in freshwater is effect-based monitoring or effect-based methods (EBM). Like the name suggests, this monitoring approach is based on the detection and quantification of effects caused by chemicals found in a sample (Brack et al., 2019[23]). This type of monitoring uses bioanalytical methods, or bioassays. Bioassays are biological test methods performed using *in vitro* (cell-based or cell-free) or *in vivo* (whole organism) models to detect effects in a concentration-dependent manner on toxicological endpoints of concern (Brack et al., 2016[38]; Robitaille et al., 2022[4]). They consist of testing the biological activity of a sample using responses of (sub)cellular systems or whole organisms (Brack et al., 2016[38]). Box 2.3 contains a simple explainer of bioassays.

Box 2.3. What is a bioassay? A simple explainer.

A bioassay is nothing more than a cell, fish or frog embryo, or animal used to test whether a chemical, or water, is toxic. When something is toxic, the bioassay will "tell" so by lighting up or by giving another signal. For example, a cell or genetically modified fish that lights up when a chemical triggers a small change in a fish[1]. In animals, bioassays can show a physical change, such as a change in the number of eggs, presence of specific proteins or steroids in blood, or a change in organs (more masculine or feminine than before).

Bioassay experts often refer to "cell lines" or "animal lines". Cell lines are cells from animal organs used for testing, often originating from tumours. Such cell lines can be purchased from companies or are developed by academic laboratories. Cell lines always come from the same source, or the same "mother cell", and are reproduced so that effects and results can be compared. This is different for whole animal assays ("*in vivo*" assays), where researchers only need the same species which do not necessarily stem from the same parent. Some, more complex, bioassays can detect multiple effects.

Note[1]: Genetically modified cells or fish have been added a "green fluorescent protein" or the luciferase gene which gives the ability to firefly to generate light, which lights up when an endocrine mechanism, or other relevant effect depending on the bioassay, is activated.
Source: Authors

If a bioassay (that measures an endocrine mode of action) responds to a water sample, it indicates potential endocrine activity in the water sample. Bioassays do not directly identify the chemical triggering the activity, but they provide signal that there is a potential concern. Bioassays are often, but not always, more sensitive than chemical analysis. There is a high correlation between results found in bioassays and chemical measurements, indicating that both methods agree on the overall endocrine potential of samples (Könemann et al., 2018[39]; Escher, Neale and Leusch, 2021[40]). However, bioassays and chemistry do not correlate well at low concentrations because, first of all, bioassays can detect activity below the limit of detection (LOD) of chemical methods, and second, bioassays can detect mixtures from chemicals that are individually below their LOD.

Bioassays can be used regardless of any prior knowledge on the chemical composition of the water sample. Any chemical, known and unknown EDCs, that triggers an activity in a bioassay could be detected. Furthermore, bioassays will inform on the activity of chemical mixtures found in the sample. Since mixtures are still characterised by uncertainty, their identification is a critical added value of bioassays (Box 2.4).

Box 2.4. Mixture assessment is complex

Freshwater contains complex mixtures of naturally occurring and man-made chemicals (see Box 1.3 Chapter 1 for more on how mixtures are grouped). Such mixtures can have an affect on humans and wildlife. Assessing the composition and the potential effects of whole mixtures, such as those found in freshwater, can be difficult (Bopp et al., 2019[41]; Kortenkamp and Faust, 2018[42]). As described in this publication, different monitoring tools can be used to assess mixtures of chemicals such as for endocrine disruptors. First, bioassays detect activity, such as endocrine activity, in mixtures. Secondly, chemical analysis can determine the composition of a mixture. Finally, effect-directed analysis can help identify chemicals that caused the effect (Altenburger et al., 2015[43]; Escher, Stapleton and Schymanski, 2020[37]). While those tools are evolving, the assessment of the effect of mixtures only based on the chemical composition is still complex and poorly understood. Currently, mixture toxicity is mainly predicted using information on the effects of single chemicals and assuming that when multiple chemicals found in the mixture have similar effects, their effects will be additive (Luo et al., 2022[34]; Bopp et al., 2019[41]).

There are many initiatives that aim to better understand the impact of environmental (Luo et al., 2022[34]). For water risk assessment, the EU project SOLUTIONS aimed to develop several tools and methods for the monitoring and assessment of mixtures (Brack et al., 2015[44]). Other projects developed *in silico* methods to characterise mixture toxicity by improving the knowledge between chemical composition and *in vitro* and *in vivo* bioassay results (Luo et al., 2022[34]). This could help predict effects solely based on chemistry. The initiative EDC-MixRisk compiles data and epidemiological studies to better understand the impact of EDC mixtures on health. EDC-MixRisk particularly focuses on children and foetuses. The PANORAMIX initiative looks at improving the use of methods such as bioassays and EDA, the development of effect-based trigger values and the modelling of chemical mixtures for human biomonitoring (Vinggaard et al., 2022[45]). More information on the risk assessment of mixtures can be found in the OECD series on testing and assessment No. 296 (OECD, 2018[46]).

Source: (Bopp et al., 2019[47]; Kortenkamp and Faust, 2018[42]; Altenburger et al., 2015[43]; Escher, Stapleton and Schymanski, 2020[37]; Luo et al., 2022[34]; Brack et al., 2015[44]; Vinggaard et al., 2022[48]; OECD, 2018[49])

For endocrine activity and endocrine disruption, bioassays are designed to detect endocrine-specific endpoints (Table 2.1). The most studied endpoints are the EATS modalities: Estrogen, Androgen, Thyroid and Steroidogenesis. Estrogen modalities are well studied. Modalities for invertebrates are also gaining traction: Juvenile Hormones (Jh) and ecdysteroids (Ec) (OECD, 2018[50]). Thyroid disruption is notably known for disrupting metamorphosis in amphibians. Effects on the glucocorticoid receptor and transthyretin (TTR) displacement have also been observed in freshwater, but these effects are less well studied (OECD, 2022[51]). For water testing, the most common endpoint evaluated involves the interaction of chemicals with hormone receptors, especially nuclear receptors for:

- Estrogen (ER),
- Androgen (AR),
- Thyroid hormones (TR),
- Progesterone (PR),
- Glucocorticoids (GR).

Other bioassays look at the synthesis of hormones (steroidogenesis assays) or at the hormone transport in blood (transthyretin binding assay) (Robitaille et al., 2022[4]). In whole organisms, endpoints such as

fecundity, growth, metamorphosis for amphibians and biomarkers (e.g. vitellogenin, female egg yolk precursor) can be measured (Table 2.1).

Table 2.1. Overview of bioassays standardised* based on EATSJh modalities

Modalities	Bioassays	Standardised protocol	Type	Endpoint
Estrogen (E)	ERTA (Estrogen Receptor Transactivation Assay)	OECD TG 455, Water: ISO 19040-3:2018	*In vitro*	Receptor transactivation
	YES (Yeast Estrogen Screen)	Water: ISO 19040-1:2018, 19040-2:2018	*In vitro*	Receptor transactivation
	EASZY (Detection of Substances Acting Through Estrogen Receptors Using Transgenic cyp19a1b-GFP Zebrafish Embryos)	OECD TG 250	*In vivo* (fish embryo)	Receptor transactivation
	REACTIV (Rapid Estrogen Activity Tests in vivo)	OECD TG under development	*In vivo* (fish embryo)	Receptor transactivation
	Estrogen receptor binding affinity	OECD TG 493	In vitro	Receptor binding
	Uterotrophic Assay**	OECD TG 440	*In vivo* (immature or ovariectomised female rat)	Weight of uterus
Androgen (A)	ARTA (Androgen Receptor Transactivation Assay)	OECD TG 458	*In vitro*	Receptor transactivation
	RADAR (Rapid androgen disruption adverse outcome reporter)	OECD TG 251	*In vivo* (fish)	Receptor transactivation
	AFSS (Androgenised female stickleback screen)	OECD GD 148	*In vivo* (female fish)	Spiggin level
	JMASA (Juvenile Medaka Anti-Androgen Screening Assay)	OECD TG under development	*In vivo* (fish)	Papillary development in male (sexual secondary characteristics)
	Hershberger Assay**	OECD TG 441	*In vivo* (castrated male rat)	Weight of male sexual organ
Thyroid (T)	XETA (Xenopus Eleutheroembryonic Thyroid signaling Assay)	OECD TG 248	*In vivo* (frog embryo)	Receptor transactivation
	AMA (Amphibian metamorphosis assay)	OECD TG 231	*In vivo* (frog)	Weight, length of body part, development stage, thyroid histology
Steroidogenesis (S)	H295R steroidogenesis assay	OECD TG 456	*In vitro*	Synthesis of estrogen and testosterone
Reproduction (EAS)	FSTRA (Fish short-term reproduction assay)	OECD TG 229	*In vivo* (adult fish)	VTG level, secondary sexual characteristics, fecundity (number of eggs), gonad histology
	21-day fish assay	OECD TG 230	*In vivo* (adult fish)	Idem as FSTRA except for fecundity and histology
Juvenile hormones (Jh)	SJHASA (Short-term juvenile hormone activity screening assay using Daphnia magna)	OECD TG under development	*In vivo*	Number of offspring and sex ratio

Note 1: This table refers to ISO methods and OECD Test Guidelines (TG) for level 2 and 3 of the OECD conceptual framework (OECD, 2018[50]) which are the more applicable bioassays for water testing. It is important to note that OECD Test Guidelines are not standardised for the analysis of water samples, while the ISO methods presented are specifically designed for the purpose of water testing.
Note 2: Bioassays in rats are not commonly used for assessing EDCs in freshwater (Robitaille et al., 2022[4]), but could probably be used to assess drinking water. See also Section 2.6.4 on animal testing.

Box 2.5. Incorporating bioassays in California's policy for recycled water

In 2009, the California State Water Board (SWB) adopted the Recycled Water Policy to "increase the use of recycled water in a manner that is protective of public health and the environment" (State Water Board Resolution No. 2009-0011). Southern California Coastal Water Research Project Authority (SCCWRP), a joint power agency has assisted the SWB to develop a management strategy for contaminants of emerging concern (CECs).

Since 2010, SCCWRP facilitated an international panel of experts to review existing CECs data and identify novel technologies to improve CEC monitoring. The panel recommended to supplement conventional targeted chemical monitoring with *in vitro* cell bioassays. Based on the recommendations of the expert panel, SCCWRP worked on the standardisation of cell bioassay protocols, guidance for developing a CEC monitoring programme and performed case studies for ambient and recycled water (Dodder, Mehinto and Maruya, 2015[52]; SCCRWP, 2014[53]; Mehinto et al., 2015[54]). SCCWRP and the expert panel also proposed a tiered monitoring framework that incorporates *in vitro* bioassays as a first step to identify sites requiring further chemical and biological analyses (Maruya et al. 2016). The last panel convened to address CECs in recycled water, recommended the incorporation of two *in vitro* bioassays in the state recycled water policy. The SWB followed these recommendations and in 2018, the policy was amended to include *in vitro* bioassays for Erα and AhR with reporting limits set at 0.5 ng/L E2 or TCDD equivalent respectively (California State Water Board, 2018[6]). To support implementation of the policy, workshops and guidance documents were produced to educate and train the utilities and testing laboratories (NWRI, 2020[55]). In 2020, recycled water utilities to started quarterly bioassay monitoring, for a period of 3 years. During this phase, no specific follow-up actions have been mandated.

Lessons learnt

While much progress has been made, SCCWRP highlights the need for international collaboration and consensus to facilitate the implementation of EBMs more broadly. Test guidelines are often insufficient as they do not include sample processing, data analysis and interpretation, and are limited to one or two vendors. Standardised protocols (from sample collection to data analysis) with quality assurance criteria and reporting requirements, vetted through interlaboratory comparison exercises, are needed to demonstrate robustness of bioassays for relevant sample matrices and for diverse laboratories (academia, industry, government). There is also a need for performance-based validation of bioassays to enable more vendors to provide products. Finally, there is a need for guidance for the development of monitoring thresholds and risk management.

Finally, outreach and communication are key. Stakeholders were engaged throughout the projects via advisory committees. SCCWRP hosted multiple workshops with academics, vendors, and testing laboratories as guest speakers for stakeholders including policy makers, utilities, and private laboratories. SCCWRP also offered laboratory demonstrations and hands-on practice.

Source: Presentation of Dr. Alvine C. Mehinto, Head of the toxicology department of SCCWRP, California, United States at the OECD Workshop on Developing Science-Informed Policy Responses to Curb Endocrine Disruption in Freshwater, 18-19 October 2022 (OECD, 2022[51])

Bioassays can also be informative in risk assessment and thresholds similar to chemical standards can be developed. These types of thresholds are generally referred to as effect-based trigger values (EBT) (Escher et al., 2018[56]). Effect-based trigger values are the threshold values, or water quality indicators, for bioassays. EBTs help interpret whether the effects detected in a bioassay are acceptable or not (Neale et al., 2023[57]). More information on setting EBTs for bioassays is given in Section 2.6.1.

For water quality monitoring, it is recommended to use a set of different bioassays to obtain a complete picture of the different effects present in a water sample (Neale, Leusch and Escher, 2020[58]). After all, a single bioassay can only detect one or a few modalities, whereas a set of bioassays - applied at the same time, covering multiple modalities or endocrine endpoints - make the water quality assessment more comprehensive. A set of bioassays is referred to as a "battery of bioassays". There is no standard recommendation for a battery of bioassays, and different methods are used by various countries. It should be noted that, generally, batteries of bioassays comprise more effects than endocrine activity, depending on the monitoring purpose (Escher, Neale and Leusch, 2021[40]). Some suggest that a minimal battery of bioassays should include testing for ER, AhR and oxidative stress, adding genotoxicity in drinking water research (Escher et al., 2014[59]; Neale et al., 2022[60]; Rosenmai et al., 2018[61]).

Limitations

While the interest in bioassays for water quality monitoring is growing, bioassays largely remain non-standardised tools, except for several whole organism tests that are not favoured for routine water quality monitoring due to concerns related to animal testing. This situation hinders their widespread adoption for water quality regulation and policy. Gaps that hinder the mainstreaming of effect-based monitoring approaches are the following:

1. Effect-based trigger values (EBT) need to be in place to determine the level of risk of each observed effect. However, most bioassays do not have a harmonised or internationally agreed standard or trigger value that determines to what extent the observed effect is (potentially) harmful (Escher et al., 2018[56]). This remains up to the discretion of individual water authorities, academia, industries, and bioassay developers. This gives rise to a patchwork of trigger values and diagnostic tools. Moreover, it is currently dependent on the formal identification of EDCs which can be a long and tedious task. Sections 2.6.1 discusses effect-based trigger values in more detail.
2. There is a lack of standardisation for bioassay methods, sample collection and preparation, result analysis, and the calculation of biological equivalent concentrations (BEQ). Such standardisation methods are available for chemical assessments, but the options are limited when it comes to water quality assessment. For water monitoring, standardised ISO methods are only available for specific estrogenic bioassays (ISO 19040 series), the calculation of BEQ (ISO 23196:2022) and water sampling (ISO 5667 series) (Table 2.1). Developing performance standards for bioassays can level the playing field for vendors wishing to enter the bioassay market and accelerates the validation of methods (see also the case study of California, Box 2.5). Finally, there is a need for technical guidance for regulators and utilities on how to apply bioassays (Neale et al., 2022[60]). Platforms, such as the Water Safety Portal (WHO and IWA, n.d.[62]), could host case studies and guidance documents. Section 3.5.2, Chapter 3, discusses standardisation in more detail.
3. Countries have different levels of bioanalytical capacity. Laboratories with the capacity to process and analyse (water quality-related) bioassays are scarce in many countries. This also includes the infrastructure for animal facilities for *in vivo* bioassays or cell culture laboratories for *in vitro* bioassays. Laboratory infrastructure is discussed in Section 2.6.3.
4. There is still a lack of specific, validated bioassays for several modes of action (European Environment Agency, 2020[63]; Brack et al., 2018[64]; Robitaille et al., 2022[4]). For example, estrogenic bioassays have more methods than any other endpoints (Table 2.1). In contrast, the thyroid modality has no test guidelines for *in vitro* bioassays. There is a need to invest in method validation for other endocrine endpoints to consider a broad range of effects related to endocrine disruption (Martyniuk et al., 2022[65]) (see also Box 3.8 on the Pepper platform). The EURION initiative (European Cluster on Identification of Endocrine disruptors) aims to bridge the gaps for non-EATS pathways, such as for metabolic disease, thyroid, neuroendocrine hormones, and for the female reproductive system (Martyniuk et al., 2022[65]; EURION, n.d.[66]). Method validation is

a long, costly, and tedious process. Section 3.5, Chapter 3, discusses validation and makes recommendations for improvement of the validation process.

5. There is still a lack of confidence in the ability to extrapolate the results from *in vitro* bioassays to their outcomes in humans or ecosystems (see also Box 2.6 on Adverse outcome pathways). More work needs to be done on quantitative *in vitro* to *in vivo* extrapolation. This could also help decrease animal use in the long. This aligns with the objective of programmes for the evaluation of single-chemicals such as the ToxCast/Tox21 of the US (Dix et al., 2007[67]; Krewski et al., 2010[68]), the EU-ToxRisk and ONTOX in the EU (Daneshian et al., 2016[69]; Vinken et al., 2021[70]), and the OECD guidelines for the evaluation of EDCs (OECD, 2018[50]). Moreover, *in vitro* bioassays do not mimic the exact effects happening in a whole organism. This includes, for example, the bioavailability of compounds, the uptake, metabolism, distribution and excretion (ADME) of substances, the impact of chronic exposure or even the sensitivity. Research is ongoing to increase the realism of *in vitro* bioassays (Robitaille et al., 2022[4]). It should be noted that, for the purposes of water quality monitoring, a bioassay does not need to represent the exact impacts on whole organisms, just like targeted chemical analysis does not represent the exact impact on whole organisms. The purpose is to get an indication of potential risks present in a water sample.
6. For ecosystem protection, bioassays need to be developed to include a diverse range of species. Most bioassays are designed for human receptors (Robitaille et al., 2022[4]). While hormones are generally conserved across species, proteins such as hormone receptors have evolved independently, which could lead to some differences in sensitivity. For ambient water quality monitoring aiming to protect aquatic ecosystems, it would be ideal to have access to *in vitro* bioassays representing a higher diversity of species.
7. Current test guidelines for individual bioassays do not give a full picture of water quality as this would require a battery of bioassays (Di Paolo et al., 2016[2]; Brack et al., 2019[23]). Developing a battery of bioassays requires specialised expertise.

Box 2.6. Adverse outcome pathways

An Adverse Outcome Pathway (AOP) describes a logical sequence of causally linked events at different levels of biological organisation, which follows exposure to a stressor and leads to an adverse health effect in humans or wildlife. AOPs have been used as a tool to formulate pathway linkages among molecular events and toxicity (see also Figure 1.8, Chapter 1). Chemicals initially interact with a molecular target (the “molecular initiating event” or MIE). The MIE initiates a biological cascade of events; triggering effects in cells, tissues and organs (Key Events) that potentially result in an adverse outcome in an individual or population. The description of this cascade of biological events is called an AOP.

AOPs are conceptual frameworks that help to build biologically supported links between data measured at different biological levels and in different tests. AOPs can organise available data, identify information gaps, direct next steps for safety testing, and develop novel approaches for chemical safety testing that, in some cases, may reduce the need for testing chemicals in animals. This approach combining results from multiple methods can be used to predict an adverse outcome *in vivo* from methods that can be conducted quickly, at low cost, and do not use animals (called predictive toxicology).

The OECD hosts the Adverse Outcome Pathway Knowledge Base (AOP-KB) (OECD, n.d.[71]): a resource for research, test method development, and regulatory decision-making. Endocrine-related AOPs in the AOP-KB are for instance: Androgen AOPs, Oestrogen AOPs, and Thyroid AOPs.

Source: Cited from (OECD, n.d.[72]; OECD, 2017[73]; OECD, n.d.[71])

2.3.2. *In situ* wildlife monitoring

While bioassays are interesting for routine risk management, they might not completely capture the ecological consequences of endocrine disruption (Windsor, Ormerod and Tyler, 2018[74]). *In situ* wildlife monitoring methods survey species in the wild for any significant physical, molecular or behavioural changes, which could indicate changes in the Predicted No-Effect Concentration (PNEC).

By analysing water samples only in a laboratory setting, water regulators may overlook impacts that are happening in the wild. For example, fish surveys helped identify reproduction issues in various water bodies across the world close to wastewater treatment plants and industries (Jobling et al., 1998[75]; Marlatt et al., 2022[76]; Sumpter, 2005[77]; Hewitt et al., 2008[78]). Those studies led to the identification of compounds found in wastewater, such as EE2, which could lead to endocrine disruption. Another example of the necessity of *in* situ wildlife monitoring is the observation of the development of male sex organs, known as imposex, in sea snails (Ellis and Agan Pattisina, 1990[79]; Smith, 1981[80]; Beyer et al., 2022[81]). Imposex was later linked to tributyltin (TBT), a biocidal agent in boat paint, which led to its ban (Beyer et al., 2022[81]). Increased wildlife monitoring would benefit research both into bioaccumulation/bioconcentration and into the differences between species, especially invertebrates, in which data are scarce (Fernandez, 2019[82]). Moreover, currently available bioassays would have overlooked the activity of TBT, as its main mechanism of action (via the retinoid X-receptor) is not assessed in most bioassays (Beyer et al., 2022[81]).

In situ surveys rely on the study of indicator species. Those indicator species are used to assess the changing quality of an environment in relation to pollution (Siddig et al., 2016[83]). Species selected as indicators are ideally sensitive to changes in their environment, are local and commonly distributed on the territory of interest, representative of their ecosystem, and well documented. Species can also be selected based on their cultural or economic importance (Hutchinson et al., 2006[84]). Moreover, wildlife monitoring programmes ideally evaluate more than one species to have a better representation of an ecosystem. An example of a such programme that was able to assess endocrine disruption is the Environmental Effects Monitoring (EEM) programme in Canada (Box 2.7). EEM surveys fish to ensure the protection of fish health and their habitat under the Fisheries Act regulations (Environment Canada, 1998[85]) in part to protect the fishing industry and for conservation.

For the selected indicator species, specific biomarkers are measured. Biomarkers act as indicators of a change in a biological organism. In the study of contaminants, biomarkers aim to evaluate either exposure (i.e. evaluate if the organism was in contact with a contaminant) or effect (i.e. evaluate if the organism was affected negatively by its environment) in a given organism (Hutchinson et al., 2006[84]). Any measurable change can be called a biomarker, ranging from physiological (e.g. body and organ mass, tissue histology, sexual secondary characteristics) to molecular change (e.g. protein production and gene expression) (Hutchinson et al., 2006[84]). It should be noted that biomarkers may have different meanings depending on the context and species at hand (Dang and Kienzler, 2019[86]). One of the most widely used biomarkers in associated with endocrine activity is the presence of vitellogenin (VTG) in the blood or liver of organisms. VTG is a precursor of the egg yolk, making it a biomarker for females as males do not produce eggs. It can be used, for example, to detect if a male fish was exposed to estrogenic substances as the production of VTG will have increased (Hutchinson et al., 2006[84]). The EEM programme in Canada studied biomarkers comprising age, weight-at-age, condition factor (weight/length^3), and relative weight of the liver and gonads (Box 2.7).

