Organisation for Economic Co-operation and Development

OECD Workshop on Developing Science-Informed Policy Responses to Curb Endocrine Disruption in Freshwater, 18-19 October 2022

Summary note

This summary note[1] presents the key messages and discussion highlights of the workshop on Developing Science-Informed Policy Responses to Curb Endocrine Disruption in Freshwater, 18-19 October 2022, held in Paris, France, and online. The workshop was convened by the OECD Environment Directorate.

The ultimate objective of the workshop was to harvest policy guidance, founded on scientific knowledge and country practices, to manage endocrine disruption in the freshwater environment. The objectives of the workshop were to:

- Build a policy-relevant knowledge base on endocrine disruption in freshwater and its impacts on ecosystems and humans
- Identify lessons learnt and good practices from country case studies on monitoring and policy approaches on freshwater
- Explore policy options to advance from monitoring and data collection to mitigating endocrine disruption.

[1] Disclaimer: Please note that the information in this summary note derives from the workshop speaker presentations and participant discussions. This information has not been independently verified by the OECD nor agreed by all participants. The workshop was held under Chatham House Rule; thus, this summary note does not identify individual speakers.

Key messages

- Endocrine disrupting chemicals (EDCs) are chemicals that can alter the function of the endocrine system of humans and wildlife. EDCs can trigger adverse effects in intact organisms or their offspring. In humans, EDCs are associated with disease outcomes in the reproductive system and metabolic syndromes such as obesity and cardiovascular diseases. In wildlife, similar effects are occurring which can have negative impacts on population of species - raising concerns for the integrity of ecosystems. EDCs have been detected widely in freshwater.
- Monitoring endocrine activity in freshwater emerged as a key policy solution. This can be achieved through effect-monitoring approaches that detect the effect of a water sample (e.g., surface water, effluents, recycled water, drinking water) on organisms. Bioassays are the main tool for effect-based monitoring. Compared to regular chemical analysis, bioassays have the benefit of capturing effects at very low doses and incorporating the effects of the often-complex mixtures of chemicals present in the environment. Bioassays are increasingly used as a pre-screening or early warning tool to detect endocrine activity in water. Effect-based methods are also used for policy evaluation and setting regulatory standards for effluents.
- Effect-based monitoring for assessing endocrine activity in water delivers best in combination with chemical analysis, which can identify and address the causes. Chemical analysis also more readily aligns with existing regulations. Lastly, non-targeted chemical screening can support in detecting new emerging chemicals. There was strong agreement that chemical tools remain essential in a robust water quality monitoring programme.
- The technological and scientific readiness to implement effect-based monitoring tools is quite high. There was a clear consensus among workshop participants that bioassays for estrogen modalities are at the most advanced state of development and acceptance. Nonetheless, there is a large discrepancy in knowledge between other modes of action. Moreover, there is still a gap between the use of bioassays in research and their actual implementation in policy.
- The economic case for controlling EDCs in freshwater is not clear-cut. The additional costs of introducing effect-based monitoring tools differ per region and country, depending on bioassay availability and laboratory capacities. Intensified monitoring will likely increase the need to implement mitigation actions, such as upgrading wastewater treatment plants. Society can bear part of these costs and might be willing to pay if such actions avoid negative health outcomes, but industry, which manufacture and market these substances, has a role to play. The cost of inaction, or acting after the damage is done, is likely more expensive than preventive measures.
- It appears that different countries and research projects have different ambitions. Ambitions vary from raising awareness in selected industries (e.g. wastewater treatment plants, paper mills, agriculture); developing new standards or methods for regulation of water quality; or engaging with the wider community. Current monitoring programmes, some of which are systematic and others ad hoc, have been successful in mitigating the source of pollution.
- There is a trend towards regulating estrogenic effects in freshwater, either by integrating endocrine disrupting effects into existing environmental quality standards or by setting separate effect-based standards on estrogenic effects. Further developments will depend on improved knowledge on modes of action other than estrogenic effects, a societal debate on trigger values for regulatory purposes, laboratory capacity and infrastructure to perform bioassays, and analyses to make the economic case for effect-based monitoring and mitigation actions.

