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Demonstrating Synergies in Combined Natural and Engineered Processes for Water Treatment Systems

Deliverable D4.3

Fast / innovative monitoring systems for various contaminants in cNES

COLOPHON

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List of abbreviations

AOP	Advanced oxidation process
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistance genes
BAC	Biologically activated carbon
CALUX	Chemical Activated Luciferase eXpression
CEC	Contaminant of emerging concern
CFU	Colony forming unit
cNES	Combined natural and engineered treatment system
CSO	Combined sewer overflow
CW	Constructed wetland
DOC	Dissolved organic carbon
DMSO	Dimethylsulfoxide
ESBL	Extended spectrum β-lactamase producing (bacteria)
EBT	Effect-Based Trigger value
EDC	Endocrine disrupting compounds
GAC	Granular activated carbon
HACCP	Hazard Analysis and Critical Control Points
HC	Hierarchical Clustering
LOD	Limit of detection
LOQ	Limit of quantification
MAR	Managed Aquifer Recharge
MRSA	Methicillin-resistant *Staphylococcus aureus*
NF	Nanofiltration
NTS	Non-target Screening
RSF	Retention soil filter
qPCR	quantitative polymerase chain reaction
SAT	Soil Aquifer Treatment
TRL	Technology readiness levels
TTC	Threshold of Toxicological Concern
VRE	Vancomycin-resistant Enterococci
WWTP	Wastewater treatment plant

Executive Summary

Water systems worldwide are confronted with thousands of known and unknown emerging compounds as well as difficult to analyse microorganisms. Furthermore, water systems and treatment technologies can transfer or even amplify antibiotic resistant bacteria and / or their genes. Therefore, water service providers, especially providers of drinking water and irrigation water, face a major challenge and are under great pressure to deliver safe and affordable water services to a growing population.

Water quality and treatment performance are generally required to be assessed for a limited set of individual parameters using classic tools and methods. These methods might result in an incomplete assessment, considering the scope, sensitivity and/or speed of detection. Room is now given in the Drinking Water Directive to develop a risk based monitoring program. Customizing monitoring gives the freedom to exclude irrelevant parameters and apply alternative tools, but requires tools to evaluate the output of these tools. Innovative tools can be used for this as long as they are robust, come with risk based thresholds and are accepted by regulators.

The AquaNES project catalyzes innovations in water and wastewater treatment processes and management through improved combinations of natural and engineered treatment systems (cNES). With natural treatment steps being generally less controllable and adaptable, requires tools to assess water quality gain additional importance. They should be able to monitor fast changes or to provide an integrative and effect based interpretation of the water quality. This way the support insights into whether the combinations of engineered and natural treatment steps might introduce new risks such as the development of antibiotic resistance. Therefore, three innovative detection methods have been tested at selected demonstration sites. Thereby providing handles to assess and control water quality in combined natural and technical water treatment systems and safe (re)use of the treated water.

This deliverable summarises the results of their application in the AquaNES demonstration sites. The selected tools are CALUX bioassays for integrative measurement of chemical contaminants and their effects are demonstrated by Biodetection Systems (BDS), the BACTcontrol for fast detection of microbial contamination by MicroLAN and qPCR techniques are applied to monitor antibiotic resistance genes are applied for specific and sensitive detection of antimicrobial resistance genes by Berliner Wasserbetriebe (BWB). In addition to the CALUX bioassays, chemical non-target screening that covers a wide array of chemical contaminants is applied parallel to compare contaminants and effects.

Ten relevant cell-based CALUX bioassays were selected for water quality monitoring in cNES systems. These assays covered a wide array of relevant biological endpoints. For five bioassays health based water quality criteria were available. For the other five bioassays, these values were not available. Therefore, thresholds were derived from a large number of previously measured responses in similar water types from an in house database of BDS. These risk- and data-based thresholds enabled to compare and interpret the responses at the demonstration sites, and to evaluate potential risks. Additionally a framework is developed that translates the outcomes of the bioassays to advice in (additional) monitoring activities. The tool provided robust results that showed limited variation between seasons. The outcomes of the bioassays appeared to be sensitive, robust and sensible, as they reflected relevant toxicological endpoints. The treatment showed significant improvement in water quality (reduction of effects) and the treated water did not exceed defined thresholds. At one demonstration site, the output was compared to extensive non-target chemical screening, illustrating the complementarity of non-target screening to effect based analysis.

The CALUX bioassays are suitable for commercial implementation for the assessment of treatment efficiency as well as water quality assessment of sources or effluents. Nevertheless, a health-based threshold is preferred above a "relative" threshold derived from previously measured responses. Currently, the regulatory acceptance is a bottleneck for wide spread application in environmental monitoring, since current monitoring is on a voluntary basis besides other monitoring activities. So there is an urge to develop risk based thresholds for all bioassays applied in this particular study. This also paves the way towards regulatory acceptance, considering the availability of risk-based thresholds that are particularly suited for risk based monitoring approaches risks based monitoring. Considering the TRL (Technology Readiness Level), the CALUX bioassays are technically ready for the market, but are still (partially) need risk based thresholds and require regulatory acceptance to be adopted in water quality assessment and treatment assessment. This translates to a TRL of 8, with the regulatory acceptance and adoption as major bottleneck for wide spread use and application, leading to a TRL of 9.

The BACTcontrol was used to study two different parameters, being the detection of *E. coli* and the generic microbial activity. The BACTcontrol was tested at six different demonstration sites with different water treatment schemes for wastewater treatment and drinking water treatment. The selected sites were: Langen Erlen (CH), Holsterwitz (DE), Budapest (HU) (all drinking water production), Agon Countainville (FR), Rheinbach (DE) (wastewater treatment) and Ovezande (NL) (sub surface storage for irrigation). Within the demonstration sites various technical issues were observed such as clogging by particles and precipitation of salts and freezing of tubing. Additionally, some unexplained peaks responses were observed at Basel that could not be explained by other means of monitoring microbial contamination. These issues illustrated the importance of on-site technical support for regular checks and troubleshooting, housing of the monitoring system and remote data monitoring. Clogging by precipitation of salts could be solved by using H_2O_2 as cleaning agent. Clogging by particles requires additional work that is currently tested by introducing pre-filtration steps. When clogging issues were overcome, stable continuous measurements were obtained with sufficient sensitivity to monitor microbial contamination in various types of water. Furthermore, correlations were observed between activity measurements and parallel cell counts at both water treatment for the production of drinking water and treated wastewater. This illustrates the potency to apply this technique for (near) continuous water quality monitoring. This translates to a TRL of 8, with stable controlled operation and prevention of clogging as the major challenge, especially for more turbid waters.

The qPCR method to detect antimicrobial resistance development was tested at demonstration site 12, Berlin. This is a wastewater treatment plant consisting of a conventional activated sludge treatment, combined ozonation treatment and constructed wetlands. The water along the treatment lines was screened for both antibiotic resistant bacteria and specific gene fragments that indicate antimicrobial resistance. The measurement of antimicrobial resistance genes showed a higher sensitivity than the analysis of the antibiotic resistant bacteria using traditional culturing methods. However, the detection of bacteria (traditional culturing method) and genes (qPCR method) conceptually differs, since the DNA fragments measured can originate from both living and dead organisms, while culturing based measurements require living organisms. Therefore, the results of the two techniques are not directly comparable but complementary. The combination reveals the fate of the alive and dead bacteria and their genes in treatment schemes. There are no quality criteria for presence of antimicrobial resistance in relation to human health risks. Consequently, the technology as well as the regulatory readiness level is still in a "validation phase". The further adoption of these techniques requires the definition of risk based quality criteria for both dead and alive bacteria so the TRL level is at 5-6.

1 About this document

1.1 Purpose of this document

Adding to the commonly applied monitoring systems, this deliverable demonstrates three innovative monitoring systems for cNES relevant parameters and enabling fast interventions. They address effects of complex mixtures of micro pollutants (e.g. endocrine disruption) that affect organisms in the environment and thereby threat functionality of aqueous ecosystems, fecal (microbial) contamination, being the major human health threat and antimicrobial resistance development that is considered an increasingly serious human health threat by the WHO. The tools enable quantitative detection of biological effects induced by chemicals present in a sample, faster detection of fecal contamination by detecting *E. coli* and Coliform bacteria and quantitative detection of antibiotic resistance genes in dead and alive microorganisms. The document describes how and to what extent the tools can

- characterize yet (unknown) endocrine disrupting compounds,
- enable faster and/or more comprehensive assessment of the presence of fecal contamination and
- detect antibiotic resistance genes

at one or more of the 13 water treatment sites of the AquaNES project where combinations of natural and engineered treatment technologies are demonstrated (Table 1).

Table 1 AquaNES demonstration sites

River Bank Filtration schemes for the production of drinking water	
Demonstration Site No.1	Havel River, Berlin, Germany
Demonstration Site No.2	Elbe River, Holsterwitz, Dresden, Germany
Demonstration Site No.3	Danube River, Budapest, Hungary
Demonstration Site No.4	Warta River, Poznan, Poland
Demonstration Site No.5	Ganga River, Haridwar, India

Managed Aquifer Recharge & Soil Aquifer Treatment schemes for water storage & quality improvement	
Demonstration Site No.6	Lange Erlen, Basel, Switzerland
Demonstration Site No.7	Shafdan WWTP, Tel Aviv, Israel
Demonstration Site No.8	Agon-Coutainville, France
Demonstration Site No.9	Waddinxveen[1], Rotterdam, the Netherlands

Constructed wetlands and other natural systems for improved wastewater treatment	
Demonstration Site No.10	Thirasia and Antiparos Islands, Greece
Demonstration Site No.11	Rheinbach, Erftverband, Germany
Demonstration Site No.12	Berlin, Germany
Demonstration Site No.13	Packington, UK

[1] At a later stage another Dutch site (Ovezande) was selected for experimental work

1.2 Structure of the deliverable

Chapter 2 introduces the relevance of fast and innovative tools in water quality assessment, and distinguishes different types of tools and different applications and benefits. Chapter 3 describes the concept of on CALUX Bioassays, discusses testing activities performed within the AquaNES project and evaluates the relevance and prerequisites of this innovative tool for water quality assessment. At one demonstration site the bioassays are combined with non-target screening chemical analysis in order to reveal similarities, differences and especially the complementarity of these two tools that integrate chemical water quality of complex and undefined mixtures of chemical contaminants. Chapter 4 describes the application of the BACTcontrol, a sensor that can analyse microbial contamination within short timeframes, enabling fast indication of microbial contamination. Chapter 5 studies the potency of quantitative polymerase chain reaction (qPCR) techniques to monitor the presence of antibiotic resistant bacteria or DNA at demonstration site 12 (Berlin wastewater treatment at Schönerlinde). The main purpose of this study is to demonstrate if and how such DNA based techniques can be applied to monitor antimicrobial resistance in the urban water cycle and how outcomes are related to other means of measuring microbial resistance. Chapter 6 provides a generic discussion on the tested tools.

1.3 Relation to the project objectives

The AquaNES project demonstrates the robustness and benefits of combined natural and engineered water treatment technologies. Water quality assessment in general, and especially treatment efficacy of natural treatment steps, that are generally less controllable and adaptable, requires tools to assess water quality in order to monitor fast changes, provide an integrative and effect based interpretation of the water quality and monitor whether the natural treatment steps might introduce risks such as the development of antibiotic resistant bacteria or their genes. Therefore, three innovative and/or fast detection methods have been tested at selected demonstration sites. Thereby providing handles to assess and control water quality in combined natural and technical water treatment systems and safe (re)use of the treated water.

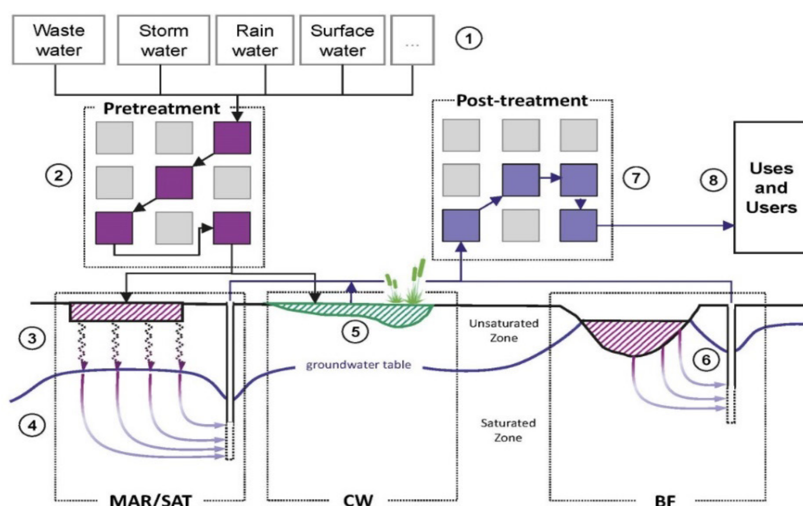

Figure 1 Schematic overview of combined natural and engineered treatment technologies within AquaNES
Legend: 1 Sources, 2 Engineered pre-treatment (Site 2, 6-13), 3/4; Managed Aquifer Recharge/Soil Aquifer Treatment (MAR/SAT) (Site 6-9), 5 Constructed Wetland (CW) (Site 10-13), 6 Bank Filtration (BF) 7 Engineered post-treatment (all sites), 8 Uses and Users such as drinking water for consumers, irrigation in agriculture and public space and emission to surface water.

2 Innovative, fast and integrated water quality assessment

Water quality assessment is a complex task with many aspects. It includes assessment of (1) potential contamination of sources of the water, (2) evaluation of treatment efficiency for this (potential) contamination, (3) and the evaluation of treated water. Furthermore, the water quality assessment should be (4) tailored for the system hydrology and dynamics and (5) related to the intended use of the water. Water quality for wastewater treatment emitted to surface water, reuse for irrigation or other purposes and the production of drinking water should comply with predefined water quality standards given in various legislations. However, not all required parameters within these legislations are relevant for each situation. Furthermore, other parameters that are not included might be relevant. Additionally, required parameters cannot always be related to an effect or associated risk. Finally, determination of parameters can take longer than what is required to take timely action.

Various innovative and fast detection methods have been developed over the past decades. These methods might be able to improve monitoring for water quality assessment. This can provide a better and more problem oriented monitoring, in line with the Hazard Analysis and Critical Control Points (HACCP) principle which is included in the revision of Annex II of the Drinking water Directive and the regulation on wastewater for reuse purposes (Commision 2015).

2.1 Definition of relevant water quality parameters

The European Union has a defined set of water quality standards for various water types listed in the European Drinking Water Directive, the European Wastewater Directive, the Groundwater Directive the water framework Directive, etc. (European Commission 1991, European Commission 1998, European Commission 2000, European Commission 2003, European Commission 2003, commission 2006, European Commission 2006, European Commission 2006, European Commission 2006, European Commission 2008, European Commission 2010, Commision 2015). Also outside the European Union governmental organizations set water quality standards (see for example https://www.epa.gov/wqc). Non-governmental organizations such as the WHO set (non-regulatory) water quality criteria (Wirtz 2009, WHO 2011, Moermond and Smit 2016). These quality standards enable water quality assessment for drinking water, irrigation water or effluents emitted to surface water (European Commission 1998, European Commission 2000). However, many contaminants lack quality criteria, and not all are criteria health or risk based. For example, there are no regulatory criteria set for pharmaceuticals in the European Drinking Water Directive nor in the Water Framework Directive (Moermond and Smit 2016). This means that not all parameters that are considered relevant for a specific site, treatment or intended use. Therefore water quality can't always be properly evaluated based on criteria set by legislation alone. For chemicals lacking criteria, a generic threshold of toxicological concern (TTC) can be developed for human health risks (Kroes, Galli et al. 2000, Mons, Heringa et al. 2013). This threshold is based on a statistical approach where the distribution of effect based water quality criteria of a large training set is used to define the 5th percentile of distribution of safe exposure levels, assuming the same distribution for chemicals with and without criteria (Kroes, Galli et al. 2000, Mons, Heringa et al. 2013, Baken and Sjerps 2016). Such approaches provide guidance where regulatory frameworks fail to provide standards.

2.2 Innovative tools in water quality assessment

Water quality is determined by numerous parameters. Here we discuss the variety of water quality criteria. One can distinguish physical characteristics, the presence of organic and inorganic particles

and dissolved molecules or agglomerates, presence of microorganisms and presence of other organisms (i.e. biodiversity) (Rutgers, Mesman et al. 2004). Within AquaNES, various combined natural and engineered water treatment technologies are combined with the purpose of producing water that is of good quality and safe for its intended use. The treatment design and use of treated water requires fast detection methods since residence times of water in the treatment components can be hours to days, and information is required on short notice to enable to stop or adapt treatment timely. Technologies also require to be sensitive since water quality criteria can be set at low concentrations or levels, and sufficient resolution is needed to register trends towards quality criteria and exceedances. Furthermore, the chemical water quality is determined by a plethora of chemicals, so measurements of individual chemicals do not provide the full picture, and integrative approaches are required to enable a more complete water quality assessment. Not all relevant chemical and microbial parameters can be monitored at desired frequencies and sampling locations for technical and practical reasons. However, relevant indicators can be used to trigger additional monitoring in tiered approaches. Fast and or innovative tools can provide these requirements, thereby improving water quality assessment.

Chemical and microbial water quality assessment tools are developed at a high pace. While the advantages of innovative techniques are evident, water quality is generally assessed for a limited set of individual parameters using rather classic tools and methods. The limited set of individual regulated chemical water quality parameters, however, provides an incomplete picture of water quality and treatment performance. Innovative techniques can cover a wider array of contaminants, can be more sensitive, can enable faster detection, can provide indicators for further analysis and can integrate assessment of the effects of contamination by complex mixtures. For example, most micro-pollutants included under regulatory frameworks are parent compounds. As these parent compounds are metabolised, they are transformed to other compounds, and drop out of sight and control of regulatory frameworks, while persistent transformation products can be relevant in both in amount and potential effect (Lambropoulou and Nollet 2014). Furthermore, persistent mobile (very polar) organic chemicals (PMOC) (ter Laak, Sjerps et al. 2015) are often ignored in monitoring and regulation, as these compounds are not well covered by current isolation and separation techniques, while their mobile and persistent nature makes them very hard to remove from water (Reemtsma, Berger et al. 2016). Additionally, environmental and human health effects and risks are not caused by individual chemicals but by the composition of the complex mixture. Bioassays allow to study toxic effects of complex mixtures for specific endpoints.

Microorganism loads in water sources can have a very dynamic character, as emissions are erratic and can be associated with rain events or local contamination. This requires frequent or event specific monitoring and fast detection. Classic plating techniques require days to obtain results and are labour intensive. They might therefore not provide the speed and efficiency needed in water treatment systems. Innovative microbial sensors may fill this gap by providing the required speed and efficiency. They can be used as fast indicator microbial water quality assessment tools.

Detection of microbial resistant bacteria and genes can be classified as a microbial response to chemical contamination with a specific indirect risk. The presence of antimicrobial agents within the water system or its use by humans and livestock can result in the development (selection) of resistant microbes in waste materials of these users. Both the presence of the anti-microbial agents in the users themselves as well as the emissions of these antimicrobial agents through human and veterinary consumption can lead to the emission and further development of antimicrobial resistance in the water cycle, respectively.

– Room is now given in the European Drinking Water Directive to develop a risk based monitoring program (Commision 2015). Customizing monitoring gives the freedom to exclude irrelevant parameters and opens opportunities for alternative parameters and tools. However, the acceptance of tools by users and regulators requires demonstration and evaluation these tools (Guillén, Ginebreda et al. 2012). Requirements for acceptance and application are (1) the definition of health/risk based trigger values in order to evaluate samples and (2) collection of reference data on water types. The application of these tools within the demonstration sites intents to illustrate the potential for application in water treatment and its technology readiness level. Additionally, it enables to evaluate treatment efficiency of the innovative treatment schemes tested at the 13 demonstration sites. It is thereby a step towards the application of such tools in water quality assessment in a regulatory setting.

2.3 Selected Innovative methods to determine water quality within AquaNES

Integrated approaches - Effect based monitoring: Biological effects of environmental complex mixtures can be monitored by a suite of bioassays such as isolated receptors, cells, biological tissues, whole organisms or ecosystems for very specific to very generic effect endpoints. The advantage is that such approaches cover a wider array of chemicals and outputs can be linked to biological effects (Oulton, Kohn et al. 2010).

Integrated approaches – non target and suspect screening: Non-targeted chemical approaches analyse integrate responses of complex mixtures by scanning for all chemicals that can be isolated, separated and detected by available techniques. Such approach covers a far wider array of chemicals compared to targeted approaches.

Automated Bacteria Monitoring / sensing microbial contamination– Microbial contamination can be detected by several analysers based on the detection of microbial (enzymatic) activity of for example faecal bacteria such as *E. coli*. This enables rapid batch at-line detection of microbial contamination and can function as an indicator and warning system.

qPCR techniques – Quantitative Polymerase Chain Reaction (qPCR) techniques enable to copy and identify specific DNA or RNA fragments of interest. This can be applied to monitor the presence of antimicrobial resistance genes within an environmental sample. There is a difference between the assessment of DNA or RNA vs. currently applied plating techniques that enable the assessment of intact microorganisms. qPCR is also able to detect DNA fragments of dead or destructed microorganisms.

Within the AquaNES project examples of these four techniques are demonstrated. These tools cover the three relevant aspects of water quality assessment, being fast indication / detection, sensitivity and integrative (effect based) water quality assessment.

3 CALUX bioassays

3.1 Study design

Setting a testing framework for the assessment of the efficiency of these combined (novel) treatment technologies requires the selection of relevant bioassays. To address the selection of relevant CALUX bioassay, a first round of water sampling and bio-analyses using a wide panel of 18 CALUX bioassays, was conducted involving all 13 AquaNES demonstration sites (round 1).

Based on the results of this first study and information provided in literature (Brand, de Jongh et al. 2013, Van der Oost, Sileno et al. 2017, Escher, Aït-Aïssa et al. 2018), a selection of the 10 most relevant CALUX bioassays was made. Besides that, the Cytotox assay was applied to evaluate if the results of the other assays were not compromised by death of the exposed cells. Six of the demonstration sites were invited to enter the second round (round 2) of sampling and CALUX analyses based on the outcome of round 1 and to cover different combinations of natural and engineered treatment technologies. In the second comprehensive study, the selected bioassays were used to evaluate the performance and efficiency of the innovative technologies in relation to the whole water treatment process. Furthermore, for each of the selected bioassays and based on both literature information and experimental derived data, bioassay-specific effect-based trigger values (EBTs) were developed and a concept action plan was drafted and proposed. Finally, water samples from one of the water treatment sites were analysed using both effect-based bioanalysis and non-target chemical analysis using high resolution mass spectrometry.

3.2 Materials and methods

3.2.1 CALUX bioassays

The CALUX (Chemical Activated Luciferase eXpression) bioassays group comprises human bone cell lines (U2-OS) or rat hepatoma cells (H4IIE), incorporating the firefly luciferase gene coupled to "responsive elements" as a reporter gene for the presence of compounds activating these responsive elements (Figure 2) (Murk, Legler et al. 1996, Sonneveld, Jansen et al. 2005, Van der Linden, Heringa et al. 2008, Pieterse, Felzel et al. 2013, Van der Burg, Van der Linden et al. 2013, Van der Linden, von Bergh et al. 2014). Cells that are exposed to compounds of interest not only express proteins that are under normal circumstances associated to responsive elements, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted (Figure 2). The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds (Table 2).

Table 2 Overview CALUX in vitro bioassays for water quality determination

Toxicity endpoints relevant for water monitoring	Specific pathway	Most promising bioassay(s)
Xenobiotic metabolism	PXR receptor agonists AhR receptor agonists	HG5LN PXR assay, PXR HepG2 assay, PXR CALUX, DR CALUX, AhR geneblazer
Hormone-mediated mode of action	(anti)estrogenic activity (anti)androgenic activity (anti)glucocorticoid activity (anti)progestin activity	ERα CALUX, YES assay AR CALUX, AR-MDA-kb2 GR CALUX, GR-MDA-kb2 PR CALUX
Reactive mode of action	Gene mutations Chromosomal mutations DNA damage response	Ames fluctuation assay, ToxTracker Micronucleus assay, ToxTracker UMUc assay, Vitotox P53(+/-S9) CALUX
Adaptive stress response	Oxidative stress pathway	Nrf2 CALUX, AREc32 assay
Developmental toxicity	Focus point endocrine disruption	Various nuclear receptor activation assays, H295R assay)

If samples are cytotoxic, results of the other CALUX bioassays cannot be used as their outcomes can be compromised by the generic toxicity to the exposed cells. Therefore, the cytotoxic potency of all the samples under investigation is tested using the cytotox CALUX bioassay. The cytotox CALUX bioassay constitutively expresses luciferase and hence, light is constantly emitted. A dose-dependent reduction of emitted light is indicative for cytotoxic effects of the samples under investigation.