The data collected during *in situ* wildlife monitoring can be used to assess the health of selected species or the ecosystem in general. This risk assessment will normally involve the comparison of the site of interest to a reference site (e.g. upstream of discharge) which is considered not polluted. If a significant change is detected in the health of selected species between both sites, it can be necessary to take action. For example, in the EEM programme, trigger values were established over time for specific fish biomarkers. When the values are exceeded, this triggers an investigation procedure by industry, which can lead to actions to mitigate the problem (see details in Box 2.7).

Box 2.7. An industry-funded monitoring programme leading to action: the case study of the Environmental Effect Monitoring (EEM) programme in Canada

In the early 1990s, research identified that fish at one Canadian pulp mill effluent discharge site had smaller gonads (ovaries and testes). These effects were similar to those documented in fish downstream of Swedish pulp mills in the late 1980s (McMaster et al., 1992[87]; McMaster et al., 1991[88]; Munkittrick et al., 1992[89]; Sandström, Neuman and Karås, 1988[90]). To allow the government to assess (over time) whether the same effects occurred at most mills or just a few, Environmental Effects Monitoring (EEM) in Canada was incorporated in the Fisheries Act regulations (Environment Canada, 1998[85]).

EEM is a programme used to assess the adequacy of current effluent regulations in Canada that goes beyond chemical assessment and toxicity testing. EEM is a cyclical (every 3 years), industry-funded assessment of specific measurements of wild fish health upstream (reference fish) and downstream (exposed fish) for effluents from pulp & paper mills, and metal and diamond mines. The monitoring and decision-making are focussed on whether wild fish are growing, surviving, reproducing normally, and whether they have enough to eat.

Monitoring strategy

Under EEM, the same measurements must be taken at each pulp & paper mill across Canada. The endpoints that EEM measures in the wild fish are indicators of growth, health, and reproductive potential: age, weight-at-age, condition factor (weight/length3), relative liver weight and relative gonad weight. These specific measurements are taken in two species of wild fish, with 20 adult males and 20 adult females sampled for each species. EEM also assesses whether the fish have good habitat and enough to eat, by assessing the benthic community structure (the numbers and types of invertebrates that live in the sediments). Other parts of EEM assess contaminants in fish tissues and provide chemical and chronic toxicological information on the effluent (Environment Canada, 2010[91]). Methodologies are contained in guidance documents issued by the Government (Environment Canada, 1998[85]; Environment Canada, 2005[92]).

Trigger values (critical effect sizes)

Deciding the fish “trigger values”, or “critical effects sizes”, for further action was important to EEM. Trigger values the amount of change in wild fish health needed to act. A 25% change in fish age, weight-at-age, relative liver of gonad size, or a 10% change in fish condition factor would be the trigger values (Lowell et al., 2005[93]). These values were chosen based on a comprehensive literature review and abundant data from 125 pulp mill sites over 4 cycles (12 years), with 2 fish species at each site (Munkittrick et al., 2009[94]).

Who does the work?

EEM sets out what is required and who does what at each stage of the process. A 3-year monitoring cycle includes one year for planning (and approval by government) of sampling design, one year for field sampling (data collection), and one year for reporting the findings (Environment Canada, 2010[91]; Environment Canada, 2012[95]). The industry pays for the monitoring, which is typically accomplished by hiring a consultant to design and complete the monitoring, analyse the data, and submit the report. The federal government provides guidance on how to do EEM, how to analyse the data, and the report format. The government also assesses the initial individual field sampling plans, collects the data after each monitoring cycle, and analyses national patterns (Environment Canada, 2010[91]; Environment Canada, 2005[92]).

Decision tree

A decision tree is used to decide on the next steps based on the findings of the previous EEM studies at a given site (Environment Canada, 2010[91]). Decisions can be made to drop to less frequent monitoring (every 6 years) if there are no effects observed over two monitoring cycles of 3-years. If significant effects are observed two cycles in a row, and if they exceed the trigger values, then more detailed studies to assess the extent and magnitude of the change are launched in the next 3-year cycle (Environment Canada, 2010[91]). For that, the next 3-year cycle will study more fish at more locations downstream to see how far the effect goes and how much of a change is seen. This 'Extent and Magnitude' phase is optional, as in reality, pulp mills that discovered their effluents were negatively affecting fish wanted to solve the problem by launching 'Investigation of Cause' studies. Investigation of cause can be studied in individual mills, assessing the areas of the facility where the potent effluents come from and which chemicals are causing the changes in fish (Dubé and MacLatchy, 2000[96]; Dubé and MacLatchy, 2001[97]; MacLatchy et al., 2010[98]; Shaughnessy et al., 2007[99]; Belknap et al., 2006[100]; Hewitt et al., 2008[78]).

Use of monitoring information

The extensive monitoring information provided by the EEM programme can be used to improve national practices (e.g., data reporting, adapt methods to difficult environments) and to assess national patterns. For pulp mill effluents, the first decade of EEM studies (from 1992 through 2003) were combined to give an assessment of national patterns of their effects in wild fish across Canada (Lowell et al., 2005[93]; Munkittrick et al., 2002[101]). The two dominant patterns were eutrophication (larger fish, larger organs) and metabolic disruption (a type of endocrine disruption where fish were growing larger and putting more energy into growth, but their ovaries and testes were smaller, so putting less energy into reproduction).

Developing mitigation action through research collaboration

To address the two observed dominant patterns, a national collaboration between industry, government, academia and the private sector investigates changes in fish by pooling funds and research efforts (Kovacs et al., 2007[102]). This collaboration led to the development of several laboratory fish bioassays to be able to observe the effects seen in the field (Martel et al., 2010[103]; Parrott et al., 2010[104]; van den Heuvel et al., 2010[105]). In this case, pulp mill effluents that caused small gonads in wild fish also stopped egg production in adult minnows (measured after 1–3-week exposures in the lab) (Kovacs, Martel and Ricci, 2007[106]; Martel et al., 2010[103]; van den Heuvel et al., 2010[105]). The developed fish reproduction bioassay was then used for testing effluents and to identify classes of chemicals which could be linked to the observed effects (Martel et al., 2010[103]; Environment Canada, 2014[107]). Those studies revealed that the lowered egg production caused by exposure to many pulp mill effluents correlated well with biological oxygen demand (BOD) of the effluent, measured as mg/L oxygen consumed in 5 days) (Kovacs et al., 2013[108]; Martel et al., 2017[109]). Mills with low BOD generally had effluents that did not impact egg production (Kovacs et al., 2013[108]; Martel et al., 2017[109]). This resulted in advice given to the mills to target their BOD to be lower than 20 mg/L and on ways to reduce the problem chemicals by ensuring in-mill processes, spill control and treatment systems were functioning optimally (Kovacs et al., 2013[108]; Kovacs et al., 2011[110]; Martel et al., 2011[111]; Martel et al., 2017[109]; Environment Canada, 2014[107]). A follow-up study will help confirm whether reductions in BOD release resulted in the improvement of endocrine disruption (reduced investment of energy into reproduction) in fish downstream (Environment and Climate Change Canada, 2019[112]; Environment and Climate Change Canada, 2020[113]).

Lessons learnt and challenges

One of the main strengths of EEM lies in its consistency in monitoring. The same endpoints are assessed consistently (every 3 years) across all sites (pulp mills or metal/diamond mines). This consistency helps provide enough data at each site for risk assessment, but also to determine national patterns. Another strength of the EEM is its decision tree approach which clarifies the decision process and gives incentive to industry to improve their treatment by decreasing monitoring from every 3 years to every 6 years.

The other novel aspect of EEM is the "Investigation of Cause and Investigation of Solutions" component. If mill effluents were causing deleterious effects, they had to find out the cause and fix the problem. This could be done individually or jointly by several mills. For pulp & paper mills, when all the stakeholders pulled together, they overcame obstacles of working in isolation, pooled their resources and expertise, and found solutions.

Some of the obvious lessons from EEM were that good data collection and good science take time. Patience was required to plan and complete the work, and to wait 6 years for the EEM results to come in from the first 2 cycles. For pulp mill effluents, the patterns of effects shown in cycle 3 (after 9 years) revealed the national pattern of metabolic disruption in fish downstream. This, combined with the trigger values, was what launched the investigations into causes and solutions. Another lesson learnt during the 'Investigations of Cause and Solutions' studies is that the specific, causative chemicals did not need to be known if a solution for their removal was found. Moreover, without looking specifically for endocrine disruptors, EEM has detected endocrine disruption in wild fish living downstream of pulp mill effluents over the past 30 years.

Source: Case study provided by Dr Joanne Parrott, Dr Mark McMaster, Dr Mark Hewitt, Environment and Climate Change Canada (ECCC)

Limitations

As with all monitoring methods, *in situ* wildlife monitoring has limitations:

1. There is a need to develop more biomarkers for all modes of action of EDCs. For example, VTG, one of the most used biomarkers for endocrine disruption, is not adapted for all species, such as invertebrates (Windsor, Ormerod and Tyler, 2018[74]) and can present problems of variability (Hutchinson et al., 2006[84]). Biomarkers need to include more mechanisms of action for endocrine disruption, covering all key characteristics of EDCs (La Merrill et al., 2020[114]) (see also Box 1.2, 'Ten key characteristics of endocrine disrupting chemicals'). Omics (transcriptomics, proteomics and metabolomics) can help in the discovery of new biomarkers and could eventually help risk assessment in the future (Martyniuk, 2018[115]).
2. *In situ* wildlife monitoring often looks at one or a small subset of indicator species which can mischaracterise the impact on the whole ecosystem. There is a need to include more species in those surveys to increase the understanding of the food-web and cascade of consequences of EDCs on the trophic system, as well as to take into account biodiversity in groups of species such as fish and invertebrates (Fernandez, 2019[82]; Saaristo et al., 2018[116]; Windsor, Ormerod and Tyler, 2018[74]). Moreover, whilst hormones are generally conserved among most species, the effects of EDCs can differ among species (Hutchinson et al., 2006[84]). Hence, looking at only a few selected species might bias risk assessment.
3. Wildlife surveys are generally field intensive, expensive, time consuming and involve mostly lethal or invasive sampling for species. New technology like environmental DNA (eDNA) (Box 2.8) could help survey the presence of species by reducing the burden of field work as well as reducing lethal and invasive methods. The former point can be of high importance when dealing with endangered species.

4. The data developed by wildlife monitoring programmes can be difficult to link to EDCs or pollution. The EEM programme in Canada illustrates this challenge (Box 2.7). It took several years to gather the evidence on pulp mill effluent effects on fish health and to develop a fish bioassay before being able to mitigate the cause. Moreover, data interpretation within species may require additional evidence. For instance, non-EDCs could trigger a change in fish and changes in fish species can be linked to pathways other than estrogen, androgen and steroidogenesis (Dang, 2014[117]).
5. There is a need to develop tools that assess the risks of pollution at the ecosystem level rather than at the species level. Biological indices are a common tool to indicate impacts at the ecosystem level. For microbial ecosystems, the Pollution-Induced Community Tolerance (PICT, (Tlili et al., 2016[118])) helps risk assessment by predicting the effect of a chemical or mixture based on the tolerance of the community in comparison to reference site. For invertebrates, the Species at Risk (SPEAR) index predicts the impact of pesticides on invertebrate communities based on species sensitivity to pesticides (Schäfer et al., 2007[119]; Hunt et al., 2017[120]). The improvement of such tools and the inclusion of other species such as vertebrates could help accelerate and facilitate risk assessment of pollution in ecosystems.

Box 2.8. Building confidence in the application of emerging environmental DNA (eDNA) and RNA (eRNA) tools for biodiversity assessments

Organisms leave all sorts of traces of their genetic material either in the form of DNA or RNA in their habitat. This genetic material found in ecosystems is referred as environmental DNA (eDNA) or RNA (eRNA). The analysis of eDNA or eRNA shed from organisms into their environment is changing the way that biodiversity assessments are done. By sampling water or sediments, these biomolecules can be isolated and analysed to provide rapid, non-destructive, accurate, and cost-effective biodiversity information in comparison to current time-constrained, physical search methods. These new tools can be particularly interesting to detect cryptic, at-risk, and invasive species. eDNA analysis can inform the presence and sometimes abundance of species in an ecosystem, while eRNA analysis is showing promise in distinguishing live versus dead sources of eDNA and indicating physiological state of species. For instance, eDNA can provide early indications of successful remediation efforts in recovery of fish populations and warnings of population decline, for example in relation to water quality.

However, inconsistent practices and poorly designed eDNA/eRNA detection tools currently threaten their uptake. Unacceptably high false negatives and false positives can compromise effective management decision-making on industrial practices and land and water management.

Canada is investing in making eDNA and eRNA practices more accurate and standardised through the iTrackDNA programme. iTrackDNA is a four-year, large scale applied research project launched in 2021 funded by Genome Canada, Genome British Columbia, and Genome Quebec that is addressing these concerns with researchers and end-users of eDNA and eRNA methods across sectors, including federal and provincial governments, First Nations, and natural resource-based industries. It is building end-user capacity through innovative, accessible, socially responsible genomics-based analytical eDNA tools for effective decision-making by: 1) supporting the creation of a targeted eDNA/eRNA detection national standard through the accredited Canadian Standards Association; 2) building eDNA kits to detect 100 priority invertebrates, fish, amphibians, birds, reptiles, and mammals in Canadian coastal and inland ecosystems; 3) applying 10 eRNA kits for determining animal biosurveillance, biosanitation, and bioremediation effectiveness; 4) generating decision support software for modelling regional biodiversity changes integrating Indigenous Ecological Knowledge; 5) developing an eDNA training, certification, and inter-laboratory validation framework for consultants, researchers, regulators, and managers; and 6) producing a guidance document on eDNA-based methods integration into management, policy and regulations.

The activities within the iTrackDNA project aim to build and augment the global community of practice through national eDNA standards that serve as a foundation for international standards and transformative testing. This can support eDNA applications in coastal and inland ecological surveys and biosurveillance for mining, forestry, energy, and infrastructure projects.

Source: Case study provided by Dr. Caren Helbing, University of Victoria, Canada

2.4. Effect-directed analysis: a combination of bioassays and chemical analysis

When EBMs, such as bioassays, have detected endocrine activity in a water sample, the source of this activity is often unknown. An additional step of analysis is needed to identify the chemical(s) causing the activity. This can be done through effect-directed analysis (EDA).

EDA is a method in which a sample is first separated into multiple fractions. Those fractions are then analysed in parallel by both non-targeted chemical analysis and bioassays. The results for each method are then put together to identify culprit chemicals found in those fractions where biological activity is detected (Brack, 2003[121]). EDA can be used to detect a range of EDCs, including new and emerging hormone-like contaminants (Houtman et al., 2004[122]; Simon et al., 2013[123]; Muschket et al., 2018[124]; Hashmi et al., 2018[125]; Gwak et al., 2022[126]; Houtman et al., 2020[127]; Zwart et al., 2018[128]).

Several case studies demonstrate the usefulness of EDA in identifying the culprit chemicals. A study in Korea (Gwak et al., 2022[126]) looked at the efficiency of different steps of treatment in a WWTP, applying bioassays for ER, AR, GR and AhR. The treatment removed all activity except for estrogenicity. After further investigation with EDA, the researchers found that the activity was caused by the pharmaceuticals arenobufagin and loratadine. The activity was confirmed by exposing the same *in vitro* bioassay to the pure molecule. Another case study is the Holtemme River in Germany, where anti-androgenic activity was suspected to cause decreased reproduction in fish (Muschket et al., 2018[124]). With EDA, fluorescent dye (4-methyl-7-diethylaminocoumarin) was identified as the source of the activity. The activity of the dye was further confirmed *in vivo*. Both cases demonstrate that the identification of chemicals is an important tool for risk management and abatement actions, as illustrated in Box 2.9.

Box 2.9. From fisherman concerns to mitigation action: a French case study applying effect-directed analysis

In 2008, fishermen observed gonad abnormalities in fish (wild gudgeons species, or *Gobio gobio*) near the Dore River in France. The concern was raised to authorities and was brought to the attention of the *Institut national de l'environnement industriel et des risques* (Ineris).

A research programme was launched to first confirm the fishermen's observation and to look at differences in fish upstream and downstream of a pharmaceutical industrial site and WWTPs (Sanchez et al., 2011[129]). In 2008 and 2009, this led to *in situ* monitoring of wild gudgeons on key indicators of fish health (gonad histology, VTG, and others), as well as the evaluation of the fish population by looking at the presence of 9 fish species in total. The study of the gudgeons confirmed the presence of bloated gonads in some fish, as observed by the fishermen. The study also showed that the male gudgeons had high levels of VTG in their blood. Moreover, the sex ratio of the population of gudgeons was drastically affected, with the level of intersex fish reaching as high as 80% in one of the downstream sites. Finally, the survey of the fish in the river showed that the density and the diversity of fish was

decreasing downstream of the industrial site, indicating an endocrine disruptive impact on the overall ecosystem and not only on one fish species.

In 2009, another study followed which used bioassays and targeted analysis to identify the chemicals causing the effects in water using passive sampling (Creusot et al., 2014[130]). The battery of bioassays used was extensive, including assays for receptors for estrogen (ER), androgen (AR), glucocorticoid (GR), mineralocorticoid (MR), progesterone (PR), aryl hydrocarbon (AhR) and pregnane X (PXR). All endocrine activities were detected at downstream sites and varied over the course of 6 months. Significant activities were observed of GR, PR and anti-MR. The chemical analysis on steroids and pharmaceuticals revealed the presence of mainly cortisol, cortisone, dexamethasone, spironolactone, 6-alpha-methylprednisolone, canrenone, hydrocortisone, prednisolone and prednisone.

Moreover, EDA was performed to establish causality between the effects detected and the chemicals identified. EDA highlighted that a few more chemicals still needed to be identified. While the effect on reproduction of the identified compounds is not fully characterised, it is suspected that they were the cause of effect observed in the first study.

In 2014, based on these results and at the order of the public authorities, the company equipped its plant with an advanced treatment strep (activated carbon on a fluidised bed) to eliminate the active pharmaceutical ingredients in its discharge. This treatment acts as a filter, the effectiveness of which has since been measured by monitoring endocrine activities in the natural environment.

This is an example of a regulatory decision taken on the basis of innovative research tools (bioassays, EDA) that were not regulated or even standardised at the time.

Source: (Creusot et al., 2014[130]; Sanchez et al., 2011[129]) and Dr. Sélim Aït-Aïssa, INERIS

2.4.1. Limitations

Currently, EDA is relatively costly and laborious to be used for routine monitoring (Brack et al., 2018[64]). However, advancements have been made in this regard with novel high-throughput techniques (Houtman et al., 2020[127]; Zwart et al., 2018[128]), which should make it more available to other users in the years to come. Another remaining challenge will be to increase the chemical analytical capacity as some of the activity detected is not always followed by chemical detection (Hashmi et al., 2020[131]; Houtman et al., 2020[127]; Zwart et al., 2018[128]). As an example, EDA was used to explain endocrine activity (ER, AR, GR, PR) in the Danube river (Hashmi et al., 2018[125]; Hashmi et al., 2020[131]). In general, EDA was able to explain the activity detected by bioassays, however part of the GR activity was not explained (Hashmi et al., 2020[131]). The authors hypothesised that it could be a method artefact or that the chemicals causing the effects are in very low concentration, but their additive effect can still be seen.

2.5. Selecting a monitoring method for EDCs

Policy recommendations

In many OECD countries, water quality monitoring and assessment programmes increasingly adopt new monitoring methods in addition to substance-by-substance monitoring. These methods have advantages. They are often more sensitive, detect effects caused by substances that are not routinely monitored, detect the effect of mixtures, or provide an overall snapshot of the chemical pressures on

water. Some of the new methods include bioassays, effect-directed analysis, non-targeted analyses based on mass spectrometry, and environmental DNA methods. Governments may benefit from considering the following recommendations when introducing new monitoring methods for water quality:

- Maintain current methods of substance-by-substance chemical analysis for routine monitoring and as a regulatory water quality standard. Chemical analysis remains essential in a robust water quality monitoring framework and readily aligns with existing regulations and practices. Chemical analysis also supports the adoption of new methods.
- Supplement existing substance-by-substance monitoring with bioassays, where appropriate and applicable. Bioassays serve as an early warning method of potential harmful pollution of ambient water, drinking water sources, effluents, and recycled water. A set, or "battery", of different bioassays is commonly recommended to capture different types of effects, including non-endocrine disrupting effects. The modes of action to be monitored by bioassays depend on the monitoring purpose, water type, the type of sources of EDCs in the environment, and the types of bioassays available on the market.
- While bioassays measure effects present in water, they do not detect the sources contributing to these effects. Additional analyses, such as effect-directed analysis, must be performed to point towards the responsible chemical(s).
- Non-targeted analytical methods, such as high-resolution mass spectrometry (chemical composition) or eDNA (biological composition), detect "known unknowns" and "unknown unknowns" in water. Such methods are useful in developing a baseline of the chemical composition of a water source or in detecting accidental spills. Critical water sources can be prioritised, such as pollution hotspots or sinks hotspots (e.g., due to low dilution capacity or intensive land-based activities), biodiversity hotspots, drinking water sources, confluences or sites of cultural importance. It requires an initial investment in technology and human resources.
- If the adoption of new methods is not feasible, adapting current practices of substance-by-substance analysis or *in situ* wildlife monitoring can be considered. With regard to chemical analysis, additional substances with endocrine active properties could be monitored. Adjusting environmental quality standards to also include the endocrine properties of substances, most likely leading to lower threshold values, could also be considered for substances that are already routinely monitored. However, the additional cost per sample can be significant and bioanalytical methods may be less resource-intensive if the analytical infrastructure is in place.

The previous sections described existing and upcoming methods for monitoring EDCs in freshwater. Each method has its strengths and limitations (Table 2.2). As there are probably infinite options of monitoring programmes, this section proposes a set of questions that should be asked during the process of designing a monitoring programme. While these questions do not necessarily provide definite guidance on the monitoring programme design, they can inform on avenues to explore. The ideal environmental monitoring system combines multiple methods of monitoring to strengthen and exploit synergies as they provide important complementary information (Brunner et al., 2020[132]; Hollender et al., 2019[26]). Some countries, therefore, apply a combination of methods. The second part of this section provides country cases of combinations of monitoring methods.

Table 2.2. Comparison of methods for water quality monitoring

	Targeted chemistry	Non-targeted analysis	Bioassays	*In situ* wildlife monitoring
Monitors presence of individual chemicals	Yes	Yes	No	No
Monitors biological endpoints (effects)	No	No	Yes	Yes
Sensitivity (detects at ng/L)	Low - Medium	Medium	High	Represents reality
Detects mixture toxicity	No	No	Yes (for a specific endpoint/MoA)	Yes
Detects by-products	No	Yes	Yes (only quantifies effects[1])	Yes
Detects unknown chemicals	No	Yes	Yes (only quantifies effects[1])	Yes
Threshold value	Environmental Quality Standard (EQS), reporting limit, concentration level, etc.	None (does not detect concentration levels[2])	Effect-based trigger values	% of change in population against a reference site population
Information on health for chronic exposure	No	No	No	Yes
Information on bioavailability and metabolism	No	No	Yes (only at cellular level for *in vitro* assays)	Yes

Note[1]: Bioassays only quantify the effect of (mixtures) of chemical activity. An additional analysis, 'effect-directed analysis', is required to identify specific chemicals.
Note[2]: NTAs do not allow quantification of concentrations, except in the case of suspect screening analyses when coupled with the use of standards.
Source: Authors

2.5.1. Guiding questions in designing a monitoring programme

Before being able to monitor EDCs, it is important that the monitoring strategy and programme is designed for the intended purpose. In a perfect world, every type of water and source should be monitored for all chemicals and effects with the best available techniques. However, choices need to be made based on multiple factors such as cost, time, available expertise, equipment and environmental conditions such as temperature, weather and geography. Hence, it is important to first confirm the intent of the programme. This section proposes a set of questions that that guide the process of designing a monitoring programme.

