

# Factsheet

## Use of effect-based monitoring for the assessment of risks of low-level mixtures of chemicals in water on man and the environment

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6<sup>th</sup> April 2020

### What are bioassays?

In the context of Effect-Based Monitoring for water quality assessment, it is common practice to apply *in vitro* assays using mammalian cell lines, bacterial strains or low complexity *in vivo* bioassays. Bioassays are commonly applied to water samples, including wastewater, recycled water and drinking water.

### Why use bioassays?

There is increasing concern about the presence of micropollutants in the aquatic environment, with micropollutants detected in both source and treated drinking water. Further, treatment processes such as disinfection can result in the formation of disinfection by-products (DBPs) and other micropollutant transformation products. The complex mixture of chemicals in water means that targeted chemical analysis alone cannot assess the total chemical burden. Bioassays are recommended to complement chemical analysis for water quality monitoring as they can detect all chemicals in an environmental or water sample that are active in an applied bioassay, including both known and unknown chemicals (Brack et al. 2019). They can also account for mixture effects and group chemicals that elicit the same mode of action. Effect-based monitoring is often applied as a screening tool, but bioassay results can be used as input for risk-based monitoring programs.

### Which bioassays to use?

Fit-for-purpose bioassay test batteries can be designed based on 1) expected effects from chemicals detected in source and treated waters, 2) compliance requirements or 3) applied treatment technologies. Modularly built batteries of reporter gene assays that target relevant modes of action (e.g., estrogenicity, genotoxicity) are recommended, with overall cytotoxicity of a water sample also quantified. Assessment of cytotoxicity is important as cytotoxic concentrations may mask specific effects. Other important considerations include practicality, assay robustness and ability to be run in high-throughput mode (e.g., 96-well plate, 384-well plate) and to perform concentration-response assessment, i.e. to test the water extract at different concentrations.

**Targeting micropollutants:** Previous experience with wastewater effluent and surface water used for drinking water production have shown that assays indicative of hormone receptor-mediated effects (e.g., activation of the estrogen receptor, ER), activation of xenobiotic metabolism (e.g., aryl hydrocarbon receptor, AhR), reactive toxicity (e.g., genotoxicity) and apical effects (e.g., cytotoxicity) are the most relevant assays for detecting organic micropollutants in those samples (Escher et al 2014).

**Targeting disinfection by-products (DBP):** Assays indicative of adaptive stress responses, such as the oxidative stress response (e.g., assays that measure induction of the antioxidant response element, ARE), and mutagenicity (e.g., Ames assay) are recommended to be applied to assess the formation of DBPs during treatment processes. In fact, Hebert et al. (2018) demonstrated that the contribution of DBPs to the oxidative stress response can be determined and differentiated from the effects of micropollutants by comparing the effect in these assays before and after disinfection.

While a large number of bioassays have been applied to water samples (e.g., Escher et al 2014), test batteries containing a small number of relevant bioassays can be applied as a more practical option. A practical bioassay battery for wastewater or recycled water would include assays indicative of activation of ER, oxidative stress response and activation of AhR (Figure 1). These assays represent effects commonly detected in water and are indicative of different stages of the cellular toxicity pathway. In the case of drinking water, an assay indicative of genotoxicity or mutagenicity (e.g., Ames assay, umuC assay or micronucleus assay) should also be included. A more comprehensive test battery could include any assay previously found to have a response in water samples and could also include assays indicative of apical effects in whole organisms (e.g., fish embryo toxicity, daphnia immobilization and algal growth inhibition) (Figure 1) (Neale et al. 2017). These bioassays encompass effects from multiple toxicity pathways, so they are a useful complement to assays indicative of specific effects and can help to safeguard against missing any unexpected effects. A comprehensive test battery could also include any newly developed assays for neurotoxicity.

While a bioassay test battery of three to four assays is recommended in most situation, at locations with no access to laboratory facilities, it may be possible to apply very simple cytotoxicity assays, such as bacterial toxicity assays. However, it should be noted that such assays only provide information about non-specific effects and should be complemented with assays indicative of specific effects. Further information on possible bioassay systems is given in Prasse et al. (2015) and Dingemans et al. (2019). A decision-making tool for the selection of bioassays will be developed in [Deliverable 3.2](#).

