

Bioassays for Evaluating Water Quality

Screening for total bioactivity to assess water safety

Background

Water quality assessment and characterization techniques typically rely on analyses and data for individual contaminants. Essentially, individual chemical concentrations in water are measured or modeled. These results are then compared to the known concentrations at which the chemicals have been shown to cause adverse health outcomes usually mortality, growth, or fecundity). This approach is helpful to approximate the risk posed by known chemicals; however, it may have limited usefulness.

Real-world exposures generally do not occur from individual chemicals; they typically occur as mixtures of different chemical compounds that can change over time. This presents challenges in understanding the risks posed to human and environmental health: (1) many chemicals lack toxicity data even if measurable, which means there is a lack of information on the effects to exposed organisms; (2) although individual compounds can accurately be measured at low concentrations, information is lacking on the presence and concentrations of other compounds; and (3) chemicals in a mixture tend not to work alone, but together additively, synergistically or antagonistically. For example, many chemicals can act as estrogens and often co-occur in environmental samples. If the risk estimate is based on one estrogen, we would underestimate the risk posed since it is the total estrogenic potency (or potential) that is important. To better understand the risk posed by these complex samples, we need methods that can characterize potential cumulative effects on the organisms without necessarily needing to know all the components of the samples.

Bioassays are a potential solution for assessing complex samples since they screen for total bioactivity for a given pathway or mode of action (MOA), such as estrogen receptor activation, in the samples. Overall, they can account for the three challenges listed above, and can



pathways or MOA. EPA has made considerable progress in the development of these assays as effects-based monitoring tools, and has applied them in a growing number of water quality assessments.

What are Bioassays?

The term 'bioassay' represents a variety of assays. Despite their variety, bioassays tend to have several characteristics in common. Generally, bioassays target a biological change that occurs *in* an organism rather than a change that occurs *on* an organism. These endpoints can be on the cellular level, such as cellular receptors being activated or genes, proteins, or metabolites changing in abundance in response to the water sample.

Bioassays can also target higher biological levels, such as cell growth, tumor development, or changes in morphology and function of organs. They can be *in vitro* (outside a living organism), monitoring natural responses of cells in culture or responses from genetically modified cell lines. The former often look for changes within a particular targeted cell type (e.g., kidney or bladder) as an indicator of an adverse outcome (e.g., cancer); whereas, the latter often target the activity of some class of toxicant (e.g., activating an estrogen dependent

pathway). Alternatively, bioassays can utilize in vivo systems (inside a living organism), such as exposure to an embryo or a whole fish.

Advantages to using Bioassays

Both in vitro and in vivo systems have advantages and disadvantages when using bioassays. One advantage of bioassays is their ability to detect the cumulative toxicity of mixtures of both known and unknown chemicals in a sample. Many attributes of bioassays make them an appealing option for a number of environmental applications. For example, some in vitro assays are amenable to high-throughput testing, suggesting their potential to be used in rapid screening and monitoring approaches. Whether the bioassays are high throughput or not, they have the ability to detect toxicity and provide needed biological context, providing some measure of risk in terms of the potential for an adverse effect.

Present and Future Applications

Currently, bioassays are used alone or are applied in conjunction with other technologies, such as analytical chemistry. When used with analytical chemistry, they can direct analytical methods or give some biological meaning to the concentrations of chemicals measured. Bioassays have been very useful in characterizing impacts to surface waters and determining potential impacts of wastewater discharges. Additionally, they have provided useful input to established adverse outcome pathways (AOPs), connecting exposure to the toxic effects. The combination of bioassays, chemical analysis, and AOPs provides a powerful approach in a host of water quality applications.

An area of active research is the development of suites of bioassays that each target different biological pathways. Alternatively, other bioassays are being developed that focus on specific MOA, such as neurotoxicity, developmental toxicity, or hepatotoxicity. Applications are not limited to the biological activity of chemicals in water, but also include the development of salivary immunoassays that can evaluate human exposure to some pathogens.

Environmental samples can be evaluated against suites of assays to give an integrated measure of the toxicity of the biologically active substances, identify potential adverse effects, and to assign some degree of risk posed. This is similar to the approach used in whole effluent

toxicity testing, where a complex sample has been shown to be toxic using traditional means, yet the drivers or the specifics of that toxicity remain unknown. Bioassays can be used to identify the pathways leading to toxicity, which can provide a means to prioritize chemicals that work along that pathway in the uncharacterized sample. They can also be used to target a specific protection goal (e.g., evaluate removal of hormonally active compounds prior to water reuse). This concept can be applied to any unknown water sample in many regulatory contexts, such as screening finished drinking water for carcinogenic potential, reclaimed water, or wastewater effluent for the potential to cause adverse outcomes.

Comparative studies indicate that some of the more well-established techniques respond in a predictable and reproducible fashion. Progress has been made to evaluate assay response in relation to the occurrence and concentrations of both known and unknown constituents in water samples. Additional work is ongoing to provide guidance on the management and interpretation of bioassay data to facilitate decision making. As bioassays evolve and new applications are identified, it is envisioned that they could increasingly be used as a standalone technology, potentially even replacing analytical methods in some applications.

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Additional EPA Information

- **Water Research:** epa.gov/water-research
- **Chemical Interaction with Biological Systems:** epa.gov/chemical-research/research-understanding-chemicals-interactions-biological-systems
- **Research on Evaluating Chemicals for Adverse Effects:** epa.gov/chemical-research/research-evaluating-chemicals-adverse-effects
- **AOP Research Brief:** epa.gov/chemical-research/adverse-outcome-pathway-aop-research-brief