Making the case for policy responses to manage endocrine disruption in freshwater: Introduction to endocrine disrupting chemicals

Endocrine active chemicals are exogenous chemicals that can interfere with the endocrine system. Endocrine disruptive chemicals (EDCs) are a subset, which cause adverse health effects in intact organisms or their offspring. EDCs can be found in multiple classes of chemicals (e.g.: natural and artificial hormones, pesticides, plasticizers, flame retardants, etc.)[2]. They can be released in freshwater bodies throughout the life-cycle of the chemical from its production, distribution, usage and disposal (e.g. in wastewater or landfills). The variety of chemicals and effects, as well as the multiple entry points of EDCs complicates policy implementation to address the issue for freshwater.

Endocrine disruption impact ecosystem and human health

Even if the issue of EDCs in freshwater is complex, there is a clear need for action to improve the health of ecosystems and humans. Multiple studies have shown direct impacts of EDCs on aquatic species such as the decrease in reproductive success, a delay or altered growth and development, as well as altered behaviour (e.g. reproductive behaviour). Indirect impacts of EDCs on aquatic systems have also been measured through the disruption of the trophic chain: even if only one species is affected in a given ecosystem, it will affect the cycle of predation in the system and hence the feeding and the survival of every species. This was well exemplified by a Canadian study where a whole lake was exposed to a small concentration of ethinylestradiol (EE2), an active compound in the contraceptive pill. The presence of EE2 directly affected the population of fathead minnow: its biomass in the lake was decreased by 99%. In the years following the start of the experiment, the population of insects and zooplanktons increased, while the population of lake trout, a key predator in the lake, decreased. This illustrates that direct effects measured in the lab on a species can have major indirect impacts on ecosystem health.

The impact of EDCs on human health is also characterised by disruption of organ growth and development, decrease in fertility, increase in hormone-related cancers and metabolic syndrome (diabetes and obesity). For EDCs in freshwater, the main route of exposure for humans is through drinking water. EDCs have been detected in drinking water around the world. It should also be considered that 2 billion people still do not have access to safely managed water. Exposure could also occur through bathing and leisure activities, as well as the consumption of seafood. New concerns also arise from the use of recycled water for watering crops for human consumption.

The economic case and the willingness to pay to avoid negative health outcomes

The workshop discussed the economic rationale to manage EDCs in freshwater. A participant highlighted that regulating EDCs can be a financial burden to regulators and industry. Other participants argued that the cost of inaction, and acting after the damage is already done, is often way more expensive than preventive measures.

Two subsequent questions arise. One relates to who should bear the costs. A participant stressed that it is not always society who should bear the costs, and producers or polluters can also cover the costs of monitoring and mitigation action. The OECD project on “Surveys of willingness-to-pay to avoid chemicals-related health effects” (SWACHE) indicated that people are willing to pay a significant amount to reduce chemicals-related health risk. Some surveyed health outcomes can be linked to exposure to EDCs such as fertility loss, very low birth weight, thyroid dysfunction and non-fatal cancer. The results could support

[2] This summary does not distinguish between endocrine active substances and endocrine disrupting chemicals for the sake of brevity. However, all workshop participants acknowledge that they are not the same.

decisions on whether chemicals management systems are worth implementing. The results of the SWACHE project presented during the workshop have yet to be published.

The other question – which was not discussed at length in the workshop – relates to the actual cost of taking measures. Monitoring EDCs can lead to options considered expensive, such as upgrading water treatment, banning some substances, or regulating reuse of treated wastewater. Such measures can be costly for specific groups or the population as a whole.

Monitoring using effect-based methods

Monitoring efforts have largely focused on chemical monitoring and analysis of water quality. However, countries and authorities are more and more adopting additional monitoring approaches that detect the effects of a water sample (e.g., surface water, effluents, recycled water, drinking water) on cells and organisms. Such effect-based approaches can help determine the toxicity of water and, subsequently, any needed regulatory action to decrease or eliminate a hazard.

