CASE STUDIES

OXIDATIVE STRESS RESPONSE AS A NOVEL ENDPOINT IN WATER QUALITY MONITORING

Recently, the AREc32 bioassay was presented as a novel tool to determine effects mediated via the oxidative stress response pathway. The latter pathway is an important part of cellular defense against different reactive chemicals, such as disinfection byproducts. A similar assay is also included in the CALUX panel.

The AREc32 bioassay has been validated and applied at a pilot installation of Waternet, a Dutch drinking water utility. The pilot installation resembles the full scale plant and includes treatment of raw water with ozone and activated carbon. The results illustrate that the AREc32 assay is highly reproducible and sensitive to a number of reference compounds. After validation, a number of raw water samples from the various treatment barriers at the pilot installation of Waternet were tested.

The results show that ozone treatment increases the oxidative stress response as measured with the AREc32 assay. In addition, activated carbon can reduce the oxidative stress response to a marginal level. It was observed that after an extra activated carbon step, the oxidative stress response increased again. This can possibly be explained by release of certain compounds that subsequently cause a bioassay response. The results below indicate the thresholds that are a human health concern. Moving forward, a comparison with analytical chemical results will be required to demonstrate the added value of the AREc32 bioassay.

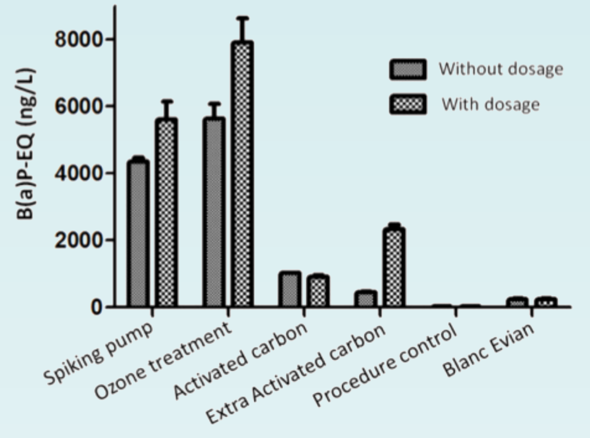

Figure 3. Induction of various water samples expressed in B(a)P equivalents

BIOSCREENING OF MAR WATER SAMPLES

Managed aquifer recharge (MAR) involves building infrastructure and/or modifying the landscape to intentionally enhance groundwater recharge under controlled conditions for water storage. There are many potential sources of recharge water including storm water (excess or redirected), treated wastewater and water from watercourses or aquifers (structures such as ponds, basins, galleries and trenches) that add to the aquifer by. Various types of MAR sources (ground-, surface- and effluent- water), investigated as part of the DEMEAU project, were bioscreened in selected in vitro reporter gene assays (CALUX-panel), in the yeast estrogen screen and in the combined algae assay assessing both photosystem II-inhibition and effects on algae growth for risk assessment.

The screening pointed out relevant toxic endpoints and also distinguished between clean and polluted sites (See Figure 4 for a summary of results with the CALUX panel). For estrogenic anti-androgenic and glucocorticoid activities, the data are modified according to the currently available trigger values. Trigger values for the other endpoints, as well as the comparison of the chemical and biological data, are currently being established. Such studies greatly demonstrate the usefulness of effect-based methods to identify samples where further chemical analyses are needed to reveal the identity of the compounds causing the measured effects.