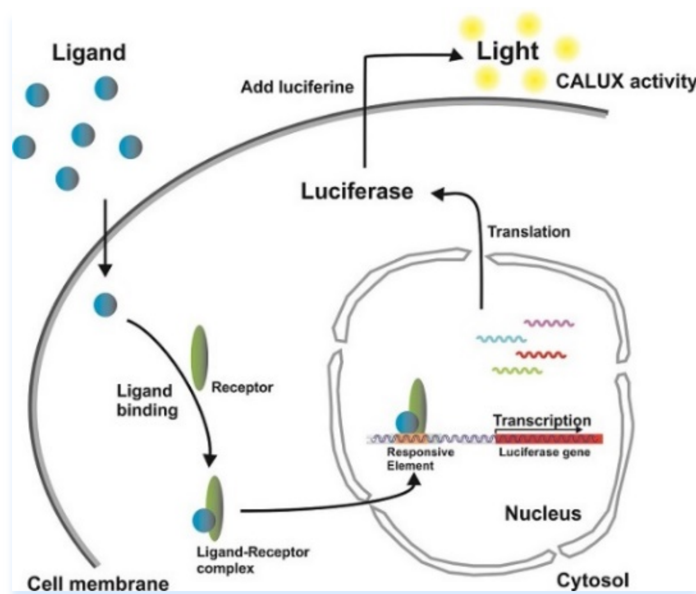

Figure 2 Illustration of working principle of CALUX bioassay

A wide panel of CALUX bioassays, each addressing specific biological endpoints such as estrogen activity and genotoxicity, have been developed. Not all of the available CALUX bioassays are relevant for monitoring water quality. Relevant bioassays were selected based on the results a wide-panel CALUX screening (18 bioassays; see Table 3) of water samples obtained from all participating water treatment

sites (round 1). The selected effect-based bioassays were used to further assess the efficiency of various innovative treatment technologies (round 2).

Table 3 CALUX bioassays applied to evaluate water treatment technologies during the first sampling campaign

Assay	Responsive towards	Reference	Cell type
Cytotox CALUX	cytotoxicity	TBT	U2OS
ERα CALUX	hormone-mediated MoA (estrogen activity (ERα receptor))	17β-estradiol	U2OS
anti-ERα CALUX	hormone-mediated MoA (anti-estrogen activity (ERα receptor))	Tamoxifen	U2OS
AR CALUX	hormone-mediated MoA (androgen activity)	DHT	U2OS
anti-AR CALUX	hormone-mediated MoA (anti-androgen activity)	Flutamide	U2OS
GR CALUX	hormone-mediated MoA (glucocorticoid activity)	Dexamethasone	U2OS
anti-GR CALUX	hormone-mediated MoA (anti-glucocorticoid activity)	Ru486	U2OS
PR CALUX	hormone-mediated MoA (progestin activity)	Org2058	U2OS
anti-PR CALUX	hormone-mediated MoA (anti-progestin activity)	Ru486	U2OS
PPARα CALUX	peroxisome proliferators	Rosiglitazone	U2OS
PPARδ CALUX	peroxisome proliferators	Rosiglitazone	U2OS
PPARγ CALUX	peroxisome proliferators	Rosiglitazone	U2OS
PAH CALUX	xenobiotic metabolism (metabolic instable; PAH-like)	B[a]P	H4IIE
DR CALUX	xenobiotic metabolism (metabolic stable; dioxin-like)	2,3,7,8-TCDD	H4IIE
PXR CALUX	xenobiotic metabolism	Nicardipine	U2OS
Nrf2 CALUX	oxidative stress inducers	Curcumine	U2OS
P53 CALUX (-S9)	genotoxicity (without metabolic activation)	Actinomycin D	U2OS
P53 CALUX (+S9)	genotoxicity (with metabolic activation)	Cyclophosphamide	U2OS

3.2.2 Demonstration sites

In total, 13 water treatment sites participated in the AquaNES project. In Table 1 participating demonstration sites are listed. For the first sampling round, all demonstration sites were invited to collect and send 2 water samples to BDS. The water samples should at least be collected before and after the innovative treatment technology train studied at each site. The additional sampling of a third water sample was requested (if possible) which represent the input water (influent) of the water treatment site. This sample was considered to be the most contaminated sample and was used as benchmark for the CALUX analyses.

Following evaluation of all CALUX analysis results of the water samples obtained during the first sampling round, 6 demonstration sites were selected to participate in the second round of the present study. These sites were selected because they showed relevant changes in responses before and after treatment in round 1 and covered both surface water and wastewater as source and (intended) use irrigation, and drinking water. During the second round, the six participating sites were requested to send 18 water samples. For each of the 6 participating treatment sites, a combined spatial/temporal sampling scheme was constructed (Annex 3).

3.2.3 Sampling, storage and shipment of water samples

Prior to sampling of water at the demonstration sites and shipment of the samples to BDS, Amsterdam, the Netherlands, a protocol for sampling, storing and shipment was drafted by BDS and send to all

participating demonstration sites (Annex 1). In addition, a sampling form was send to the demonstration site to be filled in at the moment of sampling. Upon arrival of the samples at BDS, each of the samples received a unique BDS sample code. For the first round of the AquaNES project, 2 to 4 samples were received from all demonstration sites (Annex 2). For the second round of the study, 6 selected treatment sites collected a minimum of 18 water samples during multiple sample campaigns (Annex 3).

3.2.4 Sample processing

The water samples were extracted by means of Solid Phase Extraction (SPE) according to BDS protocol p-bds-096. In short, the water samples were extracted by loading SPE columns (OASIS HLB SPE cartridges, 500 mg, 6 cc, Waters 186000115) with approximately 500-1000 ml of water and eluted with 10 ml of methanol followed by 10 ml of acetonitrile. Both fractions were pooled and evaporated under a gentle stream of nitrogen. The final extracts were re-dissolved in 150 µl of dimethylsulfoxide (DMSO) after which serial dilutions in DMSO were prepared. Water samples collected by site 6 (Lange Erlen, Basel, Switzerland) were extracted and re-dissolved in DMSO by the Fachhochschule Nordwestschweiz (FHNW; Campus Muttenz, Muttenz,Switzerland). The processing of the samples was according to the SOP used at BDS. Samples were shipped to BDS as extracts in DMSO.

For determination of the various CALUX activities, CALUX cells were seeded in 96 wells plates in assay medium. Following exposure of the CALUX cells to serial dilutions of the sample extracts in triplicate, the induction of luciferase production is quantified by measuring luminescence following addition of the substrate luciferin. On each 96-well plate, a complete calibration curve for each respective bioassays is also analysed using the relevant reference compounds. In Table 4 the exposure conditions for the various bioassays, are given. Analysis results of the test samples are interpolated in the calibration curve for quantitative determination of (ant)agonistic potential of the test samples. Only dilutions that do not show any signs of cytotoxicity (relative induction in the cytotox CALUX bioassay > 80%) are used for final evaluation of CALUX analysis results. Final results are expressed as µg, ng or pg reference compound equivalents per litre of processed water.

The bioassays were performed according to standard BDS protocols p-bds-083 (Culturing U2OS CALUX cells), p-bds-04 (Analysis of Ah-receptor mediated luciferase activity in DRCALUX cells), p-bds-066 (Analysis of luciferase activity in the PAH CALUX bioassay), p-bds-085 (Analysing samples with U2-OS CALUX bioassays using sigmoidal dose response curves (with 0.1% or 1% DMSO)), p-bds-070 (Harvesting the cells and measurement), and p-bds-084 (Calculating U2OS CALUX results using sigmoidal dose response curves). BDS protocols are available upon request.

Table 4 BDS CALUX cell culture information

Assay	(anti)ERα, (anti)AR, (anti)GR, (anti)PR, PPARα, PPARδ, PPARγ, PXR	Cytotox, Nrf2, P53 (+/-S9)	PAH, DR
Cell type	U2OS	U2OS	H4IIE
Species	Human	Human	Rat
Confluence	10000 cells per well	10000 cells per well	>95% confluence
Medium used	DMEM/F12	DMEM/F12	αMEM
Additions to assay medium	– Stripped FCS – Non essential amino acids	-Stripped FCS -Non essential amino acids	-FCS
%DMSO	0.1%	1%	0.8%
Exposure time	24 hrs	24 hrs	4 hrs (PAH), 24 hrs (DR)

3.3 Results and discussion of testing at sites

3.3.1 Round 1

During the first round of the AquaNES study, water samples from all 13 participating water treatment sites were tested on a wide panel of 18 effect-based CALUX bioassays (see table 2). In Figure 3, the final analysis results are presented in a heat-map, expressed as fold-induction above the LOQ of each respective bioassay. The quantified CALUX analysis results for all bioassays tested and all demonstration sites are given in Annex 4 (a-m).

As can be observed in the heat-maps presented in Figure 3, the level of micro-pollutants that exhibit bioactivity, is most pronounced in the demonstration sites that utilise constructed wetlands (WP3 demonstration sites). These sites use wastewater as influent that is expected to have relatively high micro-pollutant levels. In contrast, demonstration sites from WP2 use other, less contaminated, sources of influent (such as surface waters) which is reflected in lower bio-activity levels particularly in influent samples. For demonstration site 1-4 (WP1), a third sample representing the feed water of the water treatment plant, was not received. Such a sample would be a well or surface water sample expected to contain low levels of micro-pollutants.

In general, treatment of the water samples decreased the bioactivity in the samples at all demonstration sites. In most cases, the bioactivity in water samples is already significantly decreased before the water passes the innovative treatment technologies under investigation. Following water treatment using the innovative treatment technologies, CALUX activities are further reduced indicating further removal or degradation of bioactive micro-pollutants.

With respect to the selection of bioassays, the CALUX analysis results indicate clearly that anti-ERα, PR and P53 (-S9) activity was hardly demonstrated in the water samples from any of the demonstration sites. Therefore, these bioassays will not be used during the second campaign of the study. From the remaining bioassays, DR and PAH CALUX activity (metabolic stable and instable compounds able to activate the Aryl Hydrocarbon receptor) was observed in multiple demonstration sites. However, these activities seemed to be rather consistent in most samples analysed and no clear effect of water treatment was observed. Furthermore, these bioassays suffered in many cases from background activity obscuring the final results. These bioassays therefore do not seem appropriate to be used for the second campaign.

All other bioassays demonstrated activity in the tested water samples and can be used for evaluating the treatment efficiency. Most of the relevant bioassays are designed to detect compounds with an endocrine mode of action. In addition, a number of bioassays representing other modes of action (induction of xenobiotic metabolism, lipid metabolism, genotoxicity, oxidative stress response and cytotoxicity) also were able to detect activity in the tested water samples and demonstrated efficiency of water treatment in the present pilot study. Together this battery of bioassays covers a rather wide variety of toxicological endpoints. Based on the results of this first study and information provided in literature (Van der Oost, Sileno et al. 2017, Escher, Aït-Aïssa et al. 2018), 11 bioassays considered to be most relevant for water quality assessment are selected and presented in Table 4.

A River Bank Filtration schemes for the production of drinking water

Site	Sample	Cytotox CALUX	AR CALUX	anti-AR CALUX	ERa CALUX	anti-ERa CALUX	GR CALUX	anti-GR CALUX	PR CALUX	anti-PR CALUX	PPARa2 CALUX	PPARd CALUX	PPARg2 CALUX	PAH CALUX	DR CALUX	PXR CALUX	Nrf2 CALUX	P53 CALUX (-S9)	P53 CALUX (+S9)
site 1	2017_11_27_ site 1_feed NF	0.5	0.5	0.5	3.1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	8.8	0.5	3.75	0.5	0.5	0.5
	2017_11_27_ site 1_permeate NF	0.5	0.5	0.5	1.9	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.92	0.5	0.5	0.5	0.5	0.5
site 2	WW Hosterwitz influent (river (Elbe) water)	0.5	0.5	0.5	1.3	0.5	0.5	1.1	0.5	0.5	0.5	0.5	0.5	40	1.7	2.3	3.3	0.5	0.5
	WW Hosterwitz effluent (treated water)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	2.8	0.5	0.5
site 3	Treatment plant influent water (above slow filter)	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	15	1.1	1.6	2.6	0.5	0.5
	Treatment plant effluent water (RO permeate)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	40	0.5	0.5	3	0.5	0.5
site 4	Mosina treatment station influent	0.5	0.5	1.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	7.0	25	7.6	7.1	0.5	1.9
	Mosina treatment station effluent	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	17	2.2	1.6	0.5	0.5
site 5	Ganga	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	31	13	0.5	0.5	0.5	0.5
	SP1 RBF	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	25	0.5	0.5	0.5	0.5	0.5
	SP4 A0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	18	6	1.3	1.2	0.5	0.5

B Managed Aquifer Recharge & Soil Aquifer Treatment schemes for water storage & quality improvement

	Cytotox CALUX	AR CALUX	anti-AR CALUX	ERa CALUX	anti-ERa CALUX	GR CALUX	anti-GR CALUX	PR CALUX	anti-PR CALUX	PPARa2 CALUX	PPARd CALUX	PPARg2 CALUX	PAH CALUX	DR CALUX	PXR CALUX	Nrf2 CALUX	P53 CALUX (-S9)	P53 CALUX (+S9)
1 - raw river Wiese wate	0.5	0.5	1.1	1.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	23	5.1	1.3	0.5	0.5	0.5
2 - pre-treated river Wie	0.5	0.5	1.9	0.5	0.5	0.5	2.9	0.5	0.5	0.5	0.5	0.5	18	2.5	0.5	0.5	0.5	0.5
3 - after AOP treatment	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	2.2	0.5	1.2	0.5	0.5	0.5
SHAF_R1	110	22	13	520	0.5	6.4	0.5	0.5	23	34	4.3	10	800	45	41	0.5	0.5	0.5
SHAF_OZA500	0.5	0.5	1.3	18	0.5	5	0.5	0.5	1	0.5	0.5	0.5	110	37	4.8	2.5	0.5	0.5
SHAF_OZOAOZ	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	74	10	1.4	2.3	0.5	0.81
raw water WWTP inlet	14	63	0.5	710	0.5	0.5	12.5	0.5	7.3	26	4.2	11	11000	130	15	3.6	0.5	2.5
raw water WWTP outlet (before Mare a Sorre)	3.3	0.5	2.1	26	0.5	1.4	2.3	0.5	3.2	0.5	0.5	1.3	54	17	11	5.7	0.5	0.5
raw water WWTP outlet (Mare a Sorre)	3.3	0.5	1.6	9.2	0.5	1.1	1.9	0.5	1.6	0.5	0.5	1.1	16	18	5.6	3.1	0.5	0.5
Nootdorp BASSIN	1.4	0.5	0.5	1.8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	26	4.8	2.6	9.1	4.5	3.4
Nootdorp ASR	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	28	4.8	1.5	0.5	0.5	0.5
Nootdorp OPPW	2.5	0.5	2.5	1.25	0.5	0.5	5.7	0.5	1.6	0.5	0.5	0.5	120	9.3	0.5	0.5	0.5	0.5
Nootdorp VLOTTER(KIST)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	45	2.7	3.1	1.3	0.5	0.5

C Constructed wetlands and other natural systems for improved wastewater treatment

		Cytotox CALUX	AR CALUX	anti-AR CALUX	ERa CALUX	anti-ERa CALUX	GR CALUX	anti-GR CALUX	PR CALUX	anti-PR CALUX	PPARa2 CALUX	PPARd CALUX	PPARg2 CALUX	PAH CALUX	DR CALUX	PXR CALUX	Nrf2 CALUX	P53 CALUX (-S9)	P53 CALUX (+S9)
site 10	No. 1	16	41	3.9	1500	0.5	10	0.5	0.5	0.5	11	3.8	14	430	35	12	25	0.5	16
	No. 2	44	56	35	1500	0.5	4.3	4.9	0.5	71	3.75	3.4	4	26	30	15	19	0.5	110
	No. 3	0.5	0.5	0.5	4.3	0.5	2.3	0.5	0.5	0.5	2.9	0.5	0.5	0.5	23	2.8	4.8	0.5	1.2
site 11	Inflow WWTP	220	56	37	400	4.8	0.5	47	0.5	110	12	13	0.5	1200	32	45	0.5	0.5	0.5
	Pilot RSF Rheinbed Inflow	0.5	0.5	1	5.7	0.5	3.4	0.5	0.5	0.5	0.5	0.5	0.5	100	25	12	7.6	0.5	0.5
	Pilot RSF Rheinbed Outflow	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	24	0.5	0.5	1.0	0.5	0.5
site 12	WWTP Schonerlinde. Primary sedimentation	12.5	0.5	0.5	550	0.5	6.4	0.5	0.5	11	34	5.1	12	1600	65	15	8.5	0.5	2.8
	WWTP Schonerlinde. Secondary sedimentation	1.4	100	1.25	4.7	0.5	6.3	0.5	0.5	0.5	0.5	0.5	0.5	140	28	4.6	1.4	0.5	0.5
	WWTP Schonerlinde. Ozonation	0.5	0.5	0.5	0.5	0.5	1.8	0.5	0.5	0.5	0.5	0.5	0.5	0.5	8.3	1.3	3.6	0.5	0.5
	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite	0.5	0.5	0.5	0.5	0.5	1.8	0.5	0.5	0.5	0.5	0.5	0.5	15	7.6	2.1	3.1	0.5	0.5
site 13	S13-1	8.8	100	13	200	0.5	16	0.5	11	3.2	20	0.5	0.5	190	32	13	16	0.5	9.5
	S13-2	3.5	0.5	3.9	6.5	0.5	0.5	0.5	0.5	1.1	0.5	0.5	0.5	34	3.2	13	0.5	0.5	0.5
	S13-3	0.5	0.5	0.5	1.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	18	8.5	6.7	2.9	0.5	0.5

Figure 3 Heat-map of round 1 CALUX analysis results for all effect-based bioassays performed and for all participating water treatment sites expressed as fold-induction above the LOQ of each respective bioassay. Results below LOQ are represented by a value of 0.5

Table 5 Selection of effect-based CALUX bioassays considered to be relevant for the evaluation and monitoring of innovative water treatment technologies during round 2 of the AquaNES project

Assay	Responsive towards	Reference	Cell type
Cytotox CALUX	cytotoxicity	TBT	U2OS
ERα CALUX	hormone-mediated MoA (estrogen activity (ERα receptor))	17β-estradiol	U2OS
AR CALUX	hormone-mediated MoA (androgen acitivity	DHT	U2OS
anti-AR CALUX	hormone-mediated MoA (anti-androgen activity)	Flutamide	U2OS
GR CALUX	hormone-mediated MoA (glucocorticoid activity)	Dexamethasone	U2OS
anti-PR CALUX	hormone-mediated MoA (anti-progestin activity)	Ru486	U2OS
PPARα CALUX	peroxisome proliferators	GW7647	U2OS
PPARγ CALUX	peroxisome proliferators	Rosiglitazone	U2OS
PXR CALUX	xenobiotic metabolism	Nicardipine	U2OS
Nrf2 CALUX	oxidative stress inducers	Curcumine	U2OS
P53 CALUX (+S9)	genotoxicity (with metabolic activation)	Cyclophosphamide	U2OS

For the selection of demonstration sites for the second round of analyses, the criteria are whether the sites were expected to be in operation during the planned sampling rounds over various seasons, the practical availability of water samples, the origin of source water and the type of innovative treatment technology. Initially it was foreseen to select of 2 sites from WP 1-3, however, WP 1 (River Bank Filtration schemes for the production of drinking water) revealed rather low responses in the round 1 bio assays, so only one site with the highest responses (site 4) was selected. Additionally, site 6 (WP2, Managed Aquifer Recharge & Soil Aquifer Treatment schemes for water storage & quality improvement) was selected, as this site was considered more closely related to demonstration sites from WP1 (also river water passing soil as (pre) treatment for drinking water treatment) and basically different from other sites in WP2 having wastewater as source. Since site 9 (Waddinxveen / Ovezande, the Netherlands) from WP2 was not operational yet, Site 7 and 8 were selected from WP2. Finally,sites 11 and 12 of WP3 (Constructed wetlands as post treatment of communal wastewater) were selected as these sites have the most comprehensive analysis of organic micro-contaminants and on–site support. The final selection of 6 demonstration sites is presented in Table 6.

Table 6 AquaNES demonstration sites selected for the second round of the AquaNES project with their treatment trains

Site No.4	Warta River, Poznan, Poland	Bank filtration (aquifer effluent) – aeration-high rate filtration-ozonation - GAC filtration
Site No.6	Lange Erlen, Basel, Switzerland	River water-sand filtration-advanced oxidation - soil/BAC filtration - infiltration (MAR)-GAC/UV disinfection
Site No.7	Shafdan WWTP, Tel Aviv, Israel	WWTP effluent - biofiltration - advanced oxidation (ozonation/electro pulse) - SAT
Site No.8	Agon-Coutainville, France	WWTP effluent (activated sludge) - reactive beds – UV - sand dune infiltration (MAR)
Site No.11	Erftverband, Germany	WWTP effluent (activated sludge) - retention soil filter (with/without GAC)
Site No.12	Berlin, Germany	WWTP effluent-ozonation - filtration (sand/GAC; sand/anthracite; BAC) or constructed wetlands

3.3.2 Round 2

The selected participants were requested to collect 18 water samples according to a spatial and temporal sampling scheme in order to evaluate the treatment efficiency of individual treatment technologies applied at each site and evaluate possible temporal seasonal changes. In Annex 3, sample information on the samples collected at each site is provided. In addition, a schematic representation of each of the sites in given, indicating the various sampling points. In Table 7, an overview of the samples received and analysed is given.

Table 7 Summary of number of samples received and analysed from participating sites

Site	Shipments received	Samples received	Samples analysed
4	3	18	18
6	3	31	31
7	3	19	19
8	4	18	18
11	6	19	19
12	3	20	20
Total	**22**	**125**	**125**

All quantified CALUX analysis results for samples received, are presented in Annex 5. The final analysis results are also presented in a heat-map, expressed as fold-induction above the LOQ of each respective bioassay (Annex 5 a-f). As could be observed during the first round of the present study, the level of micro-pollutants in demonstration sites that utilise wastewater as water source (sites 11 and 12) is most pronounced, whereas demonstration sites (sites 4 and 6) using other sources of influent (such as surface waters) are less contaminated reflected by lower bio-active responses.

In general, all treatment trains applied at selected sites, containing the innovative combinations of natural and engineered treatment technologies, are effective in reducing the biological response caused by chemicals in the water when comparing bioactive responses in influent and effluent waters. However, removal of such compounds was not always linear along the treatment process suggesting that some treatment steps lead to the formation or incomplete removal of bioactive compounds. Especially compounds causing oxidative stress and inducing (xenobiotic) metabolism are still present at most sites after various treatment steps along the treatment train. In some case, an increase in Nrf2 CALUX bioactivity was observed after specific treatment steps (site 8, sample point S5-4 FRE4 Sand Dune Aquifer). Ozonation is known lead to an increase of oxidative species and hence increased Nrf2 CALUX activity. However, this could not be demonstrated in this study. Endocrine activity and in particular estrogenic activity was observed in influents of all sites except for site 6. The estrogenic activity decreased when passing through the various treatment trains studied and only in waters from site 8 and 12, residual estrogenic activity was observed after the final treatment step.

The obtained bioassay results suggest that the use of lines of different treatment technologies is an efficient approach to reduce bio-active substances. Not a single technology is capable of reducing bioactive responses completely but the combined use of innovative technologies significantly reduced bioactive responses in water samples as compared to activity in source water samples. The efficiency of different treatment trains as applied by the participating sites are, however difficult to compare. As indicated before, the various sites use very different source waters. Furthermore, in some cases the waters have been treated prior to entering the innovative treatment train resulting in different contaminants loads.

The sampling schemes at the sites does not allow for evaluating of possible time-trends. Although some minor differences between sampling campaigns at the various sites were observed, no clear dependence of contamination levels on time or season were observed.

From all analyses performed, it can be concluded that the panel of effect-based bioassays used in the present study is very much suitable for the monitoring and evaluation of the efficiency of innovative water treatment technologies and the monitoring of the water treatment process as a whole. It provides sufficient sensitivity / resolution to observe effects of treatment, and data suggest rather robust results since various sampling rounds provided similar patterns. However, for assessment of water quality for (re)use as drinking water, irrigation water or emission into sensitive surface waters or sub-surface storage, effect-based trigger values (EBTs) have to be developed to be able to indicate acceptable risk for complex mixtures as they occur in waterbodies. Furthermore, these trigger values need to be adopted in regulatory water frameworks, as this paves the way towards widespread use. At this moment the results of effect-based bioassays are only benchmarked against each other. No benchmark against a widely approved measure of chemical water quality has been adopted and therefore, the EU Water Framework Directive (WFD) does not allow bioassays for monitoring waterbodies. In contrast, official EU limit values for dioxins and dioxin-like compounds in food and feed samples have been put in place and the regulations allow bioanalytical methods for screening of food and feed samples (European Commission 2012, European Commission 2012).