Regular chemical monitoring is currently unable to address the issue by itself

Chemical monitoring is common across countries. However, there was a consensus from participants that current methodologies are not well suited for EDCs for multiple reasons:

- EDCs can have effects in concentrations below nanogrammes per litre (ng/L) (e.g. Swiss quality standard for EE2 is 0.035 ng/L). Most current routine chemical analyses are unable to detect known EDCs at those concentrations.
- Chemical analyses rely on lists of known chemicals that are usually regulated or prioritised. Currently, countries monitor only a few or no EDCs. For example, under the Water Framework Directive (WFD, 2000/60/EC) in the European Union prescribes to Member States the monitoring of 45 priority substances, of which some are considered EDCs or endocrine active substances. However, well-known estrogenic substances (estrone (E1), 17B-estradiol (E2) and ethinylestradiol (EE2)) were not accepted on the Priority Substances List in 2013 and were instead added to the Watch List. Moreover, the environmental quality standards (EQS) for the substances on the Priority Substances List have generally been developed without considering their endocrine disruptive effects (see the case study of France).
- Freshwater contains complex mixtures of chemicals, which include EDCs. While individually these EDCs might have insignificant effects, together they can create “something from nothing”. Mixture effects from EDCs are not yet fully understood. Hence, the use of analytical chemistry alone is not sufficient to address mixtures of EDCs in freshwater.

Effect-based approaches and bioassays are essential in regulating endocrine disruption

One way to circumvent the limitations of analytical chemistry to monitor EDCs is to use an effect-based approach, or effect-based methods (EBM). This type of approach uses bioassays: bioanalytical tools that allow the quantification of an effect. For endocrine disruption, bioassays can help to quantify effects related to specific modes of action (MoA)[3]. Bioassays can be *in vitro* (in a test tube, outside an organism) or *in vivo* (living animals). The most discussed type of bioassay in the workshop were those that quantify the activation of the estrogen receptor (ER). Similar bioassays are found for the receptor of other hormones such as for androgens (AR), thyroid hormones (TR), glucocorticoids (GR) and progesterone (PR).

[3] A description of the processes that ultimately leads to physical changes in organisms that result into negative health effects, starting from the substance that triggered an event. (OECD 2012)

For monitoring EDCs, bioassays have various advantages over traditional chemistry:

- Bioassays are more sensitive than chemical analyses. Multiple studies presented at the workshop showed bioassays could still detect effects while nothing was detected using chemical analyses.
- Bioassays do not require prior knowledge on the substances contained in the water sample. Hence, bioassays will detect effects triggered by any substance present in the water sample, even if the substance is unknown or not on an official monitoring list. This will also include metabolites of chemicals that can be active but are not necessarily measured.
- Bioassays can inform on mixture effects as the activity detected is a result of the whole mixture.
- Bioassays could be applied to implement policy in a similar way to chemical analysis. For that, thresholds of effect - more commonly called effect-based trigger values (EBT) - could be set in a similar way to standards and norms for single chemicals, as described later in this summary.

Among participants, there was a consensus that bioassays would add a lot of value in the monitoring of endocrine disruption. Moreover, most participants argued that bioassays and chemical analyses should not be mutually exclusive, and a combination of both methods makes a robust monitoring system. Moreover, the correlation between chemistry and bioassays is usually high except at low doses, since chemical analytical detection limits are too high. This means that chemical concentrations, if available, often confirm the results of bioassays.

While there was some consensus among the different parties present (academia, government, and industry) on the benefits of bioassays, there were diverging opinions on their readiness for implementation. The divergent opinions on the readiness of bioassays are well summarised by the presented results of a survey done by the Global Water Research Coalition on the use of EBM. Most survey respondents agreed that bioassays would improve water quality monitoring. However, the survey showed that there are still many barriers to the implementation of bioassays. The main barrier was the cost of EBM. Some participants suggested that EBM have a cost similar to chemical analysis, though this may vary per region. EBM becomes particularly expensive when introduced in addition to existing monitoring programmes, rather than as (partial) substitution. The survey also highlighted other barriers linked to the lack of: (1) support from authorities, (2) standardisation and guidelines for methods and result interpretation, (3) development of trigger values, (4) communication about the added value of EBM, (5) knowledge, (6) feedback and validation, (7) links between EBM and the health of an organism or population, and (8) trained staff and facilities to perform EBM.