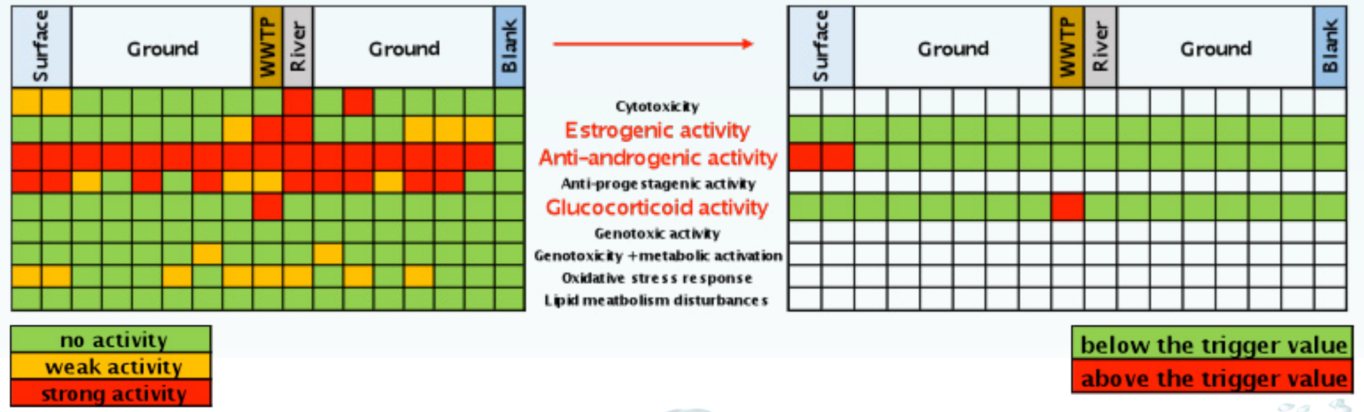

Figure 4. Summary activity profile of the tested water samples in the in vitro CALUX bioassay panel (on the left) and the modified profile (on the right) considering available trigger values (for estrogenic, anti-androgenic and glucocorticoid activity). Samples that showed lower activity than the pertinent trigger value, became "green" in the table on the right indicating low risk despite of the measured (quantifiable) activity.

BARRIERS AND SOLUTIONS

Typically, regulatory acceptance of emerging technologies is a slow process, and currently hampers the use of such modern bioassays for compliance testing and regulatory purposes. To address this barrier, **demonstration and validation** studies are being carried out in an effort to bring bioassays under increased movement toward **regulatory acceptance**. In addition to validation, another step to improve regulatory acceptance of effect-based bioscreening is the derivation of human- and ecosystem health- based **guideline values**. Such thresholds serve to act as a filter mechanism where detailed evaluation is only performed in samples exceeding the predetermined trigger value.

Beyond regulatory acceptance, increased **public (end-user) and governmental acceptance** is also desired. While some scientists and end-users view bioassays as a potential replacement of more costly techniques, such as chemical analysis, there is still a knowledge gap among many scientists, policymakers, and end-users in the applied field. In addition, a general precaution with regard to novel techniques often prohibits application of such emerging technologies, especially where investment in lab infrastructure and/or personnel training is a prerequisite to implementation.

Discussing and clarifying these critical issues and misconceptions are extremely critical in order to promote widespread application of bioassays. Moreover, in order to facilitate the operational use of these tools for decision-makers, **dissemination techniques** are also essential..

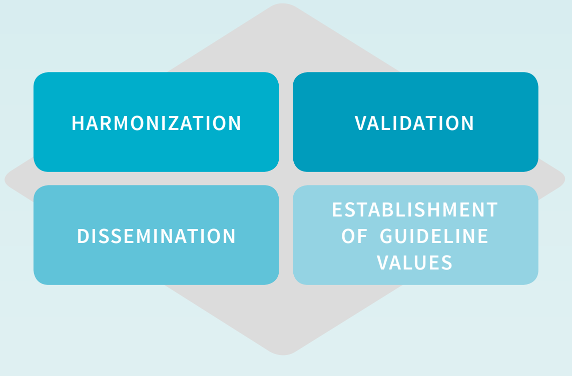

Figure 2. Crucial steps towards the better regulatory- and end-user acceptance of the bioassay technology in water quality monitoring

DISSEMINATION EVENTS

A tailored workshop on "***In-vitro* bioassays, as innovative tools for water quality assessment**" (29 January 2015; Paris, FR) in conjunction with practical demonstration of the above-mentioned workflow (Figure 1) will enable experts and decision makers from reference laboratories, water lab utilities, regulatory bodies, and from organisations in charge of risk assessment to become familiar with the added value of bioassays in safeguarding of water quality.

Another workshop is organised for spring 2015 (Utrecht, NL), where **regulatory acceptance and barriers of the bioassay technology** will be discussed in depth. This workshop aims to target policy makers, regulators, end-users, as well as standardisation institutions.

SYNERGIES WITH TECHNOLOGIES

In addition to providing cost-effective means for safety evaluations and water quality assessments of emerging and unknown pollutants, synergistic application of bioassays can also be extended to other promising DEMEAU technologies, including:

- **Managed Aquifer Recharge (MAR)**, which enables the storage of water in periods of good resource quantity and uses natural degradation of pollutants. A selected panel of Bioassays can be used for MAR samples as constant monitoring tool for (1) safeguarding good water quality and (2) investigating temporal trends in toxic activities that can be influenced by either accidental or biological sources of pollution;
- **Hybrid ceramic membrane filtration (HCMF)**, which combines ceramic membranes with processes such as coagulation, pre-coats of powdered activated carbon or ion exchange pretreatment and can remove a broad spectrum of pollutants; and
- **Hybrid advanced oxidation processes (HAO)**, which are good candidates to treat surface water and municipal wastewater effluents (the main source of emerging pollutants) and offer flexible solutions to treatment processes for water purification. For both HCMF and HAO, bioassays can be used to evaluate their proper functioning. This is done by screening the total toxic potency of the released product (i.e. drinking water or wastewater effluents).