1. What type of water will be studied in the monitoring programme?

As mentioned throughout this report, there are many types of water to monitor, such as wastewater, recycled water, surface water, groundwater, and drinking water. For human health concerns, it is relevant to look at source waters (particularly when a region relies on a single source), drinking water, recycled water used for irrigation, fish products, or recreational water. Australia and California, United States, set up specific monitoring programmes to ensure safety of recycled water (Escher, Neale and Leusch, 2015[133]; California State Water Board, 2018[6]). Monitoring recycled water is ever more relevant, as certain regions are increasingly using recycled wastewater due to the droughts associated with climate change. Even if there is no immediate risk for human health, monitoring can be a powerful communication tool to inform policy makers on water quality (OECD, 2022[51]).

For wastewater, programmes can be designed to survey and regulate the release of pollution from municipal wastewater treatment plants, but also effluents of specific types of industry (e.g., pulp & paper mills, pharmaceutical manufacturing, mining, see for example the EEM Programme in Canada, Box 2.7).

For all water types, it is also important to consider the limit of quantification required for the choice of methods. For example, drinking water, which is generally obtained from a cleaner source and highly

treated, will have low to undetectable levels of contaminants in comparison to wastewater. Hence, some methods might not be sensitive enough to capture contaminants found in drinking water. To not waste resources, it should be ensured that the limit of quantification (LOQ) of the selected method is relevant for the type of water to guarantee the usefulness of the results. Selecting the most sensitive method - with the lowest LOQ – is not necessarily the best option, as sometimes very low levels of endocrine activity do not pose a risk to humans or ecosystems.

2. Is the programme developed to protect human health or ecosystem health?

This question relates to the protection goal of the monitoring programme: human health or ecosystem health. It can inform on the prioritisation of water type as seen in the previous question. More importantly, this choice will impact the calculation of threshold levels or trigger values. Threshold values are derived based on toxicological risk data, either considering the risk to human health *or* to ecosystem health (such as benthic organisms, freshwater biota, or critical species). Different species have different tolerance levels to contaminants. For exposure assessment it is important to realise that aquatic organisms are exposed 24/7 to surface water, while human drinking water uptake is estimated to be approximately two litres per day. A threshold level is therefore heavily influenced by the underlying toxicological risk data and protection goal. When both human and ecosystem health are prioritised, the lowest Predicted No-Effect Concentration (PNEC) value can be useful.

3. What is the purpose of the monitoring programme?

It is important to define the purpose and the level of ambition of the monitoring programme. When limited prior knowledge is available, a programme could aim to collect baseline data and identify potential hotspots, such as through targeted chemical analysis (Box 2.1), non-target screening (Box 2.2), or bioassays combined with effect-directed analysis. Other monitoring strategies can be applied to identify hotspots, such as the SIMONI strategy in Box 2.10 (van der Oost et al., 2017[134]). Monitoring initiatives can react to acute situations, such as observed abnormalities in fish physiology or behaviour or concerns raised by the public (Sanchez et al., 2011[129]) (Box 2.9). In such situations a more extensive programme, combining different methods, may be more appropriate to establish a cause and effect relationship, to generate trust in the results and to justify follow-up action. Other monitoring programmes assess if water is fit for purpose (recycled water, drinking water, recreation). In such cases a routine method that embeds an early warning system may be appropriate (Box 2.10). Lastly, monitoring programmes can be used to enforce regulation or permits by setting threshold levels, such as trigger values, quality standards, or concentration levels. In such cases, regulatory “lock-ins” are important to consider, such as unintentional government-required animal testing (Section 2.6.4) or discriminating between methods by preselecting one or a few methods in regulatory standards (Table 2.3).

4. A risk- or hazard-based approach?

Water quality assessment is predominantly based on risk-based approaches (see also Chapter 3). As a consequence, the need to develop a threshold or trigger value that defines the acceptable level of risk will arise (Section 2.6.1). However, it can be plausible to adopt a hazard-based approach where EDCs are considered a hazard at any concentration. The threshold level will correspond to zero, i.e. no concentration is allowed in water. The choice between risk-based or hazard-based approaches impacts the selection of methods (e.g. highly sensitive methods for hazard-based approaches), analysis of results and the prioritisation of sampling method.

5. Who is responsible for what in the monitoring programme?

There is a need to define who is doing what and who bears the cost of the monitoring programme. For example, who is doing the analysis and the design of the study? Who is paying for the analysis? Who is

reviewing the results? What in-house capacity is available? While this might be less consequential for small research-based programmes with their own specific research fund, this can play an important role for routine monitoring. The EEM programme in Canada is an example of a monitoring programme where the role of each stakeholder is well defined in Box 2.7. The industry is responsible for monitoring and covers the cost for the conduct of the study, while the government provides guidance documents and assesses the design and the results of the study.

6. Are vulnerable groups or endangered species considered?

For human health, it is important to consider populations that are particularly vulnerable to EDCs (Section 3.4.4, Chapter 3). For ecosystem health, there might be a need to prioritise the protection of endangered species or species of cultural or economic importance. This could impact the choice of species to be studied in a *in situ* wildlife monitoring campaign, the selection of threshold or trigger value, and site selection.

7. What is the appropriate monitoring frequency?

Determining the desired type of monitoring can help define the frequency of measures and the feasibility based on available resources. Currently, there are four main types of monitoring (Neale et al., 2022[60]). The first type of monitoring is a 'system assessment' which aims to determine the baseline of contamination of the selected water. This type of monitoring can be done as a first screen or repeated over long periods of time (month or years). The second one is 'validation monitoring' which evaluates the efficacy of a measure to reduce pollution, like a wastewater treatment plant. This monitoring might be done once to a few times. The third type is 'operational monitoring', used to evaluate if water treatment infrastructure is operating well to ensure constant quality of the treatment. However, this might be more difficult for chemicals such as EDCs since, in general, the methods described in section 2.2 and 2.3 require analysis that take more than a day. Finally, 'verification monitoring' verifies the compliance of treatment plants. This is often done on quarterly or biannual basis for various parameters and could include monitoring methods for EDCs for all the methods described.

2.5.2. Integration of monitoring tools and assessments

As seen in previous sections, various types of monitoring approaches exist, and while each has its advantages and disadvantages, together they make a very strong monitoring toolbox (Table 2.2). Even though one monitoring approach might be selected over another (e.g. for reasons of cost, time, effectiveness), it is ultimately recommended to combine methods as each provides important complementary information (Brunner et al., 2020[132]; Hollender et al., 2019[26]). Since the information given by each method is of a different nature, one might need tools to integrate all the different datasets. Moreover, methods do not have to be used all at the same time but can be integrated in different stages. For example, one method might be used for pre-screening and follow-up methods can be used to further investigate the issue. Examples of ways to integrate monitoring methods are given in this section.

Early warning routine monitoring: bioassays, chemical analysis, and EDA

Bioassays and NTA are increasingly used as a pre-screening or early warning tool to detect endocrine activity in water. Neither method, however, reveals the culprit chemical. When the potential culprit chemicals are known, targeted analysis (Section 2.2.1) can help identify potential chemicals that trigger the detected activity. In some cases, the activity might not be explained by known chemicals and more investigation is needed. This can be done with the help of effect-directed analysis (EDA) (Section 2.4).

The Smart Integrated Monitoring (SIMONI) approach of Waternet, the water authority of Amsterdam, the Netherlands, applies bioassays as an early warning system for surface water quality (Box 2.10). The

monitoring programme revealed that the main sources of contamination were landfills, sewage overflow, sewage water effluents and agriculture. SIMONI comprises two Tiers of monitoring. Tier 1 is a routine risk identification by applying two methods: relatively simple bioassays performed on passive samples, and chemical analysis of grab samples is conducted for metals, ammonium, and other substances. The results of Tier 1 are analysed against effect-based trigger values and threshold values. If these values indicate an increased risk, targeted research is prompted in Tier 2. Tier 2 combines broad spectrum chemistry, *in vivo* bioassays, and effect-directed analysis. When there are concerns for human health, non-targeted analysis and advanced bioassays may be applied.

Box 2.10. SIMONI, integrating monitoring methods to assess environmental risks in surface water

Waternet is a company that manages water in the region of Amsterdam in the Netherlands. To assess water quality, Waternet has developed the Smart Integrated Monitoring (SIMONI) strategy to integrate bioanalytical and chemical monitoring for micropollutants, including EDCs (van der Oost et al., 2017[135]; van der Oost et al., 2017[134]).The SIMONI approach is composed of two Tiers, that integrate both chemistry and toxicological results to evaluate the water quality. The first Tier is a hazard identification that includes multiple toxicological endpoints: in situ toxicity in daphnids (mortality 1 week), general toxicity bioassays in laboratory (cytotoxicity assays in cells, luminescence in bacteria, growth inhibition in algae, immobilisation [mortality] in daphnids), responses on specific endpoints using CALUX® (Chemically Activated LUciferase eXpression) bioassays (endocrine disruption: ER, anti-AR, GR, anti-PR; xenobiotics metabolism: DR, PXR, PAH; lipid metabolism: PPAR; genotoxicity: p53 and oxidative stress: Nrf2), and antibiotics activities (5 classes WaterSCAN assay). Effect-based trigger values (EBT) were developed for all applied bioassays in order to create toxicity profiles of sites, using bioassay effect/EBT ratios. The result of all bioassay effect/EBT ratios is then used to calculate a SIMONI risk indication (SRI), which is a measure for the overall ecological risk. The SRI has three categories: increased risk (SRI≥1), acceptable risk (SRI: 0.5-1) and low risk (SRI ≤0.5). If an increased risk is detected, the water sample will be analysed further in Tier 2, which is a customised risk assessment which can include broad spectrum chemistry, EDA (Houtman et al., 2020[127]) and *in vivo* biological tests to confirm and identify the risk. By using SIMONI, Waternet identified hotspots in the region of Amsterdam: greenhouse areas, sewage overflows, landfill runoff and wastewater treatment plant effluents. Mitigation actions to reduce the source of pollution had a mixed success for greenhouse areas: it led to a reduction of environmental risks at one out of two greenhouse areas.

Source: Dr Ron von der Oost, toxicologist, Waternet (water company and water authority for Amsterdam and surrounding area)

Switzerland developed an online toolbox of monitoring methods to support cantons in selecting the appropriate combination of methods for surface water quality monitoring (Box 2.11).

Box 2.11. The Swiss Modular Stepwise Procedure: a toolbox of monitoring methods

Steroidal estrogens (E1, E2, EE2) and pharmaceuticals (diclofenac, a non-steroidal anti-inflammatory) are part of Switzerland's water quality watchlist. The Predicted No Effect Concentration (PNEC) for water were established at 0.4, 3.6, 0.035 and 50 ng/L for E2, E1, EE2 and diclofenac respectively (Swiss Federal Council, 1998[136]).

To screen and monitor these and other substances in water, the Swiss Ecotox Centre stresses combining chemical analysis and bioassays. To support cantonal agencies in the selection of the appropriate monitoring methods, the 'Modular Stepwise Procedure' toolkit was developed, containing methods for the analysis and assessment of surface waters in Switzerland (VSA Platform for Water Quality, n.d.[137]). The Modular Stepwise Procedure includes guidance for many methods, ranging from chemical analysis to effect-based methods to novel methods such as eDNA.

Source: (Swiss Federal Council, 1998[136]; VSA Platform for Water Quality, n.d.[137]) and presentation of Dr Eszter Simon, Scientific Officer, Federal Office for the Environment, Switzerland, at the OECD Workshop on Developing Science-Informed Policy Responses to Curb Endocrine Disruption in Freshwater, 18-19 October 2022 (OECD, 2022[51])

Responding to observed abnormalities in wildlife: in situ wildlife monitoring, chemical analysis, and EDA

Abnormalities in wildlife can be observed by routine wildlife monitoring, or even from observations by local communities. *In situ* wildlife analysis of specific physical endpoints is generally the first step. This analysis is typically conducted in the potentially contaminated site and a reference site. Bioassays can then be applied to confirm if effects are caused by chemical pollution. Mapping pressures (such as municipal or industrial effluents, landfills, agricultural activities) can guide on the selection of relevant substances for targeted analysis to identify the culprit. Laboratories carrying out the chemical analysis should be sufficiently equipped to report back on low detection limits, i.e. nanogram/litre concentrations. Effect-directed analysis is another tool to identify the culprit. A workflow to respond to observed abnormalities is well described by Sanchez et al. and Creusot et al. (2014[130]; 2011[129]) (Box 2.9), following a case of observed adverse effects in wild fish living near pharmaceutical manufacture discharges in France.

Abnormalities can arise from unregulated substances and regulators may have limited powers when guidelines do not exist. High confidence in the assessment results is paramount for industry and regulators to recognise the problem and to justify follow-up action. Thorough research, however, can increase the lag time between observation and action. Pre-defined protocols and methods could reduce this lag time.

2.6. Success factors of an effect-based monitoring programme (bioassays)

Policy recommendations

Effect-based methods, notably bioassays, are a promising monitoring tool to characterise the potential risks present in water, including risks posed by substances that not routinely monitored and mixtures of substances. Whilst increasingly adopted, there are still barriers in applying bioassays for the purpose of water quality monitoring. The following considerations can help governments overcome these barriers and benefit from the full potential of bioassays:

- Some of the barriers to adopting bioassays for water quality monitoring are: costs and budgets, access to laboratories with bioanalytical capacity, the setting of threshold values or trigger values, the availability of appropriate bioassays on the market, and the communication of monitoring results (particularly when the outcomes are uncertain).
- A transition phase can be instrumental in overcoming some barriers. During this phase, authorities can develop a knowledge base, derive and refine threshold values and effect-based trigger values, and develop a mature market for bioassays. Most countries and authorities that currently use bioassays have gone through a transition phase.
- Clear policy signals can be sent that confirm the acceptance and further development of new water quality monitoring methods.
- In the long-term, bioassays could be relevant for regulatory purposes – for instance as environmental quality standards, water quality criteria or environmental norms. The practical implementation of such standards, such as deriving trigger values and enforcing compliance (especially those effects attributable to mixtures), would need to be tested.
- Designing a monitoring programme that unintentionally stimulates animal testing, using *in vivo* bioassays, should be avoided.

This section covers success factors related to implementing bioassays as monitoring method. It discusses setting water quality standards and trigger values; options to minimise the costs; access to laboratories; considerations in relation to animal testing; and water sampling. These success factors can facilitate cost-effective deployment of bioassays for policy purposes in a range of contexts.

2.6.1. Setting water quality standards and trigger values

This paragraph discusses the options for setting threshold levels for concentrations of endocrine disrupting chemicals or endocrine disrupting effects. Threshold values are commonly used in setting environmental quality standards or as a condition in a discharge permit. There are roughly three types of thresholds: 1) water quality criteria for chemical analysis, 2) effect-based trigger values for bioassays; and 3) trigger values for *in situ* monitoring of wild species; (Been et al., 2021[138]; Neale, Leusch and Escher, 2020[139]; Escher et al., 2018[56]; van der Oost et al., 2017[134]; James, Kroll and Minier, 2023[140]). The three types of standards discussed in this section are complementary to one another, and a mix of standards can be appropriate.

This publication does not provide any definite guidance on the appropriate threshold values. Rather, it discusses the considerations when setting water quality standards for endocrine activity or disruption. Ultimately, determining the acceptable level of risk is a complex decision, usually made by government regulators (in consultation with scientists, stakeholders, industry, and other groups).

Water quality criteria for chemical analysis

Typically, substances are regulated on a substance-by-substance basis. However, current substance-by-substance regulation does not always capture the endocrine properties of chemicals in water quality criteria or standards. Regulators normally work with a calculation method for deriving water quality criteria or environmental quality standards, considering many environmentally harmful properties of substances, such as acute toxicity. In practice, the calculation methods do not fully consider the endocrine disrupting properties of substances (James, Kroll and Minier, 2023[140]) (see also Box 2.12).

France is exploring how to adjust existing water quality standards considering the endocrine properties of substances that are already prioritised on the Environmental Quality Standards list. The French National Institute for Industrial Environment and Risks (Ineris) found that 70% of the Environmental Quality

Standards of the substances that are potentially endocrine active or disruptive did not consider endocrine activity as part of the method, although there are substance-specific data suggesting or evidencing such activities (James, Kroll and Minier, 2023[140]) (see also Box 2.12). France therefore developed a method to derive Environmental Quality Standards that better reflect the endocrine disruptive properties of substances, which is further described in Box 2.12.

Box 2.12. Considering endocrine disrupting properties within derivation of Environmental Quality Standards (EQS) under the Water Framework Directive

The EU Water Framework Directive (EU, 2000[7]) aims to achieve or maintain good quality status of aquatic ecosystems. To prevent the environment from chemical pollution, it introduced EQSs: threshold values for chemicals concentrations in water bodies not to be exceeded for the protection of human health and the environment. The Technical Guidance for deriving EQSs therefore indicates that effects related to endocrine activity and endocrine disrupting properties must be taken into consideration in the derivation of an EQS (European Commission, 2018[141]). It is not prescriptive, though, on how this should be achieved.

To palliate to this lack of directive, Ineris, the institute in charge of EQS derivation in France, first looked at how consistently EDC properties have been taken into consideration in the derivation of EQSs until now. The analysis indicates that EDC properties are only incompletely and heterogeneously taken into consideration (James, Kroll and Minier, 2023[140]). Based on existing EDC lists, 94 out of 180 chemicals analysed (52%) showed on evidence of endocrine disruptive properties. The remaining 86 chemicals are listed at least once to have endocrine activities. Out of these 86 chemicals:

- the EQSs of 14 chemicals (8%) appropriately consider their endocrine disrupting properties;
- the EQSs of 12 chemicals (7%) consider their endocrine disrupting properties, but the rationale was not clear enough;
- the EQSs of 60 chemicals (70%) did not consider endocrine activity in spite of substance-specific data suggesting or evidencing such properties.

Hence, it was found that the Technical Guidance is not prescriptive enough and leads to an inadequate and heterogenous consideration of endocrine properties of chemicals.

Based on these findings, Ineris proposed a more explicit methodology to better protect ecosystems from EDCs (James, Kroll and Minier, 2023[140]). As EDCs represent a specific hazard due to their inherent toxicological properties (low dose effects, non-monotonous dose-response relationships, delayed and transgenerational effects), Ineris suggests that specific effect thresholds should be considered to account for any risk to the environment and health. Ineris therefore proposes a decision tree that guides experts in deriving EQSs that consider endocrine disrupting properties of chemicals (see (James, Kroll and Minier, 2023[142]) for the decision tree). This decision tree also provides guidance on reflecting endocrine disrupting effects in ecotoxicological and toxicological datasets, and on adjusting the assessment factor where appropriate.

This methodology has been first applied in derivation of EQS values for two River Basin Specific Pollutants (RBSPs) in France in the context of the revision of a national legislation listing EQSs for RBSPs (James, Kroll and Minier, 2023[140]). In the future, this ad hoc methodology could be adopted as a standard guidance for deriving EQSs. Overall, this method is expected to contribute to a better assessment of the possible risks caused by EDCs occurring in surface waters by improving EQSs.

Source: Dr Alice James Casas, design and research engineer, Ineris, France, and (Ineris, 2023[143]; Ineris, 2023[144])Source:

Effect-based trigger values or threshold values for bioassays

Effect-based trigger values (EBTs) are the threshold values, or water quality indicators, for bioassays. EBTs help interpret whether the effects detected in a bioassay are acceptable or not. While bioassay results provide a lot of information already, as the detected responses can be compared in time and space, they do not assess the potential risk as such, because not all levels of activity are a risk to humans or aquatic species, particularly given that some bioassays could be very sensitive to low doses of contamination (De Baat et al., 2020[145]). "Exceedance of an effect-based trigger value signals the presence of a hazard, induced by one or more potentially harmful compounds. However, this does not necessarily mean there is a risk" (Been et al., 2021[138]; van der Oost et al., 2017[134]). It rather means that below the trigger value, the chance of adverse effects on humans or environment is low (Been et al., 2021[138]; Neale et al., 2023[57]). Trigger values are most used as a pre-screening value for further analysis (van der Oost et al., 2017[134]), but can also be used as a regulatory standard for water quality (California State Water Board, 2018[6]).

Trigger values are essential to determine whether there is a (potential) risk and whether follow-up action is required. Trigger values should therefore be established at a realistic level, as low trigger values can lead to many "hits" or unnecessary follow-up actions (i.e. the trigger value was too rigid) and high trigger values may overlook issues of concern and not lead to appropriate follow-up actions (i.e. the trigger value was too tolerant) (Been et al., 2021[138]; Dingemans et al., 2018[146]). Establishing trigger values at just the right level has been subject of a long-standing scientific and policy debate and it appears to be one of the major barriers towards implementing EBMs (OECD, 2022[51]).

Some jurisdictions, such as California, apply effect-based threshold values instead of effect-based trigger values for bioassays. Though there is some nuance between the two, this report uses these terms interchangeably. One difference is that California's threshold values for bioassays are tiered, meaning that if the bioassay result is five times above the threshold value, it triggers different actions than when it is ten times above the threshold value, and so on.

Effect-based trigger values can be established for specific types of bioassays, "assay-specific trigger values", or for specific endpoints regardless of the brand of bioassay, "generic trigger values" (Neale, Leusch and Escher, 2020[139]; De Baat et al., 2020[145]). There are advantages and disadvantages to each approach (Table 2.3). De Baat et al. (2020[145]) recommend using assay-specific trigger values, as these are more accurate because each bioassay is. However, from a public policy perspective, generic trigger values are more appropriate for regulatory purposes such as setting environmental quality norms or discharge permits. Generic trigger values, combined with bioassay performance standards, allow any bioassay provider to enter the market and makes it easier to substitute bioassays with an equivalent (due to costs, shortages, laboratory capacity and other reasons). For example, California favoured an endpoint-specific approach to be able to easily replace bioassays with alternatives that are, for instance, more economical or more easily deployable by laboratories.

Table 2.3. Advantages and disadvantages of generic and assay-specific trigger values

Comparison of setting effect-based trigger values for regulatory purposes, considering scientific uncertainties, required infrastructure and regulatory implications of each approach.