To evaluate the present CALUX bioassay analysis results and quantify the water quality along the treatment trains studied, bioassay specific trigger values are required. Recently, a number of bioassay-specific effect-based trigger values have been derived for drinking and environmental waters using a statistical/theoretical approach (Brand, de Jongh et al. 2013, Van der Oost, Sileno et al. 2017, Escher, Aït-Aïssa et al. 2018). However, no EBTs are currently reported for the AR, GR, PPARa, PPARg and P53(+S9) CALUX bioassays used in the present study. In an attempt to derive EBTs for these bioassays, a practical approach was applied using CALUX bioassay analysis results from a large set of water samples (drinking water, waste water and environmental water). The distribution of these CALUX bioanalysis results were evaluated and a practical EBT was derived based on the percentage of samples showing analysis results below this EBT. Since the data set used for deriving this EBT was based on (mostly) coded water samples and therefore, the water source was unknown, the percentage used for deriving the practical EBT was set at 80% to prevent bias. Using this practical approach, EBTs were derived for all CALUX bioassays applied in the present AquaNES project. Comparison of bioassay EBTs reported in literature with their practical derived counterparts showed values in the same range. This indicates that practical derived EBTs for bioassays for which no EBT was reported, can be used for evaluation and quantification of water quality along the treatment trains studied in the present study.

In Figure 4, an example for the determination of a practical derived effect-based trigger value for estrogenic compounds is given, where 80% of the analysed samples have an estrogenic activity below the effect-based trigger value. The obtained EBT for estrogen activity (1.6 ng 17β-estradiol eq./l water) is comparable to the EBTs for estrogen activity reported in literature (0.1 – 3.8 ng 17β-estradiol eq./l water) (Brand, de Jongh et al. 2013, Van der Oost, Sileno et al. 2017, Escher, Aït-Aïssa et al. 2018). In Figure 8, effect-based trigger values the biological endpoints used in the present AquaNES study are given. In case trigger values for specific bioassays were available in literature, these EBTs were used. For all remaining CALUX bioassays, trigger values were derived according to the practical approach. In addition, an action plan for water treatment plant operators is proposed in case bioanalysis results exceed the derived trigger values. In Table 8, this proposed action plan is presented. This action plan

provides a reasonable level of protection while leading to risk management measures at a realistic number of sites only.

Applying trigger values and the proposed action plan on the quantified CALUX analysis results of water samples obtained during the second round of the present study, allows for a better evaluation of the impact of the various innovative technologies on treatment efficiency . In addition, it also allows for actual water quality assessment. Heat-maps of quantified CALUX analysis results for sites 4 and 12 are presented in Figure 5 and the trigger values and action plan is applied. These two sites were selected as they covered drinking water treatment and wastewater treatment. Using this approach, it becomes clear that quantifiable bioactivities do not necessarily mean that water quality is poor. Only when effect-based bioactivities exceed trigger values, possible action is required. In Annex 6 a-c, heat-maps of EBTs and the proposed action plan applied on quantified CALUX analysis results from all sites are presented. Based on this interpretation of analysis results, only water obtained from the Sand Dune Aquifer sampling point from site 8 shows elevated Nrf2 CALUX activity at levels at which action might be needed.

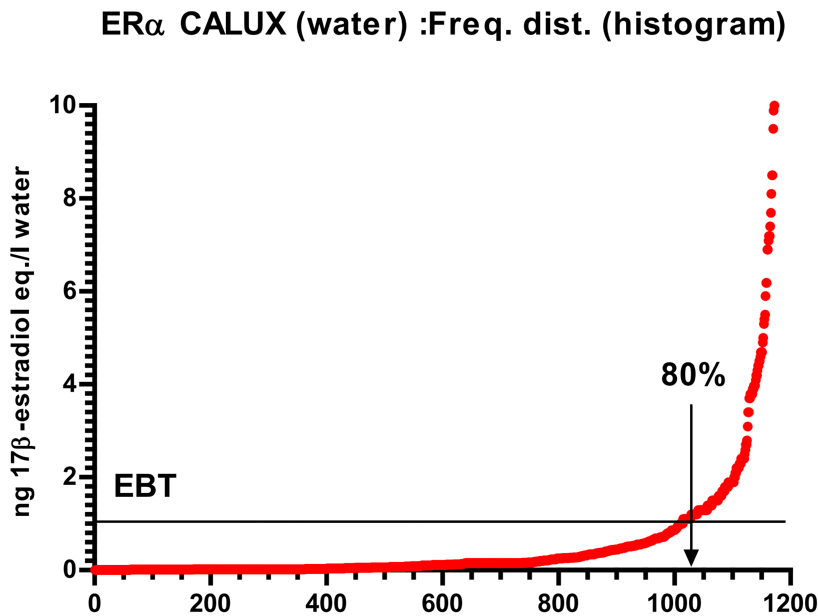

Figure 4 Derivation of a trigger value for estrogen activity using the practical approach

Table 8 Proposed action plan for assessment of water quality, based on EBT for CALUX bioassays applied during round 2 of the AquaNES study.

Assay	Unit	EBT	Reference	1*EBT	3*EBT	10*EBT	100*EBT
Cytotox CALUX							
AR CALUX	ng DHT eq./l	32	Besselink	32	96	320	3200
anti-AR CALUX	ug Flutamide eq./l	14	Escher et al. (2018)	14	42	140	1400
ERa CALUX	ng 17b-Estradiol eq./l	0.1	Escher et al. (2018)	0	0.3	1	10
GR CALUX	ng Dexamethasone eq./l	56	Besselink	56	168	560	5600
anti-PR CALUX	ng Ru486 eq./l	1.2	Escher et al. (2018)	1	3.6	12	120
PPARa CALUX	ng GW7647 eq./l	22	Besselink	22	66	220	2200
PPARg2 CALUX	ng Roziglitazone eq./l	91	Besselink	91	273	910	9100
PXR CALUX	ug Nicardipine eq./l	43	Escher et al. (2018)	43	129	430	4300
Nrf2 CALUX	ug Curcumine eq./l	20	Escher et al. (2018)	20	60	200	2000
P53 (+S9) CALUX	ug Cyclophosphamide eq./l	1100	Besselink	1100	3300	11000	110000

CALUX result < 1*EBT (or LOQ) no further action required

1*EBT < CALUX result < 3*EBT quality check data, continue to monitor every three months, until 1 year and the EBT < 1

3*EBT <CALUX result < 10*EBTdata check, immediate re-sampling and analysis to confirm EBT. It is also required to quantify specific target compounds which are known to cause the effects observed in the respective bioassay

CALUX result < 100*EBT all of the above plus enhance source identification program. Also monitoring in the distribution system closer to the point of exposure to confirm attenuation of CEC is occurring and to confirm the magnitude of safety factors associated with removal efficiency, dilution and post-treatment.

Site 4

12/03/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	1	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	18	13	LOQ	LOQ	LOQ	LOQ
ERa CALUX	LOQ	LOQ	0.1	LOQ	LOQ	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	62	4.8	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	17	35	40	13	10	8.5
Nrf2 CALUX	1000	140	LOQ	LOQ	15	22
P53 CALUX (+S9)	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ

21/08/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	0.8	3.2	LOQ	LOQ	0.8	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	24	22	16	LOQ	6.4	LOQ
Nrf2 CALUX	74	59	70	60	37	LOQ
P53 CALUX (+S9)	1900	LOQ	1900	LOQ	LOQ	LOQ

08/10/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	0.1	0.3	LOQ	0.2	LOQ	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	23.0	23.0	15.0	10.0	20.0	7.2
Nrf2 CALUX	LOQ	130	LOQ	26	LOQ	42
P53 CALUX (+S9)						

Site 12

23/01/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	LOQ	1.8	LOQ		LOQ	LOQ	LOQ
AR CALUX	155	LOQ	LOQ		LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	4.6	LOQ		LOQ	LOQ	LOQ
ERa CALUX	0.4	1.8	0.1		0.4	0.3	0.1
GR CALUX	110	210	71		87	21	25
anti-PR CALUX	40.0	LOQ	LOQ		LOQ	LOQ	LOQ
PPARa2 CALUX	400.0	LOQ	LOQ		LOQ	LOQ	LOQ
PPARg2 CALUX	1300	LOQ	LOQ		LOQ	LOQ	LOQ
PXR CALUX	LOQ	25	12		19	LOQ	8
Nrf2 CALUX	760	180	110		77	79	51
P53 CALUX (+S9)	LOQ	10000	LOQ		LOQ	LOQ	LOQ

17/04/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	53.0	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	430	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	1.1	2.6	LOQ	LOQ	1.8	LOQ
ERa CALUX	1.0	45	LOQ	LOQ	LOQ	LOQ	LOQ
GR CALUX	160	15.0	LOQ	LOQ	42.0	LOQ	50.0
anti-PR CALUX	54	LOQ	LOQ	LOQ	4.1	LOQ	LOQ
PPARa2 CALUX	140	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	81	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	80	100	93	LOQ	37	35	11
Nrf2 CALUX	810	320	130	LOQ	190	110	140
P53 CALUX (+S9)	570	2200	LOQ	LOQ	LOQ	LOQ	LOQ

16/07/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	25	2.8	0.7	LOQ	LOQ	LOQ	LOQ
AR CALUX	130	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	21	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	51	1.0	0.1	LOQ	LOQ	LOQ	LOQ
GR CALUX	130	110	48	LOQ	41	24	22
anti-PR CALUX	40	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	420	LOQ	20	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	1100	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	100	72	48	8.3	30.0	33.0	31.0
Nrf2 CALUX	740	200	170	LOQ	190	82	110
P53 CALUX (+S9)	25000	LOQ	LOQ	LOQ	1500	LOQ	LOQ

Proposed action plan

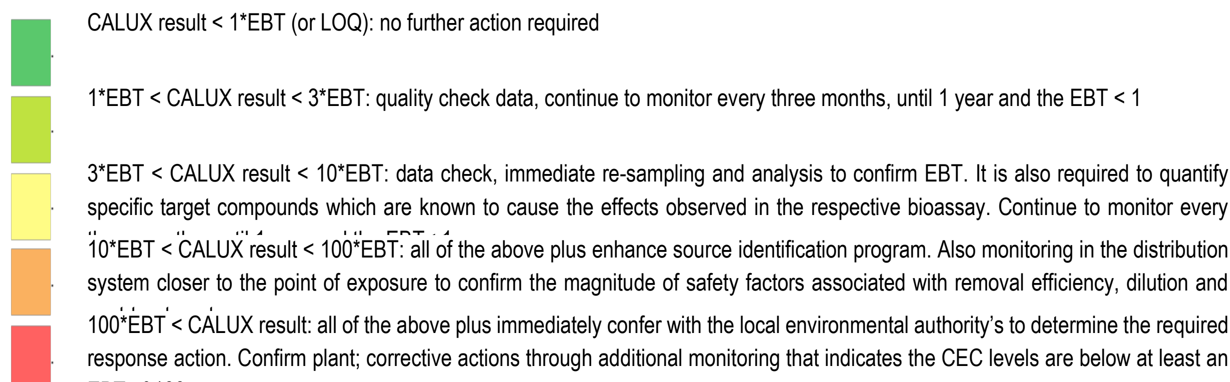

Figure 5 Heat maps according to proposed water quality assessment scheme of bioassay responses at demonstration site 4 (river bank filtration for drinking water, Poznan, Poland) and site 12 (wastewater treatment with constructed wetlands and filters, Berlin, Germany)

3.3.3 Integration of effect-based bioanalysis and chemical non-target screening to globally asses chemical water quality at site 12

Advancements in high-resolution mass spectrometry (HRMS) based screening methods have enabled a shift from target to non-target analyses to detect a much wider array of chemicals in (water) samples (Hollender, Schymanski et al. 2017). Non-target screening has therefore become a promising tool to evaluate the changes of chemical water quality during water treatment (Nürenberg, Schulz et al. 2015). However, the wealth of data resulting from non-target screenings renders structural identification, let alone toxicological evaluation of all compounds, virtually impossible. Consequently, prioritisation is required to define which of the unknown compounds need to be studied/identified first. This can happen on different levels: the abundance of an unknown feature in the sample, the matching of a feature with a suspect list entry, the trend profile of a feature's intensity across treatment steps and/or its correlation with a biological effect, for example reflected in a bioassay response. A feature represents a given compound and consists of a unique combination of an accurate mass and a retention time. Without identifying the feature, information on its response, measured in instrument counts (Sjerps, Vughs et al. 2016) or response relative to an internal standard (Parry and Young 2016), can be automatically extracted. Through suspect screening against a suspect list, potential candidates that match a feature based on their accurate mass can be found and ranked according to their occurrence or toxicity such as evaluated in CALUX bioassays (Brunner, Dingemans et al. 2019). As *in vivo* toxicity data is limited, *in vitro* bioassay data can be used as a proxy, such as the ToxCast database that includes high throughput *in vitro* toxicity information of >8000 environmentally relevant compounds and >1500 bioassays (Schroeder, Ankley et al. 2016). To more comprehensively assess changes in water quality, the trend profiles of feature intensities across treatment steps can be considered through application of data science methods that reveal patterns in the data. These profiles allow distinction between persistence, elimination and formation of a feature during treatments and prioritisation based thereupon (Schollée, Schymanski et al. 2016). Ultimately, trend profiles can be integrated with the bioassay read out profiles resulting in a fit for purpose method to monitor water quality in samples and across treatment steps.

Here, we performed non-target screening analyses on the extracted water samples from site 12 from April and July 2018 in technical triplicates, using an Orbitrap Fusion mass spectrometer (Thermo Scientific). Figure 28 in chapter 5 gives the treatment scheme of site 12.

Detected features were matched against the Water Framework Directive priority list and the SusDat database of the European Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances (NORMAN) consisting of more than 40.000 chemicals relevant for environmental monitoring. A novel data analysis workflow was applied to efficiently interpret the wealth of data generated that combined the points mentioned above. Thereby including integration of the biological effect data generated in the CALUX bioassays and the chemical monitoring data. The primary sedimentation effluent was excluded from the data analysis as the amount of features detected exceeded the processing power of the available IT infrastructure. Detailed material and methods can be found in Annex 7.

First, overall feature numbers and intensities, as well as suspect matches against the NORMAN SusDat and the WFD priority lists were determined, the results of which are shown in Table 9 and Figure 6. As expected and consistent with the CALUX assay results, the secondary effluent that was the influent for the ozonation showed most features in both sample types and post-GAC filter samples the least, respectively. Apart from the GAC filter step, there was a no or a limited reduction in feature numbers

observed through technological and natural treatment steps. Note that the post-ozonation steps constructed wetlands, sand BAC filter and sand anthracite filter are performed in parallel as is indicated by the numbering. Post-GAC treatment succeeds the sand anthracite filter treatment. Summed feature intensities, however, did show significant decrease after ozonation of roughly a factor 3.6 in April and a factor 1.5 in July, respectively. Treatments steps 3a, b and c show limited removal in April (factor 1.0-1.5) and slightly higher removal in July (factor 1.3-1.7).

The GAC treatment after the sand anthracite filter reduces the intensities by a factor 5.2 in April but only by a factor 1.4 in July. This suggests that the longer operation and or higher temperatures in July improve removal by biologically active filters while the Activated carbon sorption capacity is reduced during the same period of operation (saturation). In general this illustrates that the performance of a treatment train can change over operation time and due to conditions and treatment settings. As feature numbers do not reflect the abundance of a given feature in the sample this could either mean that the features persist at lower concentrations in the samples that the features initially present are transformed into new features, or that previously sorbed features are later released by competition of sorption sites.

Table 9 Numbers of detected features, SusDat suspect and WFD priority substance matches across all samples.
Treatment steps 1,2,3 are sequential. 3a, b and c are performed in parallel. Step 4c succeeds 3c. Color codes guide the eye, red indicates higher numbers and green lower numbers relative to the distribution observed number of features per feature-class

	Ozonation influent	Ozonation effluent	Constructed Wetlands	Sand BAC Filter	Sand Anthracite Filter	PostGac Filter
Treatment step	1	2	3a	3b	3c	4c
All Features						
April	26235	23389	24187	22370	25567	8561
July	26394	26228	25567	25648	25691	17598
SusDat suspect matches						
April	13151	11748	12221	11073	12748	4146
July	13203	13087	12764	12755	12859	8820
WFD suspect matches						
April	41	41	41	38	41	11
July	41	42	41	41	41	26

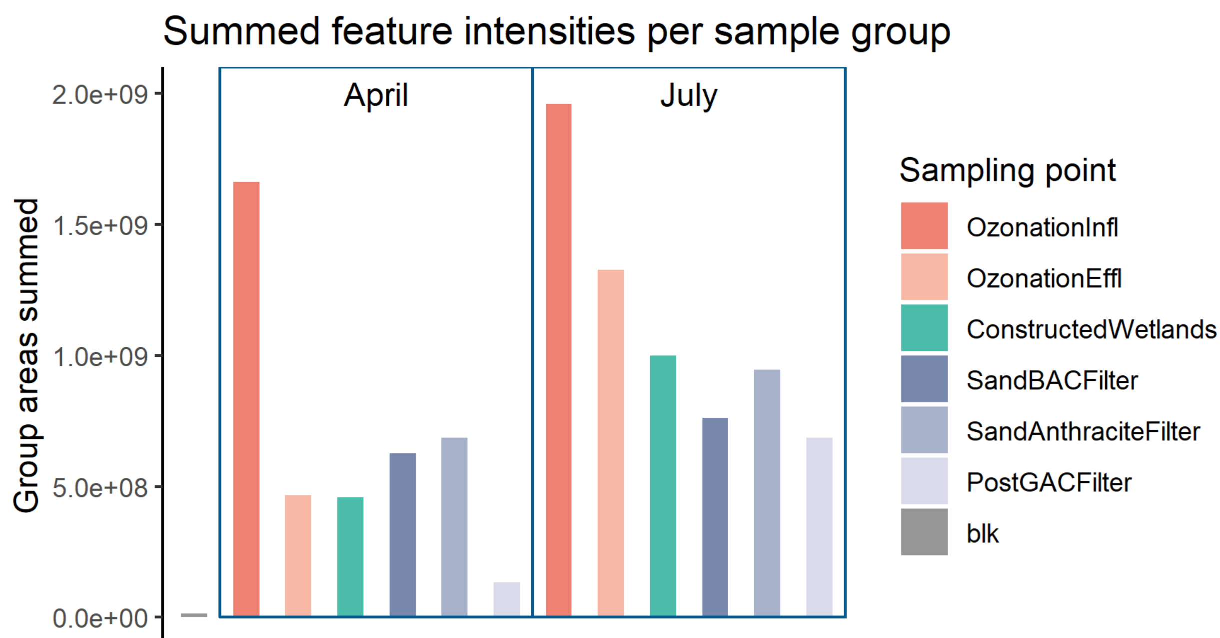

Figure 6 Summed feature intensities per sample group on a log scale, corrected for extract concentration

Next, the multivariate analysis technique principal component analysis (PCA) was applied for a qualitative overview and to group and characterize samples and features. Through reduction of the data complexity PCA can reveal relationships between samples when the principal components are depicted in a so called scores plot (Schollée, Schymanski et al. 2016)). Two thirds of the variance in the data could be explained by the first two principal components as shown in the Screen plot in Annex 7. Therefore, only the first two components were considered in the following. Figure 7 shows the distribution of the Site 12 water samples according to the first two components, referred to as dimension 1 and 2. The technical triplicates cluster together indicating good measurement reproducibilty. Dimension 1 is separating the ozonation influent from the other samples. It could thus reflect overall signal intensities. Feature numbers increse along this dimension, with the blank samples on the far left and ozonation influent samples on the far right. Dimension 2 could be representing the seasonal influence, i.e. the variability between April and July samples. In addition, it could be explaining variability introduced by transformation processes with the parent compounds present in the ozonation influent (negative Dimension 2) and transformation products in the ozonation effluent and other treated samples (positive Dimension 2, decreasing).

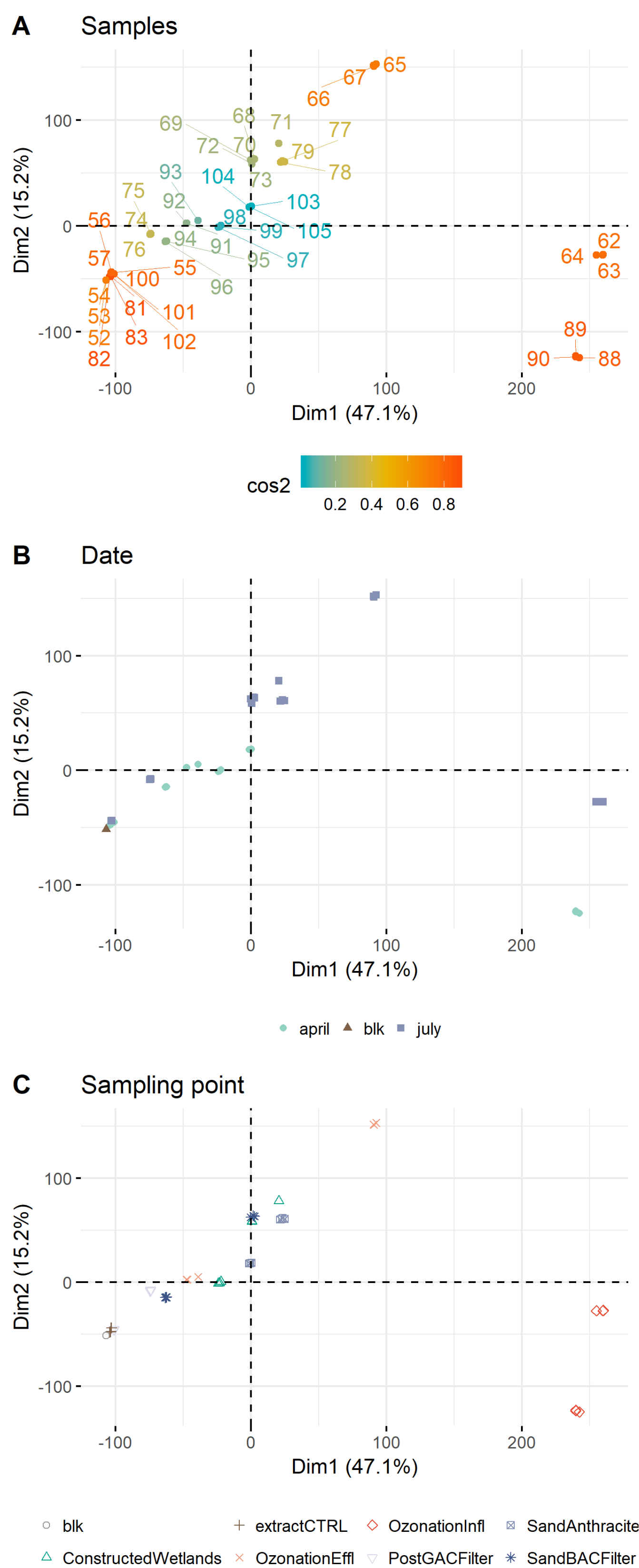

Figure 7 PCA graph of individuals of water samples from site 12, April and July.
Samples are coloured according to their cosine similarity, a measure for the difference of samples (cos^2) (A), date (B), and sampling point (C)

To investigate changes due to treatment steps, features were consequently plotted according to their changes between two samples and the significance thereof in so called Volcano plots (Cui and Churchill 2003). In these plots features on the left side of the y-axis are those that are removed by a given treatment step and features on the right those that are formed. Such a Volcano plot is shown in Figure 8 for the changes in features due to ozonation (left panel) and constructed wetland treatment (right panel) samples of the April sampling round. All features above the red line that indicates significance (p-value < 0.05) and on the left side of the y-axis represent compounds that are removed through the respective treatment technology. On the contrary, the features on the right side of the y-axis are introduced during these treatment steps and are either formed from parent compounds present in the influent or in the case of the constructed wetlands, stem from the wetlands themselves. As the features are coloured according to their retention time which can serve as a measure for polarity of a compound, the Volcano plot can reveal differences in the physicochemical properties of the chemicals before and after treatment. Visual inspection suggests that the influent sample of the constructed wetlands is more hydrophobic than the wetlands effluent. This is in line with the current understanding that more polar substances are less readily removed in such water treatment steps, as they are less prone to sorption.