Effect-based trigger values and environmental quality standards

Effect-based trigger (EBT) values provide a threshold for the assessment of bioassay test results. EBT can be embedded into regulation, acting similarly as EQS or drinking water quality standards (DWQS) for analytical chemistry. In both cases, if the effect or concentration detected is higher than the given threshold, there is a risk. If the values are below the threshold, the risk is considered low. The main method to derive EBT values for a bioassay is to adapt legal guidelines for single chemicals in biological equivalents (BEQ) for a given mode of action (e.g. estrogen equivalent).

During the workshop, there was a consensus on the need for trigger values as a tool to analyse bioassay results and as a powerful communication tool to inform policy makers on water quality. However, there were disagreements on the readiness of EBT values:

- Depending on countries, there are currently few to no standards or legal guidelines for EDCs in freshwater. Some examples include standards in the state of California in the United States for use with bioassays, and in Switzerland, but not for use with bioassays (see case studies below).
- Some participants noted that current methods to derive those standards don't generally consider endocrine disruption, and are thus higher than they should be. For EDCs, the inclusion of some characteristics into quality standards are debated, such as effects at low-dose, non-monotonic

dose responses for some EDCs, effects depending on specific temporal windows, delayed effects, and transgenerational effects. For those reasons, some participants argued that EQS for EDCs should be distinguished from other EQS.

- The contradiction between threshold values in different sectors was often pointed out by participants, some of whom suggested a harmonisation of frameworks. For example, in the EU, the Plant Protection Products Regulation (1107/2009/EU) and Biocidal Products Regulation (528/2012/EU) take a hazard-based approach to EDCs which suggests there should not be a threshold for EDCs (i.e. the quality threshold is equal to zero). However, the WFD has thus far largely adopted a risk-based approach, which means a certain level of risk is considered acceptable and a non-nil threshold can be set for risk assessment.
- Some argued that the link between *in vitro* and *in vivo* results is still uncertain; i.e. when effects are observed *in vitro*, it is not necessarily evident that the same effects will be triggered *in vivo*. This uncertainty can reduce confidence in the use of bioassays.

A recent French report showed that, under the WFD, the approach to deriving EQSs for identified or suspected EDCs is heterogeneous. This report analysed the 86 EQSs for surface water pollutants under the WFD regarding whether endocrine disruptive activity was considered in the EQS and whether the available toxicological data for risk assessment was used appropriately. The report concluded that 70% of the EQS do not appropriately consider endocrine disruption. This shows that there is a need to adapt the approach to deriving EQS for EDCs.

The same French report proposes a method to adapt the EQS derivation in Europe to integrate endocrine active effects into an EQS. The proposed methodology has three main steps: (1) evaluate the state of the knowledge on the endocrine disrupting property of the chemical; (2) evaluate if more data can be integrated to the evaluation or if more research is required; and (3) add assessment factors to the calculation of the EQS based on whether the compound is an identified or suspected EDC and based on the reliability of the data.

To go forward with bioassays, the participants strongly suggested that policy makers decide on setting trigger values, even if the values are not perfect in the first instance and might need to be revised later – as is the case in California. Policy makers, first of all, need to decide whether to set a threshold for EDCs or not (a “zero-tolerance” approach). If a threshold is deemed appropriate, they should try to adapt the derivation of their legal value to take into account the EDC endpoint. However, the process of setting the trigger value might delay the adoption of bioassays for monitoring.

Bioassays are not all equal

One gap highlighted by the workshop is that there are many differences between bioassays, and that they are not equal in various ways.

- **Development and validation of bioassays.** Bioassay development and EDC research have traditionally focused on the EATS (Estrogen, Androgen, Thyroid and Steroidogenesis) modalities. However, other hormones and endocrine axes are currently neglected. One example is the glucocorticoid receptor, for which many studies presented during the workshop detected 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 standardized *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.
- **Not every bioassay is relevant for freshwater monitoring.** Another aspect that needs to be taken into account when developing a monitoring strategy based on bioassays is that not all

bioassays are relevant for water testing. For example, only few compounds known to interact with the thyroid receptor are present in water. Hence, a thyroid receptor transactivation bioassay might be a waste of resources, while there are more compounds active in the transthyretin (TTR) displacement bioassay.