	Generic trigger values	Method-specific trigger values
Description	Effect-based trigger values defined per endpoint, i.e., each effect has its own effect-based trigger value, regardless of the method used.	Effect-based trigger values defined per bioassay, i.e., each "brand" of bioassay has its own effect-based trigger value.
Scientific considerations	Trigger values may not accurately capture the potency of a water sample as assays differ in sensitivity and chemical potency is not well captured in a generic trigger value. Lower scientific trust in this type of method, runs risk of being numbed by uncertainty. Potentially results into a "patchwork" of bioassays used, which lowers comparability of results in time and space.	Trigger value accurately captures the potency of a water sample as it considers the differences in sensitivity of bioassays. Higher scientific trust in this method. Consistency of methods across time and space.
Infrastructural considerations	Relatively easy to replace one bioassay with another. Bioassay selection based on existing laboratory infrastructure, expertise, and market availability. More resilient against market shortages as assays can be replaced. May need enhancement of laboratory infrastructure and expertise for many types of bioassays.	Replacement with alternative methods requires new trigger values. May need enhancement of laboratory infrastructure and expertise depending on bioassay.
Regulatory considerations	More appropriate for regulatory purposes, as generic trigger values are non-discriminatory towards different methods and technologies. Interpretation of the potential risk may be flawed (overestimation or underestimation of risk), potentially undermining mitigating actions and public communication. New methods entering the market will need to adhere to performance standards established or endorsed by an authority.	Potentially discriminatory by preselecting one or a few methods in regulatory standards and discharge permits. Raises a barrier for new methods to enter the market, could create unintentional monopolies. The regulated method could be considered as endorsed by government. Relatively low uncertainty surrounding the interpretation of risk, supports in implementing of follow-up actions and public communication. Each method requires its own EBT that needs to be assessed or accredited by an authority.

Source: Authors, based on (OECD, 2022[51]; Working Group Chemicals, 2021[147]; Neale, Leusch and Escher, 2020[139]; De Baat et al., 2020[145])

Trigger values are commonly expressed in terms of concentration levels (nanogramme per litre) of the biological equivalent concentration (BEQ) of a reference chemical (e.g. estrogen equivalents for estrogenic bioassays). The BEQ allows comparing the activity of a chemical or mixture by comparing it to a reference chemical. For example, the BEQ for estrogenic activity translates the levels of activity caused by a mixture of chemicals, such as of contraceptive pills and sex hormones, in the concentration level of estrogens. The concentration levels refer to a concentration of a reference chemical for the effect-based trigger value, but in fact, it expresses the cumulative effect of all chemicals present in a sample, including unknown chemicals. Box 2.13 contains a simple explainer of BEQs.

Box 2.13. Biological Equivalent (BEQs) – a simple explainer for non-ecotoxicologists

A BEQ is a value that represents the intensity of an effect in a bioassay. For example, some bioassays glow, or "light up", when an effect is triggered by a chemical (Box 2.3). The light or colour gets brighter when the effect is stronger. In other words, the more chemical is added to a bioassay, the stronger the light. This causal link between the concentration of a reference chemical and the intensity of light is called the BEQ. The BEQ therefore says something about the concentration of an unknown chemical in a sample that is equivalent to the effect observed in a particular bioassay. A BEQ is expressed in the concentration value of a reference chemical.

What is a reference chemical that is an essential part of a BEQ? An example. Bioassays that detect estrogenic effects, react to a diverse set of chemicals that are active on the estrogenic axes. It might be estrogen (E2), it might be the contraceptive pill (EE2), or something else. However, with bioassays, we do not know upfront which chemical caused the effect. The most studied chemical for estrogenic effects is E2, and even though there are many other active chemicals, estrogen is generally used as a reference chemical for estrogenic bioassays. The BEQ value is therefore expressed in estrogen (E2) equivalents, but this does not mean that estrogen caused the effect: it is simply a reference chemical. Other chemicals, such as the contraceptive pill, may have caused the effect, sometimes in combination with other chemicals. But this is still expressed in terms of the estrogen equivalent.

Source: Authors

Effect-based trigger values can be set for the protection of human health or ecosystem health, each of which yields different trigger values (Been et al., 2021[138]). In many cases, the trigger values for the protection of ecosystems can be more stringent, as most aquatic organisms are physically smaller than humans, which can make them more susceptible to pollutants, and humans naturally have higher concentrations of hormones in their bodies. In addition, an aquatic organism is continuously exposed to freshwater, or at least for a large portion of its life.

There are roughly four ways of deriving an effect-based trigger value:

1. Using available toxicological data on safe levels (for humans or wildlife) of a reference chemical relevant to the bioassay (Been et al., 2021[138]). This yields a threshold value that is similar to concentration levels applied for chemical analysis, e.g. E2 for estrogenic activity. The next step is to transform this threshold into a BEQ to be able to use the thresholds in the data analysis of bioassays. For many substances, a water quality threshold level already exists, for example in drinking water guidelines or environmental regulation. In such cases, existing guideline values can easily be adapted for a selected bioassay, this is called "read-across" (Escher et al., 2018[56])(see Table 2.4). If multiple chemicals have been identified as highly potent substances for one type of activity, the integration of all their existing guidelines in the calculation of one EBT should be considered. If there is no regulatory value available, a value can still be derived by looking at available BEQ data on known potent chemicals, and then estimate the BEQ level that is hazardous to no more than, for example, 5% of aquatic organisms (using the Species Sensitivity Distribution) (van der Oost et al., 2017[134]). Another example of such an approach is a recent study that used PNEC for the risk assessment of 56 WWTPs across 15 European countries (Finckh et al., 2022[148]).
2. Comparing *in vivo* and *in vitro* bioassay responses for a selected chemical and determining at which moment an effect can be detected *in vivo*. The *in vitro* equivalent of the *in vivo* tipping point is then selected as the trigger value. This was done for estrogenic activity by comparing effects in fish embryo and *in vitro* bioassays of multiple cell-lines (Brion et al., 2019[149]).

3. When no toxicological data is available, a simple method was recently proposed to calculate an EBT. This method consists of setting the threshold at the concentration generating 10% of effect for the reference compound of the selected bioassay (Neale et al., 2023[57]). This method enables to differentiate activity from the noise of the method and the resulting EBTs are at worst at one order of magnitude of the ones designed using previously mentioned methods. Hence, they can provide a good first approximation when no data is available.
4. If there is no toxicological data or possibility to derive a trigger value in the laboratory, EBTs can be determined in the field. This can be done by acquiring data on various sites and water types for which the expected water quality can be classified. Based on the level of activity observed for each water type or site, a threshold can be established at the level that allows us to distinguish between expected water qualities.

Table 2.4. Different reference points to derive effect-based trigger values

Context	Reference point	Source
Water recycling, Australia	Existing water quality guideline values for protection of human health	(Escher, Neale and Leusch, 2015[133])
Water recycling, California, US	United States and international potable water use guidelines for human intake	(Drewes et al., 2018[150])
SOLUTIONS research project, EU	Environmental quality standards of the EU Water Framework Directive	(Escher et al., 2018[56])

Source: (De Baat, Van Den Berg and Pronk, 2022[151])

Trigger values are unavoidably associated with uncertainties due to incomplete knowledge about the composition of a sample, the quality of underlying data and the dynamics of mixture effects (Working Group Chemicals, 2021[147]). The Working Group Chemicals under the European Water Framework Directive developed a decision framework to derive effect-based trigger values based on the breadth and quality of knowledge and data available on the risks associated with the relevant effects and chemicals (Working Group Chemicals, 2021[147]). It prioritises four methods of deriving a trigger value. The trigger values derived in Tier 4 are the most robust and based on complex methods; the ones in Tier 1 are the least robust and based on simple methods.

- Tier 4: derived based on *in vivo* and *in vitro* studies that have been calibrated against one another. In addition, chemical-mixture effects and risks have been quantified based on monitoring data.
- Tier 3: derived based on *in vitro* studies, and data from chemical monitoring and mixture risk assessments.
- Tier 2: derived based on data for existing environmental quality standards for single compounds, enriched with data on other compounds that trigger the bioassay.
- Tier 1: derived based on data of a reference compound that has the most potent effect in a bioassay, ideally based on an existing environmental quality standard.

Trigger values for in situ *monitoring of wild species*

Programmes that monitor fish and other species in the wild require methods that measure a (statistically significant) change in fish health. The trigger values adopted by the Canadian Environmental Effects Monitoring (EEM) programme are called “Critical Effect Size Triggers”. The EEM programme is a comparative method based in part on five “core fish endpoints”, namely age, weight-at-age (growth rate), relative gonad size, relative liver size and condition (weight/length^3). To assess the effects of pollution, a comparison is made between fish living in habitats exposed to effluent pollution and the reference fish that live in reference or unexposed habitats. If a statistically significant difference is detected on one of the five endpoints, this gives lead to further investigation on the potential impact of effluent pollution. By means of

illustration, the Critical Effect Size Triggers are a ≥ 25% change in relative gonad or liver size, or a 10% change in condition factor (for more on Canada's EEM programme, see Box 2.7).

2.6.2. Costs of an effect-based monitoring programme

Analysing the costs of new water quality monitoring methods is not straightforward and depends on several factors. The cost components of water quality monitoring, excluding method development costs, comprise (Kienle et al., 2015[152]; Drewes et al., 2018[150]):

- Sampling and pre-treatment of samples.
- Capital expenditure on laboratory equipment.
- Laboratory consumables and test products, such as kits and/or cell lines. Note that the costs of bioassays that require a license are, at the moment, relatively higher than license-free bioassays.
- Labour required to maintain, prepare and operate the samples and analysis.

The outsourcing of services can affect the costs of a monitoring programme. Distance to the nearest qualified laboratory is an issue in some countries where samples need to be shipped domestically or abroad for analysis.

Comparing the cost-effectiveness of methods is ambiguous, though some general statements can be made. Targeted chemical analysis of well-regulated or routinely-monitored chemicals benefits from economies of scale which reduces analytical costs (Working Group Chemicals, 2021[147]). Such economies of scale have not yet been reached with bioanalytical methods and non-targeted chemical analysis. In a way, bioassays can be more cost-efficient than targeted chemical analysis as they respond to a group of substances, which is inherently impossible with substance-by-substance methods. Moreover, anecdotal evidence suggests that the required infrastructure and equipment for EBMs (e.g. incubators, sterile hood and plate-readers) have lower cost than for analytical chemistry (e.g. mass spectrometer). However, EBMs may need additional methods, such as effect-directed analysis, to identify the culprit chemical with certainty. Moreover, comparing the different bioassay approaches, *in vitro* methods are generally more cost-effective than *in vivo* methods, as they can be more easily scaled up by automation and high-throughput technologies (Drewes et al., 2018[150]; Working Group Chemicals, 2021[147]). Lastly, non-target screening is a relatively expensive method due to the need for specialised experts and the capital cost of equipment.

The costs of bioassays differ per cell line provider, laboratory, type of services outsourced (depending on in-house capacity), and country (Kienle et al., 2015[152]; Drewes et al., 2018[150]; Working Group Chemicals, 2021[147]). In the Netherlands, the implementation of a complete set of bioassays (including, but not limited to, assays detecting endocrine activity) costs about EUR 800-1100, which comes down to around EUR 100 per bioassay (De Baat, Van Den Berg and Pronk, 2022[151]). The cost of estrogenic effect monitoring has been estimated at approximately EUR 140-200 per sample within the European Union (Working Group Chemicals, 2021[147]). It is generally expected by the experts who contributed to this publication that costs associated with bioassays will diminish as demand increases.

A smart monitoring programme design can potentially reduce costs. For example, the same samples can be used for multiple purposes, such as chemical analyses and effect-based analyses (Wernersson et al., 2015[5]). Moreover, it could be worthwhile researching if some routine chemical analysis can be partially replaced with bioassays that capture the same chemicals. However, this has not been widely explored in the literature and requires further research. Lastly, cost-effective choices can be made in determining the comprehensiveness of a battery of bioassays. Knowledge about the environmental pressures can direct the selection of a battery of methods. For instance, estrogenic effect assays could be prioritised if a water body is primarily exposed to sewage effluents. The Water Quality Guidelines in the Netherlands distinguish between a "basic battery" of six bioassays and an "additional battery" (De Baat, Van Den Berg and Pronk,

2022[151]). In Canada, the frequency of Environmental Effects Monitoring is reduced if there are no effects observed over two consecutive monitoring cycles (Box 2.7).

2.6.3. Laboratory access and capacity

In some countries, very few to no laboratories have the expertise or the infrastructure to perform and analyse bioassays. To make bioassays more widely available for regulators, various types of laboratories could be considered, including research laboratories, contract laboratories, water utility/authority laboratories, and medical laboratories (OECD, 2022[51]). Medical laboratories often have long-standing experience with bioanalytical methods but may need additional guidance on water sample preparation and treatment. Outsourcing bioanalytical analysis to university laboratories may not be appropriate for water safety analysis, as it requires specialised expertise and certification. Various steps of the analytical process, from sample preparation to analysis, can be outsourced to the test method developer or cell line supplier. Interlaboratory comparison should be performed to ensure the robustness of methods across laboratories (industry, academia, government facilities), platforms/vendors and relevant sample matrices (OECD, 2022[51]).

Non-targeted screening and effect-directed analysis also require highly specialised equipment and experts. These methods may not be available to every country and at every budget. International collaborative research projects and outsourcing analysis to laboratories abroad are common practice to overcome the barrier of limited laboratory access.

2.6.4. Considerations in relation to animal testing

In many cases, *in vivo* bioassay methods are a form of animal testing. Fish species are commonly used in freshwater and effluent testing (Robitaille et al., 2022[4]). Designing a monitoring programme or regulatory standard that unintentionally stimulates animal testing should be avoided, particularly if non-animal methods are available. In some countries, invertebrates and fish and frog embryos are accepted methods that reduce animal suffering. By regulating or integrating bioassays into regulatory practices or test guidelines, countries run the risk to lock in practices of animal testing for regulatory compliance.

There are many reasons why animal testing is used in water monitoring. For some endpoints, *in vivo* methods may be the only method sufficiently sensitive or reliable to make statements on toxicity of a water or effluent sample. *In vivo* methods can also be used as a second-step test to confirm effects found *in vitro* settings. Regulatory standards can also be a driver for animal testing. Governments sometimes require that companies use *in vivo* methods to monitor compliance with regulatory standards, such as in the oil and gas industry (Hughes, Maloney and Bejarano, 2021[153]).

It is worth considering that *in vivo* tests are more expensive and time-consuming. Mittal *et al*. (2022[154]) estimate that traditional, animal-based, ecotoxicity tests for a single chemical "cost USD $118,000, require 135 animals, and take 8 weeks", while New Approach Methods cost "USD $2,600, require 20 animals (or none), and take up to 4 weeks to test 16 (to potentially hundreds of) chemicals" (Mittal et al., 2022[154]).

Moreover, bioassays should not be considered as a tool that *exactly* represents what is happening in the water sample. Rather, bioassays should be valued on par with targeted chemistry: as a proxy for the state of our water quality. It cannot be expected that bioassays be closer to reality than chemical analysis. Bioassays simply provide additional pieces of information that inform on potential risks. Using *in vivo* bioassays for compliance monitoring therefore most likely overshoots the purpose of a routine water quality monitoring programme, particularly when *in vitro* assays are available. In this context, there is a concern that the international definition of endocrine disruptors may become a driver for regulatory animal testing, as it implies that an adverse health effect needs to occur in an intact organism: "An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (IPCS, 2002[155]).

For the purposes of (routine) water quality monitoring, permanent compliance with the definition may be unnecessary as *in vitro* tests, combined with *in silico* methods, can provide valuable information on the effect on the mixture effects of all EDC present in a sample (Escher, Neale and Leusch, 2021[156]).

Some recommendations can be made to avoid unnecessary animal testing for freshwater and effluent quality testing:

- Avoid developing regulations or standards that lock in government-required animal testing, and instead design flexible standards that allow for alternative methods in the future. This also includes the development of effect-based trigger values. If an *in vivo*-based-effect-based trigger value is embedded in regulation, it may lead to government-required animal testing.
- In most OECD countries, the use of animals for scientific or regulatory testing is regulated and reported to the public. However, testing for water quality regulation is sometimes beyond the scope of animal testing statistics. Sharing data of animal testing for water quality regulation can help avoid unnecessary animal testing and ensure humane treatment of animals in unavoidable cases.
- Embed the 3R principles of Replacement (avoiding animal testing), Reduction (limit the number of animals exposed to animal testing), and Refinement (limit the suffering and distress of animals) in water monitoring and regulation (Russel and Burch, 1960[157]). Concrete ways of embedding the 3R principles in water practices is adding an article on “Choice of methods” in regulation or guidelines, prioritising non-animal methods in validating test guidelines. A lot can be learnt from chemicals regulation and practices (Scholz et al., 2013[158]).

2.6.5. Sampling strategies and sample preparation matter

For assessing risk in freshwater, the sampling strategies and the sample preparations matter. The development of guidelines and standard operating procedures would facilitate the use of bioassays (Neale et al., 2022[60]). The sampling strategy is first designed depending on the objective of the sampling campaign (Escher, Neale and Leusch, 2021[159]). This objective will depend on the water to test (e.g. surface water, drinking water, wastewater) and the information sought (e.g. assess efficiency of treatment, find hotspots in surface water). Clearly identifying the objective of the sampling will help determine what is necessary for the rest of the sampling strategy. International or national guidelines exist for sampling water from different sources (e.g. ISO 5667 series, (European Commission, 2009[160])) to help determine how to perform the sampling (e.g. number of samples, type of bottle, conservation of samples). While they might not be specific to endocrine disruptors, they can still guide on the strategies to be used.

The method and timing of water collection also matters. The traditional method of collecting water samples is referred to as grab sampling, i.e., taking a sample of water directly at the site (Escher, Neale and Leusch, 2021[159]). However, those samples represent only one moment in time for the selected site and might not be representative of the contaminants that can be found generally at the site. To mitigate this issue, composite samples are often done for water treatment plants (Escher, Neale and Leusch, 2021[159]). For that, water samples will be collected throughout 24 hours and mixed to form one composite sample. Composite samples take into account the variation of contaminants during the day. In research, there is a growing interest in passive sampling (Luo et al., 2022[34]; Escher, Neale and Leusch, 2021[159]) to increase the representativeness of a site over time. Passive sampling uses a device that contains a sorbent which collects chemicals over a chosen period at a given site.

After the collection, samples will need a pre-treatment before being able to use them for chemical analysis and *in vitro* bioassays. This pre-treatment or sample preparation is necessary to concentrate the sample for the analysis, but also to remove components that might interfere with the analysis (Luo et al., 2022[34]; Robitaille et al., 2022[4]). As the sample is modified in this process, some chemicals can be lost (see Annex 2.A). Hence, methods are often judged on their capacity to retain chemicals of interest, called ‘recovery’.

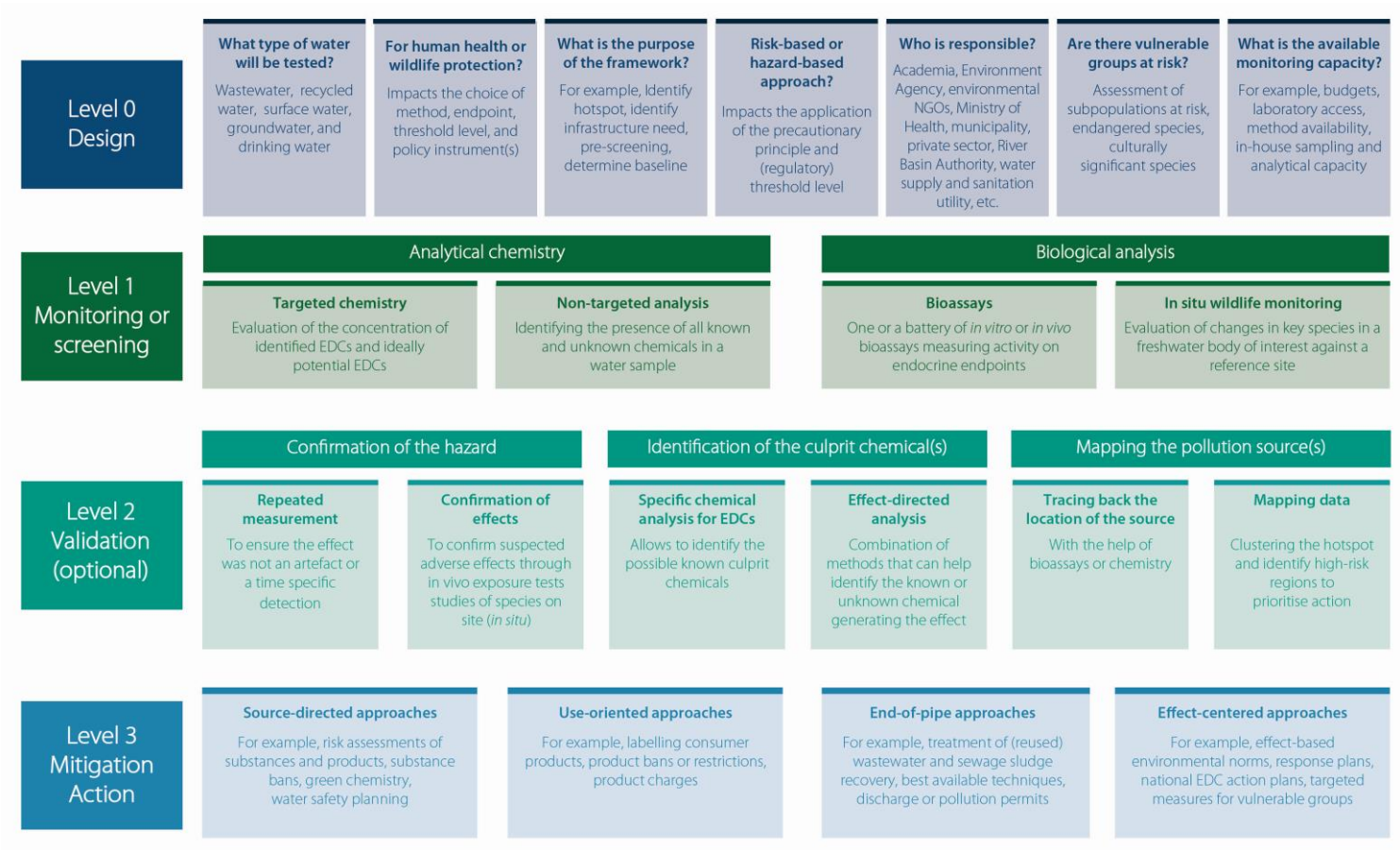
For sample preparation, there is a clear need for standardisation, as well as a need for increasing the capacity of processing samples (Paszkiewicz et al., 2022[25]; Luo et al., 2022[34]; Metcalfe et al., 2022[19]; Robitaille et al., 2022[4]). Embedding guidelines for water sample preparation within existing international test methods or guidelines, such as ISO Standards or OECD Test Guidelines, is worth considering.

2.7. Chapter conclusion

This chapter described the available methods for monitoring EDCs and endocrine activity in water, based on case studies from across OECD countries. It also discussed potential barriers and uncertainties in monitoring EDCs and endocrine activity. Figure 2.2 presents a conceptual framework summarising the monitoring possibilities and follow-up actions in four Levels. Level 0 guides through the design of the monitoring programme with the help of questions as described in Section 2.5.1. Level 1 addresses the choice of methods, which can a single method or a combination of methods (described in Sections 2.2 and 2.3). Level 2 provides an overview of all the validation methods to confirm the hazard, identify the culprit chemical or map the sources (described in Section 2.5.2). Finally, Level 3 describes potential action that can be taken after either a threshold was exceeded (Level 1) or a risk was confirmed (Level 2), which will be discussed in the next Chapter.

