Factsheet

Global Water Research Coalition

Effect-Based Monitoring in Water Safety Planning

Factsheet for water operators

This factsheet provides an overview of the key information on the use and interpretation of Effect-Based Monitoring (EBM) for operators.

Why are new tools needed for water quality monitoring?

With an estimated 350,000 chemicals and mixtures registered for commercial production and use, water bodies globally contain a complex mixture of chemical contaminants (e.g., pesticides, pharmaceuticals and personal care products, flame retardants, surfactants, industrial chemicals), and their transformation products.

Targeted chemical analysis is typically used for water quality monitoring; however, only a small fraction of chemicals potentially present in the water will be detected by this type of analysis. Further, some chemicals may be present below the analytical limit of detection but may still contribute to a biological effect resulting from exposure to complex low-level mixtures of chemicals via different exposure routes (so-called "cocktail effect").

What is "effect-based monitoring"?

This expression encompasses many analytical tools based on a different approach compared to targeted chemical analyses: They aim to characterize chemicals by their biological activity or toxicity, and not by their chemical structure. Effect-based methods (EBM) (also referred to as bioanalytical tools or bioassays) are used to determine the potency of chemicals, chemical mixtures, or water samples to cause adverse effects on whole organisms (*in vivo*, for example with fish, crustaceans or algae), or cells, cultured tissues, or isolated enzymes (*in vitro*).

Compared with *in vivo* assays, the advantages of *in vitro* bioassays are multiple: they generally show less variability, are easier to implement, faster, cheaper, and have a lower ethical cost. *In vitro* bioassays can also provide information about specific modes of action, such as estrogenic activity or genotoxicity, while *in vivo* assays integrate the effects from multiple toxicity pathways and provide essential information on apical effects.

This factsheet focuses on the use of *in vitro* assays for health and environmental risk assessment. However, *in vivo* assays are relevant in ecotoxicity studies, since they rely on organisms present in the environment.

Are *in vitro* bioassays mandatory ?

At present EBM has not been included in water regulations, except in California where *in vitro* bioassays responsive to activation of estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) are used to monitor recycled water quality intended for groundwater recharge and reservoir water augmentation. However, the benefits of these tools have been acknowledged by regulatory agencies such as the WHO or Australian authorities in the context of water reuse. The inclusion of ER bioassays in the European Water Framework Directive is also currently under consideration.

Despite the absence of regulatory pressure, EBM should be considered by utilities willing to investigate water safety beyond the limits of current regulations as it can also provide answers to consumers concerned about the risk

How to apply effect-based monitoring?

In the last ten years, there have been tremendous scientific developments in this field and *in vitro* bioassays are moving from academia to applied research. Many bioassays are now offered as a service to water utilities by different laboratories and universities. Even though the methods applied for sample preparation are similar to targeted chemical analyses, cell culture techniques require specific know-how and equipment, and are not within the reach of all routine laboratories.

1) Which bioassays should be applied?

While many bioassays are available, a practical test battery of bioassays representative of effects commonly detected in water extracts and aligned with relevant steps of adverse outcome pathways is recommended.

As multiple assays indicative of the same endpoint are available, relevant endpoints have been grouped into three test batteries based on assay sensitivity, and on the context and purpose of the monitoring. This is summarized in the table below.

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Test Battery	ER	Oxidative stress	AhR	Geno-toxicity
1 – WW	X	X	X	
2 – NPR	X	X	X	
3 – DW	X	X	X	X

Wastewater (WW), Non-potable reuse (NPR), Drinking water (DW).

These three batteries can be applied to assess product quality, assess water treatment efficacy and understand treatment processes.

For research applications, more bioassays may be included in a test battery and screening might be possible with fewer bioassays. More details can be found in reference **(1)**.

Examples of common assays for recommended endpoints

ER: Yeast Estrogen Screen, ER-CALUX, ER-GeneBLAzer, MELN, MVLN
Oxidative stress : AREc32, ARE-GeneBLAzer, Nrf2-CALUX
AhR: AhR-CALUX, AhR-CAFLUX, H4IIE-luc
Mutagen / Genotoxicity: Ames test, umuC, SOS Chromotest, Micronucleus

2) What sampling strategy and sample preparation is recommended?