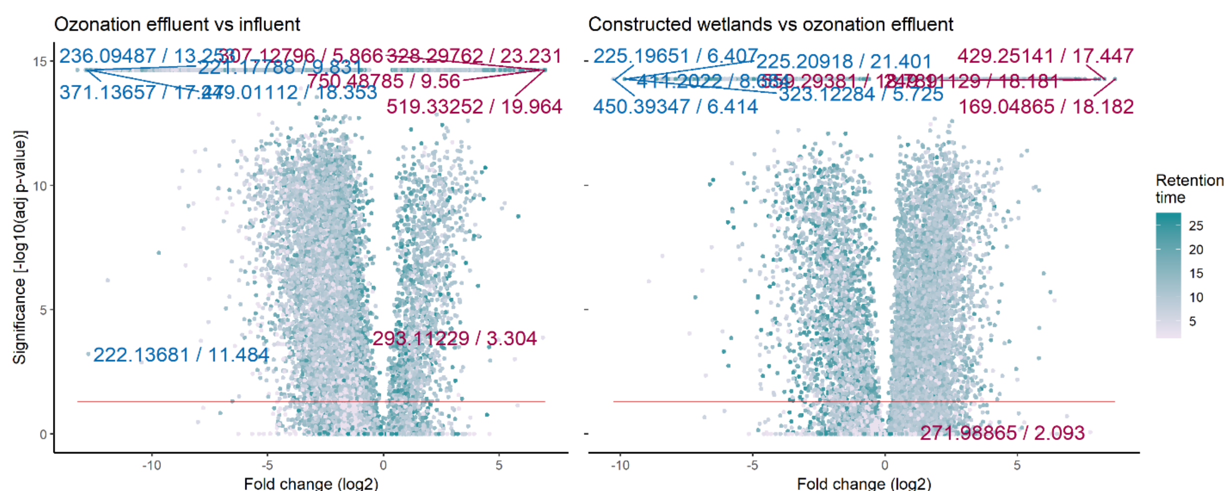

Figure 8 Comparison of feature intensities between ozonation effluent and influent (left panel) and ozonation effluent and constructed wetlands (right) samples (April sampling round)
The changes between feature intensities (log2 fold change, x-axis) are plotted against the significance (p-value) in a Volcano plot. The five most intense features of the respective before (blue) and after treatment samples (red) are labelled. The features are coloured according to their retention time as a measure for polarity.

The high number of features (> 25000) detected in the non-target screening data calls for prioritisation of relevant features of which the structure should subsequently be identified. Which features are categorized as relevant strongly depends upon context. In the scope of AquaNES, focus could be on the features that are persistent across treatments as these pose a risk to the final water quality, as well as the features that are different in constructed wetlands treatment compared to other treatments.

The Volcano plot shown in Figure 8 assists in prioritizing features based on their changes and intensities. The five peaks that show the greatest increase in intensity during ozonation and constructed wetland passage, respectively, are coloured in red, those that show greatest removal are coloured blue. These Top5 features can serve as a starting point for identification.

Alternatively, Hierarchical Clustering (HC) can facilitate prioritisation efforts. HC is a strategy that can cluster samples and features based on their similarity and thus reveal trend profiles of features

between samples or is able to cluster groups of features that show similarities in their distribution over samples, through treatment trains or seasons. This means that clusters of features that are persistent, are formed during a specific treatment step or do not change across treatments each fall in their own cluster. Here, we performed HC on the NTS data set based on Euclidean distances after data normalization, integrated the chemical NTS data with the effect-based data from the CALUX bioassays and visualized the clustering output in the heat map shown in Figure 9.

In this heat-map the relative intensity of each feature (vertical) for each sample (horizontal) is shown ranging from blue (lowest intensity) to red (highest intensity). Based on these intensities the samples are clustered; as expected the technical triplicates cluster together, however, April and July samples do not in all cases, indicating some seasonal changes in water quality. Ozonation influent is clearly separated from the treated samples. Based on this heat-map, feature clusters can be selected for the selection of indicators for treatment performance or further identification studies. Features that show high intensities in the ozonation effluent but not in the influent potentially represent ozonation transformation products (oxidation by-products), while features that still show high intensities in the Post-GAC filter samples are thus not removed by the multi-step treatment.

Furthermore, through integration of the non-target screening data with the CALUX assay readouts, feature clusters that show high intensities when a CALUX response is observed can be determined and prioritized for subsequent identification, as the potentially are responsible for the observed biological effect. Thereby, the integration of chemical non-target screening data with effect-based bioanalysis can assist in more efficient prioritisation and a more comprehensive assessment of chemical water quality and changes during treatment.

The combination of the assessment of number of features, total intensity of the response and various statistical techniques to analyse similarities and dissimilarities between samples in treatment trains and at different sampling occasions. This enables us to view the obtained data in multiple ways and assess the qualitative as well as quantitative impact of treatment steps as well as differences between sampling rounds. Furthermore the correlations of the output with effect based sampling results that integrate all chemicals that have a specific biological effect is a first step towards identifying chemicals responsible for observed effects.

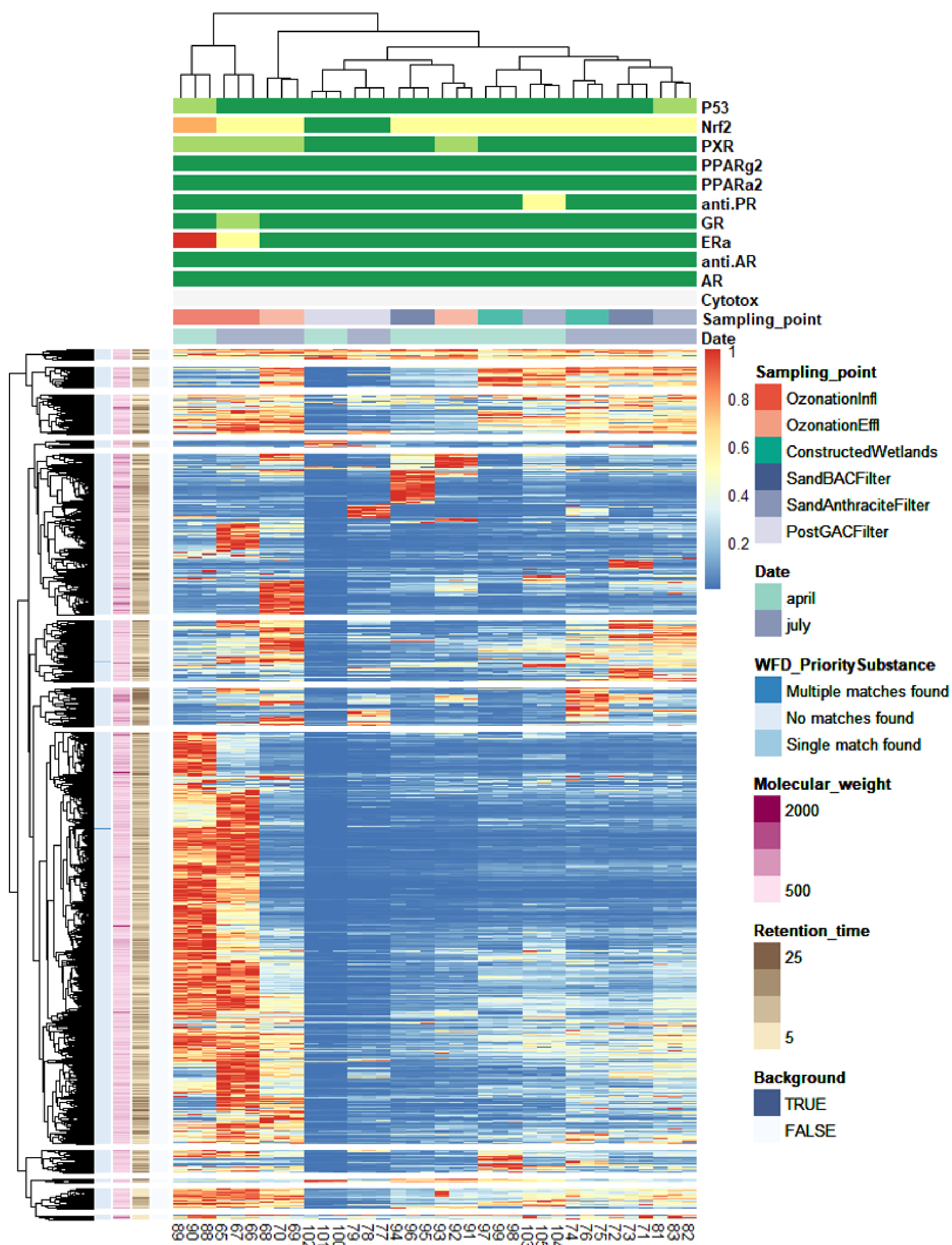

Figure 9 **CALUX bioassay response data integrated with non-target screening data. Hierarchical clustering of normalized non-target screening data based on Euclidean distance**

3.4 Conclusions / lessons learned

- Most effect-based CALUX bioassays demonstrated activity in water samples obtained from the demonstration sites.
- The results from effect-based CALUX bioassays can be used as an indicator parameter for removal of bioactive substances by various water treatment technologies and compare treatments to each other.
- Influent obtained from demonstration sites using wastewater for treatment showed highest bioactivity.
- Development of effect-based trigger values (EBTs) is required for the assessment water quality and implementation of effect-based bioassays in regulatory water frameworks for risk assessment.
- EBTs contribute to a realistic evaluation of efficiency of novel innovative water treatment technologies and assessment of the water quality.
- The development of an action plan for water treatment plant operators based on EBT, enhances the applicability of effect-based bioassay for assessment of water quality.
- Comparison of chemical analysis and bioassays showed that they are complementary techniques. Their integrated results enable aid efficient prioritization of organic micro-pollutants and ultimately a more comprehensive assessment of chemical water quality and changes during treatment

4 BACTcontrol

4.1 Working principle / technology

BACTcontrol™ system is an automated online instrument for the detection of microbiological (enzymatic) activity in water. The system is used to monitor microbial water quality for various types of water that are intended to be (re)used for irrigation or human consumption. This system can monitor microbial activity, and notice changes (events) within a time frame of 1-2 hours. Registration of an event can initiate in depth microbiological analysis as well as potential measures to prevent microbial contamination from spreading in the system or ending up in the produced water. Its fast detection makes the technique suitable for on line water quality monitoring and complimentary to off-line cultivation methods.

BACTcontrol monitors the enzyme activities of β-glucuronidase (GLUC), β-galactosidase (GAL), β-glucosidase (GLUCAN) and alkaline phosphatase (ALP), enzymes of *E. coli*, coliforms, enterococci and total bacterial activity, respectively.

A schematic overview of the BACTcontrol device is shown in Figure 10 and Figure 11. The main element of the device is the reactor, which consists of two chambers separated by a ceramic filter with a pore size of 0.45 µm. In the reaction chamber the water sample is concentrated by the filter, the temperature and reaction are stabilized while the sample is constantly stirred by a magnetic stirrer and the enzymatic activities are measured by the fluorescence detector during the incubation period. Prior to each measurement, the water sample is pumped from the water source through the reactor chamber at flow rates from 1 to 24 ml per minute, the time needed for the filtering depends on the volume that has to be filtered, the turbidity of the water and the condition of the filter. The sampled water volume is also measured by the pump during this process.

After the sample has been pumped through the reactor and concentration has taken place, the temperature is adjusted, and the reaction buffer is injected into the reactor chamber. Different buffers are used for the detection of *E. coli*, coliforms, enterococci and total bacteria activity. They contain the different substrates that are hydrolysed to 4-methylumbelliferone (MUF).

- 4-methylumbelliferyl-β-D-glucuronide (MUG) by GLUC
- 4-methylumbelliferyl-β-D-galactopyranoside (MUGal) by GAL
- 4-methylumbelliferyl-β-D-glucopyranoside (MUGlu) by GLUCAN
- 4-methylumbelliferyl-β-D-phosphate (MUP) by ALP

Serial detection of *E. coli*, coliforms, enterococci and TA is possible.

MUF fluoresces after excitation via UV irradiation (λ_{ex} 360 nm; λ_{em} 450 nm).

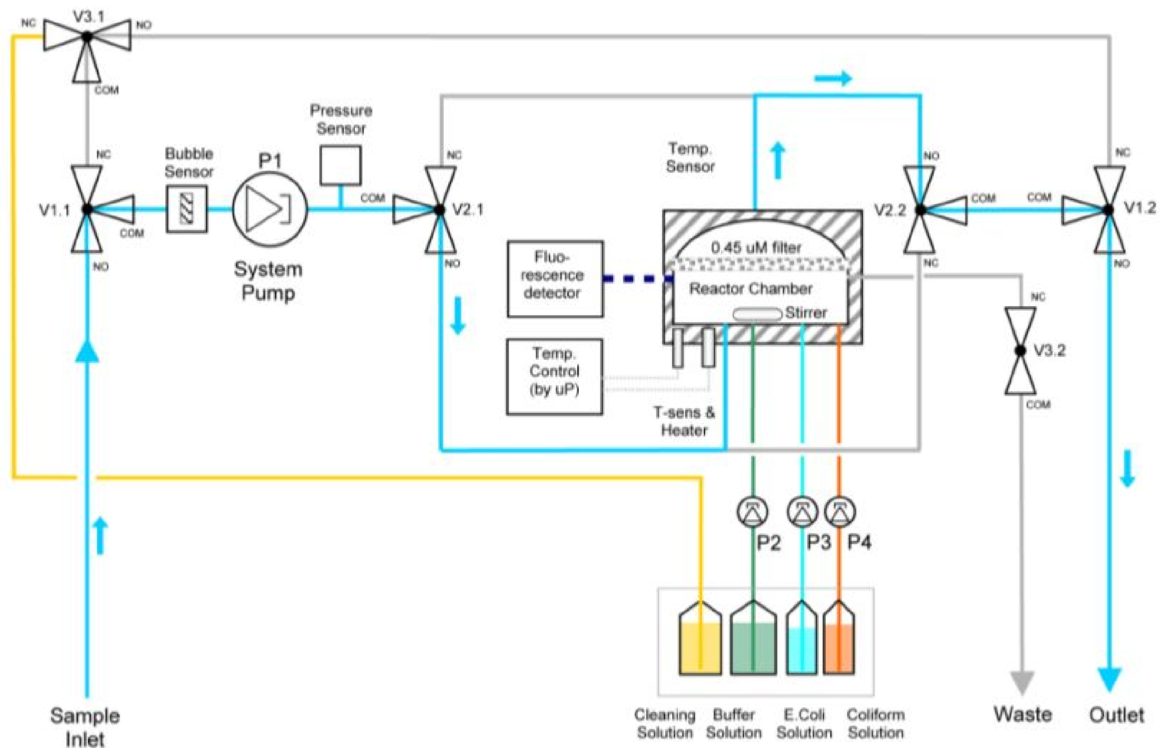

Figure 10 Schematic overview of the BACTcontrol system. The blue line shows the flow from inlet to outlet.

A schematic view of the enzymatic reaction is shown in Figure 11 below.

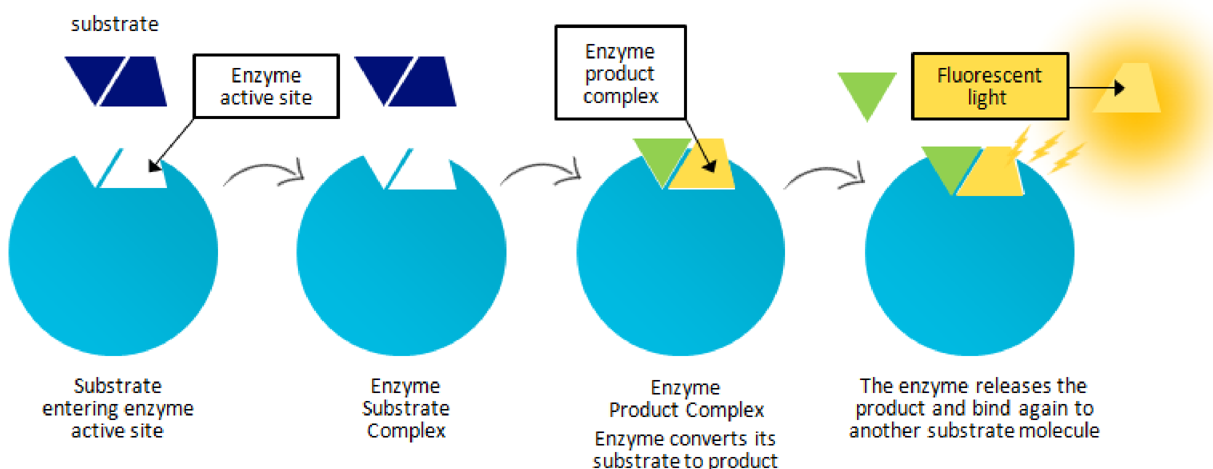

Figure 11 Schematic overview of the enzymatic reaction

After setting the temperature inside the reaction chamber to the optimum temperature (i.e. 44 ± 0.1 °C for GLUC, 36 ± 0.1 °C for GAL, 37 ± 0.1 °C for GLUCAN, 45 ± 0.1 °C for ALP), the sample in the reaction chamber is stirred for 20 minutes, followed by a 20 minute measurement of the fluorescence intensity that are performed without stirring.

The fluorescence intensity of the fluorometer has been calibrated using a standard with a concentration of 1000 nM MUF. This calibration allows the fluorometer to measure and quantify the production rate and define the hydrolysis rate of the substrate by converting the fluorescence intensity into MUF production per time and volume (pmol MUF * min * 100 ml^{-1}).

The increase in fluorescence is automatically saved to the BACTcontrol computer and the slope of the signal in the steady state phase is used to calculate the enzymatic activity by ordinary least square linear regression analysis. Furthermore, the software calculates a limit of detection for each measurement performed. For this statistical approach, the measurement is regarded as significant if the average signal during the measurement exceeds the standard deviation in relation to the theoretical zero line of the reaction by factor three. The limit of detection calculation is determined after the stabilization.

4.2 Testing of the device at AquaNES demonstration sites

BACTcontrol has been applied at six demonstration sites. Table 10 shows the locations and details of the tested water type and testing period. The sections below give details on the experiences and results of these tests.

Table 10 Test locations of the BACTcontrol

Site	Water type and use	Treatment technology	Samples	Parameter tested	Period of testing
No. 6 Lange Erlen, CH	Drinking water production from surface water using artificial groundwater recharge	UV/H_2O2 treatment of sand-filtered surface water before soil infiltration	Sand filtered surface water Effluent from soil columns	Total activity	July – December 2017 Spring 2018
No. 2 Hosterwitz, DE	Drinking water production from bank filtrate and infiltrated surface water	River bank filtration assessment and subsequent flocculation and filtration steps	Surface water before and after flocculation Bank filtrate Effluent from ultrafiltration	Total activity, colony count, coliform count and ATP	March-April 2019
No.3 Budapest, HU	Drinking water production from bank filtrate and infiltrated surface water	River bank filtration assessment and subsequent disinfection steps	Bank filtrate Ozonation Sand filtration UV disinfection	Total activity and cell count	March-April 2019
No. 9 Ovezande, NL	Infiltration of harvested rainwater for reuse	Filtration	Filtered storm water	Total activity	February - March 2019
No. 8 Agon Coutainville, FR	Secondary effluent of municipal WWTP	Conventional activated sludge (CAS) process	Secondary effluent	*E. coli* activity and total coliforms	Spring 2017
No. 11 Rheinbach, DE	Polished secondary effluent of municipal WWTP	CAS followed by retention soil filter	Feed and outflow of the retention soil filter	*E. coli* activity and total coliforms	July 2018 to February 2019

4.2.1 Lange Erlen: Determining total activity in surface water treatment processes

The Lange Erlen site produces drinking water for the city of Basel (CH) from the river Rhine (surface water abstraction). The treatment train encompasses screening, filtration and subsequent soil infiltration. Re-abstracted groundwater is treated by granular activated carbon and is finally UV-disinfected.

The AquaNES intervention around this drinking water production chain consists of a UV+H_2O_2 pre-treatment of Rhine filtrate before soil infiltration. To this end, a pilot plant (reactor and column set-ups) was operated to investigate the effectiveness of the advanced oxidation process for the removal of micro-pollutant and any effects on biodegradation and sorption. The soil infiltration of the full-scale was represented by two types of columns: one containing soil from the real infiltration sites mixed with sand, the second system contained used granular activated carbon from the full-scale plant and served as a biologically active carbon (Figure 12).

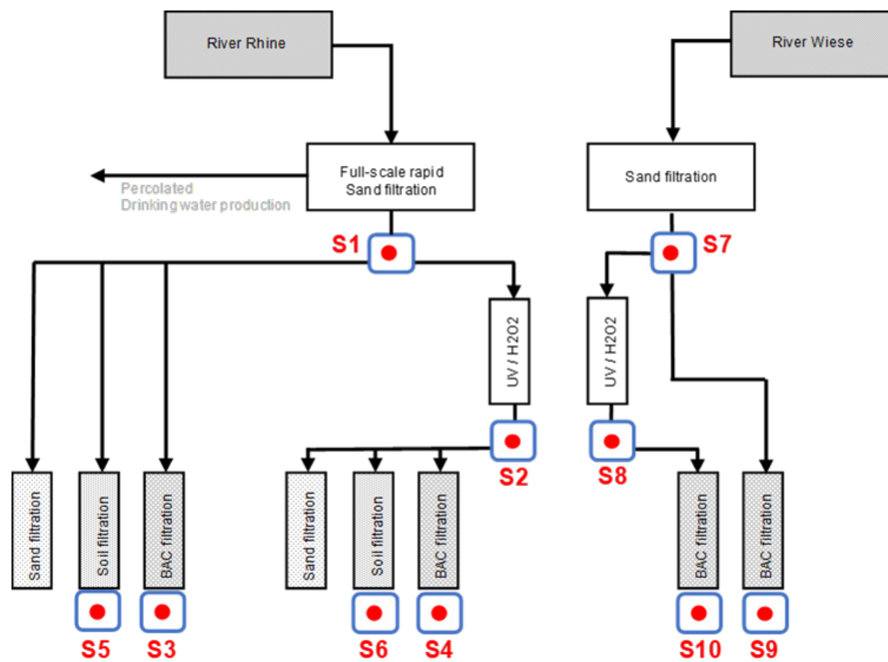

Figure 12 Flowsheet of the pilot plant in Lange Erlen

The BACTcontrol device was used for continuous monitoring of the feed water of the process (point S1 in above scheme). It was installed in the experimental hall next to the UV reactor (Figure 13). A hose was fixed permanently to the device. The feed consists of sand filtered Rhine water, so called Rhine filtrate. This line of the pilot plant is fed from the full-scale sand filter.

Periodically samples were taken manually of the UV/H2O2 treated water (point S2) and the sampling ports of the soil columns (S5 and S6) by connecting the sampling hose via some fittings.

Figure 13 View of the BACTcontrol device installed at the Lange Erlen pilot plant.

4.2.1.1 Results

4.2.1.1.1 Variability / fluctuation of inflow water quality

The total activity in the Rhine filtrate varied between 260 and 400 pmol/min for the first days of operation. It then increased steeply to values around and above 1200 pmol/min and peaked in 2250 pmol/min. During these three days values raised and dropped frequently. After a week of operation, the values stabilised between 250 and 500 pmol/min. Furthermore temporary disturbances were observed. Attempts to link the variations to external influences, as e.g. heavy rainfall impacting on the water quality failed. It is also not expected to see such effects in the tested system as the scheme operates a full-scale sand filter and the intake of surface water is stopped anyhow at elevated turbidity. The only coincidence detected was related to the operational state of the full-scale sand filter. When it was switched on again after some days of stagnation, a slight increase in bacterial activity was observed, maybe due to material or biomass mobilization from the filter.

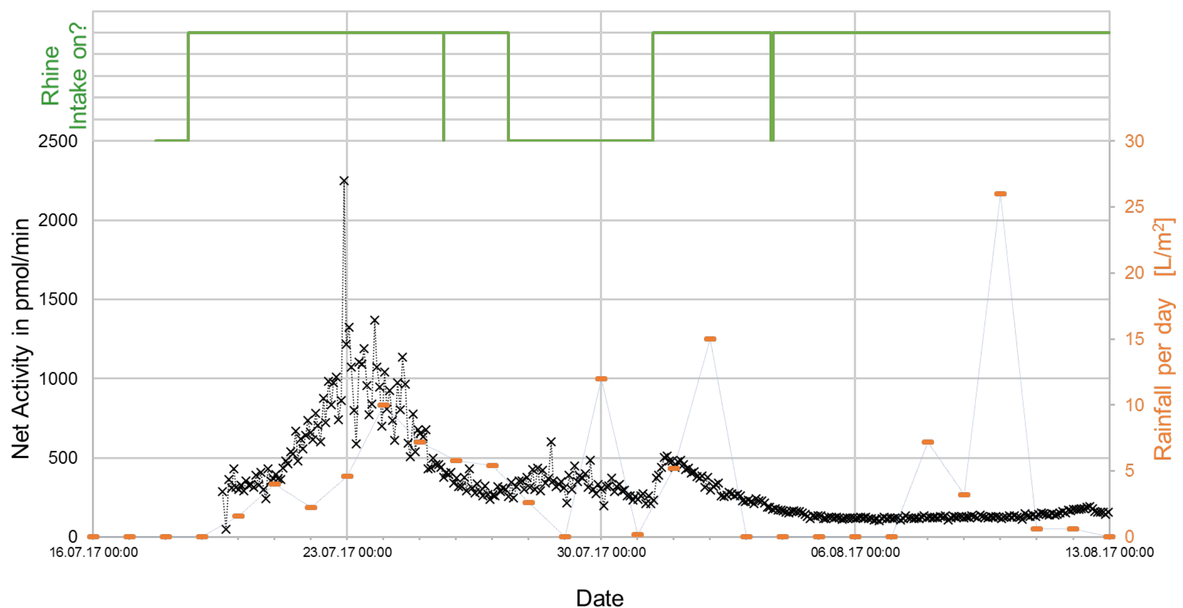

Figure 14 Total bacterial activity measured in Rhine filtrate, during first month of measurement

The variations and decline in values were not reflected by any other online-measured parameter (such as turbidity – see Figure 15 – UVA254 or electrical conductivity – not shown). Turbidity is on average 0.5 FNU or well below. The period with the highest values and biggest fluctuation in turbidity occurs from 26 July to 2 August. Any outliers do not occur on days with microbial activity peaks. The period 3-13 August, which shows stable values for microbial activity at around 120 pmol/min is also characterised by a narrow spread of turbidity values around 0.3 FNU (Figure 15).