It is important to note that monitoring is not a pollution reduction measure in itself (OECD, 2019[161]). Monitoring can support in prioritising or justifying action, but uncertainties will persist – particularly given continued international manufacturing and trade of existing and new substances, the further release of EDCs and other CECs into the environment, and challenges arising from environmental change and degradation - such as climate change, biodiversity decline, land degradation and desertification. These pressures only increase the imperative for governments to avoid “decision paralysis” and identify options for near-term preventive action for the safety of humans and the environment. The next Chapter sets out such instruments to manage EDCs in freshwater.

Figure 2.2. Conceptual framework for monitoring EDCs in freshwater



Source: Authors

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Annex 2.A. Losing chemicals in the sample preparation process

As water samples are modified in the sample preparation process, some chemicals may be lost. For targeted chemistry, the sample preparation will be selective to the chemicals desired and will have high recovery (Metcalfe et al., 2022[19]). However, for bioassays and non-targeted chemistry, the preparation process aims to keep as many chemicals, while preventing the matrix interference during the analysis (Paszkiewicz et al., 2022[25]; Luo et al., 2022[34]; Robitaille et al., 2022[4]). This means that, even with a lot of effort, some chemicals will inevitably be lost. For example, for *in vitro* bioassays, the most used method is solid-phase extraction (SPE) (Luo et al., 2022[34]; Robitaille et al., 2022[4]) which are columns containing sorbents similar to the one used in passive sampling. The water will be passed through the sorbent which will trap certain chemicals. One important notion to understand is that while sorbents (e.g., HLB) are designed to catch as many chemicals as possible, it is not possible to retain all, though multiple solid-phase extraction methods with different sorbents can be used for a given sample. Most methods used currently (Luo et al., 2022[34]; Robitaille et al., 2022[4]) will use sorbents that keep mostly hydrophobic molecules, i.e. molecules that do not like water which encompass a majority of EDCs (Escher, Neale and Leusch, 2021[159]; Robitaille et al., 2022[4]). However, some chemicals, such as metals, will be lost in the process (De Baat et al., 2020[145]). It is important to take this limitation into account as some EDCs will be removed in the sampling process. Perchlorate, which can disrupt the thyroid axis, is a case in point (Pleus and Corey, 2018[162]; Niziński et al., 2021[163]).

Notes

[1] Joint ED-list by Belgium, Denmark, France, Netherlands, Spain and Sweden.

3 Policy options to reduce and manage endocrine disruption in freshwater

This chapter presents policy options to tackle endocrine disruptors in water bodies, drinking water and wastewater. It documents policy approaches that intervene throughout the life cycle of endocrine disrupting chemicals (EDCs) at the source, during use, and at the end-of-pipe. In addition, it proposes interventions that are centred around the adverse effects of EDCs. Effect-centred approaches are well-suited to respond to emerging monitoring methods, such as bioassays, that do not instantly identify the culprit chemical. Lastly, this chapter makes the case for international actions as the adverse impacts of EDCs are a global concern in need of global solutions.

3.1. Introduction

As presented in Chapter 1, endocrine disrupting chemicals (EDCs) are compounds that can disrupt the endocrine system and cause adverse effects in intact organisms or their offspring. EDCs are produced by various industries (e.g., pharmaceuticals, pesticides, personal care products, electronics) and will eventually make their way into the environment where they can impact human health and ecosystem integrity. Since EDCs are emitted from different sources, monitoring EDCs in freshwater is important to prioritise mitigation actions. Monitoring methods have been discussed in Chapter 2, which recommends supplementing chemical analysis with bioassays and other methods where appropriate and applicable.

This chapter presents the different policy options that can be used to address EDCs, supported by country case studies. It takes into consideration that some EDCs are unknown, whilst endocrine disrupting effects have already been observed in freshwater. This chapter pays particular attention to measures that can support a further diffusion of the test methods reviewed in Chapter 2.

Section 3.2 discusses principles that underlie policy decisions, such as principles on the accepted level of risk or hazard. Section 3.3 documents existing policy approaches that intervene throughout the life cycle of EDCs at the source, during use, and at the end-of-pipe. Section 3.4 proposes interventions that are centred around the adverse effects of EDCs. Effect-centred approaches are well-suited to respond to emerging monitoring methods that do not instantly identify the culprit chemical, such as bioassays. Lastly, Section 3.5 makes the case for international actions as EDCs are a global concern in need of global solutions.

3.2. Principles underpinning policy decisions

The type of policy action is preceded by an agreed approach to the accepted level of risk. This is ultimately a political decision, informed by societal debate. Such a debate is particularly important for EDCs, as some substances are suspected of having endocrine active properties, but this may not be acknowledged or established with certainty. The same holds true for mixture effects. Moreover, endocrine disruptive substances fall under different legislative spheres, which may limit the toolbox of water regulators in addressing (suspected) endocrine disrupting effects in water (Figure 3.1).

Figure 3.1. Regulations relevant to EDCs and their impacts on water quality

Note: Product regulations can cover a vast array of products, e.g., agricultural inputs, cosmetics and personal care products, pharmaceuticals.
Source: Authors

3.2.1. Principles that guide decision-making under uncertainty

Uncertainty is inherent to risk management of chemicals. Policy principles can provide guidance on decision-making under uncertainty. The types of uncertainty vary, depending on the compound and policy approach. It can include uncertainty in hazard or exposure assessments, uncertainty in the economic costs and benefits of a decision, uncertainty in the enforcement and effectiveness of the risk management approach, uncertainty in the safety of a substitution, and others (OECD, 2022[1]).

A more proactive policy approach, based on the precautionary principle, could be considered when the environmental and human health risks are uncertain and the potential consequences of inaction are high. For example, it is worth considering a precautionary policy approach when there is a long-term risk to the environment, health, or the economy. The damage caused at the population and ecosystem levels can take years to repair and can be experienced across generations. This is particularly relevant to EDCs.

Risk versus hazard approaches

Chemicals can be managed based on risk or hazard. Decisions of chemicals management can be guided by the adverse impact of the chemical regardless of human or wildlife exposure to the chemical. This so-called hazard-based decision-making "focuses on addressing the inherent hazards of chemicals through substitution or other approaches, rather than calculating an acceptable level of risk" (UNEP, 2019[2]). The alternative is a risk-based approach, where decisions are prioritised based on exposure to the chemical. "This includes identifying use patterns that may create widespread exposure across a population, or intense exposure for a subset of the population" (UNEP, 2019[2]).

Hazard-based approaches to meet water quality objectives are best achieved through chemical related regulation. Realistically, the water community has limited control over the governance of substances and products, and water policies often enter the 'regulatory stage' at the end-of-life of substances. Chemical related regulations are the gatekeeper of chemicals on the market, and product regulations determine their use. Implementing a hazard approach is even more complex in transboundary contexts, as water is a recipient of wastes and chemicals from other jurisdictions with different regulations or enforcement. Moreover, the impacts can occur decades after the phasing out of chemicals, as some EDCs are legacy chemicals that can persist in the aquatic environment, such as in sediments (Kurek et al., 2019[3]). Nevertheless, hazard-based approaches could be appropriate in drinking water production, wastewater reuse, or for the protection of critical ecosystems. Additional treatment can be justified based on the precautionary principle.

Water regulation traditionally uses risk-based approaches. The water community can set the acceptable level of risk and the tolerated concentrations in water, for example by setting water quality standards or effluent standards. Water quality standards are based on different parameters, such as predicted no effect concentrations (PNEC): concentrations at which there are no predicted effects to humans, aquatic organisms, or secondary poisoning of predators. When a risk is uncertain, the accepted level of risk can be lowered out of precaution, such as by specifying an additional assessment factor to existing environmental quality standards that reflect the endocrine properties, the risk of mixtures and any potential uncertainty of the chemical (James, Kroll and Minier, 2023[4]). Box 2.12 in Chapter 2 presents a methodology to take the risk of endocrine disruption into consideration in environmental quality standards.

However, risks from EDCs in water to human health and ecosystems remain difficult to quantify. Given the large number of compounds present in the aquatic environment, prioritisation frameworks can support the selection of substances to monitor and regulate. Table 3.1 presents a prioritisation framework developed by the NORMAN Network, and similar prioritisation frameworks have been developed (Götz et al., 2009[5]; Johnson et al., 2017[6]; Gaston et al., 2019[7]). Follow-up actions for monitoring and assessment are based on the state of knowledge of a specific substance (Dulio and von der Ohe, 2013[8]; von der Ohe et al., 2011[9]). Bioassays, combined with effect-directed analysis, can support the prioritisation of contaminants (Smital et al., 2012[10]). These methods have been described in detail in Chapter 2.

Table 3.1. Prioritisation of problematic EDCs in freshwater: determining actions based on the state of knowledge of hazards

Category	Description of the state of knowledge on the hazard of a substance	Appropriate action for priority substances within category
A	Substances for which there is sufficient evidence of exposure and adverse effects at environmental concentration	Integration in routine monitoring and derivation of legally binding environmental quality standards
B	Substances for which hazard assessment is based on experimental data BUT few monitoring data	Screening studies for information about current exposure
C	Substances for which there is evidence of exposure BUT hazard assessment is based on predicted toxicity (PNEC)	Rigorous hazard assessment
D	Substances for which hazard assessment is based on experimental data BUT analytical capabilities are not yet satisfactory	Improvement of analytical methods required
E	Substances for which no or few monitoring data AND hazard assessment is based on predicted toxicity (PNEC)	Screening studies AND rigorous hazard assessment
F	Substances for which toxicity data are sufficient for the derivation of an EQS and there is evidence that the exposure does not pose a hazard to ecosystems	Monitoring efforts for these compounds could be reduced[1]

Note: Based on the NORMAN Prioritisation framework for emerging substances. Note that actions should only be applied to a shortlist of prioritised chemicals within each category.
Note[1]: Reducing monitoring might have a negative consequence if the investigated or suspected EDC leads to severe effects in mixtures with other chemicals.
Source: Adapted from (Dulio and von der Ohe, 2013[8])

A hierarchy of policy principles

The following hierarchy of OECD principles can usefully guide the development of policy for the management of diffuse pollution sources. They are captured by the OECD Council Recommendation on Water (OECD, 2016[11]):

- Principle of pollution prevention – prevention of pollution is often more cost-effective than treatment and restoration.
- Principle of treatment at source – treatment at the earliest stage possible is generally more effective and less costly than waiting until pollution is widely dispersed.
- Polluter pays principle – makes it costly for those activities that generate pollution and provides an economic incentive for reducing the pollution.
- Beneficiary pays principle – allows sharing of the financial burden with those who benefit from water quality improvements. Minimum pollution regulations must be met to first ensure additionality and avoid rewarding polluters.

In addition, environmental justice is a relevant guiding principle in the case of EDCs, as some subpopulations may be more affected than others. Section 3.4.4, on minimising the impacts of EDCs on vulnerable populations, discusses this in further detail. Environmental justice can comprise (OECD, 2017[12]):

- Distributive justice - fair treatment in terms of access to natural resources, environmental services fand benefits, and environmental risk exposure.
- Corrective justice - accountability and remediation for environmental harm.
- Procedural justice - access to environmental information, judicial and administrative proceedings and participation in environmental decision making.

3.3. Policies that address the life cycle of endocrine disrupting chemicals

Policy recommendations

EDCs can be found in multiple classes of chemicals, e.g., natural and artificial hormones, pesticides, plasticisers, and flame retardants. They are released into freshwater throughout the life cycle of the chemical from its production, distribution, usage to its disposal. A policy mix that addresses all steps of the life cycle of EDCs could have the following design:

- At the source, Environmental Protection Agencies, water authorities, river basin organisations and water service providers can support initiatives that decrease the identification time of EDCs, by turning to new approach methods (NAMs). NAMs include methods such as high-throughput *in vitro* screening, omics and *in silico* methods such as Quantitative structure-activity relationship (QSAR). Group-wise assessments can also help increase the efficiency of EDC assessment and simultaneously reduce animal use for the chemical assessment. The forementioned water entities have a role to play in sharing monitoring results and raising issues relevant to (emerging) water risks.
- Water authorities would benefit from stimulating and getting involved in use-orientated initiatives even if these are not directly linked to the water sector, such as waste disposal campaigns, consumer awareness campaigns, labelling schemes, and private sector initiatives. User decisions, even if motivated by personal health reasons, co-benefit the environment.

- End-of-pipe measures should only be used in conjunction with source-directed and use-orientated measures. An over-emphasis on upgrading wastewater treatment infrastructure is not a sustainable, optimal use of limited financial, technical and natural resources. Regulators could prioritise more stringent treatment standards to those discharges that pose a particular pressure to health or ecosystems. Regulators and service providers could consider advanced treatment and monitoring with bioassays for wastewater reuse and sewage sludge recovery infrastructure.
- Regulators could consider making use of existing public databases when issuing discharge permits, such as EDLists.org, the Endocrine Active Substances Information System, Database of Endocrine Disrupting Chemicals and their Toxicity Profiles. The databases inform about the suspected or confirmed endocrine disrupting properties of released substances. Assessments based on the grouping of chemicals can also inform water discharge permits. Water permits could include a condition for additional monitoring requirements when there is a suspected risk of EDCs being released in the environment.

There are several mitigation options in the EDC life cycle that contribute to water quality improvement at the source, during use and at the end-of-pipe (Figure 3.2). Source-directed approaches impose, incentivise or encourage measures that prevent the release of EDCs into water bodies. A focus on preventive options early in an EDC life cycle, may deliver the most long-term and large-scale benefits. This, however, requires an acceleration of chemicals assessment or increased adoption of the precautionary principle. Use-orientated policy approaches include policy instruments which impose, incentivise, or encourage a reduction in the use of EDCs and their release to the environment. Users of EDC's, such as consumers or the agricultural sector, have a role to play in making choices on the use and disposal of EDCs. Some users avoid using EDCs for personal health reasons. Such decisions co-benefit the environment as less waste is generated and accumulated. End-of-pipe measures focus on removing or eliminating EDCs after their use or release into water. End-of-pipe policies involve different types of instruments that impose, incentivise, or encourage improved wastewater treatment and solid waste disposal. Relying solely on end-of-pipe measures, such as WWTP upgrades, can be costly, energy intensive and toxic transformation products may be formed. However, in combination with source-directed and use-orientated approaches, extra treatment at the level of WWTPs play a role in reducing EDCs in the aquatic environment. Buffer zones or wetlands are promising nature-based solutions in capturing some EDCs before their release into the aquatic environment.

Regulatory, economic and voluntary policy instruments are all part of the policy toolkit that is needed to manage multiple sources of pollution throughout the life cycle of EDCs (OECD, 2016[11]; OECD, 2019[13]). Figure 3.1 presents a mix of these policy instruments, in no particular order.

Figure 3.2. Selected life cycle instruments that contribute to water quality improvements

Source-directed instruments, use-oriented instruments and end-of-pipe and end-of-life instruments

Note: The presented instruments are non-exhaustive
Source: Adapted from (OECD, 2017[14]; OECD, 2019[13]; OECD, 2021[15]; OECD, 2022[1]; OECD, 2023[16])

The difficulty with managing the life cycle of EDCs is that, first, many chemicals are not identified as endocrine disrupting or endocrine active even though effects have been detected in water, and second, EDCs comprise numerous product groups (Table 3.2). Addressing the life cycle of each of these sources and uses is beyond the scope of this publication. This report therefore refers to the policy recommendations made in other recent OECD reports on contaminants of emerging concern, which are equally relevant and applicable in the case of EDCs. Table 3.2 provides an overview of the OECD reports that cover in more detail the measures to manage the life cycle of specific substances or product groups. The following paragraphs highlight three policy approaches particularly relevant to EDCs: A source-directed approach: Risk assessment of substances and products, A use-oriented approach: Labelling consumer products, End-of-pipe measures: Wastewater reuse and sewage sludge recovery.

Table 3.2. Relevant OECD resources on managing the life cycle of substances and product groups

Substance or product group	Relevant OECD policy studies
Consumer products (e.g., children's products, electronics, textiles)	Policies to reduce microplastics pollution in water (OECD, 2021[15])
Cosmetics, personal care products	Pharmaceutical residues in freshwater (OECD, 2019[13])
Food contact materials (e.g., plastic food containers, food wrappers, baby bottles)	Workshop report on flexible food-grade plastic packaging (OECD, 2023[16])
Industrial chemicals	Government risk management approaches used for chemicals management (OECD, 2022[1])
Metals	Government risk management approaches used for chemicals management (OECD, 2022[1])
Pesticides	Diffuse pollution, degraded waters (OECD, 2017[14])
Pharmaceuticals (for humans and livestock)	Pharmaceutical residues in freshwater (OECD, 2019[13])
Synthetic and naturally occurring hormones	Pharmaceutical residues in freshwater (OECD, 2019[13])

Note: Even though the above-mentioned reports are not specific to EDCs, they present relevant source-directed, use-oriented or end-of-pipe measures of product groups that can end up as endocrine active or endocrine disrupting pollutant in freshwater
Source: (OECD, 2017[14]; OECD, 2019[13]; OECD, 2021[15]; OECD, 2022[1]; OECD, 2023[16])

3.3.1. A source-directed approach: Risk assessment of substances and products

This section explores how EDCs are assessed and how the water community could contribute to the prioritisation of substances. Chemical related legislation is the gatekeeper to chemicals entering the market, by assessing whether a substance poses a risk to function(s) of the endocrine system. Source-directed approaches are therefore dependent on risk assessments of substances, and specific regulations for consumer products, agriculture and pharmaceuticals.

Risk assessments and chemicals management are promising source-based policy measures. They are best combined with other source-directed policy instruments that reduce the production of EDCs (listed in Figure 3.2).

A first approach to reduce EDCs in the environment is to evaluate their risks to health and biodiversity. To ensure science-based regulation of EDCs, various countries and regions have developed frameworks for the evaluation of the endocrine active or disrupting properties of chemicals (IPCP, 2017[17]). Those include programs such as the Endocrine Disruptor Screening Program (EDSP) by the US-EPA (EDSTAC, 1998[18]), the Extended Tasks on Endocrine Disruption (EXTEND) by Japan's Ministry of the Environment (MoE) (Box 3.1) and the EU guidance for the identification of EDCs under the REACH (Andersson et al., 2018[19]). To provide guidance on evaluation of chemicals for endocrine disruption, the OECD continuously develops and standardises test guidelines for the identification of EDCs (OECD, 2018[20]).

While those programs are well described and OECD guidance documents are available (OECD, 2018[20]), it is still important to mention that those processes are data- and time-intensive. The US-EPA's EDSP illustrates the time-intensity of the EDC screening process (Maffini and Vandenberg, 2022[21]; U.S. EPA, 2021[22]). It started the evaluation of 52 chemicals in 2005. In 2015, all 52 chemicals were analysed through the EDSP first Tier of testing (U.S. EPA, n.d.[23]). From those 52, 18 were recommended for further testing in Tier 2, of which the results are still pending. In 2013, a second list of 109 chemicals was prepared for testing, containing 41 pesticides ingredients and 68 chemicals targeted by the Safe Drinking Water Act (U.S. EPA, 2013[24]).

Many countries aim to decrease the identification time by turning to new approach methods (NAMs) which include methods like high-throughput *in vitro* screening, omics and *in silico* methods such as Quantitative structure-activity relationship (QSAR). Examples are the ToxCast and Tox21 programmes in the US (Dix et al., 2007[25]; Krewski et al., 2010[26]), and the EU-ToxRisk and ONTOX (Daneshian et al., 2016[27]; Vinken et al., 2021[28]). Other countries, such as Japan (Box 3.1) are evaluating the appropriate use of NAMs, as challenges remain in deviating from traditional toxicological risk assessment approaches. The use of

Integrated Approaches for Testing and Assessment (IATAs) can help to combine the information gathered from different methods. The OECD provides guidance and case studies on the use of IATAs (OECD, 2017[29]).

While IATAs and NAMs can provide quick information, challenges remain in advancing evaluation of EDCs. For one, the common definition of EDCs states that an EDC "consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (WHO-UNEP, 2013[30]). This requires proof of adverse effects with high weight-of-evidence. The data acquired need to be able to show that the chemical or mixture (1) generates adverse effects in an intact organism or its progeniture, (2) acts via an endocrine mode of action and (3), that the adverse effects is caused by the endocrine mode of action (Kassotis et al., 2020[31]; James, Kroll and Minier, 2023[4]). It is worth pointing out that for routine water quality monitoring, as presented in Chapter 2, whole animal testing overshoots the intended purpose of establishing a risk profile of the chemical present in the water sample.

Group-wise assessment of chemicals can help increase the efficiency of EDC assessment while reducing animal use. Some chemicals are analogues: similar structures may ignite similar biological activities (Swedish Government Inquiries, 2019[32]; OECD, 2017[33]). The grouping of those similar chemicals can be justified with tools such as QSAR. Group-wise assessments can be done to avoid assessing every endpoint for every chemical. Furthermore, this could help prevent harmful substitutions for which acquiring a sufficient knowledge base to enable regulation can take years. As an example, the European Chemicals Agency grouped 148 bisphenols for risk assessment and recommended the restriction of 30 of them in relation to their potential of disrupting the endocrine system and causing reprotoxic effects (ECHA, 2021[34]). To learn more about the grouping of chemicals, please refer to the OECD guidance on the subject (OECD, 2017[33]). Assessments based on grouping chemicals can also inform water discharge permits, for example by including a condition for additional monitoring when there is a suspected risk of EDCs being released in the environment.

Water managers have access to public databases that inform about the suspected or confirmed endocrine disrupting properties of a substance. Such databases can support on the prioritisation of problematic EDCs in activities such as monitoring, permitting and designing policy interventions. The website EDLists.org, initiated by Belgium, Denmark, France, the Netherlands, Spain and Sweden, informs stakeholders about the current status of substances identified as endocrine disruptors or suspected of having endocrine disrupting properties (edlists.org, n.d.[35]) Other databases compile available toxicity data on EDCs and EASs, such as the Endocrine Active Substances Information System (European Commission, 2022[36]) and the Database of Endocrine Disrupting Chemicals and their Toxicity Profiles (Institute of Mathematical Sciences, n.d.[37]) (Karthikeyan et al., 2019[38]; Karthikeyan et al., 2021[39]).

Box 3.1. Japan's Extended Tasks on Endocrine Disruption 2022

Already in 1998, Japan's Ministry of Environment (MoE) put itself at the forefront of EDC research and method development with the launch of the Strategic Programs on Environmental Endocrine Disruptors '98 (SPEED'98) (MoE Japan, 1998[40]). The programme was followed by Extended Tasks on Endocrine Disruption (EXTEND) 2005, 2010, 2016 and 2022 (MoE Japan, 2005[41]; MoE Japan, 2010[42]; MoE Japan, 2016[43]; MoE Japan, 2022[44]).

Over the years, the programme led to the development of knowledge of the presence of EDCs in Japan, as well as the development of methods for the identification of EDCs. Those methods include the OECD Test Guideline 240 for the Medaka Extended One Generation Reproduction Test (MEOGRT) (OECD, 2018[45]) and OECD Test Guideline 241 on the Larval Amphibian Growth and Development Assay (LAGDA) (OECD, 2018[46]) developed in collaboration with the US.

Moreover, in EXTEND 2016, Japan implemented its 2-Tier framework to assess EDCs with in vitro bioassays as pre-screening, followed by short-term in vivo bioassays (Tier 1). Tier 2 applies long-term in vivo bioassays to confirm positive results obtained in Tier 1. All the bioassays used in the framework have been developed by Japan to assess effects in non-mammalian species (fish, frog, daphnia). Through this framework, over 200 substances detected in Japanese aquatic environment have been tested with at least in vitro bioassays in Tier 1. Furthermore, six substances have been fully evaluated with the MEOGRT. Through its programme on EDCs, Japan is also involved in international initiatives such as developing OECD Test Guidelines and international research programmes.