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Figure 1: Ivan Pel and Hamilton Robotics
Figure 2 and 4: Dr. Eszter Simon
Figure 3: Dr. Merijn Schriks

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DEMEAU

DEMONSTRATION OF PROMISING TECHNOLOGIES TO ADDRESS EMERGING POLLUTANTS IN WATER AND WASTE WATER

BIOASSAYS

NOVEL EFFECT-BASED MONITORING APPROACHES IN WATER QUALITY MONITORING

Efficient monitoring and adequate treatment of water sources for potentially harmful emerging pollutants are essential for avoiding direct health risks to consumers. Current monitoring strategies rely exclusively on chemical analysis. However, chemical analysis only identifies specific, targeted compounds, and provides no information on the biological effects of the pollutants. As such, chemical analysis is rather limited in scope.

MEASURING THE TOXICOLOGICAL EFFECT OF ENVIRONMENTAL SAMPLES

Bioassays have emerged as a new, promising technology to address this gap in monitoring strategies. Specifically, bioassays offer the ability to measure biological effects of individual compounds present in water samples, and thus are particularly useful for assessing the harmful effects of complex mixtures of unknown pollutants. Bioassays hold the potential to serve as an additional, complementary technology for use in conjunction with chemical analysis, and therefore present a promising opportunity for introduction and integration into current water monitoring strategies.

MONITORING OF WATER QUALITY: FOR FUTURE USES AND EVALUATION OF WATER TREATMENT TECHNOLOGY

Recommendations from DEMEAU, a project funded under the 7th Framework Programme for Research and Technological Development of the EU (FP7), suggest that bioassays could play an important role in prescreening water sources to help identify polluted sites. According to DEMEAU, bioassays also have the potential to be used to evaluate the effectiveness of water treatment technologies already applied.

This project has received funding from the European Union's Seventh Framework Programme for Research, Technological Development and Demonstration under the Grant Agreement no. 308339.

INTRODUCTION

In light of the increasing number of chemicals entering water bodies, as well as recent advances in (bio)analytical measurement strategies, new technologies for improving and optimising conventional monitoring programs offer the potential to increase the rigor and scope of current water quality monitoring. Though targeted chemical analysis is routinely used in water quality monitoring and well-accepted in regulatory frameworks, its scope is restricted to a relatively small selection of compounds. **Integration of (bio)analytical techniques, such as bioassays, into novel monitoring programmes** present opportunities for measuring the integrated (eco)toxicological effects of chemicals found in aquatic ecosystems and/or sources of drinking water—regardless of the structure, concentration and identity of such chemicals.

One of the aims of the DEMEAU project is to demonstrate effect-based monitoring strategies and the usability of effect-based bioanalytical tools. As such, DEMEAU's work package on bioassays focuses on implementation of bioassay technologies in the context of water quality and safety assurance.

BACKGROUND

Bioanalytical tools offer the potential to effectively (pre) screen for chemical pollutants, while also offering important complementary tools to use in combination with chemical analysis. Bioassays, in particular, allow the identification of the observed biological effects caused by environmental chemicals and the mixtures that contain them. Recent technological developments have provided powerful quantitative *in vitro* bioassays to effectively measure a wide range of major classes of toxicants (i.e. acutely toxic compounds, endocrine disrupting substances and genotoxic agents) in the water cycle. Bioassays utilise living animals, plants (*in vivo*), tissues or cells (*in vitro*) to determine the biological activity of a substance or environmental sample containing a mixture of both known and unknown substances.

CONTAMINANTS

These modern bioassays are capable of effect-monitoring of a broad range of known contaminants exerting the following toxic endpoints reported as relevant to assessment of aquatic environments. These contaminants include polycyclic aromatic hydrocarbons (PAHs), phenol derivatives, detergents, pesticides, pharmaceutical residues and, personal care products, plasticizers, etc.

Toxic endpoints	Possible adverse health and/or ecotoxicological effects
Endocrine disruption Agonistic and antagonistic - Estrogenic - Androgenic - Progestagenic - Glucocorticoid effects	Tumor development Birth defects (Sexual) developmental disorders
Xenobiotic metabolism	Reproductive and developmental problems, interfere with hormone action and cancer
Oxidative stress	Inflammation, sensitisation and neurodegenerative diseases
Genotoxicity / DNA damage	Tumor development
Cytotoxicity	General toxicity
Inhibition of the photosystem II	Photosynthesis inhibition linked to reduced algae/plant survival and growth
Acetylcholinesterase inhibition	Neurotoxic effects of a certain group of insecticides(organophosphates and carbamates)

BIOASSAYS IN PRACTICE

COST-EFFECTIVENESS: SIMPLE AND RAPID ASSESSMENT OF THE SAFETY OF WATER SAMPLES, HIGH-THROUGHPUT SCREENING CAPACITY

In practice, bioassays follow relatively simple protocols and **allow for a rapid, high-throughput screening of adverse effects** that can occur in waste streams and in the environment. As the biological response of different living organisms confronted with a novel chemical substance is diverse and depends on their sensitivity to toxicants, the selection of bioassay(s) predetermines the type of toxicants eventually identified.