Test Battery	Water Type	Bioassays
Minimal (e.g. no laboratory access)	All	Bacterial toxicity
Practical	Wastewater/ recycled water	ER, Oxidative Stress, AhR
Practical + DBP	Drinking water	ER, Oxidative Stress, Mutagenicity/genotoxicity, AhR
Comprehensive	All	ER, AR, GR, PR
		Oxidative Stress, p53, NF-κB
		AhR, PPAR, PXR
		Fish embryo toxicity, Daphnia immobilization, Algal growth inhibition

xenobiotic metabolism; hormone receptor-mediated effects; adaptive stress responses; apical effects  
 AhR: aryl hydrocarbon receptor; AR: androgen receptor; ER: estrogen receptor; GR: glucocorticoid receptor;  
 PPAR: peroxisome proliferator-activated receptor; PXR: pregnane X receptor; PR: progesterone receptor

**Figure 1:** Options for bioassay test batteries for drinking water, wastewater and recycled water. In many cases, several alternative bioassays indicative of the same endpoint may be available.

**Which samples to collect?**

Which samples to collect will depend on the purpose of the sampling campaign (Figure 2). To assess product quality, only the produced water either at the outlet of the drinking water treatment plant or at the customer tap needs to be collected. This would be treated effluent or recycled water in the case of wastewater treatment or water reuse, respectively. To assess treatment efficiency, it is necessary to collect a sample of the source water feeding the plant (e.g., surface water or ground water for drinking water treatment) and a sample of the treated water. To understand the impact of critical processes, such as advanced oxidation or disinfection, samples can be taken throughout the treatment train to evaluate which processes are contributing to effect removal. Further, samples can be collected throughout a drinking water distribution system after re-chlorination points to evaluate DBP formation. Grab sampling is suitable for collecting drinking water samples, though 24 h composite sampling is more appropriate for wastewater samples to capture the diurnal variability in the micropollutant load.