Grab samples were also analysed by flow cytometry, heterotrophic plate counts and ATP measurements, yet not always in the experimental period.

Table 11 **Cell counts (flowcytometry) and microbial activity (BACTcontrol). Data for dates only where both methods were applied**

Date	Total cell [cells/mL]	Intact cell [cells/mL]	Microbial activity [pmol/min]	HPC [CFU/mL]
10.07.2017	1’171’800	1’054’600	Not measured	740
15.08.2017	2’054’800	1’827’000	138 ± 10	Not measured
05.09.2017	2’385’200	1’757’800	182 ± 22	Not measured

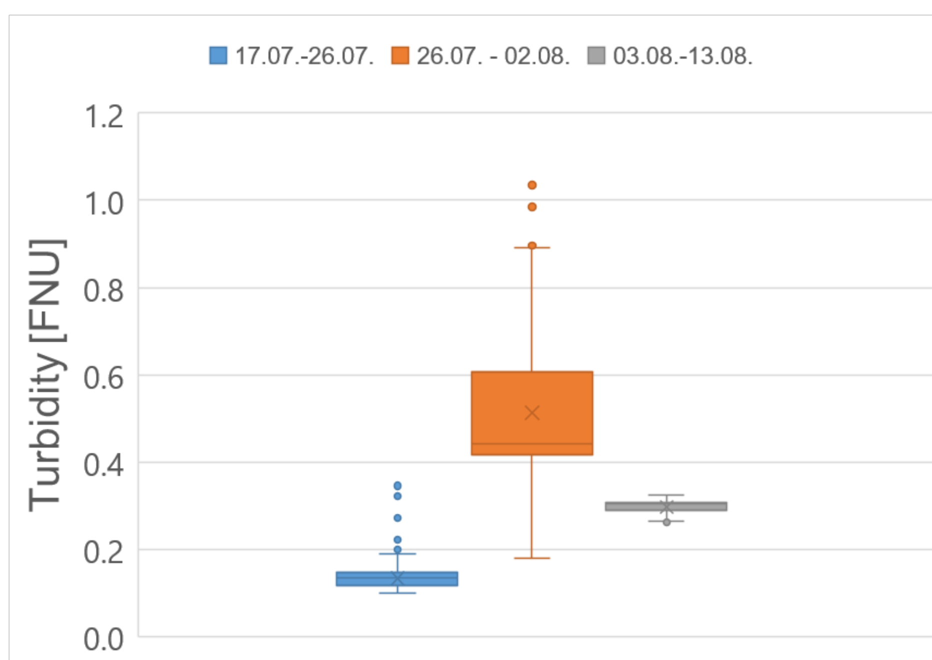

Figure 15 **Results of online turbidity measurement during device set-up and period from 17 July to 5 August first month of operation for selected periods (Measuring interval: 5 minutes)**

During the subsequent months of operation (August-September 2017) a continuous stable measurement was impaired by relatively frequent maintenance efforts (refilling chemicals and replacing filter) and adaptation of operational settings (Figure 16).

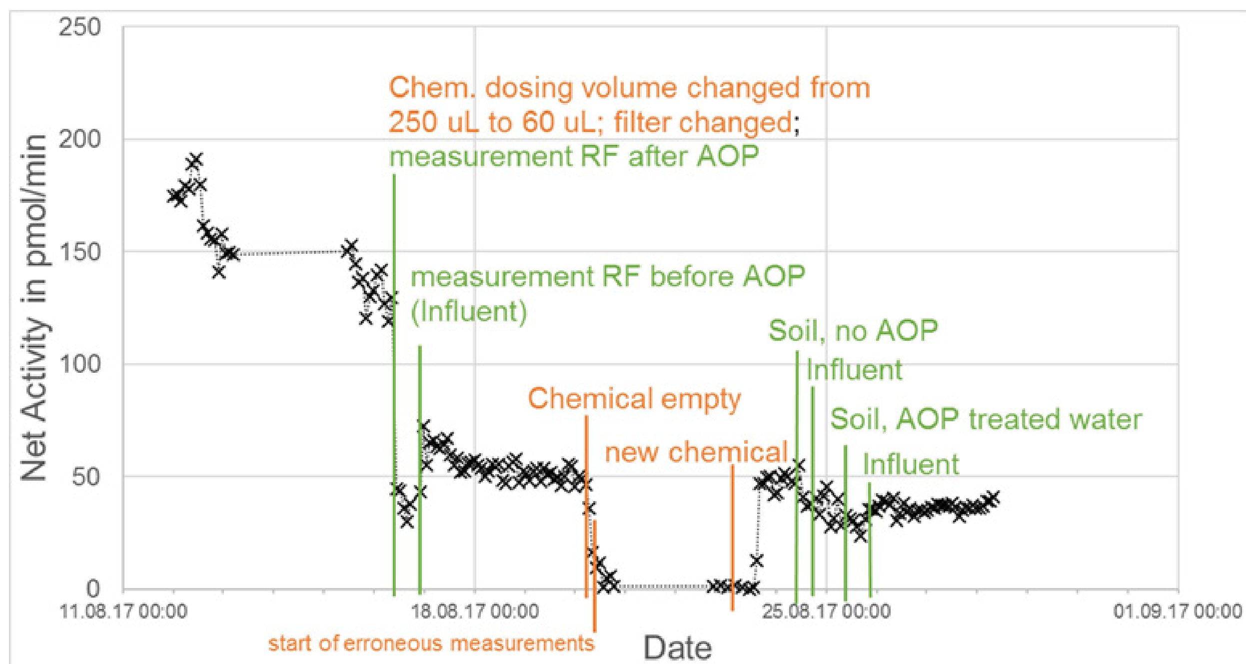

Figure 16 **Total activity measured in Rhine filtrate between August and September 2017 (data gaps are due to either manual sampling activities or technical problems as indicated in the figure)**

4.2.1.1.2 Disinfection effect of the AOP treatment step

The BACTcontrol was also tested to observe a disinfection efficiency of UV/H_2O_2 treatment. The applied dose of 6000 J/m^2 is very high compared to minimal doses of 400 J/m^2 normally applied in drinking water disinfection.

For this comparison several consecutive measurements of the inflow (Rhine filtrate) were compared to several consecutive measurements in the AOP treated stream (outflow) on the same day (Figure 17). The reduction is thus not calculated from paired values but averaged from inflow and outflow data.

Measurements with the BACTcontrol device on two separate occasions gave quite different results. The detected reduction was 70 % and 30 %. These measurements were obtained with differing activities of 130 and 300 pmol/min, respectively. ATP measurement and cultivation methods showed a much higher disinfection of 94-97 %. Lower and variable disinfection, as measured by the BACTcontrol might be due to suboptimal or varying sample volume and reagent dosing.

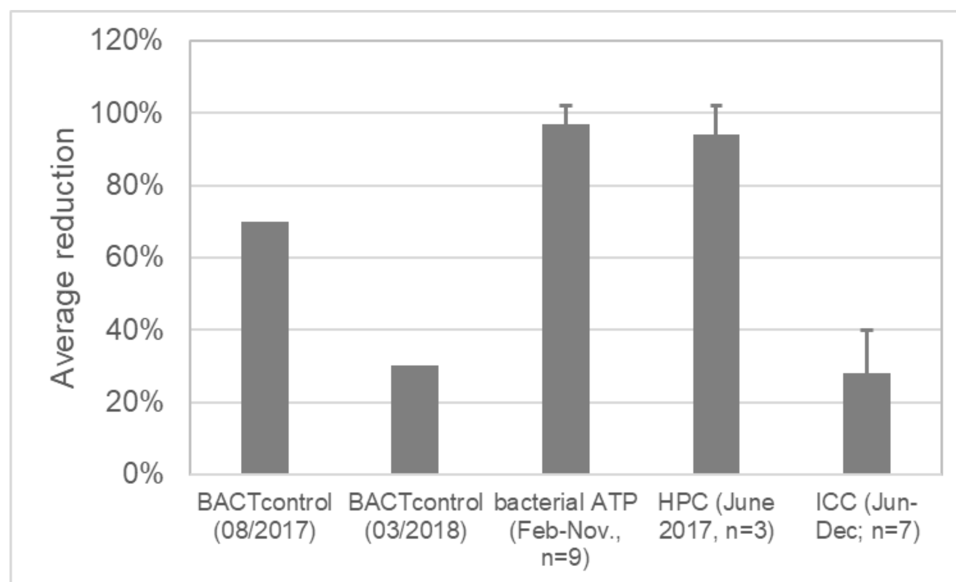

Figure 17 Disinfection effect of the AOP treatment step as detected by different microbiological measurement methods (HPC: heterotrophic plate count; ICC: intact cell counts by flow cytometry)

4.2.1.1.3 Long-term effect of natural treatment step in combination with advanced oxidation

During spring 2018 the effluent of the soil columns was sampled to detect differences in the bacterial activity. The soil columns were fed with water that either had or had not received AOP treatment.

For this purpose the effluent of the two columns was measured several times a day. The results are depicted in Figure 18. Whilst values for the non-AOP pretreated column were around 323 pmol/min. on average, those of the AOP column fluctuated strongly with frequent peaks exceeding 1'200 pmol/min. and even up to 2'000 pmol/min.

This finding for the soil column receiving AOP treated water could not be properly explained. It is unclear whether this really reflects strongly varying bacterial activity, caused by uneven flushed out biomass from the column, or whether it is rather an artefact related to the measurement. However, when switching the lines, no air bubbles or particles were detected in the tubes nor in the reaction chamber. Such effect has not been observed in earlier measurements in August 2017 (data not shown).

Interestingly, the effluent of the baseline of the soil column (sampling point S6) and all measurements of the BAC column receiving AOP treated water (sampling point S10) showed similar levels that were on slightly lower than the levels of the soil treatment of water that was not pretreated with AOP. It thus cannot be confirmed the peaks are related to the AOP pre-treatment.

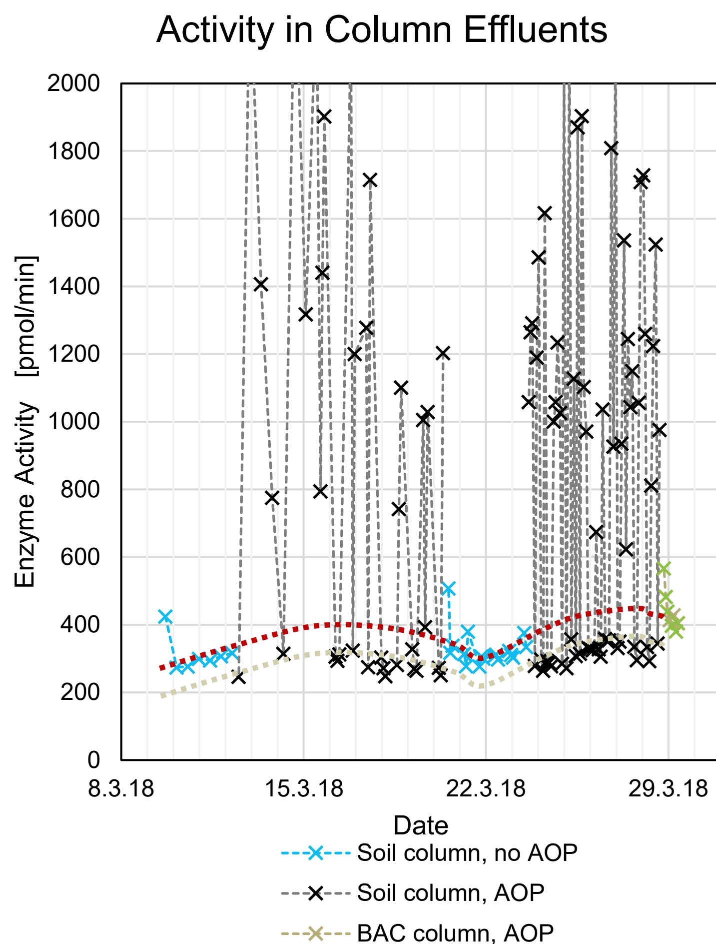

Figure 18 Total activity detected in the outflow of the soil columns and the BAC (Biologically Active Carbon) column
(The red and greenish dashed lines depict the hypothetic trend/data for the column not measured when the other columns were sampled. This baseline differs between both soil columns by approx. 70 pmol/min)

4.2.2 Hosterwitz (Dresden) and Budapest: measuring coliforms and microbial activity

The TU Dresden team applied BACTcontrol to determine *E. coli* and coliforms normalized for their biomass and intact cells at site no. 2 Hosterwitz, DE and site no. 3 Budapest, HU, in the spring of 2019, respectively. There was a strong emphasis on robust operation and technical support as well as parallel assessment using other monitoring methods. The sampling was well coordinated following well described procedures and careful planning.

4.2.2.1 Results

Figure 19 show that BACTcontrol results, normalized for microbial biomass are in line with the colony count and ATP measurements. Furthermore, the reduction of bacteria and their activity along the treatment train follows the same trend.

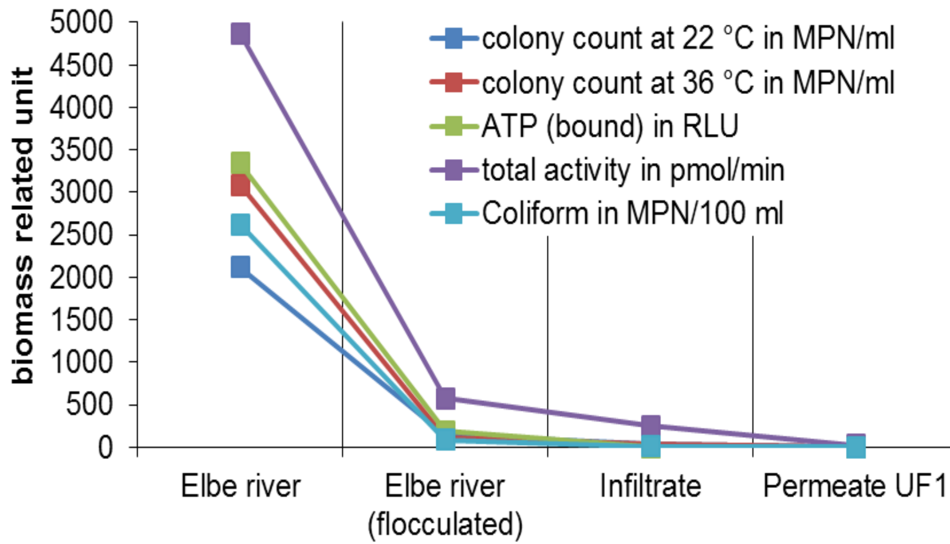

Figure 19 **Comparison of BACTcontrol (total activity in pmol/min) to standard monitoring and ATP method at Hosterwitz, DE (AquaNES Site, no. 2)**

In Budapest, the BACTcontrol measurements of total enzyme activity are compared to intact cell counts. Figure 20 show a similar pattern of both parameters, but residues of enzyme activity seem to remain at higher levels along treatment trains than the intact cell counts. This presumed discrepancy can be explained by the fact that enzymes can maintain activity even when cells are broken / dead.

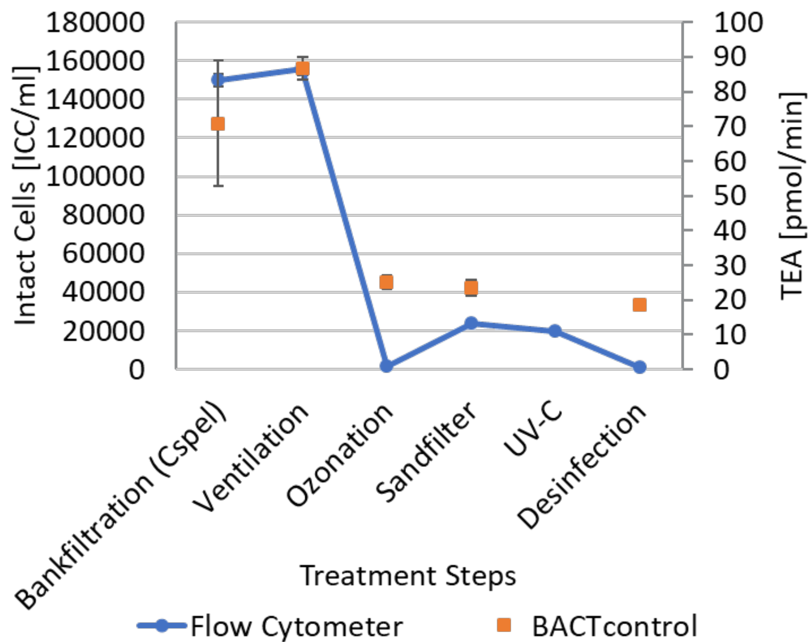

Figure 20 **Comparison of BACTcontrol to flow cytometry at Budapest, HU (AquaNES Site no. 3)**

Application of results

The observed correlations between BACTcontrol measurements and more traditional methods for the detection of microbial contamination illustrate the potential applicability of the BACTcontrol for monitoring microbial water quality as well as treatment performance. The results show how the enzyme activity measurements correlate with more traditional units of water quality monitoring. The correlation supports the applicability of innovative fast detection methods of the BACTcontrol. Thereby it supports acceptance within the water sector as well as in regulation. Before further implementation, the observed correlation requires standardization in order to translate the output of the BACTcontrol to typical endpoints such as cell count or colony growth currently used for water quality monitoring. Such assessments are necessary to implement innovative techniques in such a way that they are able to evaluate if regulatory quality criteria or other water quality thresholds are exceeded. Nevertheless the current results indicate that the tool can be applied for first tier monitoring of variations in water quality or performance efficiency, and to initiate further testing if trends (increase) are observed.

4.2.3 Ovezande: Monitoring of infiltration of storm water for aquifer storage and recovery

Site 9: The Ovezande site is a polder located in the southwest of the Netherlands. The underlying aquifer is prone to salinization. The infiltrated storm water fulfils two purposes, first it will reduce saline intrusion and additionally it will be reused for irrigation purposes. The technology is designed to enable high rate water treatment and subsurface infiltration during storm water events. The technical challenge for this site was to design a stand-alone, high capacity rainwater treatment unit with a low spatial footprint that decreases infiltration well clogging rate cost-effective manner. The goal was an optimal design and operation to prevent overflows and optimize fresh water storage. One of the issues with clogging is the growth of bacteria in flocs that can lead to clogging after the filtration steps. Therefore the monitoring of bacterial activity is relevant for the monitoring of microbial-induced clogging. During winter and early spring of 2019 the BACTcontrol was installed to measure the total microbial activity after filtration, as an indicator for microbial clogging potency under different post-filtration disinfection treatments.

4.2.3.1 Results

Figure 21 Test setup of the BACTcontrol at Ovezande

Clogging was observed by the penetration of small particles through pre-filtration steps (5 and 1 micron filters) and microbial growth within weeks of operation. Local UV disinfection after pre-filtration, did not improve the infiltration performance of the well, but continuous dosing of disinfectant (Na-

hypochlorite) did. Rapid pre-treatment using compact filtration is a challenge, removal of even the finest particles and prevention of biological growth are vital for the operation of the infiltration well. The BACTcontrol was able to monitor total activity during the full operational period with different disinfection steps without large technical issues of failures (data shown below of a 3 months period in Figure 22). The filtration pretreatment prevented the microfluidics from clogging BACTcontrol microfluidic device in combination with thanks H2O2 washing of the device to prevent precipitation of salts. As observed in Figure 22, initial operation of the well without disinfection lead to clogging and reduction of infiltration rates within days to a week. The UV disinfection was not able to prevent clogging. The operation with irregular chlorination also was not able to prevent clogging, and BACTcontrol measurements illustrated high microbial activity. The continuous chlorination lead to stabilization of the well capacity, preventing clogging and showing lower microbial activity. The total activity measurements performed by the BACTcontrol suggest incomplete disinfection at the dosing point for continuous chlorination. However, the activity was measured directly after chlorination, while chlorinated water is infiltrated, leading to better permeability of filters below the surface. Future assessment of microbial activity inside the wells are required to define thresholds for microbial activity that can induce clogging.

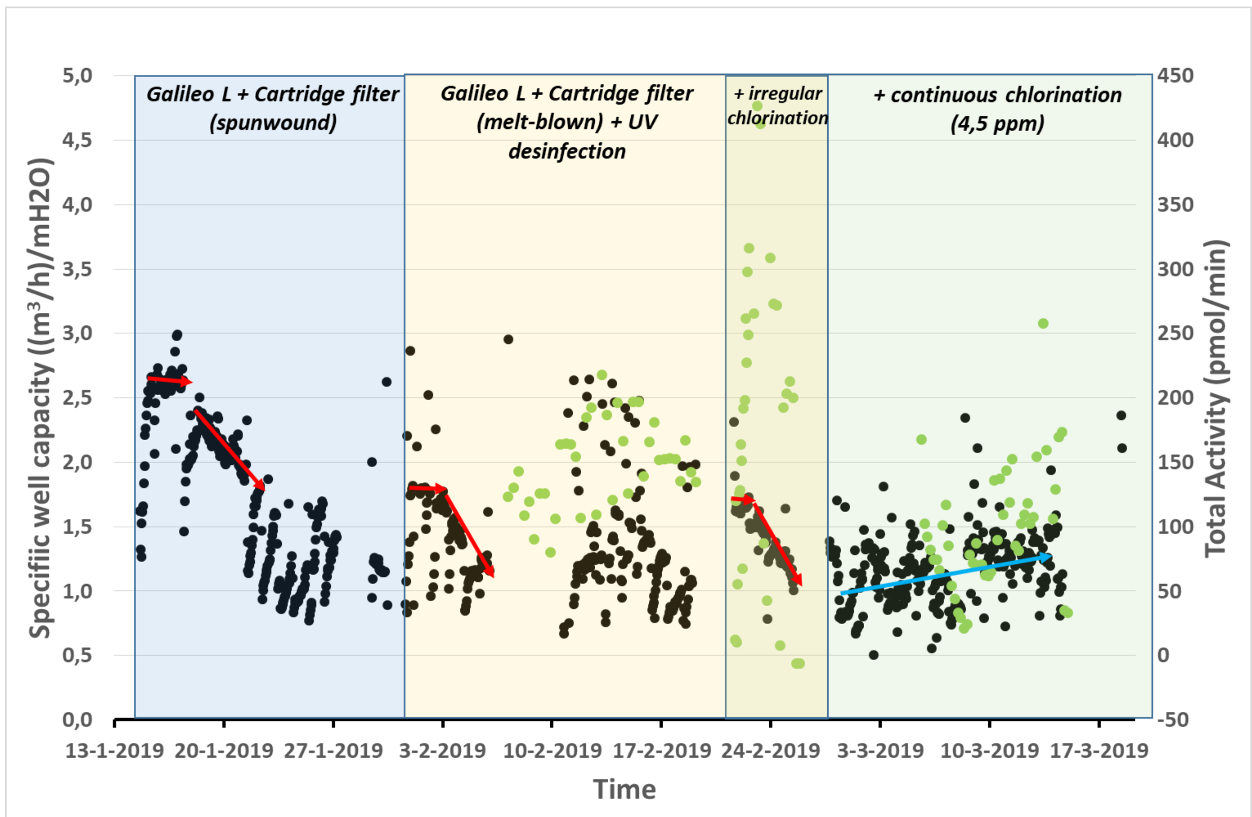

Figure 22 Specific well capacity of the Freshmaker well and measured total (biological) activity of the pre-treated rainwater in Ovezande during the pre-treatment tests in 2019.
The black dots give the specific well capacity. The green dots give the total microbial activity just after the disinfection step and before infiltration.

As a spin-off quality criteria for hardness (<2mmol/L) could be derived to prevent precipitation of salt complexes in the BACTcontrol device that hamper analysis.

4.2.4 Agon Coutainville: Monitoring faecal contamination in WWTP effluent for aquifer recharge

Site 8: The Agon-Countainville site uses secondary effluent after reed bed and sand dune filtration for golf course irrigation in a coastal area. The underlying aquifer is prone to salinization. The BACTcontrol is implemented from May to September 2017 to monitor *E. coli* and total coliforms. The main focus of this implementation is to demonstrate how innovative water quality monitoring devices such as the BACTcontrol can be linked to other monitoring data and modelling efforts. At this rather remote site (with no on-site personnel and technical staff), modelling is linked to data management and communication, in order to facilitate remote operation and quality control.

Figure 23 Test setup of the BACTcontrol at Agon Coutainville

4.2.4.1 Results:

Operation of the BACTcontrol at a remote site testing treated wastewater effluent with no availability of technical staff illustrated how technical issues such as clogging of microfluidic systems can compromise measurements, and therefore remote monitoring and data management. During a short period of time there was a big increase of fecal bacteria observed by The BACTcontrol. Unfortunately, this was not verified by parallel measurements using classic techniques. The increase might have indicated a disruption of the test system. However soon after the observed increase, the BACTcontrol clogged, hampering further data acquisition.