- **Sensitivity varies across bioassays.** One study presented at the workshop showed that among estrogenic bioassays, there can be noticeable differences in sensitivity, with yeast-based assays having the highest detection limit. Those variations need to be considered when selecting bioassays.
- ***In vitro* vs *in vivo* bioassays.** Another aspect to consider is the difference between using *in vitro* or *in vivo* bioassays. Researchers and policymakers around the world aim to reduce animal use following the 3Rs (replacement, reduction and refinement). To answer to this resolution, *in vitro* bioassays are appropriate. However, there are various opinions on the readiness of the tools, mostly linked to the capacity of *in vitro* bioassays to predict *in vivo* effects. The first reason for this argument is that *in vitro* bioassays do not take into account toxicokinetic effects. Another important aspect to consider for EDCs is that the current definition of endocrine disrupting effects requires proof of adverse effects in an intact organism via an endocrine mode of action. This means there is a need to demonstrate that (1) an adverse effect is generated by the chemical or mixture in an intact organism, (2) the chemical or mixture can act through an endocrine MoA and finally (3) the disruption of the endocrine MoA is the cause of the adverse effect. In some countries, the definition could prompt the use of *in vivo* rather than *in vitro* bioassays for monitoring and in policy. A middle ground between *in vitro* and *in vivo* bioassays are bioassays that use genetically modified embryos of fish and frogs, such as the Watchfrog bioassays. Embryo stages are non-sentient, limiting animal suffering, but still allow relatively quick assays and can take into account the toxicokinetic of chemicals found in water samples.

There was a consensus among participants that estrogenic bioassays are the readiest for monitoring and policy. Moreover, participants agreed that there was a need to develop and validate more bioassays for other endocrine modalities in freshwater.

Other considerations in adopting effect-based monitoring

- **Few chemical drivers for each MoA.** It was mentioned often that each specific endocrine mode of action usually has a small set of high-potency chemicals. For example, for estrogenicity, the main drivers are estrone (E1), 17b-Estradiol (E2), estriol (E3) and 17a-Ethinyl-estradiol (EE2). Other molecules can generate effects, but with a lower potency. When high-potency chemicals are present in a mixture, they will typically have additive effects and they will drive the overall activity of the mixture, making the contribution of low-potency molecules to the effect small.
- **Sample preparation.** While this topic was discussed only briefly during the workshop, sampling methods and their preparation for bioassay analysis can affect the activity detected. Grab samples for *in vitro* bioassays will usually be prepared using solid-phase extraction. For *in vivo* bioassays, the animal or embryo can be exposed directly to the effluent or to an extract similar to *in vitro* bioassays. Some studies presented during the workshop used passive sampling in parallel to grab sampling. While both sampling methods can give similar results, sometimes one method will detect an activity and the other will detect nothing. A French study presented at the workshop indicated that a combination of both sampling methods would be valuable.

Effect-directed analysis

Multiple presentations showed approaches using bioassays as a first step, followed by effect-directed analysis (EDA). After hotspots were identified based on analyses with bioassays, some studies, such as the cases from France and the Netherlands, used EDA to identify the compound generating the detected

effect. EDA is a method in which a sample is separated into multiple fractions, which are then analysed by both chemical analysis and bioassays to identify culprit chemicals. EDA can help confirm that the effect is generated by known compounds, but it can also help identify new compounds.

An example is the case study of the Holtemme River in Germany. In river samples, anti-androgenic effects were detected. Moreover, the fish from the river had decreased reproduction. With the use of EDA, a fluorescent dye (4-methyl-7-diethylaminocoumarin) was identified as the source of the effect. Its activity was further confirmed *in vivo* (Muschket et al, 2018).

In France, a surface water sample showed high activation of the glucocorticoid receptor. Using bioassays, analysts were able to track down the source of the contamination to a pharmaceutical company. They were then able to perform EDA and to determine that the activity detected was linked to 2 synthetic glucocorticoids and 2 metabolites. Following the discovery, the local governmental authorities were informed, and some mitigation actions were undertaken by the industry to reduce the discharge.