When **selecting the suitable assay panel for water quality monitoring**, the following criteria should be thoroughly considered: (1) the availability of standardised protocol, (2) the support and services desired; (4) short/realistic analysis time; (5) measure of accuracy (sensitivity and specificity); (6) reproducibility and repeatability, (7) straightforwardness of readouts; (8) cost effectiveness and (9) applicability to complex samples. The latter is especially important to address, as most of the currently available in vitro bioassays work most effectively when screening pure compounds, but may fail when screening more complex environmental samples.

The workflow of a bioassay analysis of an aqueous sample is illustrated in *Figure 1*. In the first step, the aqueous sample is transferred to a solvent that is suitable for the selected bioassay(s) technique. Next, the concentrated extract is used to expose the cell-based assay. Read-out modern bioassays, such as CALUX assays, use quantitative methods to assess activation of toxicity pathways, including for example, measurement of activation of luciferase activity. The activity of the tested extract is expressed as reference compound-equivalent concentration per sample unit.

In order to perform **rapid and cost-effective water quality assessment**, the high-throughput (HTP) screening capacity of the assays is very important. Using robotics, automated sample workup, miniaturised assay formats, liquid handling devices, sensitive detectors, high-speed plate readers, data processing and control software facilitates, the generation of large number of individual assay data points allows for more efficient screening, while also reducing the costs associated with chemical analysis. However, HTP screening is only applicable/cost-effective in laboratories with a certain sample throughput.

CALUX BIOASSAY PANELS: BIOASSAYS FOR EFFICIENT ANALYSIS

Because a number of emerging chemical compounds are polar and/or occur mainly in surface waters at very low concentrations (ng/L), consideration of the selection and the efficacy of the applied extraction/concentration method, in addition to the sensitivity of the applied bioassay, are crucial to address prior to the screening process. In response to this, as part of the DEMEAU project, scientists recently developed the CALUX cell panel, a type of bioassay panel **with the ability to run in an efficient and automated way** (Van der Linden et al., 2008; Van der Burg et al., 2013).

In order to avoid possible contamination and loss of analytes of interest during the sample preparation process, the applicability of "direct exposure" is currently being investigated within the CALUX technology. To do so, water samples are directly exposed to the CALUX cells without preparing an organic extract in advance. The CALUX reporter gene assays (as well as the exposure) are performed in an aqueous cell culture medium. As such, adding the water samples directly to the cells, thus far, appears to work. However, special care should be taken to avoid microbial and/or bacterial contaminations. In addition, the sensitivity of the assay is of much higher importance if this method is selected.

Figure 1. Generic workflow of water bioscreening

IMPACT

Bioassays provide the unique possibility to investigate water quality (and other matrices) based on the toxic activity of the pollutants that are present, as opposed to their specific structural nature. Bioassays are wide in their scope of water quality monitoring, and can be tailored to and adjusted for testing a range of water sources, from general toxicity tests to very specific biological activities.

The major advantages of the application of novel quantitative Bioassays include the following:

- Improved safety assessment through measurement of the effect(s) of untargeted (unknown) water contaminants, including contaminants that are the result of metabolic conversions at low concentrations (ng/L);
- Provision of antagonistic, synergistic, and/or additive effects of complex contaminant mixtures by measuring the total biological activity of a water sample;
- Cost-effective, when compared to instrumental chemical analysis;
- No use of experimental animals; and
- Relevance to human toxicity, if a human cell-based assay is applied. For example, in vitro toxicity tests conducted in human cells can help identify specific biomarkers of exposure, biologic change, or susceptibility that can be investigated directly in human populations. If e.g. fish cell-based assays are used or experiments on algae or yeast cells are conducted, also conclusions with regard to aquatic species can be drawn.

Potential users of the technology and applications include the following:

Potential users	Applications for
WATER QUALITY CONTROL AGENCIES	► The generation/validation/control of water quality objectives related to ecosystem and human health ► Assessment of the presence of toxic compounds ► Tracing hidden sources of pollution ► Setting permit criteria for the discharge of effluents ► Checking the compliance of effluent dischargers ► Determining the efficacy of pollution control measures ► Defining and standardising the interpretation of results gained within monitoring studies using bioassays ► Future implementation within the Water Framework Directive (using the derived toxicological dataset)
INDUSTRY	► Toxicity screening of waste streams before releasing them into the environment ► Pollution control measures (evaluating the effectivity of technology) ► Alarm notification for process failure
DRINKING WATER COMPANIES	► Safety assessment of source water ► Treatment technology effectiveness evaluation ► Efficient and comprehensive safety assessment of drinking water ► Failure notification alarm

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