Obtained results (data not shown) revealed the importance of on-site availability of technical staff and troubleshooting to be essential, since microfluidics in the system are prone to clogging especially when working with treated wastewater. Therefore, remote application of the BACTcontrol should be performed carefully, with well-functioning communication network to monitor performance and sufficient technical support that can solve clogging or other issues in due time. The experiences from this site were used in later testing at other sites.

4.2.5 Rheinbach: Monitoring efficacy of retention soil filters for removal of fecal indicators

Site 11: Rheinbach is a wastewater treatment plant with pilot scale Retention Soil Filters (RSF) are applied to treat (polish) effluent during dry periods and treat storm water overflow during heavy rainfall. The efficiency of this so called "RSFWWTP+" system and the effects on water quality are studied. During July 2018 to February 2019 BACTcontrol was installed to monitor fecal bacteria (*E.coli*) in treated wastewater before and after retention soil filter treatment.

Figure 24 Test setup of the BACTcontrol at Rheinbach

During application of the device some issues rose with the freezing of buffers during the winter months, illustrating how harsh weather conditions can compromise operation. All in all the BACTcontrol illustrated the variability of *E. coli* activity, furthermore it showed that the RSF treatment lead to a significant reduction of *E.coli* activity. Parallel measurements of *E. coli* and *E. coli* counts show a correlation between the two methods. Correlations appeared to be stronger before retention soil filter treatment than after this treatment, with a respective correlation coefficient of 0.77 and 0.62 respectively (Figure 25).

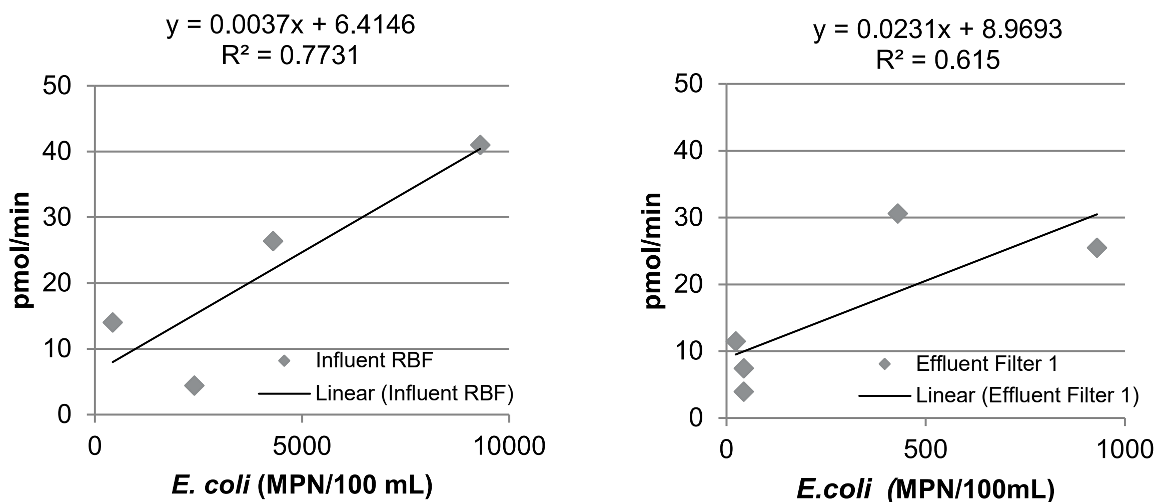

Figure 25 Correlation of *E. coli* counts and BACTcontrol measurements at influent (left) and effluent (right) of RSF filter 1

Nevertheless, 77 % and 62 % of the variation could be explained by the BACTcontrol measurements. However, the overall level of fecal contamination in inflow and outflow could not properly be detected by the BACTcontrol measurements. Whilst the cultivation method detected difference of one order of magnitude, the activity detects by BACTcontrol were in the same range.

These results support the application of the BACT control as a fast detection device to monitor fecal contamination in wastewater treatment plant effluent, as a first tier quality control tool. Obtained responses can initiate further research or induce mitigation measures if deemed necessary. Besides that the tool might also be used as a substitute in future, when the correlations between the different measuring techniques (i.e. plate counting vs. activity measurements) are sufficiently validated.

4.3 Discussion

The BACTcontrol was used for different purposes in different types of water. Various technical issues were observed such as clogging by particles and precipitation of salts and freezing (site 8 and 11). These issues illustrated the importance of on-site technical support for regular checks and troubleshooting and remote data monitoring. This means that applications in remote locations require specific measures to obtain robust operation. Furthermore, variations in sensor response could not always be explained or related to other measurements (site 6), rendering some needs for research to determine the causes of the observed results, and potentially solving this issue.

Nevertheless, observed clogging by precipitation of salts could be solved by using H_2O_2 as cleaning agent (site 9). The clogging because of turbid samples will al always be an issue for microfluidic sensors such as the BACTcontrol. Rendering its application in relatively turbid (waste)water streams or water types that are susceptible to strong variations of turbidity (e.g. storm water) difficult. Currently, pre-filtration units are tested to enable measurements in more turbid waters (results not presented in this report). Nevertheless, it was observed that the system generated stable continuous measurements for several months and provided sufficient sensitivity to monitor microbial contamination in various types of water. Furthermore, correlations were observed between activity measurements and parallel cell counts at both water treatment for the production of drinking water (site 2 and 3) and treated wastewater. This illustrates the potency to apply this technique for (near) continuous water quality monitoring.

4.4 Conclusion / lessons learned

- On site on-site technical support are essential for continuous operation of the BACTcontrol
- Clogging because of precipitation of salts can be prevented by washing
- Clogging because of particles turbidity is an issue for turbid water types, so pre-filtration steps might be necessary
- BACTcontrol measurements correlate with colony counts, cell counts and ATP measurements
- When technical issues are under control, BACTcontrol measurements can provide near-continuous measurements on microbial contamination which can be applied for monitoring performance of natural and engineered treatment and water quality control

5 Antibiotic restistence genes

5.1 Study design

Due to the frequent application of antibiotics in human and veterinary medicine, antibiotic resistant bacteria (ARB) and genes encoding for antibiotic resistance can be found in different compartments of the water cycle (Stoll et al., 2012). Bacteria can receive antibiotic resistance genes (ARG) by spontaneous DNA-mutations and vertical or horizontal gene transfer. Horizontal gene transfer is a major pathway for the dissemination of ARG in the environment and even allows a gene exchange between different strains and species (Frost et al., 2005). As shown in Figure 27 the genes can be exchanged directly between bacterial cells via mobile gene elements (conjugation), injected by bacteriophages (transduction) or taken up with free DNA (transformation).

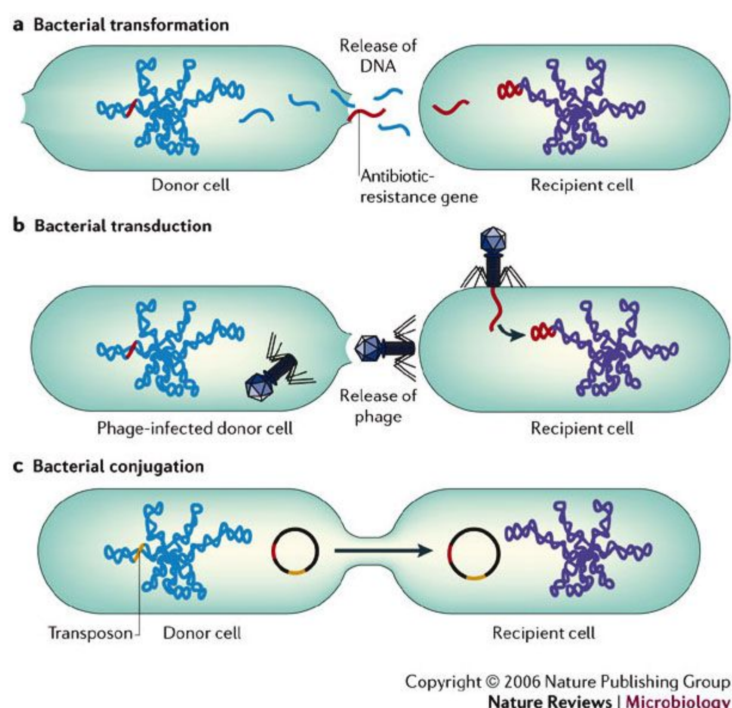

Figure 26 Mechanisms of horizontal gene transfer from Furuya and Lowy (2006)

Conventionally the presence of ARB has been analysed using microbiological culture methods. Their main disadvantages are long incubation times in the range of days and the fact that organisms in a so called viable but not culturable (VBNC) state are not considered in the quantification. It has been shown that bacteria in VBNC state are relevant since they are able to reproduce under certain conditions (Ramamurthy et al., 2014). ARG analysis based on quantitative Polymerase Chain Reaction (qPCR) technology represents an innovative approach with the potential to overcome the shortcomings of conventional culture methods.

ARG analysis in the AquaNES project is performed by BWB in cooperation with the Water Technology Centre (TZW).

5.2 Working principle

The qPCR analysis enables to quantify a certain target-DNA fragment (e.g. an ARG) in a sample by amplifying the target-DNA with Polymerase Chain Reaction (PCR) and simultaneously introducing a fluorescent probe into the DNA which later allows for optical measurement. The PCR is a repetitive process, in which the target-DNA is duplicated with each cycle. The cycle consists of the following three steps:

1. Denaturation: High temperatures (>90°C) break the hydrogen bonds between complementary bases and double-stranded DNA is separated in two single-stranded DNA molecules
2. Primer annealing: After lowering the temperature the specific primers bind to the start and the end of target-region of the single-stranded DNA
3. Elongation: At temperatures around 70-80 °C the enzyme DNA polymerase synthesizes a complementary DNA strand starting at the position of the primers. The DNA fragment is double-stranded again

The fluorescent probe gets excited after binding to the single-stranded DNA. Since the amount of target-DNA is increasing exponentially with the cycles, the measurable fluorescence increases accordingly. A sufficient level of fluorescence is required in order to guarantee accurate quantification. Knowing the number PCR-cycles the initial amount of target DNA-copies can be calculated.

5.3 Material and methods

5.3.1 Demonstration site

The ARG and ARB analysis monitoring was conducted at demonstration site 12 (WWTP Schönerlinde). As shown in Figure 28 secondary effluent is treated with ozone, followed by two vertical-flow constructed wetlands (CW) and different deep-bed filter systems.

The ozonation process is operated with a specific ozone dose of 0.7 mg O_3/mg DOC and a hydraulic retention time of approximately 30 min.

Both CW have a surface area of 11 m² each and were planted with *Phragmites australis* and *Carex acutiformis* in equal parts. They were operated under saturated conditions with filtration rates of 200 mm/d, 400 mm/d and 1000 mm/d in different phases. In CW1, technical sand is used as filter material (bed depth = 0.55 m, d = 0.2-2 mm). In CW2, coarser filter material (bed depth = 0.8 m) consisting of a homogeneous mix of lava gravel (d = 4-8 mm) and biochar (d = 8-20 mm) is tested.

All deep-bed filter columns are constructed identically with a diameter of 0.3 m but differ in their filter media. The 3 filters BAC, S/BAC and S/A which are operated in parallel contain activated carbon (d = 1.4-2.4 mm), sand (d = 0.7-1.25 mm) / activated carbon (d = 1.4-2.4 mm) and sand (d = 0.7-1.25 mm) / anthracite (d = 1.4-2.5 mm), respectively. The post-GAC filter is operated with activated carbon (d = 0.6-2.4 mm) subsequent to the S/A. The dual-media filters S/A and S/BAC are additionally equipped with coagulant dosing for phosphorous removal.

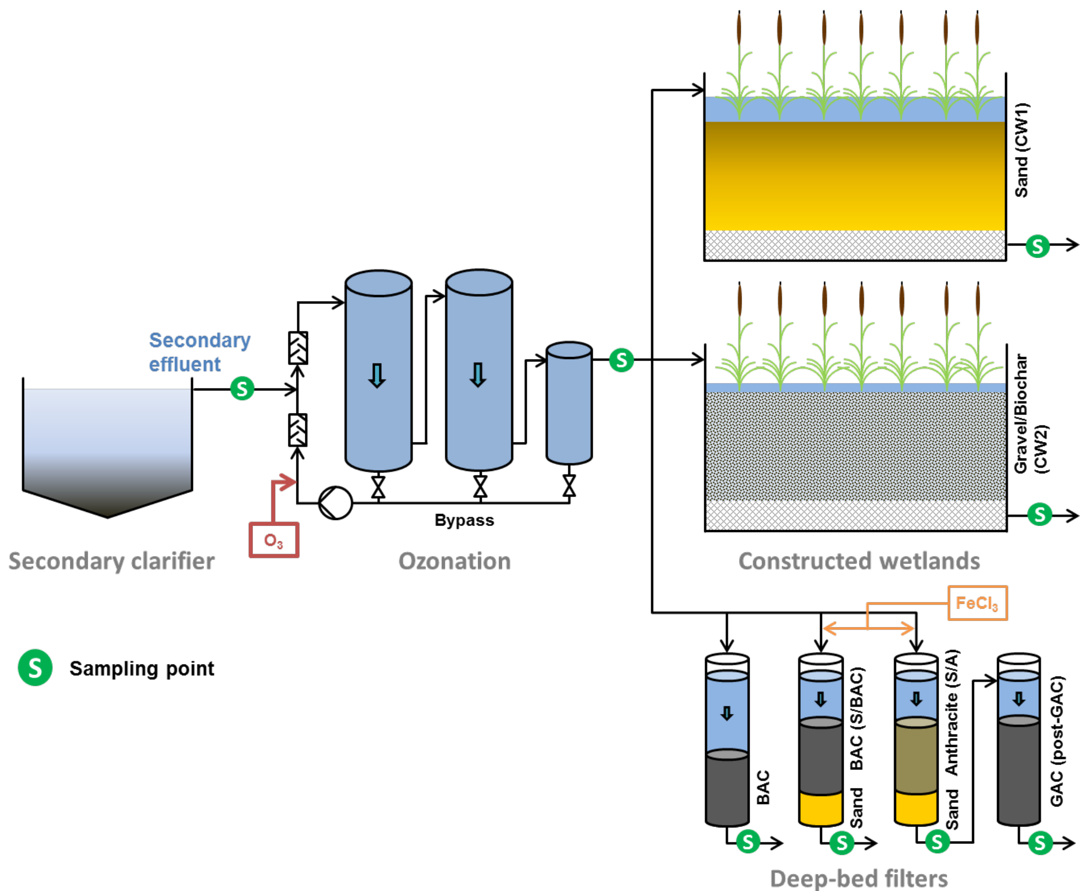

Figure 27 Simplified flow-scheme of pilot-plant at demonstration site 12

5.3.2 Sampling

A total of 10 sampling rounds were carried out between September 2017 and September 2018. In 1 out of 10 sampling rounds constructed wetlands were not included.

All samples were taken as grab samples in sterile 1L-plastic bottles. A time offset corresponding to the hydraulic retention times between the sampling points was considered in order to have corresponding influent and effluent samples. Immediately after sampling the samples were cooled and the cooled samples were shipped to the laboratory via courier and analysed / processed within 24 hours.

5.3.3 Selection of ARG and ARB

Before starting an ARG monitoring program it is essential to make a selection of relevant and representative genes. Otherwise analytical efforts will be high and for many genes results will be poor due to insignificant concentrations. The main aspects that were taken into account for gene selection of the present investigations are listed below:

- Relevant and varying levels in the environment
- Indicator genes for antibiotic group
- Medical relevance
- Data availability

Based on these criteria 2 resistance encoding genes and 2 other relevant genes were selected for the analysis:

– blaTEM: encodes for resistance to β-lactam antibiotics.
– sul1: encodes for resistance to sulphonamide antibiotics.
– class 1 integron (intl1): integrons are mobile gene elements that can capture gene cassettes, e.g. ARG, and spread among bacteria via plasmids or transposons. Gene cassettes integrated by intl1 encode almost exclusively antibiotic resistance determinants (Gillings et al., 2008) for which reason intl1 are associated with multiple antibiotic resistances.
– 16S rDNA: encodes for 16S ribosomal RNA which is present exclusively in prokaryotic cells. Therefore it can be used as an indicator parameter for total bacteria.

A set of different ARB was analysed with conventional culture methods in parallel to the ARG. The following ARB were selected for the analysis:

– Extended spectrum β-lactamase producing (ESBL) *E. coli*
– ESBL KEC (Klebsiella, Enterobacter, Citrobacter)
– Vancomycin-resistant Enterococci (VRE)
– Methicillin-resistant *Staphylococcus aureus* (MRSA)

5.3.4 ARG analysis

The sample volume is filtered through a 0.2 µm Supor®-200 membrane (47 mm diameter, Pall Life Science). Cells (and the contained DNA) are retained while free DNA passes the filter. The membranes are stored at -20 °C until DNA extraction and analysis. Total DNA is extracted directly from the membranes by using the Fast DNA® SPIN Kit for Soil (MP Biomedicals) according to manufacturer's instructions.

Gene copy numbers of the 16S ribosomal DNA and the ARG are measured by quantitative real-time PCR (qPCR). For the investigations primer sets are used as shown in Table 12, allowing the amplification of short amplicons (160-420 base pairs). All qPCRs were performed using a Rotor-Gene 6000 cycler (Corbett) with SensiMix SYBR No-Rox Kit (Bioline). The temperature profile for the SensiMix was as follows: 10 min 95 °C (initial phase), 45 cycles of 15 s at 94 °C (denaturation), 20 s at 55-68 °C (annealing) and 15-25 s at 72 °C (elongation), followed by melting curve analysis.

All samples and standards were analysed in duplicates. The presence of PCR inhibitors was excluded by analysing dilutions of the DNA samples. Calibration was performed with serial dilutions of a known quantity of linearized plasmid containing according to gene fragments. For quality control, R^2 of the standard curve as well as the amplification efficiency were determined and melting curves analysis was performed. Only qPCR experiment with R^2 values >0.980 and efficiencies between 90 and 105% were considered. Amplification products were verified via QIAxcel® Advanced system (Qiagen). Table 12, shows the main parameters applied in the qPCR analysis.

A more detailed description of the methods was published by Stange and Thiem (Stange and Tiehm 2015). The level of quantification (LOQ) in the conducted analyses was 30 copies/mL. When results were below LOQ, the value of the LOQ was used.

Table 12 qPCR parameters for the analysed genes 16S rRNA, blaTEM, sul1 and intl1

Gene	Sequence (5'-3')	Amplicon in bp	Reference	Annealing temp. Elongation time
16S rRNA	f: cctacgggaggcagcag r: attaccgcggctgctggc	160	Smits et al., 2004 (Smits, Devenoges et al. 2004)	68 °C 20 s
blaTEM	F: TTCCTGTTTTTGCTCACCCAG R: CTCAAGGATCTTACCGCTGTTG	112	Bibbal et al., 2007 (Bibbal, Dupouy et al. 2007)	66 °C 20s
sul1	F: CGCACCGGAAACATCGCTGCAC R: TGAAGTTCCGCCGCAAGGCTCG	160	Pei et al., 2006 (Pei, Kim et al. 2006)	68 °C 20 s
intl1	F: GCCTTGATGTTACCCGAGAG R: GATCGGTCGAATGCGTGT	196	Barraud et al., 2010 (Barraud, Baclet et al. 2010)	63°C 20 s

5.3.5 ARB analysis

A sample volume of 1-100 mL was filtered through a 0.2 µm pore size membrane and the membrane was placed on different selective CHROM agar plates (MAST Diagnostica) and incubated at 42°C ± 1°C for 24 h. The grown colonies where classified according the manufacturer´s instructions. Further verification tests (like PCR test and MALDI-TOF) were performed to ensure the classification.

The LOQ for ARB analysis was 1/100 mL. When results were below LOQ, the value of the LOQ was used.

Conventional *E. coli* and Coliform analyses with the Colilert® method were carried out in parallel in order to be able to compare ARB behaviour to the overall community.

5.4 Results and discussion of testing at Belin Schönerlinde

5.4.1 Behaviour of ARG

All analysed genes could be detected and quantified in the effluent of the WWTP (=influent of the pilot-plant), which is a precondition for their suitability as monitoring parameters in municipal wastewater. Figure 29 shows the median and mean concentrations of ARG measured for the different sampling points. As expected total bacteria indicator 16S rDNA was found at highest concentrations (median: $3.6 \cdot 10^6$ copies/mL). Median values for sul1 ($3.9 \cdot 10^4$ copies/mL) and intl1 ($9.6 \cdot 10^4$ copies/mL) were significantly higher than for blaTEM (76 copies/mL).

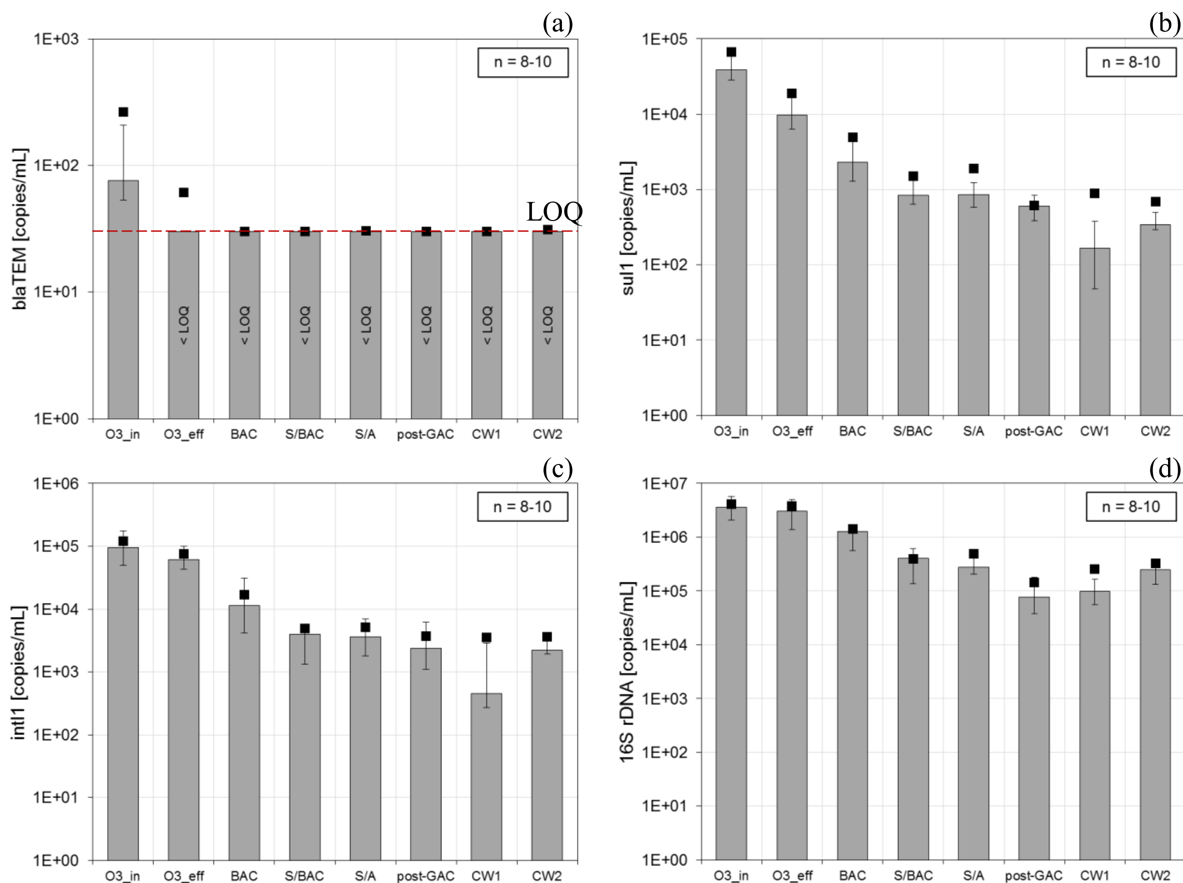

Figure 28 Concentrations for (a) blaTEM, (b) sul1, (c) intl1 and (d) 16S rDNA at different sampling points; columns with error bars = median with 25th and 75th percentile; squares = mean

5.4.1.1 Ozonation

As depicted in Figure 29, (a)/(b) ARG were removed by less than 1 log-unit during ozonation. BlaTEM was usually present at low levels in the range of 10^1-10^2 copies/mL and in 8 out of 10 samplings it was removed below LOQ which didn't allow for determining a reliable reduction. In 2 samplings influent concentrations were higher and log-reductions of 0.6 and 0.7 were calculated. Sul1 could only be removed by 0.4 log-units on average. No relevant removal during ozonation was observed for intl1 and 16S rDNA (Figure 29, (c)/(d)).