Those examples illustrate the usefulness of EDA not only to identify EDCs, but also to mitigate the source. However, it should be noted that EDA is currently time consuming and costly, which makes it less accessible until the process is automated. Moreover, a few participants pointed out that the identification of a chemical by EDA does not mean that the chemical is formally classified as an EDC which is an even longer process.

Addressing the gaps between research and regulation

While there was a consensus among participants of the workshop that effect-based monitoring would be an asset for monitoring EDCs in freshwater, there is still a gap between their use in research and their actual implementation in policy. This section highlights the gaps discussed.

- **Guidelines on effect-based trigger values**. Guidelines on trigger values exist, mostly developed by academia, with a high level of consensus among the science community. However, the policy community feels that the guidelines are not quite there yet. Arguments raised by policy practitioners include that trigger values are complex to understand; opinions diverge on the appropriate threshold levels; and some endpoints are not yet well supported by scientific evidence.
- **Validation and standardisation of methods and documentation**. There is a need for standardisation of protocols not only for bioassays, but also for the method of collection and processing of samples. Standard protocols should also inform on minimum requirements for the quality assurance of samples and the cell assay (preferably performance-based). Protocols should not be limited to one specific brand or type of bioassay to make sure the methods stay flexible for competition and to make them adaptable to the specific reality of each country. Furthermore, standard protocols should describe data analyses and their interpretation. They should include recommended methods (and software/tools) to calculate bioassay equivalent concentrations, data acceptability criteria and reporting requirements. Moreover, interlaboratory comparison should be performed to insure the robustness of methods across laboratories (industry, academia, governmental facilities), platforms/vendors and relevant sample matrices. Of note: some ISO methods for calculating estrogenic potential of water sample exist.
- **Laboratory capacity and infrastructure**. While bioassays are currently used widely in water research, those methodologies are not necessarily accessible to regulators. 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 labs should be considered, including research labs, contract labs and water utility/authority labs.
- **Availability of the technology**. The current supply of tests may not meet the need of every regulator across the world. In addition, tests are still under development for certain endpoints.

Moreover, bioassay developers and vendors are not always aware of the complexity of water samples and should be involved in the conversation.

- **Outreach and communication**. The workshop showed that there is a difference in the level of acceptance of bioassays between academia and regulators. Moreover, there is lack of communication across sectors of regulation, but also with industry. One way to solve this is by improving communication. For example, Southern California Coastal Water Research Project Authority did a lot of outreach before implementing bioassays in policy. Outreach activities included workshops and seminars for industry, policymakers and utilities, technical workgroups for industry and regulators, and lab certification and training programmes. Finally, stakeholders were engaged through project advisory committees.
- **Multilateral and cross-sectorial approaches**. As water is a cross-sectorial issue, there is a need for multilateral and cross-sectorial approaches at international, national and regional levels. This should include communication across the sectors and levels of government. As an example of such an effort, the EU has conducted a fitness check of all regulations related to EDCs. It is also working on the “one substance one assessment” initiative.
- **Maximising the value of existing data**. Many countries do not have access to widespread chemical analysis and effect-based monitoring tools. However, they may be able to benefit from the wealth of data accumulating in various ways. For instance, data collected to track progress towards sustainable development goals (SDG) include data portals on Water, Sanitation, Wastewater Treatment, Drinking Water, Ambient Water Quality, Solid Waste Management, Urban Slums, Material Consumption and Hazardous Waste. Organisations such as the European Medicines Agency host a lot of data on pharmaceuticals use. One participant highlighted that it could be worthwhile to explore whether these existing data portals could serve as a proxy for identifying EDC hotspots.

To move ahead on these fronts, participants valued both bottom-up (research-driven) and top-down (regulatory-driven) approaches. They complement each other. The former is innovative and anticipates new issues and develops methods that need attention from decision-makers. It is also useful to reach a consensus in the international community of experts. The top-down approach is needed to make the transition from the current system to the new technologies. Both approaches can help systematic, sustainable and long-term data sharing between countries.

Case studies

Case studies from different countries and regions were presented during the workshop in relation to the monitoring and management of EDCs in freshwater using a regulator's perspective. Those case studies illustrate how to use and implement effect-based methods in regulation, as well as examples of concrete actions. The case studies are listed in the presentation order of the workshop.