The EXTEND 2022 objectives include:

- Assessment of effect and method development
- Monitoring of environmental concentrations of EDCs and exposure assessment
- Risk assessment and management
- Collection of data and improving knowledge
- International collaboration and information sharing
- Assessing pesticides and pharmaceuticals for EDC potential
- Investigating New Approach Methods to reduce animal use in the assessment of EDCs
- Introducing perspectives on mixtures assessment
- Proposing methodologies and procedures for assessments under regulatory risk management of EDCs

Source: (MoE Japan, 2016[43]; MoE Japan, 2022[44]; OECD, 2018[45]; OECD, 2018[46])

3.3.2. A use-oriented approach: Labelling consumer products

Labelling schemes can be implemented to share information related to health or the environmental impact of products and packaging. Labelling can support consumers in making informed decisions on the products they use.

In 2022, the European Commission released a proposal for the creation of dedicated hazard classes for EDCs under the 'EU Regulation on classification, labelling and packaging of substances and mixture' (European Commission, 2022[47]). If adopted, this proposal requires manufacturers, importers or downstream users of substances or mixtures to classify, label and package their hazardous chemicals appropriately before placing them on the market. Besides "hazard labels" that warn consumers against

potential hazards, positive labels can inform consumers on the low risk of using a product. For example, a study in Korea found that consumers are willing to pay around $2/year for an “EDC-free” labelling policy (Kim, Lee and Yoo, 2018[48]) (see also Box 3.5 on the OECD study on the willingness to pay to avoid negative health effects due to chemical exposure).

The Nordic Swan Ecolabel is an example of voluntary product labelling. It has adopted a set of principles in awarding their Ecolabel. It restricts products that 1) have hazardous properties (hazard-based approach in principle), though under specific circumstances a small quantity of hazardous substance can be allowed (risk-based approach when necessary), 2) are identified as endocrine disruptor or potential endocrine disruptor, and 3) are to be avoided based on precautionary principle, particularly applied to groups of similar substances (Nordic Swan, n.d.[49]). The supermarket COOP Denmark completely phased out the use of EDCs from their products (Box 3.2).

There are some challenges with EDC-labelling. Only a few compounds have been identified as EDCs, while the list of suspected compounds is much longer, and unknown endocrine disrupting compounds may exist. Absence of a hazard label or presence of an “EDC-free” label could therefore lead to the incorrect assumption that a product is truly free of EDCs (Government of Belgium, 2022[50]).

Box 3.2. Voluntary action by a Danish supermarket: co-benefits for the environment through safe consumer products

COOP Denmark is one of Denmark’s largest retailers. In 2015 COOP Denmark voluntary started to remove several EDCs[1] from products and packaging. As there was no regulatory requirement to do so, the supermarket collaborated with suppliers, authorities and scientists to voluntarily phase out the EDCs from products. The process took several years. One of the main challenges was to find a cost-neutral alternative to the harmful substances.

The decision was driven by a wish to supply safe products and protect its consumers. Commercial interests also played a role, as the company wished to protect its brand and maintain high customer loyalty. While this decision was driven by the motivation to protect consumers from exposure to EDCs through food consumption and product usage, COOP Denmark may also have created co-benefits for the environment. As a percentage of consumer products still end up in freshwater through incineration facilities, landfills or litter, a safe product design may reduce endocrine disrupting effects in water.

To inform consumers about product safety COOP Denmark also worked with green labels awarded by third parties, such as Nordic Swan.

Note[1]: Per- and Polyfluorinated Compounds (PFC/PFAS0) and Bisphenols (BPA, BPS, BPFT, and others)
Source: Presentation by COOP Denmark at the Conference "Chemicals: better protecting health and the environment", organised within the framework of the French Presidency of the Council of the European Union, May 2022

3.3.3. End-of-pipe measures: Wastewater reuse and sewage sludge recovery

Urban wastewater treatment

Urban wastewater is one of the sources of EDCs in the environment as conventional wastewater treatment plants (WWTPs) are not designed to fully remove contaminants of emerging concern. A study of the removal of micropollutants in wastewater treatment processes in the Baltic Sea region showed that PFAS and pharmaceuticals are not efficiently removed by conventional wastewater treatment practices (HELCOM, 2022[51]). The study found similar concentrations both in influents and in effluents. What is

more, some compounds (such as PFOA and PFNA) occurred in higher concentrations in effluents than in influents. Metals were only moderately removed.

Advanced wastewater treatment processes, such as reverse osmosis, ozonation, activated carbon, membranes and advanced oxidation technologies, can achieve higher removal rates in comparison to conventional secondary treatment. However, a "one size fits all" treatment for EDCs does not exist and no single technology can remove all EDCs (Azizi et al., 2022[52]; HELCOM, 2022[51]). What is more, upgrading treatment processes is not always necessary, nor cost-effective nor sustainable. Some treatment methods have high carbon emissions. The production of raw materials used for treatment can also have an environmental footprint. For instance, activated carbon is an effective method in removing PFAS, but its production and regeneration comes from the burning of fossil fuels (NORMAN Network and Water Europe, 2019[53]).

Countries commonly prioritise stringent treatment standards for those discharges that pose a particular pressure to health or ecosystems. This is the practice in Switzerland (OECD, 2019[13]). The NORMAN Network suggests the following criteria to prioritise WWTPs that could benefit from additional treatment (NORMAN Network and Water Europe, 2019[53]):

- Large WWTPs service areas, and/or
- WWTPs with a high proportion of wastewater compared to the receiving water body (consider seasonal and climate change scenarios to anticipate flows with lower dilution potential), and/or
- WWTP that influence drinking water resources, and/or
- WWTPs that influence valuable ecosystems.

The removal effectiveness and cost efficiency of wastewater treatment options, and options to finance wastewater treatment plant upgrades, are discussed in detail in Section 3.5 of the OECD report on Pharmaceutical Residues in Freshwater (OECD, 2019[13]). This discussion is highly relevant in the context of EDCs.

Wastewater reuse and sewage sludge recovery

Driven by the impacts of climate change, previously water abundant countries are increasingly facing droughts. Consequently, countries are turning towards reusing wastewater for agriculture, horticulture, cooling, or aquifer recharge (Fairbrother et al., 2019[54]). What is more, in transition to the circular economy, sewage sludge is more and more recovered as a nutrient for agricultural practices. However, wastewater reuse and sewage sludge recovery may put additional pressure on water quality as both products contain contaminants of emerging concern and their transformation products, including EDCs (Domini et al., 2022[55]; Sichler et al., 2022[56]; Kumar et al., 2022[57]). Advanced treatment of sludge and wastewater, monitoring and setting appropriate water quality standards can reduce the risk of endocrine disruption.

Advanced treatment and monitoring can securely provide recycled water and can sufficiently remove EDCs. The Environmental Protection Authority (EPA) of Victoria, Australia, studied contaminants of emerging concern (CECs), including EDCs, in recycled water (OECD, 2022[58]) (see also Box 2.1, Chapter 2). The study was performed at thirty WWTPs. It detected 181 contaminants, including 15 EDCs. In general, wastewater treatment was able to reduce the EDC concentration. The best treatment was a combination of activated sludge processes with extended aeration, ultraviolet light disinfection, microfiltration, reverse osmosis, and chlorine. However, this treatment is expensive.

In some countries, sewage sludge generated during wastewater treatment is applied as fertiliser for agriculture. However, EDCs and other contaminants of emerging concern have been detected in sewage sludge. Sludge disposal can thus unintentionally lead to the spread of EDCs on land and in water. The Danish Environmental Protection Agency (EPA) identified thousands of substances in treated sewage sludge through non-targeted screening analysis (Danish EPA, 2022[59]). Perfluorooctane sulfonic acid

(PFOS), a type of PFAS, were detected on all sites. Other compounds detected were mercury, cadmium, 1H-benzotriazole, 2,6-dichlorophenol, bisphenol S, methylparaben, terbutryn and prosulfocarb. The concentrations of substances differed per site. In a follow-up study, the Danish EPA analysed three new alternatives for sludge disposal that can recycle phosphorous and minimise the emission of green-house gases (mono-incineration, pyrolysis integrated with pre-drying, and hydrothermal liquefaction) (Danish EPA, 2023[60]). The study stresses high uncertainty regarding the transformation of PFAS compounds during treatment, which can lead to the further spread of PFAS compounds during use.

Monitoring the water quality impacts of recycled wastewater can inform on the risk of negative impacts on health and ecosystems. As mentioned above, the Danish EPA applied non-targeted and suspect screening to determine micropollutants, including EDCs, in sewage sludge. California (United States) emphasised routine monitoring to ensure the safety of recycled water. California's State Water Board introduced a state-of-the-art monitoring programme including two bioassays as a water quality indicator. One bioassay monitors estrogenic effects of effluents (California State Water Board, 2018[61])(see also Box 2.5, Chapter 2).

Whilst it may be obvious to adopt recycled water quality standards based on human health protection goals, given public concerns about the safety of recycled water, more stringent criteria based on the protection of ecosystems may be the better choice. Water quality standards are more stringent for ecosystem protection than for human health, as aquatic organisms are more susceptible to chemicals than humans due to their size and permanent exposure in water. Chapter 2 provides guidance on developing water quality standards for different purposes.

3.4. Policies centred around the effects of EDCs

Policy recommendations

Endocrine disrupting effects may be observed while the culprit is not immediately evident. Intervention strategies and response plans that put effects rather than individual culprit chemicals at the centre, combine the following actions:

- Intervention strategies or response plans can reduce the lag time between observed or suspected abnormal effects, such as in wild fish, and the mitigation of the causes. In many instances, the suspected effects and culprit chemicals need to be confirmed through additional analysis. Valuable response plans cover accepted methods for collecting evidence, temporary no-regret or low-cost mitigation options, interpretation of exceeded threshold values or trigger values, roles and responsibilities of authorities and (suspected) sources of emission, and a communication plan.
- Endocrine disrupting chemicals touch many sectors. National strategies and action plans on EDCs can build bridges across sectors and send a policy signal of national priorities related to the issue. National strategies and action plans can act as a first step towards developing policy instruments and monitoring programmes. Most national action plans also contain a research agenda to reduce uncertainties and guide on the development of measures.
- Some authorities are considering introducing water quality regulation based on bioassays. Supplementing substance-by-substance water quality criteria with effect-based environmental quality norms could better capture the effect of mixtures, non-regulated chemicals, and impacts of low concentrations of chemicals. With the current state of development of bioassays and other methods, as well as the identification of endocrine disrupting chemicals, many regulators are not yet comfortable introducing monitoring programmes and regulations. A transition phase

helps to establish a knowledge base, derive threshold values for monitoring and regulation, and mature the bioassay market.

- Policies and actions could specifically consider the impact of EDCs on vulnerable populations. The risk of exposure to endocrine disrupting chemicals can be higher to certain groups within a population, such as children, adolescents, pregnant women, and lactating women. Moreover, exposure levels can differ within societies and across countries. Culturally important species or cultural keystone species could also be threatened. Interventions may therefore consider a wide scope of factors affecting vulnerability, including biological susceptibility, socio-economic vulnerability, and cultural vulnerability to EDCs. Actions could include risk assessments for vulnerable groups and populations, information campaigns targeting specific groups, and monitoring of endangered species and cultural keystone species.

Life cycle interventions, as discussed in the previous section, may not be fully fitted to EDCs and may result into a regulatory mismatch for several reasons. First, the sources of EDCs in freshwater vary widely, ranging from consumer products, industry, pharmaceuticals, agriculture, hormones, etc. Moreover, chemicals can act in mixtures, possibly when the life cycles of compounds come together. Lastly, not all EDCs are identified, suspected or even known as EDCs, while effects may already be detected in effect-based monitoring programmes and non-target screening. With the increased use of bioassays as water quality monitoring method, that do not identify the culprit chemical at once, effect-centred approaches can complement life cycle-based interventions.

Effect-centred approaches impose, incentivise or encourage measures that reduce the cumulative impacts of endocrine disruptors on humans, aquatic species or ecosystems, regardless of a compound's regulatory identification as endocrine disruptive or endocrine active. Effect-centred approaches typically respond to the results detected in water monitoring, such as bioassays, emphasize precautionary measures directed at vulnerable populations, and adopt an intersectoral approach to reduce environmental pressures from EDCs. As with effect-based monitoring, effect-centred policy approaches take the effects of endocrine disruption on human and ecosystem health as a starting point for action.

3.4.1. Effect-based environmental quality norms

It remains to be seen if the current system of environmental quality norms (EQNs; also known as environmental quality standards) is a viable option to address contaminants of emerging concern. Existing EQNs aim to determine acceptable water quality based on an assessment of individual compounds. Some of the challenges are the vast number of chemicals in the environment, the effect of mixtures, and the time it takes to develop quality norms for every single chemical (OECD, 2019[13]). Combining compound and effect-based norms may thus provide a more holistic picture of water quality (Brack et al., 2018[62]).

There are few cases of effect-based methods (bioassays) as an environmental quality norm. The California State Water Board is the first regulator to adopt reporting limits for the estrogen receptor (ERa) and the Aryl hydrocarbon receptor (AhR) for recycled wastewater, the former being associated with endocrine activity or disruption (California State Water Board, 2018[61]). The European Commission has submitted a proposal that recommends the monitoring of estrogenic activity in water bodies, and that gives way for the adoption of effect-based environmental quality norms in the future (European Commission, 2022[63]).

Environmental quality norms based on bioassays are ideally generic and based on non-animal methods (see Table 2.3, Chapter 2). Generic environmental quality norms (i.e., not pegged to a specific brand of bioassay) are more appropriate in the regulatory context because they do not discriminate between methods and they allow any bioassay-provider to enter the market. As animal methods are still used in effluent testing, regulators should avoid prescribing a regulatory standard that unintentionally stimulates animal testing, particularly if non-animal methods are available. Section 2.6, Chapter 2, explains in more

detail how to derive environmental quality norms for bioassays. A transition phase can support the implementation of effect-based environmental quality norms. Other requisites that need to be in place before adopting an effect-based environmental quality norm are a monitoring budget, laboratory capacity, sampling protocols and a sufficient supply of bioassays.

Adopting a transition phase to avoid decision paralysis

With the current state of development of bioassays, many regulators are not yet comfortable in adopting effect-based methods as an environmental quality standard. A transition phase to establish a knowledge base, derive effect-based trigger values or environmental quality norms, and develop a mature market for bioassays can be instrumental in overcoming some barriers. It gives room for trial and error before an official regulatory standard is adopted.

Concentrating efforts on estrogenic bioassays can be an appropriate first step as estrogenic assays have been validated and are widely accepted, and knowledge of these effects is relatively well established (OECD, 2022[58]). Adverse outcome pathways and bioassays for other endocrine axes, EATS and non-EATS, are less well established. For example, there are no standardised *in vitro* bioassays for the thyroid axis even though thyroid disruption is known for disrupting metamorphosis in amphibians (OECD, 2022[58]).

The European Commission's proposal for amending the Water Framework Directive suggests an intermediary phase of applying chemical analysis alongside effect-based monitoring of estrogens for a period of at least two years (European Commission, 2022[63]). This two-year period allows time to collect and compare data which can inform any future decision on the use of routine effect-based monitoring and deriving effect-based trigger values. Chemical analysis will monitor the individual compounds of E2, E1 and EE2 (comparing concentrations against threshold levels), while effect-based methods will monitor estrogenic effects.

The California State Water Board also adopted an intermediate phase for optimising the selection of effect-based methods (Box 2.5, Chapter 2). The Scientific Advisory Panel of the California State Water Board recommended a three-phased approach towards the adoption of bioassays (Drewes et al., 2018[64]):

- Phase 1: Data collection to determine the range of responses for *in vitro* bioassays and to confirm that the *in vitro* bioassays represent endpoints relevant to human health.
- Phase 2: Pilot evaluation of the effect-based trigger values, i.e., of the interpretation of the monitoring results by *in vitro* bioassays. This includes interlaboratory comparisons.
- Phase 3: Full implementation of bioassays as an integral component of routine screening/monitoring of recycled water quality.

The California State Water Board implemented bioassays (ERa and the Aryl hydrocarbon receptor, AhR) as a pre-screening tool of water quality hotspots affected by wastewater recycling (California State Water Board, 2018[61]). The selected bioassays will be tested for a period 3 years starting in 2020, after which an evaluation will take place on the relevance of the methods, and whether it is appropriate to continue, remove or substitute the current bioassays. During this period no regulatory action will be undertaken if the threshold values are exceeded (OECD, 2022[58]).

Canada’s Environmental Effects Monitoring (EEM) Programme (Chapter 2, Box 2.8) also allowed ample time for setting the right trigger values for *in situ* wildlife monitoring (Environment Canada, 2010[65]). The trigger values were not set until the results of four monitoring cycles were collected for two fish species at 125 pulp & paper mill sites, which took 12 years. Moreover, the EEM Programme has built in regulatory feedback loops. It cyclically evaluates if effluent standards are adequate in protecting fish, fish habitat and fish usability. Guidance documents are updated if needed. This demonstrates the need for patience to acquire data to set threshold levels and the importance of flexibility to respond to new findings and arising needs.

3.4.2. Response plans

Intensified monitoring of endocrine disruptors and endocrine disrupting effects will likely increase the need to intervene and implement mitigation actions. Case studies often show a lag time between observed abnormalities and mitigating action. See for example the case of France where, three years after fishermen observed changes in fish, actions were taken to eliminate the pollutant (Box 2.9, Chapter 2) (Creusot et al., 2014[66]; Sanchez et al., 2011[67]). Similarly, the Canadian EEM programme works in three-year cycles, and it can take up to six years between observation of abnormalities and mitigation actions (Box 2.7, Chapter 2) (Environment Canada, 2010[65]).

This lag time is often caused by a need to validate effects through further research, notably by confirming effects through effect-directed analysis or *in vivo* methods. Collecting proof of the causality between the culprit chemical and observed effect is also time-consuming. All the same, delaying mitigation action comes with risks to human health and ecosystem integrity, as well as economic costs. The cost of inaction, or acting after the damage is done, is likely more expensive than preventive measures. On the other hand, unnecessary action is also costly, such as disrupting business processes, sediment remediation or upgrading wastewater treatment plants.

There is value in developing a response plan, protocol or good practice guide or other approach to quickly mobilise a response to observed or suspected abnormal effects in freshwater. A response plan could cover, for example:

- Accepted methods for collecting evidence. This includes describing how a weight of evidence shall be established. It ensures that regulators follow a consistent, clear, and transparent delivery of evidence for the evaluation of water contamination. Moreover, it determines the circumstances under which *in vitro* methods, QSAR, grouping and/or international databases are accepted as evidence. This is particularly important as the international definition of endocrine disruption still leads to the expectation that “consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations” (WHO-UNEP, 2013[30]). A protocol that describes accepted methods can help to avoid unnecessary animal testing.
- Temporary no-regret or low-cost mitigation options, such as putting in place buffer zones, temporarily taking suspected effluents to tertiary treatment facilities, providing warnings to consumers to hold off consumption of suspected fish or crops.
- Guidance on the interpretation of exceeded threshold values or trigger values, and the appropriate response actions. For example, the California State Water Board works with different tiers of action depending on the level exceedance of the bioanalytical equivalent concentration (BEQ) (Table 3.3) (California State Water Board, 2018[61]).
- Roles and responsibilities of involved authorities (Ministry of Health, EPA, utilities, basin authority), industry, and the actor responsible for the emission. The EEM programme in Canada is an example of a monitoring programme where the role of each stakeholder is well defined (Box 2.7, Chapter 2).
- A communication plan that details out how the monitoring results can be interpreted and explained, particularly in relation to health concerns for human and wildlife, and that explains any follow-up actions. The communication plan may also make general recommendations on behavioural changes and making environmentally friendly choices.

Table 3.3. California's State Water Board's response actions to detected activity in bioassays

BEQ/MTL Threshold	Response action by the recycled water producer
If BEQ/MTL ratio is consistently less than or equal to 0.15 for ER-α or 1.0 for AhR	After completion of the pilot monitoring phase, consider decreasing monitoring frequency or requesting removal of the endpoint from the monitoring programme.
If BEQ/MTL ratio is greater than 0.15 and less than or equal to 10 for ER-α or greater than 1.0 and less than or equal to 10 for AhR	Continue to monitor.
If BEQ/MTL ratio is greater than 10 and less than or equal to 1000	Check the data, resample within 72 hours of notification of the result and analyse to confirm bioassay result. Continue to monitor. Contact the regional water board and the State Water Board to discuss additional actions, which may include, but are not limited to, targeted analytical chemistry monitoring, increased frequency of bioassay monitoring, and implementation of a source identification program.
If BEQ/MTL ratio is greater than 1000	Check the data, resample within 72 hours of notification of the result and analyse to confirm bioassay result. Continue to monitor. Contact the regional water board and the State Water Board to discuss additional actions, which may include, but are not limited to, targeted and/or nontargeted analytical chemistry monitoring, increased frequency of bioassay monitoring, toxicological studies, engineering removal studies, modification of facility operation, implementation of a source identification program, and monitoring at additional locations.

Note: BEQ = Bioanalytical Equivalent Concentration; MTL = Monitoring Trigger Level (nanograms/litre)
Source: (California State Water Board, 2018[61])

Several OECD documents could serve as a model for guidance documents targeting EDCs, such as the Principles on Good Laboratory Practice (OECD, 1998[68]), Best Available Techniques to Prevent and Control Mercury Releases to Land and Water (OECD, 2022[69]), and Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment (OECD, 2019[70]).

Regulatory entry-points can justify and accelerate precautionary action, such as legislative clauses permitting action based on the precautionary principle or acknowledging EDCs as a potential risk. The European Drinking Water Directive, for example, refers to endocrine-disrupting compounds at several places of the preamble – providing an entry point for action (European Union, 2020[71]).

3.4.3. National action plans on EDCs

As EDCs touch many sectors, there is a need for a cross-sectoral approach. National action plans or strategies can act as a first step towards developing policy instruments and monitoring programmes. National action plans can coordinate efforts between sectors, strengthen knowledge, and, more generally, send a policy signal on the priorities of government. National action plans are common practice in the One Health context, such as the National Action Plans on Antimicrobial Resistance (AMR) (OECD, 2019[13]; Özçelik et al., 2022[72]; Anderson et al., 2019[73]; Brack et al., 2022[74]).

National action plans are increasingly adopted for endocrine disruption. France is one of the frontrunners with its Second National Strategy on Endocrine Disruptors (Box 3.3). Other examples are Japan's EXTEND programme (Box 3.1) and Belgium's National Action Plan on EDCs 2022-2026 (Section 3.4.2) (Government of Belgium, 2022[50]). In Canada, researchers from multiple disciplines have grouped themselves in the Intersectoral Centre for Endocrine Disruptors Analysis (ICEDA) (Box 3.4).

Based on existing national strategies on endocrine disruption, and the national action plans on antimicrobial resistance (WHO, n.d.[75]; Özçelik et al., 2022[76]), the following checklist for water-relevant national strategies on endocrine disruption emerges:

- A description of the coordination, governance structure and implementation across sectors, at least involving the human health sector, chemicals' sector, agricultural sector, environmental sector

(including water and biodiversity), food safety (including drinking water, packaging, and agricultural and aquaculture products), and industry.