Ozonation is known to be an efficient disinfection process that reduces indicator parameters such as *E. coli* or Enterococci by >2 log-units. Gene analyses by qPCR showed much lower reductions which can be explained based on disinfection mechanisms of ozonation. Ozone mainly attacks surface structures of cells and causes damages in the cell membranes (Ho 2017). These damages increase permeability for ozone and intracellular components such as DNA can also be oxidized. However, with commonly applied levels of ozone DNA oxidation only takes place to a limited extent (Cho, Kim et al. 2010). As a consequence many damaged cells that contain intact DNA are present in the effluent of the ozonation. The applied qPCR method considers these damaged bacteria as well as active and VBNC ones.

It has been reported that although absolute numbers of ARG are reduced by ozone treatment of secondary effluent their relative abundance in the surviving population can increase (Alexander, Knopp et al. 2016). These findings could not be confirmed for blaTEM, sul1 or intl1 in the present investiga-

tion. Relative abundances were calculated as the ratio of ARG concentration and total bacteria indicator 16S rDNA. As displayed in Figure 30 ozonation caused a slight decrease in relative abundance for the analysed ARG. An enrichment of antibiotic resistant individuals would be particularly critical assuming that the surviving population develops in the receiving water after discharging the treated wastewater. Further research would be important to get a better understanding of the fate of ARG and ARB after their release into the environment.

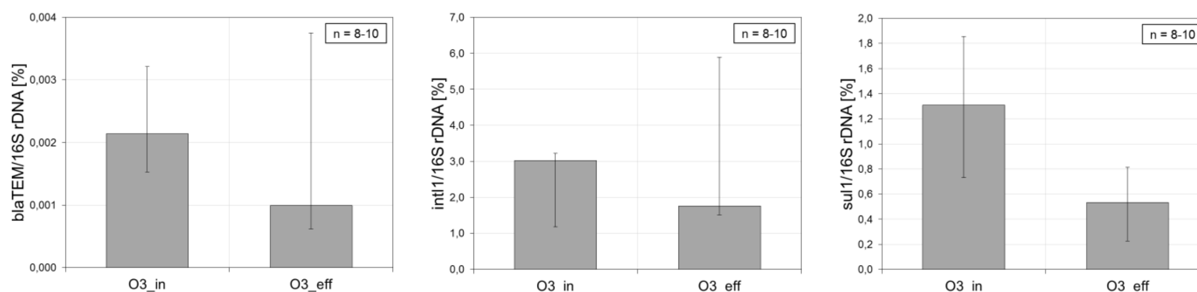

Figure 29 Relative abundance of ARG before and after ozonation expressed as ratio ARG/16S rDNA [%]; columns with error bars = median with 25th and 75th percentile

5.4.1.2 Post-treatment

Since blaTEM was usually removed below LOQ during ozonation reliable assessment of its behaviour in the post-treatment steps was not possible. However, when it appeared in quantifiable concentrations (n = 2) in ozonation effluent it was removed by >0.9 log-units in all post-treatments. The other analysed DNA-fragments were removed by ≥1 log-unit (related to ozonation effluent) in all post-treatments except for BAC filtration. Median concentrations of sul1 and intl1 after CW treatment were lower than after flocculation/filtration steps S/BAC and S/A. The observed difference was more pronounced for CW1 which performed better than CW2 for all analysed genes. The treatment efficiency of the post-GAC filter, which treats the effluent of the S/A only, is difficult to assess since only in two occasions quantifiable results were obtained after ozone treatment, prior to these treatment steps.

All post-treatments were more efficient in ARG removal compared to ozonation. In contrast to the chemical inactivation by ozone the filter systems retain bacteria in the filter bed by physical means. Thus, the complete cells including their DNA are removed from the water which explains the reduced ARG concentrations in the effluents. In case that retained cells are digested by other microorganisms and ARG are set free and dissolved in water they would no longer be detected with the applied method due to membrane filtration in the beginning of the sample treatment. Based on the present results it is not possible to make a statement about the amounts and therefore the relevance of free DNA in the filter effluents.

Figure 30 shows that levels of ARG were lower after dual-media filters that contain a sand layer compared to the single-media BAC and after the sand CW (CW1) compared to CW2 with the coarser filter material. Hence, the results of sul1, intl1 and 16S rDNA clearly demonstrate that the use of fine filter material such as sand is important for efficient ARG removal.

All genes that were not removed below LOQ (sul1, intl1, 16S rDNA) showed similar behaviour in the different treatment steps. If this can be confirmed by further studies, it could be an option to quantify only indicator genes with representative behaviour in order to reduce analytical efforts.

5.4.2 Behaviour of ARB

Since MRSA was not detected in any of the samples at the beginning of the monitoring the parameter was excluded from the analysis. The other 3 analysed antibiotic resistant bacteria (ARB) could be found in the WWTP effluent in the range of 10^1-10^2/100 mL. As shown in Figure 31 median concentrations for ESBL *E. coli*, ESBL KEC and VRE amounted to 430/100 mL, 55/100 mL and 250/100 mL, respectively.

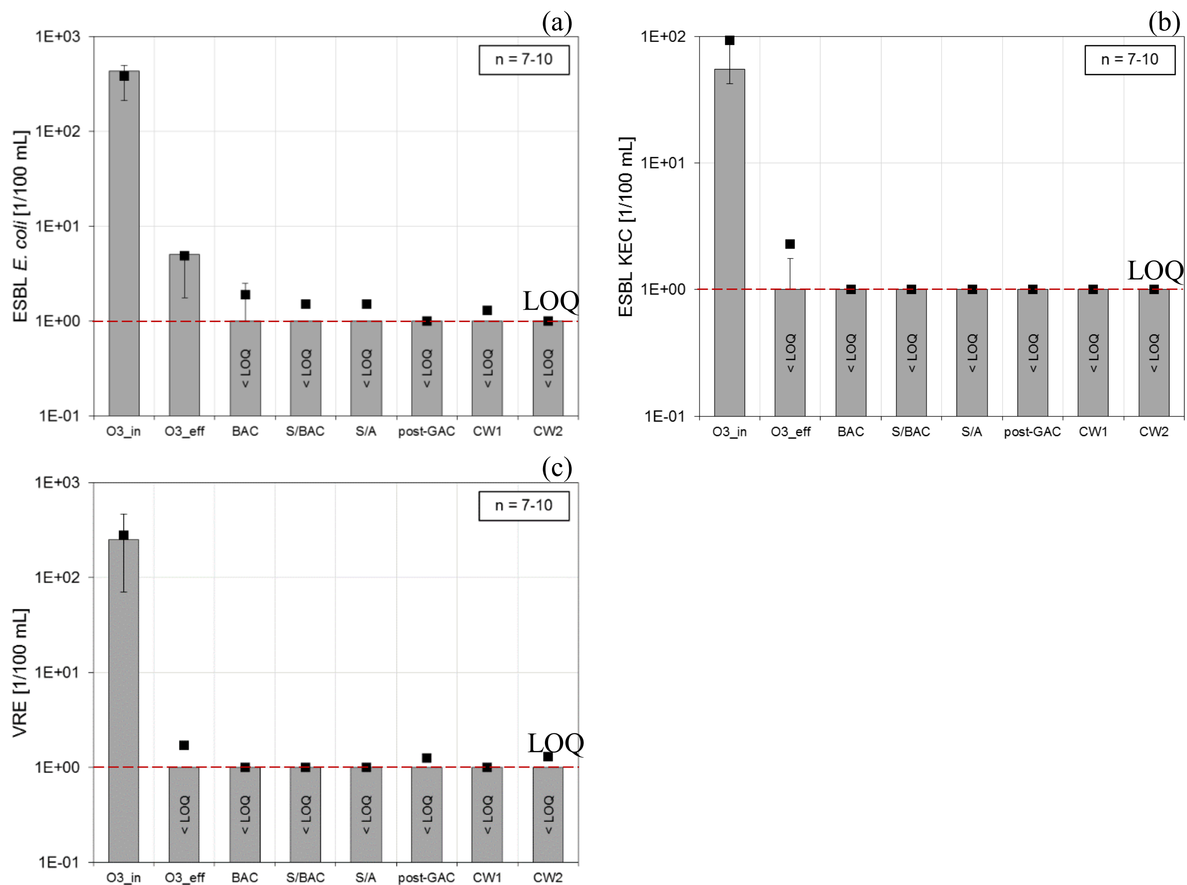

Figure 30 Concentrations for (a) ESBL E. coli, (b) ESBL KEC and (c) VRE at different sampling points; columns with error bars = median with 25th and 75th percentile; squares = mean

5.4.2.1 Ozonation

In contrast to ARG efficient reduction during ozone treatment could be observed for all analysed ARB. Levels after ozonation were usually below 10/100 mL. Calculated logarithmic reductions were slightly higher for ESBL *E. coli* and VRE (~ 2 log-units) than for ESBL KEC (~ 1.7 log-units). It has to be considered though, that influent concentrations of ESBL KEC were lower. Treatment efficiency of ozonation for the 3 ARB is therefore assumed to be comparable.

ARB are quantified with conventional culture methods where only viable cells can be detected. Bacteria with cell membrane damages caused by ozone are not quantified with culture methods which explains the observed strong effect of ozonation on ARB. One approach for achieving qPCR results that are more similar to ARB is a live/dead discrimination using propidium monoazide (PMA). PMA exclusively penetrates dead cells and makes it possible to differentiate them from intact cells in the subsequent qPCR. Jaeger et al. (Jäger, Alexander et al. 2018) studied the removal of different bacteria during ozonation using PMA-qPCR in comparison to culture methods. Observed removals for culture

methods were still higher than for PMA-qPCR which was interpreted as VBNC cells. Especially for environmental samples with a large mix of bacteria PMA staining is not trivial because PMA concentration usually needs to adapted to the analysed species (Ho 2017).

Parallel analysis of *E. coli* and Coliforms with the same water samples demonstrated that behaviour of ARB during ozonation was comparable to the overall community. Median removal for ESBL *E. coli* was 2.0 log-units compared to 2.2 log-units for *E.coli* (Colilert). ESBL KEC and Coliforms (Colilert) were both reduced by 1.7 log-units (median).

5.4.2.2 Post-treatment

Concentrations of ESBL KEC and VRE were close to or below LOQ after ozonation. The effect of the different post-treatment systems could therefore not be studied. It can only be stated that none of the post-treatments caused an increase of the 2 parameters by regrowth.

ESBL E. coli was still present at low levels after ozonation and post-treatments often removed it below LOQ. Comparing the mean concentrations after the different post-treatment steps that reduction in CW was slightly more robust than in the deep-bed filters operated in parallel.

5.5 Conclusions / lessons learnt

5.5.1 Analytical methods

ARG analysis by qPCR is a suitable monitoring tool for wastewater treatment processes that allows for fast quantification of antibiotic resistance determinants. However, for interpretation of results it is essential to consider that they are not directly comparable to ARB results due the different methodologies.

Special attention is required for the interpretation of ARG results in processes that don't retain but damage or inactivate the bacteria (e.g. ozone treatment). Here, the biggest differences to ARB results are expected. For treatment systems that retain the complete cells (e.g. filters) results for removal efficiency by ARG and ARB analysis will be more similar. An approach to exclude dead cells from ARG analysis is the PMA-qPCR. However, in complex environmental samples the use of PMA can pose a problem. Table 13 shows the applicability of the discussed analytical tools with respect to the state of bacteria.

Table 13 Applicability of analytical methods for different states of bacterial cells

State of cells	qPCR	qPCR with PMA	cultivation
Viable and culturable	X	X	X
Viable but not culturable (VBNC)	X	X	
Dead	X		

Since qPCR and cultivation methods deliver different information their relevance also depends on the studied topic. When focus is set on dissemination of antibiotic resistances it makes sense to include dead cells because they are known to contribute to ARG spread via horizontal gene transfer. If pathogenic effects of antibiotic resistant bacteria have priority results from ARB analysis might be more relevant because only viable cells contribute to pathogenicity.

5.5.2 Treatment systems

It could be demonstrated that the process combination of the studied cNES creates synergies for the removal of ARB and ARG. Since ARB were already reduced below or close to LOQ during ozonation performance of post-treatment could not be assessed reliably. However, when quantifiable concentrations of ARB were present after ozonation (e.g. ESBL *E. coli*) additional removal in post-treatments was observed. Results of ARG showed that ozone treatment without post-treatment is not effective (removal of less than 1 log-unit). Filtration steps with suitable filter media achieved additional reduction of ARG by ≥1 log-unit.

Grain size of the filter media was shown to be an important factor for efficient ARG retention. Filter media for CW or deep-bed filters should therefore not be too coarse in order to enable sufficient removal of ARG.

6 Benefits of innovative water quality assessment tools

Water quality is determined by numerous parameters. Within AquaNES, various combined natural and engineered water treatment technologies are combined with the purpose of producing water that is of good quality and safe for its intended use at all times. Within this study three fast and/or innovative tools have been applied to a selection of the AquaNES demonstration sites.

6.1 Selected Innovative methods to determine water quality within AquaNES

Within the AquaNES project integrated approaches such as effect based monitoring combined with non-target chemical screening, microbial sensors, to detect fecal contamination sensors and qPCR techniques to detect antimicrobial resistance are demonstrated.

CALUX bioassays have been applied to provide an integrated and effect based approach for monitoring water quality and assess treatment efficiency. The main advantage of these effect-based analytical tools is that the output provides specific biological/toxic effect of the total complex mixture of chemicals present in the sample. These bioassays do not enable the determination of individual chemicals present and responsible for the output of the assay as multiple chemicals can either induce or reduce responses of these assays. Potential culprit chemicals can be suggested based on known mechanisms of action (for example from literature or in vitro toxicity databases such as EPA's ToxCast database) or identified based on effect-directed analysis approaches (Zwart, Nio et al. 2018). By using both bioassays and non-target screening (chemical analyses) in parallel the outcomes are correlated to reveal potential culprit chemicals.

The experiments performed within AquaNES clearly demonstrated that CALUX bioassays are sensitive and robust bioanalytical tools that can be used to evaluate the efficiency of innovative natural and engineered treatment technologies to improve water quality. The selected suite of toxicological endpoints covered various relevant toxicological mechanisms. The combination of these assays thereby provide a rather integrated assessment of the water quality. The Achilles heel for wide-spread application of these bioassays in water quality assessment and treatment efficiency studies, is the lack of regulatory acceptable limit values or trigger values for each of the individual bioassays which enables quantified monitoring of water quality possible. In addition to such effect-based trigger values (EBTs), an action plan is lacking in case a bioanalysis result exceeds the proposed EBT. Within this study, trigger values were collected from literature or derived from available reference projects of BDS. Further research is necessary to obtain and evaluate these trigger values and to formulate clear criteria such as an action plan that can be adopted by regulatory frameworks. Within this study a framework for such an action plan is proposed to guide monitoring and further actions when trigger values are exceeded. Putting this in perspective of technology readiness levels (TRL), one can argue that the tool itself is robust and sensitive TRL 9. But that without proper trigger values and regulatory acceptance, the highest technology readiness level is not reached since there are no regulatory frameworks that opens the market for such tools. A TRL 8 therefore is more appropriate.

Microbial water quality assessment using an at-line sensor enables to assess the microbial water quality. The main advantage of the tested sensor is it fast response time (2-4 hours) compared to classical plating techniques (several days) which enable both source and product control. Fast assessment of water quality is especially of relevance for microorganisms as these microorganisms have dynamic concentrations (in sources), variable removal rates during treatment (Smeets and Medema 2006, Smeets, Rietveld et al. 2010) and can have very acute effects on people exposed to the water. Examples of such effects are people getting ill due to contaminated drinking water or surface water that is used

for recreation or irrigation, but also organisms in receiving ecosystems can be affected. The innovative microbial sensor BACTcontrol fills this gap by providing the required speed and efficiency and can be used as fast indicator microbial water quality assessment tool. Thereby this tool is of high value for water quality assessment and control. The tool was applied to study generic microbial activity and the presence of *E. coli* bacteria. Thereby it fits in current legislation and regulation frameworks that use *E. coli* as an indicator organism. Initial experiments revealed that, within a water treatment setting, controlling water quality, the system is sensitive for clogging. This is something that needs to be prevented in order to provide smooth operation of the system. This illustrates that the tool might not be suitable for waste streams with a high particle load without proper pre-filtration steps, since the presence of particles compromise continuous operation. The analytical sensitivity is insufficient to meet drinking water quality standards (European Commission 1998, WHO 2011), but one has to realize that classic plating techniques hold the same disadvantage, as their sensitivity is also insufficient to reach these levels. When clogging is prevented, the assessment of *E.coli* and microbial activity show good correlation with plating and counting techniques, however is specific cases strong temporary variation was observed and could not be validated with other measuring techniques. Therefore, the tool, presuming smooth operation, seems suitable to monitor fecal contamination and can be applied as a first tier assessment that triggers temporal measures or further research. Putting this in perspective of technology readiness levels (TRL) for the specific application in water treatment trains, one can argue that technology for *E.coli* measurements is far developed, but it requires site specific validation to ensure robust and valid results within specific treatment systems. All in all this this corresponds to TRL 8. However, for the detection of microbial activity, in some cases unexplained spikes are observed, additional studies are necessary to explain these patterns at demonstration site 6 before it can be implemented on a larger scale.

The presence of microbial resistant bacteria and genes can be classified as a microbial response to chemical contamination with a specific indirect risk. Antimicrobial resistance is a human health threat, and risks are clear in medical and veterinary settings (WHO 2014). The presence of antimicrobial agents within the water system or its use by humans and livestock can result in the development (selection) of resistant microbes in aqueous waste materials of these users. Both the presence of the antimicrobial agents in the users themselves as well as the emissions of these antimicrobial agents through human and veterinary consumption can lead to the emission of antimicrobial resistance in the water cycle, respectively. However, the health risk of anti-microbial resistance in the water cycle is still unclear, as transfer of these genes from environmental micro-organisms to pathogens and the exposure of humans via this route is largely unknown. Therefore, the WHO advices to keep the number of ARGs in the environment as low as reasonably achievable from a precautionary perspective. A further increase of resistance genes in the urban water cycle is therefore unwanted (Berendonk 2015) (Larsson 2014, Huijbers, Blaak et al. 2015) (WHO 2014). So techniques are required to be able to monitor ARGs in water treatment systems to enable the analysis of the fate of resistant bacteria and their genes in the urban water cycle. The analysis of antibiotic resistant bacteria and antimicrobial resistant genes was tested at demonstration site 12 (Berlin). It was observed that the antibiotic resistant bacteria showed a steeper removal with ozonation than the removal of resistance genes, and that further removal of resistant bacteria was not detectable, as levels dropped below the limit of quantification, while the detection of antimicrobial resistance genes provided sufficient resolution to study their behavior further down the treatment line. This illustrates that the antibiotic resistant bacteria have a different fate in the treatment from their genes, that appear to be more persistent, and that the qPCR technique has a higher resolution in the chosen experimental set up. Considering the uncertainties on the risk of the antimicrobial resistance in water treatment and the aqueous environment. The risk of the observed levels of both bacteria and their genes cannot be determined. Nevertheless, the qPCR tool can compare

treatment steps and efficiency of treatment trains, while the detection of the resistant bacteria requires higher sensitivity to provide sufficient resolution. Therefore, the technology readiness level is still in a validation stage (TRL 5-6). It requires risk based thresholds or guidance values of both the presence of antibiotic resistant bacteria and their genes, as well as further application in treatment systems to build a reference database and correlate the outcome to other indicators of antimicrobial resistance, such as the presence or use of antimicrobial agents and epidemiological evidence on resistance development in communities. So all in all, risk evaluation of the results is required before wide spread application in water treatment and the aqueous environment.

6.2 Recomendations for future application and implementation

AquaNES demonstrates the combination of natural and engineered systems. As these combinations, and especially the natural components are less controllable, water quality monitoring becomes more important. The presented innovative and fast techniques provide additional benefits to classic monitoring tools and regulations. They provide an effect based or more generic (integrated) measure of chemical water quality (CALUX bioassays, non-target screening, respectively), enable faster detection of microbial contamination (BACTcontrol), or measure water quality parameters that have not been regularly monitored before.

The presented tools each have their own benefits and challenges. For CALUX bioassays provided robust results and a framework to interpret the results. In addition, it was illustrated that the combination with non-target chemical screening techniques have great potential by combining a measurement of effects (toxicity) and contamination. However, this technique currently lacks water quality standards in regulatory frameworks. Regulatory adoption is key for wide spread implementation.

The BACTcontrol provides a fast detection of fecal contamination. Its application requires translation from the regulatory accepted indicator organism *E. coli* to its enzymatic activity. This seems feasible as presence of *E. coli* and enzymatic activity correlate. The major challenge lies in the application and smooth operation under different conditions and with different types of water. It is vulnerable for the local conditions and characteristics of the water it encounters, like many other *in situ* applied tools. These issues can be solved with for example filtration or clean-up steps, but require location specific measures and control of operation.

The results of the microbial resistance measurements provide robust data on presence of antimicrobial resistance genes, but defining thresholds of these measurements and relating them to risks requires more research on the covariance with epidemiological data on of antibiotic resistance in human populations. It needs additional research on multiple aspects of antimicrobial resistance within and beyond the urban water cycle. Correlating multiple tools that study various aspects of antimicrobial resistance such as chemical analysis, microbial analysis, effect assays on antimicrobial effects and epidemiological data on presence of resistant bacteria in (human) populations is required to enable future application and implementation in water quality monitoring and risk assessment.

7 Literature

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Annex 1 Sampling, storage and shipment of water samples for CALUX bioanalysis

p-aquanes-001 Number of Pages: 104

Sampling, storage and shipment of water samples for CALUX bioanalysis

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1 General

This part describes specific requirements for the sampling with respect to the determination of CALUX activity in water samples.

The amount of water sample to be collected, is at least 1000 ml. Five hundred ml will be used for extraction, the remaining 500 ml of water will be stored and used in case of re-analysis.

2 Bottles and material for sampling

2.1 Use clean glass bottles (borosilicate glass) with polypropylene caps with polytetrafluoroethylene (PTFE) inlays.

2.2 To avoid (photo) degradation of compounds of interest, use amber/green glass bottles.

2.3 If transparent, non-coloured glass bottles are used, wrap the bottles in aluminium foil or store them in a dark container following collection of water.

3 Cleaning of materials

3.1 Rinse brand new glass bottles three times with water before cleaning.

3.2 After rinsing with water, clean the bottles and caps at least one day before sampling by rinsing them three times with acetone or ethyl acetate (3-times 15-25 ml).

3.3 Let the residual acetone/ethyl acetate evaporate overnight at room temperature (e.g. drying oven, fume hood).

3.4 Close the bottles immediately after drying and evaporation.

3.5 Rinse all other glassware, spatulas etc. getting in contact with the sample three times with acetone or ethyl acetate (3-times 5-15 ml). Let the residual acetone/ethyl acetate evaporate.

4 Sampling procedure

4.1 Use Nitrile-gloves during sampling. Do not use any hand cream prior to sampling and avoid skin contact with the sample.

4.2 Avoid using any plastic/rubber material for sampling: tubing, funnels etc. Use material from glass, polytetrafluoroethylen (PTFE), or stainless steel only. In case the use of plastics/rubber cannot be avoided during sampling (e.g. use of pumps with plastic tubing inside), make sure the used equipment is rinsed with water thoroughly, e.g. by running the pump for 10 minutes). Use sampling form (frm-aquanes-001.docx) for indicating that the sample has been in contact with plastic/rubber during sampling.

4.3 Fill a 1-liter bottle with 1000 ml of water sample. Do not stabilize the samples with chemicals.

4.4 To avoid oxidative processes, close bottles immediately after filling.

4.5 Label each bottle of water. See §5.1 for the information required on each bottle.

4.6 If transparent glass bottles are used, wrap the bottles in aluminium foil or store them in a dark container (see §2).

4.7 Store samples until shipment at 4 °C. Do not store samples for more than 2 days before shipment of the samples!

5 Coding of samples

5.1 Following sampling, immediately label each individual sample bottle. Indicate:

- Site and location of sampling
- Type of sample (e.g. influent; effluent)
- Unique sample code
- Volume of sample
- Date of sampling
- Person responsible for sampling
- Storage temperature

5.2 In case a single sample has to be divided over several sampling bottles, also indicate this. e.g.: bottle 1 of 2, bottle 2 of 2.

5.3 Fill out the sampling form (frm-aquanes-001.docx)

6 Transport of samples

6.1 Samples should be shipped by courier. Notify the recipient prior to shipment of the samples and send the completed sample form (frm-aquanes-001.docx) by e-mail to the recipient prior to shipment.

6.2 To assure a quick delivery of the samples, please contact the courier and inquire about delivery times. Make sure the delivery is scheduled during the workweek to avoid the shipment is put on hold for the weekend. (BDS is open for deliveries on Monday - Friday, 8:00 to 17:00 pm).

6.3 Transport samples cooled as soon as possible.

6.4 Make sure that bottles are wrapped in paper or in air bubble film to avoid breakage during transport.

6.5 Place bottles in a styrofoam box (or any other box designed for cooled shipment).

6.6 Add pre-cooled cooling element to keep samples as cool as possible during shipment.

6.7 Place a copy of the sampling form (from-aquanes-001.docx) containing the information for the samples to be shipped, inside the styrofoam box.