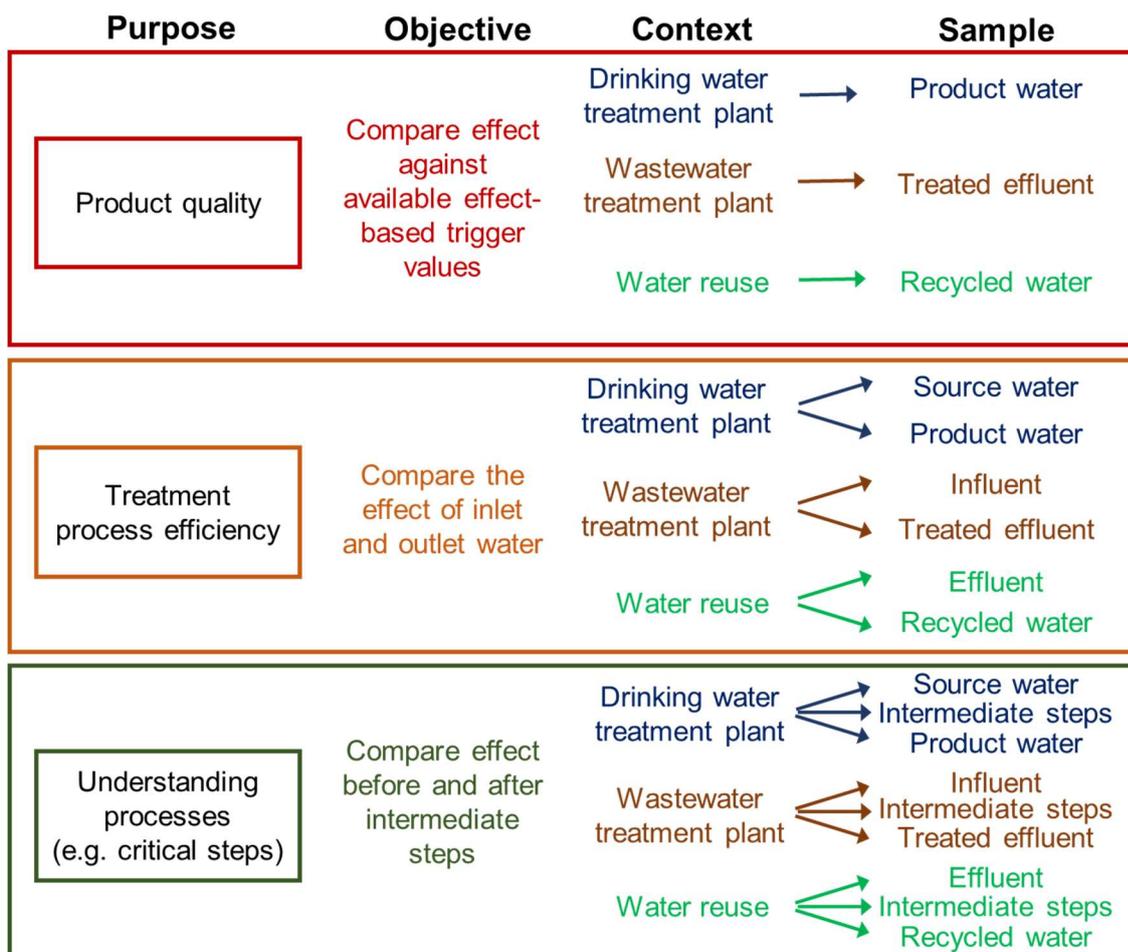


Figure 2: Examples of different sampling campaign purposes for drinking water, wastewater and water reuse with the required samples for each purpose indicated by the color-coded arrows.

### Sampling requirements

Organic micropollutants are often present at low concentrations in water samples, particularly in surface water and drinking water. Further, water contains other matrix components, such as metals and salts, which will also have an effect in the bioassay. Therefore, sample processing is often required prior to bioanalysis to enrich and isolate organic micropollutants, with solid-phase extraction (SPE) typically used. Sample processing is also often needed before chemical analysis, with common polymeric SPE sorbents used for bioanalysis and chemical analysis including Oasis HLB (Waters), Chromabond HX-R (Macherey-Nagel) and Strata-X (Phenomenex). These contain a copolymer mix that allows for extraction of a wide range of both hydrophilic and hydrophobic contaminants, with comparable effect recovery and chemical recovery reported in the literature (Neale et al. 2018, Simon et al. 2019).

Another approach is the use of passive samplers (PS), devices in which micropollutants are collected from a water environment over a longer period of time. Although the use of PS gives rise to additional uncertainty on the sampled volume and rapid fluctuations of water quality in time, this approach allows chemical and bioassay analysis of very low concentrations of chemicals. However, it should be

noted that passive sampling can distort the mixture composition in water as different chemicals will have different uptake rates.

Wastewater and effluent samples are commonly pre-filtered on a 0.45 µm filter prior to SPE as otherwise the SPE cartridge would clog. It is possible to extract the filter cake with solvents and run bioassays to capture micropollutants that are sorbed to particles. Samples from water reuse and drinking water treatment plants typically have lower particle concentrations and can be passed through the SPE cartridge without pre-filtration.

Various size SPE cartridges exist, but the 6cc/500mg sorbent versions are commonly used. From previous experience, each 6cc/500mg SPE cartridge can extract:

- 2 L of drinking water or clean surface water (e.g., source water for a drinking water treatment plant),
- 1 L of surface water or wastewater effluent, or
- 0.5 L of wastewater influent.

Rather than transporting large water samples, the first steps in sample processing can be done at the sampling site or in laboratories nearby by the sampling entities within 24 hours after collection. After sample processing, the dried SPE cartridges can be wrapped in parafilm and aluminium foil and stored at -20°C for several months. The dried cartridges can be sent to a bioassay laboratory for elution prior to bioanalysis.

Since many DBPs are volatile, a purge and trap method can be used to capture and concentrate volatile chemicals (Stalter et al. 2016). However, this is a tedious procedure that is not fit for routine monitoring applications and needs to be done onsite or within a very short transportation time. Since the toxicity contribution of volatile DBPs to the overall effects is minor and caused by easily detectable chemicals (Stalter et al. 2016), and because most routine bioassays do not allow to work with volatile chemicals, we recommend this sampling procedure only in specific cases, such as research, but not for monitoring studies.

Further information on sampling strategies and sample pretreatment options, as well as the development of a decision-making tool for selection of sampling methods, will be addressed in [Deliverable 3.3](#).

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### **Acknowledgements**

This project was funded by PUB – Public Utilities Board, Stowa- Foundation for Applied Water Research, Water Research Australia, the Water Research Commission and the Water Services Association of Australia. In-kind support was kindly provided by Veolia Environnement – Research and Innovation (VERI), SUEZ, and KWR.

The authors acknowledge the project team: Geertje Pronk, Stefan Kools, Milou Dingemans, Patrick Smeets (KWR), Jerome Enault, Jean-Francois Loret (SUEZ), Gaëlle Meheut, Magali Dechesne, Charlotte Arnal (Veolia), and Stehanie Rink-Pfeiffer (GWRC), as well as the project technical advisory committee: Dan Deere, Henner Hollert and Etienne Vermeirssen.

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