- An analysis of regulatory strengths and weaknesses, identifying regulatory gaps and science-policy gaps. Such an assessment is part of Belgium's National Action Plan (Government of Belgium, 2022[50]). The European Commission published a regulatory Fitness Check on EDCs in 2019-2020, assessing whether the different pieces of EU legislation are fit to address the human health and ecosystem impacts of EDCs (European Commission, 2020[77]). The fitness check recognised that the EU regulatory system of EDCs is overall fragmented and limited, and it urges a comprehensive simplification and consolidation.
- Research priorities and exploratory work to fill gaps, particularly on the assessment of chemicals and their impact on humans and ecosystems. Research and chemicals' assessments are an integral part of the national strategies of Belgium and France (Government of Belgium, 2022[50]; Ministère de la transition écologique et solidaire, 2019[78]) (Box 3.8).
- A set of actions targeted at the reduction of EDCs in the (aquatic) environment, ranging from no-regret measures, precautionary or hazard-based approaches for critical hotspots. Actions or investments that require additional cost-benefit analyses, such as more stringent wastewater discharge standards can also be prepared or implemented.
- Water quality monitoring, including scaling up existing programmes and developing new initiatives. This includes pilot projects or roll-out of new methods such as bioassays and non-targeted screening. Pilot projects can create "snapshots", "archives" or "digital freezes" of water samples, which can be useful for future analysis (Badry et al., 2022[79]).
- Communication activities that improve awareness of the risks of EDCs and that guide on risk-reducing actions. Communicating the results of new monitoring methods (such as bioassays) to decision-makers, industry and the public demands specific attention, as this is highly technical and prone to misinterpretation. Communication activities could also address public water quality concerns, such as concerns related to chemicals found in the environment and drinking water. Belgium's national strategy prioritises communication and outreach activities specifically targeting vulnerable populations (Government of Belgium, 2022[50]). People's willingness to pay across OECD countries to address chemicals-related health risks, including fertility loss and low birthweight, underlines the relevance of action plans and public communication (Box 3.5).
- A costed implementation plan with an indication of funding gaps, a resource mobilisation strategy, and a monitoring and evaluation plan.

The global scope of the issue lays bare how tackling EDCs from only a national level is not nearly enough. As such, a coherent, coordinated and far-reaching global strategy needs to be developed, in parallel with national policy frameworks. Developing a global approach to the issue of EDCs could be inspired by the Global Action Plan on Microbial Resistance (WHO, 2015[80]).

Box 3.3. France's Second National Strategy on Endocrine Disruptors

In 2019, France launched its Second National Strategy on Endocrine Disruptors (SNPE 2) to tackle EDCs in all spheres of society, including freshwater (Ministère de la transition écologique et solidaire, 2019[78]). It is part of the fourth national plan health environment called "My environment, My health" of the Ministry of Ecological Transition and the Ministry of Solidarity and Health. Both programmes aim to protect biodiversity, health and ecosystem integrity through prevention and pollution reduction. A French survey published in 2018 and 2022 by the Institute of radioprotection and nuclear safety (IRSN) indicated that half of the population was concerned about (IRSN, 2018[81]; IRSN, 2022[82]). The SNPE 2 encompasses a total of 50 actions to tackle EDCs, which are classified in three main goals:

1. *Training and informing*. This goal aims to prevent exposure by training healthcare professionals, workers manipulating EDCs (e.g., farmers), and workers in contact with vulnerable populations (e.g., teachers). One of the objectives is to inform parents about creating a better environment for their new-borns (1000-premiers-jours.fr, n.d.[83]). The programme also published a list of confirmed and suspected EDCs (edlists.org, n.d.[35]). Chemical assessments are also part of this goal.
2. *Protecting the environment and the population*. This goal is based on the "One Health" concept, which presupposes that human health is linked to the health of wildlife and the environment. This goal aims to continue and improve the monitoring of EDCs found in water, air and soil. It makes the data publicly available. Furthermore, this goal explores the best available techniques to address contaminated sites. Moreover, this goal funds research projects and ignites voluntary action to reduce the number of products containing EDCs and substitute EDCs with safer chemicals. Lastly, this goal reiterates France's ambition to tackle the issue at EU-level.
3. *Improving knowledge*. This goal aims to reinforce the evaluation of substances. It supports the development of tools and methods to assess EDCs and it finances public-private platforms to conduct method validation (see, for example, Box 3.8). Furthermore, research on EDCs, their mode of action and their impact on human (studies of cohorts) and ecosystem health will be continued to improve knowledge, model EDC exposure (i.e. their fate in the body and environment), and develop good laboratory practices.

SNPE 2 aims to be as inclusive as possible. The strategy considers multiple stakeholders, including politicians, experts, industry, associations for the protection of the environment and consumers, the public and more. It specifically considers vulnerable populations (babies, teenagers and pregnant women) and populations with social vulnerability (exposed workers, specific social context, geographical location).

Source: (Ministère de la transition écologique et solidaire, 2019[78])

Box 3.4. Scientists join forces across disciplines and sectors to better manage EDCs in Canada

To remove barriers and decompartmentalise knowledge between and across sectors of industry and academic disciplines, researchers in the province of Quebec, Canada, founded the Intersectoral Centre for Endocrine Disruptors Analysis (ICEDA) in 2020. ICEDA's mission is to inform, assist and provide resources to the government, non-governmental organisations, industry and the general population in the identification, recognition, quantification, and management of EDCs. To achieve this mission, ICEDA's work is divided into three axes: intersectoral collaboration, knowledge sharing and the active involvement of policymakers.

To stimulate intersectoral collaboration and update the current knowledge on EDCs, ICEDA published an open-access special issue on EDCs in the Environmental Research journal (Langlois et al., 2022[84]). The edition comprises fourteen peer-reviewed articles with topics ranging from EDC detection methods, endocrine endpoints and regulation and remediation of EDCs. The lead authors presented the highlights to federal ministerial departments on health and environment.

To raise awareness of EDCs, ICEDA also invests resources in public outreach, especially to children. ICEDA reaches out to families through children's books, teen magazines, video clips and interactive activities. Moreover, young researchers are involved in every project.

ICEDA also stimulates dialogue between academia and all levels of government (federal, provincial, and municipal) to improve policies that address EDCs. Policymakers take part in ICEDA committees to guide on the development of the scientific program. Moreover, ICEDA launched a series of workshops to discuss ways to move from science to action for EDCs in Canada. Some recommendations from the workshops were: governing bodies (including regulators), academia and NGOs, should continue collaborating on chemical related regulation and management; stronger direct links between lab-based research, NGOs and policymakers should be made; and science should be translated in understandable language for public consumption.

ICEDA has 170 members, including 71 students, from 48 institutions and 8 different countries. It received funding from the *Institut national de la recherche scientifique* and the *Fonds de recherche du Québec Nature et technologies (FRQNT)*.

Source: Case study provided by Myriam Castonguay, Coordinator of ICEDA. The Special issue on EDCs: (Langlois et al., 2022[84])

Box 3.5. Willingness-to-pay to avoid negative health effects due to exposure to chemicals

The OECD project on "Surveys of willingness-to-pay to avoid chemicals-related health effects" (SWACHE) indicated that people in OECD countries are willing to pay a significant amount to reduce chemicals-related health risk. Several health outcomes included in the SWACHE project can be linked to exposure to EDCs such as fertility loss, very low birth weight, and IQ loss. On average, people are willing to pay USD 3 050[1] to avoid the loss of 1 IQ point in their children; future parents are willing to pay USD 91 000[2] to avoid infertility; and parents are willing to pay USD 1 194 000[3] to avoid very low birth weight of their new-born (Dussaux et al., 2023[85]; Mourato et al., 2023[86]; Ščasný, Zvěřinová and Dussaux, 2023[87]).

The results of this study could support decisions on whether chemicals management options and environmental policies are worth implementing based on a cost-benefit analysis. Assessment of chemicals management options and environmental policies can be considerably improved by better estimating their costs and benefits. The SWACHE project provides data to support such analyses. Understanding people's willingness to pay to avoid negative health outcomes supports the quantification of the benefits of chemicals regulation and the cost of policy inaction.

The values estimated in these studies could feed into cost-benefit analyses of water management decisions, such as investments in additional wastewater treatment infrastructure or the remediation of sediments when such investments benefit human health[4].

It should be noted that the results are not limited to exposure to EDCs through water. The studies take into account any class of chemical and any type of medium. Moreover, this study only evaluated health endpoints. Environmental endpoints, such as biodiversity loss, are not covered generating willingness to pay value that can be used for the evaluation of a variety of environmental and health policies and not only chemicals management options.

Note[1]: USD_{2022} Purchasing Power Parity (PPP) 3 050
Note[2]: Value of a statistical case; USD_{2022} PPP 91 000
Note[3]: Value of a statistical case; USD_{2022} PPP 1 194 000
Note[4]: While the survey questionnaires employ safer chemicals products as a payment vehicle to elicit willingness to pay, the surveys successfully deliver people trade-off between a reduced risk and a higher private cost.
Source: (Dussaux et al., 2023[85]; Mourato et al., 2023[86]; Ščasný, Zvěřinová and Dussaux, 2023[87])

3.4.4. Minimising negative impacts on vulnerable populations

Some populations are particularly vulnerable to EDCs, which can take various forms: biological susceptibility, socio-economic vulnerability, and cultural vulnerability to EDCs. The risk of exposure to EDCs can be higher to certain groups within a population, such as children, adolescents, pregnant women, and lactating women. Moreover, exposure levels can differ within societies and across countries, such as communities living close to contaminated sites, or groups that eat relatively more contaminated fish - for example, due to their socioeconomic status (U.S. EPA, 2019[88]). Physical effects aside, humans can also be culturally affected by EDCs when the existence of culturally important species or cultural keystone species is threatened. This is especially relevant to indigenous peoples.

Table 3.4. Factors affecting susceptibility to chemicals or pollutants

Intrinsic factors (biological)	Extrinsic factors (exposure-related)	Extrinsic factors (biodiversity-related)
Age and life stage	Disease status	Cultural practices and needs
Gender	Socio-economic status	
Race/ethnicity	Nutrition status	
Genetic polymorphisms	Geographic proximity	
	Lifestyle	

Source: Adapted from USEPA "Factors affecting susceptibility" (U.S. EPA, n.d.[89])

To improve human exposure risk assessments, the U.S. EPA has integrated a specific guidance and toolbox of techniques to assess risks for vulnerable groups and populations (U.S. EPA, 2019[88]). The multitude of sources and exposure routes, some of them uncertain, complicates the design of policies targeting vulnerable populations. Humans can be exposed to chemicals through different routes and water is only one source of exposure (Govarts et al., 2023[90]). Food products, consumer products, air, or occupational activity also contribute. The relative contribution of each of these sources to the health of a human being is hard to establish and varies from compound to compound. Risk assessments for vulnerable groups and populations can guide the development of policies.

Some policy options to targeting biologically susceptible groups have been put to practice. Through dietary advice campaigns, Sweden discourages children, adolescents and women of childbearing age from consuming contaminated fish and fish from specific water bodies, such as the Baltic Sea and several lakes, due to the concentrations of dioxins and PCB in fish (Swedish National Food Administration, 2008[91]). The Belgian National Action Plan on Endocrine Disruptors endorses several actions to protect vulnerable groups from exposure to pesticides. Examples are information campaigns on limiting the use of plant protection products around schools, playgrounds, childcare facilities and health care facilities, and subsidies for initiatives that inform, guide or raise awareness of vulnerable groups (Government of Belgium, 2022[50]; Government of Belgium, 2018[92]).

Looking at social vulnerabilities, EDCs may constitute an environmental justice issue, although the patterns are complex. The US EPA's definition of environmental justice is: "Environmental justice is the fair treatment and meaningful involvement of all people regardless of race, colour, national origin, or income with respect to the development, implementation and enforcement of environmental laws, regulations and policies" (U.S. EPA, n.d.[93]). Socio-economic status can influence exposure to EDCs. There is evidence that marginalised communities - with lower socio-economic status - have reported higher exposures to EDCs (Ruiz et al., 2017[94]). Inequalities in EDCs exposure have been observed in the United States (Attina et al., 2019[95]; Pumarega et al., 2016[96]). Ethnic minorities are disproportionately exposed to these chemicals, hence contributing to inequalities in diseases and disability. However, the role of water pollution hotspots in creating such environmental injustices is less studied. Some compounds have been found in higher concentrations in populations with higher socio-economic status (Govarts et al., 2023[90]). Studies in Belgium and the United States found an association between fish and shellfish consumption, high socio-economic status and relatively high concentrations of chlorinated compounds (in Belgium) and PFOA, mercury and arsenic (in the United States) in the sampled populations (Morrens et al., 2012[97]; Schoeters et al., 2022[98]; Tyrrell et al., 2013[99]).

Policies should also consider the needs and practices of indigenous peoples, in particular in protecting culturally significant species and cultural keystone species on which indigenous communities depend for their social, economic, physical and spiritual wellbeing (Garibaldi and Turner, 2004[100]). Some aquatic species are of cultural significance to indigenous communities (Noble et al., 2016[101]). EDCs could threaten the abundance, size, or distribution of species, potentially including those species that are of cultural significance. In New Zealand, Māori communities and scientists conducted a 4-year research project on aquatic cultural keystone species. The project developed cultural values-based environmental assessment

and reporting frameworks, and co-management and restoration strategies (NIWA, n.d.[102]). Whilst this project was not specifically targeting the issue of EDCs, its design could be relevant in identifying pressures from EDCs on cultural keystone species. Canada is integrating environmental DNA (eDNA) tools and indigenous ecological knowledge to model regional biodiversity changes (Box 2.8, Chapter 2).

3.5. A global challenge: international actions at the forefront

Policy recommendations

A global issue of concern, endocrine disruption is appropriately addressed at an international level. International actions can also boost innovation in water monitoring and assessment. Many regulators and utilities, in OECD and non-OECD countries alike, face challenges in the roll-out of water quality monitoring based on bioassays. Access to internationally standardised bioassays relevant for water quality testing, limited bioanalytical laboratory capacity and costs are common barriers. The following repertoire can inspire global responses to endocrine disruption:

- There is need to upscale, at international level, the standardisation and verification of test methods that are appropriate for water quality testing, based on international environmental technology verification processes or the principle of mutual acceptance of data. Currently, there are only few international guidelines and standardised methods for sampling and analysis for water quality testing using bioassays. The International Organization for Standardization (ISO) provides relevant methods for water quality testing and testing the estrogenic potential of water and wastewater. OECD Test Guidelines for EDCs are also relevant, though these have not been specifically developed for the purpose of water quality testing.
- The international market of bioassays for water quality testing needs to be expanded in terms of number of suppliers, endpoint variety and geographical service areas. Stimulating the demand for and development of new bioassays, based on non-animal methods, can support in achieving a diverse supply and reasonable cost of methods. Performance standards for bioassays, ideally at the international level to create a level playing field, can ease the market entry of new methods and method providers.
- Governments, at national level, could stimulate the uptake of new methods, by developing user toolkits for water authorities or water utilities and by training commercial, governmental and/or medical laboratories to perform bioanalytical methods for water quality testing. Laboratories specialised in water quality analysis often lack in-house expertise to apply bioanalytical methods, and training programmes for laboratory experts have proven to be effective.
- Mainstream the issue of endocrine disruption on global science-policy agendas, such as agendas on chemicals management, waste management and the One Health agenda.
- International research partnerships can encourage knowledge- and data-sharing on EDCs. Research partnerships have also been instrumental in the transition to new monitoring methods and the development of regulation.

There is a strong rationale for international and regional coordination to address endocrine disruption, as EDCs 1) are transported across international basins and ecosystems, 2) may be imported into a jurisdiction (national or sub-national) through trade, and 3) create impacts that are experienced globally (Godfray et al., 2019[103]; Kassotis et al., 2020[31]). The Endocrine Society recognises EDCs as a global health issue and affirms that “health issues related to EDCs cannot be geographically compartmentalised and should be addressed by intergovernmental actions” (Endocrine Society, 2018[104]). Yet, many research and policy

initiatives, such as biomonitoring and water quality monitoring programmes, are limited to high-income countries. Low- and middle-income countries cannot afford such programmes and yet often are disproportionately exposed to products and waste (Kassotis et al., 2020[31]). This section presents four recommendations for actions at the international level.

3.5.1. International market for bioassays

The test method available on the market - bioassays in particular - do not meet the needs of regulators and water service providers across the world. The market of bioassays *for the purpose of water quality testing* is limited in terms of number of suppliers, variety of endpoints, standardisation of tests (more on this in the paragraph below), suppliers' and laboratories' awareness of the specificities of water quality testing, and awareness of regulators and water service providers on the advantages and disadvantages of bioassays. International collaboration can accelerate the development of robust knowledge and databases, stimulate markets for new (monitoring) technologies, and support standardisation at the appropriate geographical scale. Moreover, demand-driven initiatives can facilitate market access to small and medium-sized enterprises and reduce costs at a longer term. Box 3.6 describes the international market of bioassays and makes recommendations for improvement. Methods such as mass spectrometry can also be scaled up at international level, for example by training national experts and by sharing robots across regions.

Box 3.6. The demand and supply of bioassays: a blind spot for water quality testing?

One of the main barriers to adopting bioassays for water quality testing is their limited availability, even though the assays may exist for other purposes such as pharmaceutical development, chemicals assessment and food analysis. This box characterises the international market of bioassays for water quality testing.

Supply-side

The global market of bioassays for water quality testing is dominated by a small number of international companies. Bioanalytical companies provide cell lines, test kits, and licences to laboratories to use their methods. Currently, only one company provides services across the full bioanalytical chain - from sample treatment to analysis - removing the need to run bioanalytical tests by the regulator or specialised laboratories.

In addition, academia is involved in developing bioassays, but these are often not offered on the market or made available for public use. Similarly, companies outside of the environmental sector, such as pharmaceutics, are involved in the development of bioassays that are not adapted to water quality monitoring.

To facilitate the uptake of bioassays for water quality monitoring, the supply market needs to mature. The international market of bioassays specific to water quality testing needs to be expanded in terms of suppliers, bioassay variety and geographical service areas. Market diversification can, first of all, make substitutes available which makes the market less sensitive to disruptions in supply, and secondly, reduce the costs of cell lines and kits. Governments could play a role in stimulating supply by accelerating the validation of bioassays (see for example Box 3.8), standardising methods (Box 3.7), developing performance standards for new methods, transferring technologies from other sectors to the water sector, and overall by stimulating demand.

Demand-side

With increasing interest to monitor endocrine disruption and other chemical risks in water, there is an expectation that the market for bioassays for water quality monitoring will expand. Potential demand comes from wastewater treatment facilities for municipal water and recycled water, the drinking water surveillance chain (from source water to treatment), and authorities tasked with environmental monitoring. Middle-income countries form a potential market once the supply side matures and the price decreases. For these countries, bioassays could possibly become a partial substitute for expensive chemical analysis, such as for the monitoring of pharmaceuticals.

In many countries, laboratories do not have the capacity to perform biological analysis at scale. Water quality and accredited commercial laboratories often lack bioanalytical capacity, whereas medical laboratories lack knowledge of water samples. As a consequence, bioanalytical analysis is outsourced to university laboratories which cannot deliver at scale and may not have the personnel specialised in water safety analysis.

Governments could stimulate demand by training commercial, governmental and/or medical laboratories to perform bioanalytical methods for water quality testing. User toolkits for water authorities or water utilities could also stimulate demand, such as the “Deltafact” toolkit in the Netherlands. The Deltafact toolkit includes simple explainers of bioassays, a cost assessment, suggested batteries of bioassays for drinking water, surface water and wastewater, case studies and knowledge gaps (De Baat, Van Den Berg and Pronk, 2022[105]).

Source: Authors, based on correspondence and interviews with stakeholders from the water sector.

3.5.2. International standardisation and validation of test methods for water quality testing

One of the main barriers to adopting new monitoring tools, such as bioassays and non-targeted analysis, is the lack of standardisation and validation of methods. Standardisation and harmonisation at international level, based international environmental technology verification processes or on the mutual acceptance of data principle, can avoid duplication, and therefore reduce costs. For instance, the net benefits of the OECD work on Environmental Health and Safety, including the OECD Mutual Acceptance of Data (MAD) system for chemicals testing and assessment, are estimated to be more than EUR 309 million per year (OECD, 2019[106]).

Currently, only few international guidelines on how to use and analyse bioassays and prepare samples for water quality testing have been developed. ISO has standardised methods for water quality testing and testing the estrogenic potential of (waste)water (e.g., the ISO 5667 series on water sampling and the ISO 19040 series on the estrogenic potential of water and wastewater). Moreover, the OECD Test Guidelines for bioassays are a useful tool for analyses, but these need to be further tailored for purposes of water quality testing. This means that authorities interested in the implementation of such methodologies need to rely on highly trained experts to develop monitoring strategies and methods. International collaboration to develop those guidelines and standardised protocols would make new approaches clearer and more accessible. Those guidelines would need to cover topics such as sampling, sample preparation, bioassays, analysis of results and risk assessment. The Global Water Research Coalition is an international initiative that has made steps towards standardisation of effect-based methods and sampling for water. Box 3.7 presents recommendations on the standardisation of methods. Box 3.8 presents an initiative that supports bioassay developers in the validation of their test methods in an OECD Test Guideline.

Box 3.7. Standardisation of methods for water quality testing

One of the ways to enhance water quality monitoring this is to standardise and verify methods for the specific purpose of water quality testing (e.g., through setting performance standards, environmental technology verification processes, good practices, or guidance documents on adapting test guidelines for water sampling). The four methods below can particularly benefit from standardisation:

Bioassays

Accelerating the standardisation of bioassays for water quality testing is crucial for the maturation of water quality monitoring. Aspects of the standardisation process are the verification of methods, the development of performance standards for new methods, and the development of a broad range of methods that address different endpoints relevant to water quality. Initiatives that support in the validation of test guidelines for ISO or OECD, such as the *Plateforme public-privé pour la pré-validation des méthodes de caractérisation des perturbateurs endocriniens* (Pepper) (Box 3.8), can facilitate this process. Standardisation of bioanalytical methods include a description of the method, procedures, observation, data and reporting, validity criteria of the test, apparatus and materials, test documentation and report templates, and a standard operating protocol (SOP) for the test performance.

Sampling

Sampling strategies and sample preparations are key to the successful performance of bioanalytical tests, and other monitoring strategies. The development of guidelines and standard operating procedures would facilitate the use of effect-based methods, effect-directed analysis and non-target screening (Neale et al., 2022[107]). To date, sampling is standardised in ISO standards (e.g. ISO 5667 on design of sampling programmes and sampling techniques for all aspects of sampling of water, and ISO 19458 on sampling for microbiological investigations) (WFD, 2009[108]), but it has not been mainstreamed across methods. California and Switzerland have developed a Standard Operating Procedure for sampling for water quality testing by bioassays (Kienle et al., 2015[109]; NWRI, 2020[110]). Generic guidelines on testing water samples can support the standardisation of tests. It could be worthwhile exploring if existing and future OECD Test Guidelines can be complemented with a sampling protocol for those methods that are relevant to water quality testing. Lastly, passive sampling is an upcoming method that is not standardised.