- In the region of Amsterdam in the **Netherlands,** the Waternet company has developed an approach called the Smart Integrated Monitoring (SIMONI) where bioassays are used to screen for any activity in water samples. This monitoring approach integrates both chemistry and toxicological results. Toxicological endpoints include bioassays for endocrine activity (ER, anti-AR, GR, anti-PR) as well as *in situ* toxicity (daphnids mortality), general toxicity (e.g. algae growth inhibition), reactive toxicity (e.g. genotoxicity) and other specific toxicity endpoint (e.g. xenobiotic metabolism). Based on the results of the multiple analysis, the sample will be classified using the SIMONI risk indication (SRI). The SRI can be classified in three categories: increased risk (SRI≥1), acceptable risk (SRI: 0.5-1) and low risk (SRI ≤0.5). Sample with increased risk will be prioritized for customised research which can involve broad spectrum chemistry, EDA and *in vivo* bioassays. High risk samples were identified close to greenhouse areas, wastewater treatment plants, landfill runoff and sewage overflows. Mitigation actions to reduce the source of pollution had a mixed success for greenhouses: it led to a reduction of endocrine activity for one out of two greenhouses.
- **France** developed the Surveillance Prospective Network to support the WFD, anticipating new monitoring requirements (Staub et al. 2019). France performed a study called DEMO-bioassays to compare various methods to look at contaminants of emerging concern (CECs), including EDCs. Twenty surface waters were sampled using grab and passive sampling methods. The study compared *in vitro* bioassays (ER, AR, GR and AhR (Aryl hydrocarbon receptor)), *in vivo* bioassays (EASZY), targeted (E1, E2 and EE2) and non-targeted chemical analysis. The study concluded that *in vivo* and *in vitro* data were comparable for estrogenicity. The methods were also able to identify contaminated sites (Aït-Aïssa et al. 2020). The study also led to regulatory outcomes: at certain hotspots, actions were taken to reduce pollution.
- The Environmental Protection Authority (EPA) Victoria in **Australia** studied CECs, including EDCs, in recycled water. The study was performed at 30 wastewater treatment plants (WWTPs) before and after treatment using grab and passive sampling. Some toxicity assays were also performed, of which some were specific to EDCs. The study detected 181 contaminants, including 15 EDCs. In general, wastewater treatment was able to reduce 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 quite expensive. This works also highlights the need of ecotoxicological data to better derive acceptable levels of contaminants for water reuse.
- A representant of **Türkiye** mentioned that similar studies were performed in Istanbul, comparing different processes and techniques. The studies showed that alterations in wastewater treatment processes can be effective in the removal of contaminants of emerging concern. For instance, high sludge age helped to reduce EDCs in effluent.
- In a second study, EPA Victoria in **Australia** monitored pharmaceuticals, personal care products and EDCs in upstream waters, downstream waters, at the discharge point of WWTPs, and in fish tissue. The study showed that EDCs and residues of personal care products can be detected in streams and that a small set of personal care products can accumulate in fish tissue. The study highlighted the need for other types of methods, as all the chemistry analyses

available were not sensitive enough. The study also identified challenges such as access to polluting sites and concerns about follow-up regulatory actions.