The sampling strategy depends on the purpose and objective of the monitoring, as described in the test battery section. A quarterly to biannual frequency is recommended for routine monitoring. The type and volume of a sample depend on the context:

- **For wastewater**: Composite samples of 0.5 to 2 litres per sample are recommended for test batteries 1 and 2.
- **For drinking water/potable reuse**: Grab samples of 2 to 4 litres per sample is recommended for test battery 3.

Oxidant residuals must be neutralized with sodium thiosulfate and samples must be stored at 4˚C before shipment, and processed within 48h.

Sample preparation is usually done by the laboratory in charge of the EBM. Preparation usually starts with solid-phase extraction (SPE), enabling the extraction and concentration of micropollutants from the water matrix. The extract can then be used for bioassays. More details can be found in reference **(2)**.

3) How are the results expressed and interpreted?

Results are usually expressed as **bioanalytical equivalent concentrations (BEQ)**. A BEQ relates the effect of a water sample with the effect of a highly potent reference compound. For example, ER assay results are commonly expressed as ng of 17β-estradiol equivalent concentration / L (abbreviated as ngEEQ/L).

How much activity is usually found in water resources? How much removal can be expected from water treatment?

Representative BEQs from diverse water types are presented in a literature review of published EBM results in water treatment - see reference **(3,** open access**)** for more information.

Effect-based trigger values (EBTs) are used to assess whether the studied water type poses a potential risk to humans or the environment. EBTs reflect the acceptable effect level in a particular water type **(4)** and thus can be used in the same way as water quality standards or guideline values for chemical pollutants.

BEQ results should be interpreted as follows **(5)**:

If BEQ < EBT: No action is required; risks are considered negligible. The frequency of testing can be reduced if results remain below EBT after a few campaigns.

If BEQ > EBT: Further action is required if confirmed after laboratory quality control validation and re-testing.

1. **If BEQ < 10×EBT**: More frequent monitoring is recommended until BEQ is less than EBT.
2. **If BEQ > 10×EBT,** or **between EBT and 10xEBT** for more than 6 to 12 months: Further action is required: **1)** Health Authorities should be informed; **2)** an effort should be made to try to identify the chemicals contributing to the effect; and **3)** optimization of the treatment process should be considered.

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Is it possible to identify the active micropollutants?

This should be discussed with the laboratory performing the assay. For ER (and other assays for which only a few chemicals contribute to the effect) it is possible to explain the results by targeted analysis of known potent chemicals. For ER, this includes natural (17β-estradiol, estriol and estrone) and synthetic estrogens (17α-ethinylestradiol).

If the response in the assay is triggered by many low potency chemicals (which can be the case with e.g., AhR, oxidative stress and genotoxicity assays), identifying causative chemicals may not be achievable.

How does EBM fit in water safety planning?

Water Safety Plans (WSP) aim to ensure the safety of drinking water with the assessment and management of risks associated with microbial, chemical and radiological hazards. Together with chemical analysis, **EBM can be applied in WSPs to assess chemical risk**. Specifically, EBM have the potential to be applied in several of the WSP modules, including those that describe water quality in the water supply system (Module 2), identify hazards and assess risks (Module 3) and determine and validate the control measures (e.g., the efficacy of the treatment in removing toxic effects), reassess and prioritize the risks (Module 4). Details on how EBM can be applied in WSP modules, as well as where and how frequently to apply EBM are provided in references **(6** and **7)**.

And in Sanitation Safety Planning (SSP)?

SSP is a risk management approach for the safe use and disposal of wastewater, grey water and excreta and was developed by the WHO based on the WSP framework. The integration of EBM into a SSP is a logical step to assess environmental risks associated with chemical hazards.

The application of EBM in SSP is described in reference **(8)**.

References

(1) EBM in WSP. WP3.2: Medium-to-high throughput bioanalytical tools and decision-making tool for selection of bioassays. ISBN 978-3-944280-12-7.
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(3) Enault J, Loret JF, Neale PA, De Baat ML, Escher BI, Belhadj F, Kools SAE, Pronk GJ, Leusch FDL, 2023. How effective are water treatment processes in removing toxic effects of micropollutants? A literature review of effect-based monitoring data. J. Water Health 21, 235–250.
(4) EBM in WSP. WP3.4: Effect-based trigger values for different water quality classes considering hazards for human and the environment health. ISBN 978-3-944280-14-1.
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(8) EBM in WSP. Factsheet. Applicability of effect-based methods in Sanitation Safety Planning and water reuse.

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