Databases

To widely adopt non-targeted analysis (NTA) and effect-directed analysis (EDA), there is a need for standardisation and harmonisation of methods to ensure that data can be exchanged and compared, for example regarding potential culprit chemicals detected with EDA methods. Keeping global records of NTA data also ease effect-directed analysis, as databases of suspected effect-drivers are readily available. Databases of collected data for chemistry (e.g. Information platform or chemical monitoring (IPCHEM)) can help information sharing, which in turn could help regulation (Hollender et al., 2019[111]).

eDNA/eRNA methods

To adopt eDNA methods for ecological surveys, invasive species management, and regulatory purposes, methods of standardisation are needed. An example of eDNA standardisation is Canada's iTrackDNA programme (for more on this programme, see Box 2.8, Chapter 2).

Source: Authors

Box 3.8. Pepper: a public-private partnership to accelerate the validation of bioassays

The Pepper Platform (*Plateforme public-privé pour la pré-validation des méthodes de caractérisation des perturbateurs endocriniens*) is a public-private platform, based in France, that supports bioassay developers in the process of pre-validation of test methods for the identification of endocrine disruptors.

The validation of test methods, such as bioassays, ensures the quality of reproducible methods that can be used around the world and in which regulators can have confidence. Validation can result, for example, in an ISO standard or an OECD Test Guideline. While a careful validation process is necessary, the validation process is also long and costly. The validation process of an *in vitro* bioassay takes at least two years and can cost as much as EUR 1 million or more, excluding the costs of method development. To give an example of the costs involved for the validation of *in vitro* bioassays in an OECD Test Guideline, requires that three laboratories without prior experience with the method acquire the know-how to apply the method, demonstrate the repeatability, predictability and reproducibility of the results for 30 chemicals, replicating the experiments at least three times. For *in vivo* bioassays, this process is even longer and more expensive.

Pepper selects methods that are mature and that meet the FRAND (Fair, Reasonable, and Non-Discriminatory) guiding principles of the OECD Test Guidelines. This means that the method needs to be accessible for users around the world, at a reasonable price. The Scientific Committee safeguards the quality.

Pepper supports the method developer in writing a standard operating protocol (SOP). Pepper also selects two other laboratories to test the SOP. The SOP can then be adjusted, if necessary, before starting the long work of establishing the reproducibility and predictability of the method. Pepper is also involved throughout the standardisation process with the OECD, from the first proposal to the official validation.

Validation of methods can also be organised by national or international entities, such as the European Centre for the Validation of Alternative Methods (ECVAM) of the EU Joint Research Centre (JRC), the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in the US, or the Japanese Centre for the Validation of Alternative Methods (JaCVAM). The public-private model of Pepper has advantages, such as the pooling public and private resources, establishing a centre of excellence, and providing access to funding for the validation process with the aim of improving chemical evaluation.

It should be noted that, besides a handful of ISO Standards, most test guidelines are not applicable to water quality testing. There is a need to adapt existing and develop new test guidelines for water quality testing. The authors are not aware of any intermediary organisation, such as Pepper, focusing water quality test guidelines.

Source: Authors and Philippe Hubert, Director of Pepper

3.5.3. International research partnerships

International research partnerships are essential and necessary in managing EDCs. They can be instrumental in sharing knowledge and data on EDCs, reducing uncertainties, supporting the transition to implementing new technologies and in supporting regulatory processes. Endocrine disruption in freshwater is still characterised by uncertainty which affects policy making. Ideally, international research partnerships also include researchers from low- and middle-income countries. Examples of research partnerships are

the NORMAN Network (Box 3.9), the European Partnership for the Assessment of Risk from Chemicals (PARC) (Box 3.9), the Intersectoral Centre for Endocrine Disruptors Analysis (ICEDA) in Canada (Box 3.4) and the Global Water Research Coalition (GWRC). The GWRC has done extensive work on mainstreaming bioassays and establishing trigger values for water quality monitoring (Neale, Leusch and Escher, 2020[112]; Neale, Leusch and Escher, 2020[113]). The GWRC is a not-for-profit organisation with research organisations from Australia, Canada, France, Germany, Netherlands, Singapore, South Africa, United Kingdom, and the United States as members.

Box 3.9. Research partnerships in Europe: the NORMAN Network and PARC

Two European examples of research partnerships are the NORMAN Network and the European Partnership for the Assessment of Risk from Chemicals (PARC).

The NORMAN Network supports work on monitoring CECs. Its members comprise experts from academia, agencies and the private sector. The NORMAN Network resulted into an up-to-date database where members submit and share information on substances, suspect lists, ecotoxicology and monitoring data, including on bioassays and chemical occurrence. The network's mission is to exchange information on CECs, improve data quality and promote synergies among research teams for a more efficient transfer of research findings to policymakers. Various activities of the network are linked to EDCs, such as the working group on bioassays and biomarkers in water quality monitoring. The working group aims to demonstrate the applicability of EBM as well as the use of effect-based trigger values. It also provides guidance documents, interlaboratory studies and communication with regulators.

PARC (European Partnership for the Assessment of Risk from Chemicals) is an institutional partnership based on regulatory drivers. The partners are from EU agencies (EEA, EFSA, ECHA) and academia. It is co-funded by the European Commission and EU Member States. PARC has several working parties such as the ones working on a common science-policy agenda, monitoring and exposure, hazard assessment and innovation in regulatory risk assessment. PARC is currently performing a pilot study for the environmental monitoring of PFAS and EDCs. The aims are to assess the background levels, characterise relevant exposure routes from diffuse and point sources and assess the effectiveness of management actions. The study will involve targeted and non-targeted analysis as well as EBM.

Source: Presentation by Dr Valeria Dulio of the French National Institute for Industrial Environment and Risks (Ineris) at the OECD Workshop on Developing Science-Informed Policy Responses to Curb Endocrine Disruption in Freshwater, 18-19 October 2022 (OECD, 2022[58])

3.5.4. Mainstreaming endocrine disruption on international agendas

A global issue of concern, endocrine disruption is appropriately addressed at an international level. It could therefore be appropriate to mainstream endocrine disruption on international science-policy agendas, such as agendas on One Health and chemicals. This includes the agreements made at the 2022 United Nations Environment Assembly to negotiate an internationally legal binding instrument by 2024 to end plastic pollution and to establish a Science-Policy Panel on Chemicals and Waste and to Prevent Pollution (Brack et al., 2022[74]). Similarly, EDCs and water quality more broadly, could take a more prominent position on the international One Health agenda.

3.6. Chapter conclusion

This chapter presented policies to tackle EDCs in freshwater. It documents existing policy approaches that intervene throughout the life cycle of EDCs. It also proposes interventions that are centred around the negative effects of EDCs on human and wildlife health, as the culprit chemical causing such negative effects is often unknown until further analysis is done. This chapter also makes the case for multilateral actions to improve monitoring, research and global action to tackle endocrine disruption.

The large number of potential endocrine active substances and their infinite number of mixtures, the diverse sources and entry-pathways into the aquatic environment and the need to make decisions under uncertainty, make policy design all the more complex. There is no single-best policy instrument to mitigate the negative effects of EDCs in water. Only a carefully designed package of policies has the potential to comprehensively reduce risks to human health and wildlife health. In addition, water quality assessments and monitoring are instrumental in safeguarding, to the best available knowledge, the integrity of ecosystems and human health. The next chapter therefore presents an action plan that supports the transition towards new monitoring methods that better capture the impacts of endocrine disruption.

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Annex 3.A. Suggestions for an international research agenda

Understanding the impacts of EDC pollution at ecosystem level. Current research is mostly focused on the effects on individual species. At higher levels of biological organisation (i.e., the effects of EDCs to species, communities and ecosystems), our understanding declines. The cascade of consequences of EDCs on the trophic system, including the aquatic food web, is uncertain (Saaristo et al., 2018[114]; Windsor, Ormerod and Tyler, 2018[115]).

Characterising and prioritising mixtures that impact water quality. The complexity of managing and regulating chemical mixtures lies in the combined effect of chemicals, the multitude of sources (agriculture, urban wastewater, industry, landfills), and uncertainties surrounding the effects of mixtures (Kortenkamp and Faust, 2018[116]).

Cross-species comparisons - using sentinel species such as zebrafish to draw conclusions on the impacts on other organisms such as humans - are a limitation in understanding the true impacts on other species. "Laboratory species may not always be relevant to the species of concern and data from human health risk assessments may not accurately reflect the risk to fish and wildlife" (Hotchkiss et al., 2008[117]). However, this uncertainty may not hinder any monitoring efforts to detect potential risks in water, as such effects can be validated through further analysis.

Making an economic case for controlling EDCs in freshwater. The cost of inaction, or acting after the damage is done, is likely more expensive than preventive measures, but this has not been established with certainty. While there have been attempts to define the economic costs of endocrine disruption, the data is characterised by uncertainty and most likely represents an underestimate of the actual costs (Malits, Naidu and Trasande, 2022[118]; Attina et al., 2016[119]; Trasande et al., 2016[120]; Cordner et al., 2021[121]). Moreover, the economic costs have not been defined when it comes to the loss of biodiversity and ecosystem services caused by endocrine disruption. A strengthened economic rationale informs the cost-effectiveness of policy decisions such as developing routine monitoring programmes and mitigation actions, such as upgrading wastewater treatment plants.

Bioassay development and EDC research have traditionally focused on the EATS (Estrogen, Androgen, Thyroid and Steroidogenesis) modalities. However, other hormones and endocrine axes are somewhat neglected. One example is the glucocorticoid receptor, for which many studies detect activity in freshwater samples. Moreover, even within the EATS modalities, not all the axes are equally developed. On the one hand, bioassays for estrogenic effects are in a very advanced state of development and could easily be deployed. In contrast, there are currently no standardised *in vitro* bioassays for the thyroid axis as pointed out by some participants. However, thyroid disruption is well studied and is notably known for disrupting metamorphosis in amphibians. More efforts are needed to develop and validate bioassays for EATS and non-EATS modalities (OECD, 2022[58]).

4 Action plan on monitoring and assessment of EDCs in freshwater

A policy-oriented action plan can support the implementation of new methods for monitoring and assessing endocrine disrupting chemicals (EDCs). The action plan presented in this chapter is targeted at ministries, environment agencies and utilities who are interested in making a start with assessing and monitoring of EDCs in water.

The presence of endocrine disrupting chemicals in water (EDCs) is potentially harmful to human health and the integrity of ecosystems. Prevailing methods of water quality monitoring and regulation, based on the substance-by-substance analysis of chemical concentrations in water, have been effective in managing pollution. However, prevailing methods have reached their limits in achieving water quality objectives: there is a growing body of evidence that current methods do not capture the complex and diverse chemical pressures on water. More chemicals are registered than ever before, while the global production capacity of chemicals is also increasing. Moreover, the global impacts of climate change on water, notably intensified flood and drought events, could increase the chemical burden through remobilisation of chemicals from sediments, sewer overflows, increased wastewater recycling, as well as reduced dilution capacity. At the same time, we better understand the risks of chemical pollution, such as risks stemming from mixtures, by-products, metabolites, and lower concentrations of chemicals.

All in all, there is need for methods that capture a broader range of substances than currently monitored, that are more sensitive to lower concentrations, and proactively warn about emerging (potential) risks. This publication therefore recommends adopting new methods, notably bioassays, to better monitor and assess the quality of water bodies, groundwater, (recycled) wastewater and drinking water. Whilst bioassays are increasingly adopted for water quality monitoring, some regulators and utilities are still exploring the practical implementation of bioassays (and other methods).

Table 4.1 presents a policy-oriented action plan that supports the implementation of new methods for monitoring and assessing EDCs. It is targeted at ministries, environment agencies and utilities who are interested in making a start with assessing and monitoring of EDCs in water.

Table 4.1. A policy-oriented action plan on monitoring and assessment of EDCs in freshwater

Action	Objectives of the action	Lead agency (national or local)	International partners	Section
Action area 1: Water quality monitoring and assessment				
Supplement existing substance-by-substance monitoring with effect-based methods, where appropriate and applicable	Capture effects of mixtures Capture non-priority-listed substances	Environment agency (national) Water supply and sanitation utilities		Section 2.3 Biological analysis
Maintain existing databases of identified and suspected EDCs and inform utilities, water regulators and other stakeholders about their usefulness and impacts on water quality	Inform about status and potential risk of chemicals Prioritise action for water quality	Environment agency (national) Ministry of Health	Country partnerships Academia	Section 3.3.1. A source-directed approach
Encourage the adoption of non-targeted analytical methods, where appropriate and applicable	Develop a baseline or snapshot of the chemical composition of water	Environment agency (national) River basin authorities Drinking water service providers	Transboundary river basin authorities Regional sea committees	Section 2.2.2 Non-targeted analysis
Carefully manage the transition to adopting new monitoring and assessment methods, by addressing barriers, communicating results and uncertainties, and managing pilot phases	Give room for trial and error Prioritise the adoption of new methods	Environment agency (national) River basin authorities Water supply and sanitation utilities		Section 3.4.1.
Action area 2: Driving innovation for water quality monitoring and assessment				
Send clear policy signals to test method providers that confirm the acceptance and further development of new water quality monitoring methods	Standardisation of test methods Encourage suppliers to innovate	Environment agency (national) River basin authorities Water supply and sanitation utilities		Section 3.5 international actions
Stimulate demand for new monitoring methods by sending clear policy signals to regulators and utilities, developing laboratory infrastructure, and introducing user toolkits	Improve monitoring and early warning Providing guidance to users and method suppliers	Environment agency (national) River basin authorities Water supply and sanitation utilities		Section 3.5 international actions; Box 3.6 on Demand and supply of bioassay
Invest in the standardisation and verification of test methods appropriate for water quality testing at international level	Improve monitoring and early warning Capture effects of mixtures Capture non-prioritised substances Reduce monitoring costs in the long-term	Environment agency (national) Private sector (bioassay developers) Academia	OECD and ISO as global standard development organisations	Section 3.5 A global challenge: international actions at the forefront Box 3.7 on Standardisation Box 3.8 Pepper: a public-private partnership to accelerate the validation of bioassays

Stimulate supply of bioassays and other monitoring methods by developing performance standards for bioassays suitable for water quality monitoring	Improve monitoring and early warning Predictability to supplier Reduce monitoring costs in the long-term	Environment agency (national) Private sector (bioassay developers)	OECD and ISO as global standard development organisations	Section 3.5. A global challenge: international actions at the forefront Box 3.6. The demand and supply of bioassays
Action area 3: Environmental quality norms and water quality standards				
Consider and prepare to put in place the requisites for the adoption of effect-based environmental quality norms and water quality standards, based on bioassays, in the future	Protect environment and human health Capture effects of mixtures Capture non-priority-listed substances	Ministry of Environment Ministry of Health Environment agency (national)		Section 2.6. Success factors of a robust effect-based monitoring programme
Set standards for wastewater discharge and sludge management that reflect endocrine activity	Protect environment and human health Capture effects of mixtures Capture non-priority-listed substances	Ministry of Environment Ministry of Health Environment agency (national) Water supply and sanitation utilities (invest, operate, monitoring, reporting)	Transboundary river basin organisations Regional sea committees	Section 2.6.1. Setting water quality standards and trigger values
Take endocrine activity into consideration in existing environmental quality norms and water quality standards for single substances (e.g., by including an additional assessment factor for endocrine activity)	Protect environment and human health Accurately reflect the endocrine activity of substances	Ministry of Environment Ministry of Health		Section 2.6.1. Setting water quality standards and trigger values Box 2.12 Considering endocrine disrupting properties in Environmental Quality Standards
Action area 4: Policies and actions that put effects, rather than individual culprit chemicals, at the centre				
Develop national and local response plans that can quickly mobilise a response to observed or suspected abnormalities in water quality monitoring results or wildlife	Reduce damage by reducing lag time Predictability to regulator, civil society	Ministry of Health Ministry of Environment Environment agency (national) Water supply and sanitation utilities Sub national entities: River basin authorities, municipalities	Transboundary river basin organisations Regional sea committees	Section 3.4.2. Response plans
Develop or renew national action plans on endocrine disruption	Predictability of a policy signal Drive innovation Reduce uncertainties Establishing a cross sectoral (One Health) approach	Inter-ministerial (often led by the Ministries of Health and Environment) Academia	UN agencies (UNEP, WHO)	Section 3.4.3. National action plans on endocrine disruption
Reaching out, raise concerns, on EDCs		Environmental NGOs Ministries of Environment Ministries of Health		

Action area 5: International research and multilateral policy agendas				
Develop international research partnerships	Share knowledge and data on EDCs Reduce uncertainty Support the transition to implementing new technologies Evaluate effectiveness of monitoring and policies	Ministries of Health and Environment	International organisations (EU, UN, other) Transboundary river basin organisations Regional sea committees	Section 3.5. A global challenge: international actions at the forefront
Mainstream the issue of endocrine disruption in international science-policy agendas, such as agendas on One Health and chemicals	Address pollution by EDCs at the global scale Establish a cross sectoral (One Health) approach	Ministries of Health and Environment	UN agencies (UNEP, WHO)	Section 3.5. A global challenge: international actions at the forefront

Note: This action plan is my no means exhaustive. Actions may be tailored to local needs and priorities.
Source: Authors

Glossary

Term	Definition
Adverse effect	Change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub) population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences (OECD, 2019[1]).
Adverse Outcome Pathway (AOP)	An Adverse Outcome Pathway describes a logical sequence of causally linked events at different levels of biological organisation, which follows exposure to a stressor and leads to an adverse health effect in humans or wildlife. (OECD, n.d.[2])
Bioassay or effect-based method	Bioassays or effect-based methods "are bioanalytical methods using the response of whole organisms (*in vivo*) or cellular bioassays (*in vitro*) to detect and quantify the effects of groups of chemicals on toxicological endpoints of concern. (Brack et al., 2019[3]) See also: *in vivo* tests and *in vitro* tests
Biological organisation	Levels of biological organization: Atom, molecule, cell, tissue, organ, organ system, organism (individual), population, community (Villeneuve and Garcia-Reyero, 2010[4]).
Contaminants of emerging concern (CECs)	A vast array of contaminants that have only recently appeared in water, or that are of recent concern because they have been detected at concentrations significantly higher than expected, and/or their risk to human and environmental health may not be fully understood. Examples include pharmaceuticals, industrial and household chemicals, personal care products, pesticides, manufactured nanomaterials, microplastics, and their transformation products. Also commonly known as micropollutants or emerging pollutants.
EATS modalities	Estrogen, Androgen, Thyroid and Steroidogenesis modalities are the most studied endpoint for endocrine disruption. The OECD added in its revised document modalities for invertebrates: Juvenile Hormones (Jh) and ecdysteroids (Ec) (OECD, 2018[5]).
Endocrine active substance	A substance having the inherent ability to interact or interfere with one or more components of the endocrine system resulting in a biological effect but need not necessarily cause adverse effects (EFSA, 2013[6]).
Endocrine disruptor or endocrine disrupting chemical (EDCs)	"An [endocrine disrupter] is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." And, "a potential [endocrine disrupter] is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations" (IPCS, 2002[7]). Also known as endocrine disrupting chemical or endocrine disrupting substance.
Endocrine system	The chemical coordinating system in animals, that is, the endocrine glands that produce hormones (Jacsó, 2002[8]).
Endpoint	The recorded observation coming from an *in chemico* method, an *in vitro* assay or an *in vivo* assay (OECD, 2011[9]).
Effect-based monitoring / methods (EBM)	Bioanalytical methods using the response of whole organisms (in vivo) or cellular bioassays (in vitro) to detect and quantify the effects of groups of chemicals on toxicological endpoints of concern (Brack et al., 2019[10]).
Effect-directed analysis	"A tool for identifying predominant toxicants in complex, mostly environmental mixtures combining effect testing, fractionation and chemical analysis"; "Designed to direct chemical analysis toward those chemicals that actually cause hazards mostly indicated by laboratory in vitro and in vivo bioassays" (Brack, 2011[11])
Hazard-based decision-making	Decision-making in chemicals management that "focuses on addressing the inherent hazards of chemicals through substitution or other approaches, rather than calculating an acceptable level of risk" (UNEP, 2019[12])
Hormone	"The traditional definition of a hormone is a molecule produced by an endocrine gland that travels through the blood to produce effects on distant cells and tissues" (UNEP WHO, 2013[13]).
Indirect effects	"Indirect effects in ecotoxicology are defined as chemical- or pollutant-induced alterations in the density or behaviour of sensitive species that have cascading effects on tolerant species in natural systems" (Fleeger, 2020[14]).
In vitro test	The technique of performing a given experiment in a test tube, or, more generally, in a controlled environment outside of a living organism (OECD, 2018[15]).
In vivo test	Experimentation using a whole, living organism as opposed to a partial or dead organism, or an in vitro controlled environment. Animal testing and clinical trials are two forms of in vivo research (OECD, 2018[15]).
Legacy chemical	Chemicals that are banned or restricted, but still appear in environment as legacy compounds.
Mixture	A combination of two or more chemicals (liquid or solid) that do not react with each other (OECD, 2018[15]).

Mixture effect	Temporal co-exposure to any combination of two or more compounds that may jointly contribute to actual or potential effects in a receptor population (OECD, 2019[1]).
Non-monotonic dose response	Adverse effects of chemicals that exhibit greater or even opposite effects at low doses compared to those observed at high doses. This means that traditional toxicology, which hinges on the premise that high-dose toxicity testing will proportionally inform us about low-dose exposures, does not hold (Vandenberg et al., 2012[16]).
Recycled water	Former wastewater that has been treated to remove solids and certain impurities. It is only intended to be used for non-potable uses (e.g. irrigation, dust control, fire suppression); with more advanced treatment, it can be used for indirect potable reuse (i.e. discharged into a water body before being used in the potable water system). Also known as reclaimed water (OECD, 2009[17]).
Risk-based decision-making	An approach to decision-making in chemicals management based on patterns of exposure to a chemical. "This includes identifying use patterns that may create widespread exposure across a population, or intense exposure for a subset of the population." (UNEP, 2019[12])
Substance-by-substance approach	Risk approach for testing water quality based on detection of above-threshold levels of single chemicals.
Trophic levels	The classification of natural communities or organisms according to their place in the food chain. Green plants (producers) can be roughly distinguished from herbivores (consumers) and carnivores (secondary consumers) (United Nations, 1997[18]).

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OECD Studies on Water

Endocrine Disrupting Chemicals in Freshwater

MONITORING AND REGULATING WATER QUALITY

Endocrine disrupting chemicals (EDCs) are contaminants of emerging environmental and health concern that have been detected in freshwater, wastewater and drinking water. They interfere with the endocrine system in humans and wildlife, and produce adverse effects such as developmental, reproductive, neurological and immune effects. Their presence in water raises concerns for the integrity of ecosystems and biodiversity. Addressing the challenges of EDCs in water is particularly complex due to their ability to trigger adverse effects at very low concentrations, their potency in mixtures with other chemicals, and the vast range of sources and entryways of this group of chemicals into the environment. This report presents new water quality monitoring methods, such as bioassays and non-targeted analysis, that are well equipped to capture the impacts of EDCs in water. These new methods supplement the traditional substance-by-substance chemical analysis of water quality. The report also outlines policy instruments to manage the chemicals' lifecycle from source to end-of-pipe. It proposes tools and regulations that respond to the negative effects of endocrine disruption, even if the culprit chemical is still unknown. The analysis draws on case studies from OECD countries to provide practical examples and concrete policy actions.

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