- Both studies from Australia showed that monitoring data can be used to inform, guide and educate duty holders. This can in turn influence future monitoring campaigns and policy action. For example, there is a new government-led project for the development of guidance for CECs in recycled water used in irrigation and investigating uptake of CECs into edible crops.
- **Canada** has an Environmental Effects Monitoring (EEM) programme under the Fisheries Act (R.S.C., 1985, c. F-14). The objective of this programme is to ensure that effluent discharge is not causing an effect on fish, fish habitat or fish usability, and to evaluate whether end-of-pipe regulations are adequate. Industries are required to conduct a cyclical analysis of the health of fish in streams near discharge points, and to submit the results to government. The government then reviews the results to ensure the requirement are met and update the guidance documents if needed. Various aspects are assessed in those studies from fish survey, evaluation of fish habitat, such as the evaluation of fish tissue for tainting, as well as relevant supporting information (e.g.: sublethal toxicity, effluent characterization, water quality, sediment quality). For fish surveys, evaluated endpoints include age, weight-at-age, relative gonad and liver size and condition (weight/length). This programme also provides an incentive for industry to reduce their impact, as the monitoring cycle can be intensified or scaled down depending on survey results. While this programme is not specifically addressed to the management of EDCs, it was able to address endocrine disruption generated by paper mill effluents. Various studies showed significative effects on gonad size in fish of both sexes. The meta-analysis of the studies for pulp and paper mill effluent showed metabolic disruption (small gonads, larger livers, larger/fatter fish), as well as eutrophication. In a laboratory setting, a link was established between level of BOD5 (amount of dissolved oxygen consumed in 5 days by biological processes breaking down organic matter) exceeding 20 mg/L and the decrease of reproduction in fathead minnow in a shorter version (one week) of the fish short-term reproduction assay (FSTRA). The discovery made via the EEM programme led to an unprecedented multi-stakeholder collaborative effort since 2005 which involved more than 20 mills and over CAN $2.0 million.
- In **Switzerland,** quality criteria were developed for estrogenic compounds with Predicted no-effect concentration (PNEC) of 0.4 ng/L for E2, 3.6 ng/L for E1 and 0.035 ng/L for EE2. Also, a PNEC of 50 ng/L was established for diclofenac, a non-steroidal anti-inflammatory for which some endocrine activity has been observed.
- Moreover, **Switzerland** has put in place a Modular Stepwise Procedure to aid cantonal agencies. This procedure comprises various standardized methods to survey and assess the status of water bodies from hydrology and temperature measurement to the evaluation of species (e.g. fish, diatoms, macrophytes) and more recently to ecotoxicology with bioassays. Two bioassays were selected: the Yeast Estrogen Screen (YES) and a bioassay for herbicide activity. Those bioassays are currently being used for the evaluation of wastewater discharge as part of protection efforts which aim to upgrade WWTPs. Those methods can provide a cost-effective pre-screening and can be used for investigative monitoring.
- In **California**, the Southern California Coastal Water Research Project Authority (SCCWRP) manages various type of water found in the State (e.g. surface water, groundwater). California is the only example where effect-based monitoring is formalised in policies. Due to an increase in drought, there was a need to turn towards the use of recycled water for watering crops. For human safety concerns, California decided to implement bioassays as a pre-screening tool to identify sites that require further assessment. After the identification of those hotspots, further tests will be performed with analytical chemistry. The selected bioassays are for ERa and the

Aryl hydrocarbon receptor (AhR). The threshold used for ERa bioassays is 0.5 ng/L. The selected bioassays will be tested for a period 3 years from 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.

- As mentioned earlier, under the WFD of the **European Union** (EU) three estrogenic substances were included in the surface water watch list. The European Commission has now proposed EQS for them (0.017, 0.18, 0.58 ng/l respectively for EE2, E2, E1), which have been validated by the SCHEER (Scientific Committee on Health, Environmental and Emerging Risks). This could lead to the inclusion of these main estrogenic chemicals in the priority substances list, which would mean that they would be monitored on a mandatory basis across the EU and measures would have to be taken to meet the EQS.
- It was highlighted that partnerships are essential and necessary in managing EDCs, and can be instrumental in sharing knowledge and data on EDCs, supporting the transition to implementing new technologies and in supporting regulatory processes. Two **European** examples of 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 can 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 synergy among research teams to have 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 as well as communication with regulators.
    - PARC (European Partnership for the Assessment of Risk from Chemicals) is an institutional partnership that has 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 multiple working parties such as the one working on 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. Part of the strategy will involve targeted and non-targeted analysis as well as EBM.

Resources shared by participants

- The presentations are shared on https://www.oecd.org/water/science-policyworkshoponendocrinedisruptingchemicalsedcs.htm
- France: Avis relatif aux méthodes d'échantillonnage, de traitement et d'analyse des échantillons à utiliser dans le domaine de la surveillance de l'état écologique et chimique des eaux de surface - Légifrance (legifrance.gouv.fr)
- Japan: Japan: Fish Short-Term Reproduction Assay (FSTRA) (OECD TG 229)