6.8 Close to styrofoam box

6.9 Samples should be shipped to:

Annex 2 Round 1 sample information

Table 2-1 Round 1 sample information

Scheme	Site	BDS project no.	BDS sample code	Client sample code	Location	date of sampling	sampling method	volume (ml)	date of arrival
River Bank Filtration schemes	1	12820	---	2017_11_27_ site 1_feed NF	Site 1, Berlin, water works Tiefwerder	27/11/2017	Grab sampling	1000	28/11/2017
	1	12820	---	2017_11_27_ site 1_permeate NF	Site 1, Berlin, water works Tiefwerder	43066	Grab sampling	1000	28/11/2017
	2	11625	25825	A	WW Hosterwitz - Elbe water	06/03/2017	Grab sampling	1000	07/03/2017
	2	11625	25827	B	WW Hosterwitz - drink water	06/03/2017	Grab sampling	1000	07/03/2017
	3	11630	25835	RW_Q1_20170307	Baja, raw water above slow filter	07/03/2017	Grab sampling	1000	09/03/2017
	3	11630	25836	RO_Q2_20170307	Baja, RO permeate	07/03/2017	Grab sampling	1000	09/03/2017
	4	11246	25277	Mo 1	Mosina treatment station	19/12/2016	taken from tap	1000	20/12/2016
	4	11246	25278	Mo 2	Mosina treatment station	19/12/2016	taken from tap	1000	20/12/2016
	5	13309	29312	Ganga	---	---	Grab sampling	500	13/03/2018
	5	13309	29313	SP1 RBF	---	---	Grab sampling	500	13/03/2018
	5	13309	29314	SP4 A0	---	---	Grab sampling	500	13/03/2018
Managed Aquifer Recharge & Soil Aquifer Treatment	6	12263	27118	W-roh	1 - raw river Wiese water	11/07/2017	Grab sampling	586	18/07/2017
	6	12263	27119	WF-v	2 - pre-treated river Wiese water (rapid sand filter)	11/07/2017	Grab sampling	792	18/07/2017
	6	12263	27120	WF-n	3 - after AOP treatment	11/07/2017	Grab sampling	794	18/07/2017
	7	12562	28032	SHAF_R1	Site 7 - Shafdan	10/02/2017	Grab sampling	1000	06/10/2017
	7	12562	28033	SHAF_OZA500	Site 7 - Shafdan	10/02/2017	Grab sampling	1000	06/10/2017
	7	12562	28034	SHAF_OZOAOZ	Site 7 - Shafdan	10/02/2017	Grab sampling	1000	06/10/2017
	8	12472	28035	raw water WWTP inlet	Agon-Coutainville (9.00 hrs)	09/07/2017	Grab sampling	1000	12/09/2017
	8	12472	28036	raw water WWTP outlet (before Mare a Sorre)	Agon-Coutainville (11.16 hrs)	09/07/2017	Grab sampling	1000	12/09/2017
	8	12472	28037	raw water WWTP outlet (Mare a Sorre)	Agon-Coutainville (10.40 hrs)	09/07/2017	Grab sampling	1000	12/09/2017
	9	12541	28038	SRKWR1	Nootdorp BASSIN	25/09/2017	Grab sampling	2000	27/09/2017
	9	12541	28039	SRKWR2	Nootdorp ASR	25/09/2017	Grab sampling	2000	27/09/2017
	9	12541	28040	SRKWR3	Nootdorp OPPW	25/09/2017	Grab sampling	2000	27/09/2017
	9	12541	28041	SRKWR4	Nootdorp VLOTTER(KIST)	25/09/2017	Grab sampling	2000	27/09/2017
Constructed wetlands and other natural systems	10	12517	27671	No 1	10a Thirasia - wastewater influent of WWTP	19/09/2017	Grab sampling	1000	20/09/2017
	10	12517	27672	No 2	10a Thirasia - influent photocatalystic treatment	19/09/2017	Grab sampling	1000	20/09/2017
	10	12517	27673	No 3	10a Thirasia - effluent photocatalystic treatment	19/09/2017	Grab sampling	1000	20/09/2017
	11	11712	25943	1724	Pilot RSF Rheinbed Inflow	27/03/2017	Grab sampling	1000	28/03/2017
	11	11712	25944	1732	Pilot RSF Rheinbed Outflow	27/03/2017	Grab sampling	1000	28/03/2017
	11	12091	26780	Inflow WWTP	Inflow WWTP	19/06/2017	Grab sampling	1000	20/06/2017
	12	12468	28042	O1	WWTP Schonerlinde. Primary sedimentation	09/06/2017	Grab sampling	1000	11/09/2017
	12	12468	28043	O2	WWTP Schonerlinde. Secondary sedimentation	09/06/2017	Grab sampling	1000	11/09/2017
	12	12468	28044	O3	WWTP Schonerlinde. Ozonation	09/06/2017	Grab sampling	1000	11/09/2017
	12	12468	28045	O4	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	09/06/2017	Grab sampling	1000	11/09/2017
	13	12694	28141	S13-1	Packington, UK	23/10/2017	Grab sampling	1000	26/10/2017
	13	12694	28142	S13-2	Packington, UK	23/10/2017	Grab sampling	1000	26/10/2017
	13	12694	28143	S13-3	Packington, UK	23/10/2017	Grab sampling	1000	26/10/2017

Annex 3 Round 2 sample information

Table 3-1 Round 2 sample information site 4

Site	BDS project no.	BDS sample code	Sampling point	Location	date of sampling	sampling method	volume (ml)	date of arrival
4	13320	29329	S1	untreated water	12/03/2018	Grab	1000	14/03/2018
4	13320	29330	S2	before high-rate filters	12/03/2018	Grab	1000	14/03/2018
4	13320	29331	S3	after high-rate filters	12/03/2018	Grab	1000	14/03/2018
4	13320	29332	S4	after ozonation	12/03/2018	Grab	1000	14/03/2018
4	13320	29333	S5	after carbon filters	12/03/2018	Grab	1000	14/03/2018
4	13320	29334	S6	after disinfection	12/03/2018	Grab	1000	14/03/2018
4	13958	30703	S1	untreated water	21/08/2018	Grab	1000	24/08/2018
4	13958	30704	S2	before high-rate filters	21/08/2018	Grab	1000	24/08/2018
4	13958	30705	S3	after high-rate filters	21/08/2018	Grab	1000	24/08/2018
4	13958	30706	S4	after ozonation	21/08/2018	Grab	1000	24/08/2018
4	13958	30707	S5	after carbon filters	21/08/2018	Grab	1000	24/08/2018
4	13958	30708	S6	after disinfection	21/08/2018	Grab	1000	24/08/2018
4	14137	30703	S1	Untreated water	08/10/2018	Grab	1000	11/10/2018
4	14137	30704	S2	Before high rate filters	08/10/2018	Grab	1000	11/10/2018
4	14137	30705	S3	after high rate filters, before ozometion	08/10/2018	Grab	1000	11/10/2018
4	14137	30706	S4	after ozometion	08/10/2018	Grab	1000	11/10/2018
4	14137	30707	S5	after carbon filters	08/10/2018	Grab	1000	11/10/2018
4	14137	30708	S6	after disinfection	08/10/2018	Grab	1000	11/10/2018

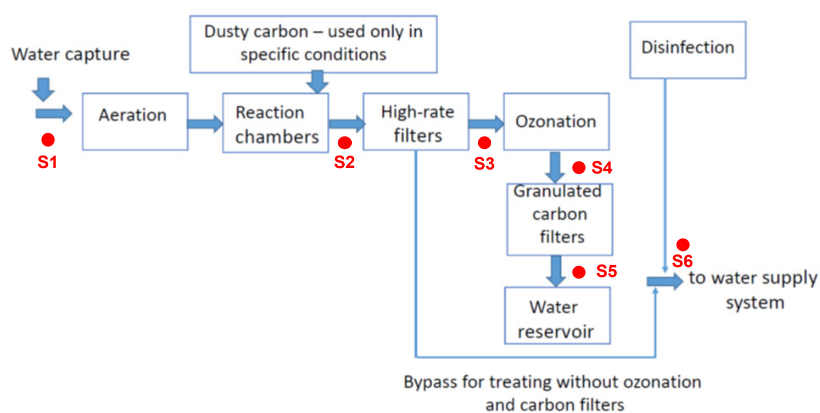


Figure 3-1 Schematic representation of water treatment site 4, indicating sampling points for CALUX bio-analyses

Table 3-2 Round 2 sample information site 6

Site	BDS project no.	BDS sample code	Sampling point	Location	date of sampling	sampling method	volume (ml)	date of arrival
6	12368	27386	S1	RF-v (before columns, no AOP)	15/08/2017	Grab	991	22/08/2017
6	12368	27387	S2	RF-n (before columns, with AOP)	15/08/2017	Grab	975	22/08/2017
6	12368	27388	S3	RF-v-AK3 (after active carbon, no AOP)	15/08/2017	Grab	912	22/08/2017
6	12368	27389	S4	RF-n-AK3 (after active carbon, with AOP)	15/08/2017	Grab	935	22/08/2017
6	12368	27390	S5	RF-v-B4 (after soil column, no AOP)	15/08/2017	Grab	980	22/08/2017
6	12368	27391	S6	RF-n-B4 (after soil column, with AOP)	15/08/2017	Grab	1017	22/08/2017
6	12368	27392	S7	WF-v (before columns, no AOP)	15/08/2017	Grab	963	22/08/2017
6	12368	27393	S8	WF-n (before columns, with AOP)	15/08/2017	Grab	968	22/08/2017
6	12368	27394	S9	WF-v-AK3 (after active carbon, no AOP)	15/08/2017	Grab	972	22/08/2017
6	12368	27395	S10	WF-n-AK3 (after active carbon, with AOP)	15/08/2017	Grab	945	22/08/2017
6	12828	28526	S1	RF-v (before columns, no AOP)	21/11/2017	Grab	999	28/11/2017
6	12828	28527	S2	RF-n (before columns, with AOP)	21/11/2017	Grab	986	28/11/2017
6	12828	28528	S3	RF-v-AK3 (after active carbon, no AOP)	21/11/2017	Grab	998	28/11/2017
6	12828	28529	S4	RF-n-AK3 (after active carbon, with AOP)	21/11/2017	Grab	975	28/11/2017
6	12828	28530	S5	RF-v-B4 (after soil column, no AOP)	21/11/2017	Grab	973	28/11/2017
6	12828	28531	S6	RF-n-B4 (after soil column, with AOP)	21/11/2017	Grab	986	28/11/2017
6	12828	28532	S7	WF-v (before columns, no AOP)	21/11/2017	Grab	1002	28/11/2017
6	12828	28533	S8	WF-n (before columns, with AOP)	21/11/2017	Grab	994	28/11/2017
6	12828	28534	S9	WF-v-AK3 (after active carbon, no AOP)	21/11/2017	Grab	976	28/11/2017
6	12828	28535	S10	WF-n-AK3 (after active carbon, with AOP)	21/11/2017	Grab	930	28/11/2017
6	13391	29530	S1	RF-v (before columns, no AOP)	19/03/2018	Grab	1026	28/03/2018
6	13391	29531	S2	RF-n (before columns, with AOP)	19/03/2018	Grab	1031	28/03/2018
6	13391	29532	S3	RF-v-AK3 (after active carbon, no AOP)	19/03/2018	Grab	1007	28/03/2018
6	13391	29533	S4	RF-n-AK3 (after active carbon, with AOP)	19/03/2018	Grab	988	28/03/2018
6	13391	29534	S5	RF-v-B4 (after soil column, no AOP)	19/03/2018	Grab	1022	28/03/2018
6	13391	29535	S6	RF-n-B4 (after soil column, with AOP)	19/03/2018	Grab	993	28/03/2018
6	13391	29536	S7	WF-v (before columns, no AOP)	19/03/2018	Grab	1082	28/03/2018
6	13391	29537	S8	WF-n (before columns, with AOP)	19/03/2018	Grab	1038	28/03/2018
6	13391	29538	S9	WF-v-AK3 (after active carbon, no AOP)	19/03/2018	Grab	1038	28/03/2018
6	13391	29539	S10	WF-n-AK3 (after active carbon, with AOP)	19/03/2018	Grab	1011	28/03/2018
6	13391	29540	11 - Blank	FHNW (Lab 5.24)	19/03/2018	Grab	988	28/03/2018

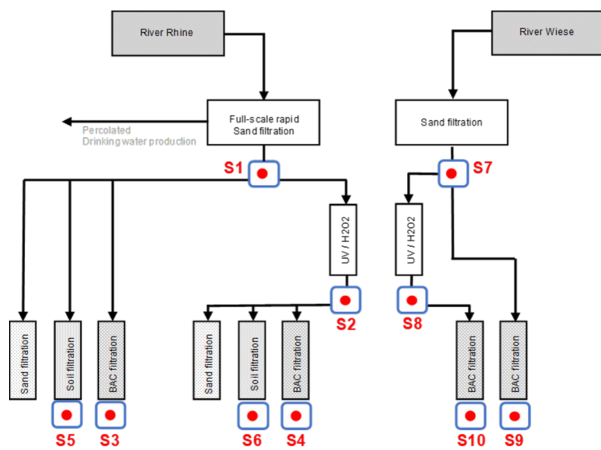


Figure 3-2 Schematic representation of water treatment site 6, indicating sampling points for CALUX bio-analyses

Table 3-3 Round 2 sample information site 7

Site	BDS project no.	BDS sample code	Sampling point	Location	date of sampling	sampling method	volume (ml)	date of arrival
7	13322	29335	S1	OZA500 (2nd effluent)	13/03/2018	Grab	1000	15/03/2018
7	13322	29336	S6	OZAITA (after ozonation)	13/03/2018	Grab	1000	15/03/2018
7	13322	29337	S7	OZOOB1 (after ozonation/SAT - observation well R1)	13/03/2018	Grab	1000	15/03/2018
7	13322	29338	S8	OZOOB3 (after ozonation/SAT - observation well R3)	13/03/2018	Grab	1000	15/03/2018
7	14497	32483	S1	OZA500 (2nd effluents)	17/12/2018	Grab	1000	19/12/2018
7	14497	32484	S4	OZAFTA (after filtration tank – ozonation)	17/12/2018	Grab	1000	19/12/2018
7	14497	32485	S6	OZAITA (ozonation product tank)	17/12/2018	Grab	1000	19/12/2018
7	14497	32486	S5	OZBACT (ozonation after BAC)	17/12/2018	Grab	1000	19/12/2018
7	14497	32487	S7	OZOOB1 (after ozonation/SAT - observation well R1)	17/12/2018	Grab	1000	19/12/2018
7	14497	32488	S2	OZFTEP (EP filtration product tank)	17/12/2018	Grab	1000	19/12/2018
7	14497	32489	S3	OZPTEP (WADIS EP product tank)	17/12/2018	Grab	1000	19/12/2018
7	14911	33948	S1	OZA500 (2nd effluent)	19/03/2019	Grab	1000	21/03/2019
7	14911	33949	S4	OZAFTA (after filtration tank – ozonation)	19/03/2019	Grab	1000	21/03/2019
7	14911	33950	S6	OZAITA (ozonation product tank)	19/03/2019	Grab	1000	21/03/2019
7	14911	33951	S5	OZBACT (ozonation after BAC)	19/03/2019	Grab	1000	21/03/2019
7	14911	33952	S9	OZOAUF (after cUF)	19/03/2019	Grab	1000	21/03/2019
7	14911	33953	S7	OZOOB1 (after ozonation/SAT - observation well R1)	19/03/2019	Grab	1000	21/03/2019
7	14911	33954	S8	OZOOB3 (after ozonation/SAT - observation well R3)	19/03/2019	Grab	1000	21/03/2019
7	14911	33955	S3	OZPTEP (WADIS EP product tank)	19/03/2019	Grab	1000	21/03/2019

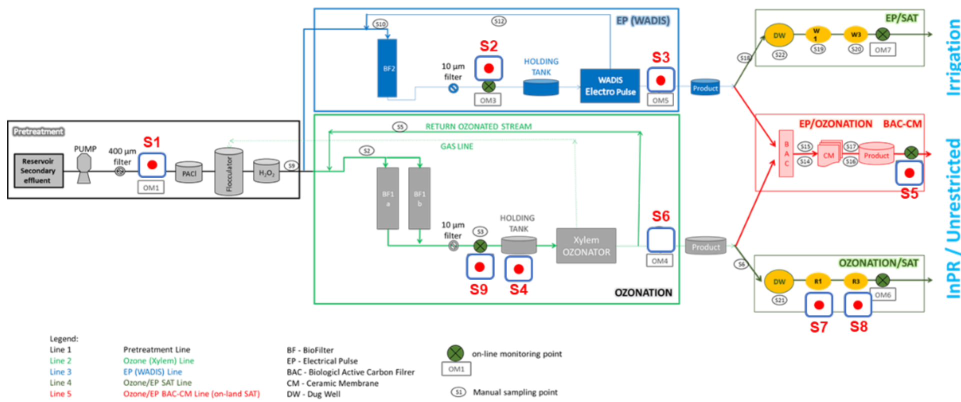


Figure 3-3 Schematic representation of water treatment site 7, indicating sampling points for CALUX bio-analyses

Table 3-4 Round 2 sample information site 8

Site	BDS project no.	BDS sample code	Sampling point	Location	date of sampling	sampling method	volume (ml)	date of arrival
8	13435	29645	S1	Inlet WWTP	11/04/2018	Grab	1000	13/04/2018
8	13435	29646	S2	Outlet WWTP	11/04/2018	Grab	1000	13/04/2018
8	13435	29643	S5-1	NP1 Agon Aquanes	10/04/2018	Grab	1000	13/04/2018
8	13435	29644	S5-2	NP2 Agon Aquanes	10/04/2018	Grab	1000	13/04/2018
8	13505	29743	S4	Golf pond	30/04/2018	Grab	1000	03/05/2018
8	13505	29745	S5-3	NP3 Agon Aquanes	01/05/2018	Grab	1000	03/05/2018
8	13505	29744	S5-4	FRE 4	30/04/2018	Grab	1000	03/05/2018
8	14195	31293	S2	Outlet WWTP	22/10/2018	Grab	1000	26/10/2018
8	14195	31289	S4	Golf pond	22/10/2018	Grab	1000	26/10/2018
8	14195	31294	S5-1	NP1 Sand Dune Aquifer	22/10/2018	Grab	1000	26/10/2018
8	14195	31290	S5-2	NP2 Sand Dune Aquifer	22/10/2018	Grab	1000	26/10/2018
8	14195	31292	S5-3	NP3 Sand Dune Aquifer	22/10/2018	Grab	1000	26/10/2018
8	14195	31291	S5-4	FRE4 Sand Dune Aquifer	22/10/2018	Grab	1000	26/10/2018
8	14471	32371	S5-1	NP1-T6 (Sand Dune Aquifer)	29/10-26/1/2018	Grab	500	12/12/2018
8	14471	32372	S5-1	NP1-T13 (Sand Dune Aquifer)	29/10-26/1/2018	Grab	500	12/12/2018
8	14471	32373	S5-1	NP1-T20 (Sand Dune Aquifer)	29/10-26/1/2018	Grab	500	12/12/2018
8	14471	32374	S5-1	NP1-T27 (Sand Dune Aquifer)	29/10-26/1/2018	Grab	500	12/12/2018
8	14471	32375	S5-1	NP1-T34 (Sand Dune Aquifer)	29/10-26/1/2018	Grab	500	12/12/2018

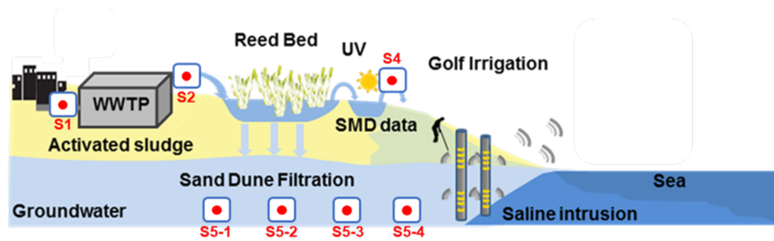


Figure 3-4 Schematic representation of water treatment site 8, indicating sampling points for CALUX bio-analyses

Table 3-5 Round 2 sample information site 11

Site	BDS project no.	BDS sample code	Sampling point	Location	date of sampling	sampling method	volume (ml)	date of arrival
11	13390	29527	S1	Inflow pilot plant	26/03/2018	grab	1000	28/03/2018
11	13390	29528	S2	Outflow filter 1	26/03/2018	grab	1000	28/03/2018
11	13390	29529	S3	Outflow filter 3	26/03/2018	grab	1000	28/03/2018
11	13523	29767	S0	WWTP Inflow	09/05/2018	grab	1000	11/05/2018
11	13523	29768	S1	Inflow pilot plant	09/05/2018	grab	1000	11/05/2018
11	13523	29769	S2	Outflow filter 1	09/05/2018	grab	1000	11/05/2018
11	13523	29770	S3	Outflow filter 3	09/05/2018	grab	1000	11/05/2018
11	13750	30156	S1	Inflow pilot plant	20/06/2018	grab	1000	21/06/2018
11	13750	30157	S2	Outflow filter 1	20/06/2018	grab	1000	21/06/2018
11	13750	30158	S3	Outflow filter 3	20/06/2018	grab	1000	21/06/2018
11	13968	30734	S1	Inflow pilot plant	28/08/2018	grab	1000	30/08/2018
11	13968	30735	S2	Outflow filter 1	28/08/2018	grab	1000	30/08/2018
11	13968	30736	S3	Outflow filter 3	28/08/2018	grab	1000	30/08/2018
11	14185	31256	S1	Inflow pilot plant	17/10/2018	grab	1000	23/10/2018
11	14185	31257	S2	Outflow filter 1	17/10/2018	grab	1000	23/10/2018
11	14185	31258	S3	Outflow filter 3	17/10/2018	grab	1000	23/10/2018
11	14296	32024	S1	Inflow pilot plant	08/11/2018	grab	1000	13/11/2018
11	14296	32025	S2	Outflow filter 1	08/11/2018	grab	1000	13/11/2018
11	14296	32026	S3	Outflow filter 3	08/11/2018	grab	1000	13/11/2018

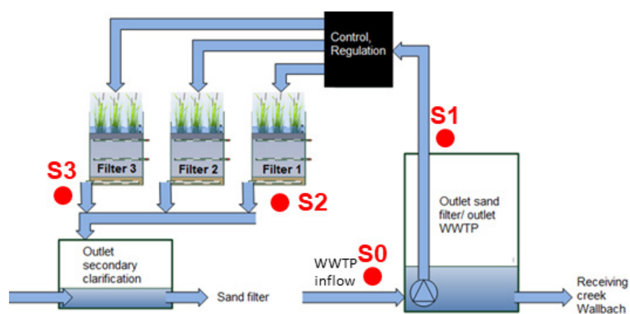


Figure 3-5 Schematic representation of water treatment site 11, indicating sampling points for CALUX bio-analyses

Table 3-6 Round 2 sample information site 12

Site	BDS project no.	BDS sample code	Sampling point	Location	date of sampling	sampling method	volume (ml)	date of arrival
12	13060	28975	S1	prim. sedimentation effl. (infl. biological treatment of WWTP)	24/01/2018	grab	1000	25/01/2018
12	13060	28970	S2	sec. sedimentation effl. (ozonation infl.)	23/01/2018	grab	1000	25/01/2018
12	13060	28971	S3	ozonation effluent	23/01/2018	grab	1000	25/01/2018
			S4					
12	13060	28972	S5	sand/anthracite filter	23/01/2018	grab	1000	25/01/2018
12	13060	28973	S6	sand/BAC filter	23/01/2018	grab	1000	25/01/2018
12	13060	28974	S7	constructed wetland 1	24/01/2018	grab	1000	25/01/2018
12	13457	29679	S1	Primary sedimentation effluent	17/04/2018	grab	2 * 1000	19/04/2018
12	13457	29673	S2	Ozonation influent	17/04/2018	grab	2 * 1000	19/04/2018
12	13457	29674	S3	Ozonation effluent	17/04/2018	grab	2 * 1000	19/04/2018
12	13457	29677	S4	Post-GAC filter	17/04/2018	grab	1 * 1000	19/04/2018
12	13457	29676	S5	Sand/anthracite filter	17/04/2018	grab	2 * 1000	19/04/2018
12	13457	29675	S6	Sand/BAC filter	17/04/2018	grab	2 * 1000	19/04/2018
12	13457	29678	S7	constructed wetland 1	17/04/2018	grab	2 * 1000	19/04/2018
12	13815	30275	S1	Primary sedimentation effluent	16/07/2018	grab	2 * 1000	18/07/2018
12	13815	30276	S2	Ozonation influent	16/07/2018	grab	2 * 1000	18/07/2018
12	13815	30277	S3	Ozonation effluent	16/07/2018	grab	2 * 1000	18/07/2018
12	13815	30280	S4	Post-GAC filter	16/07/2018	grab	2 * 1000	18/07/2018
12	13815	30281	S5	Sand/anthracite filter	16/07/2018	grab	2 * 1000	18/07/2018
12	13815	30278	S6	Sand/BAC filter	16/07/2018	grab	2 * 1000	18/07/2018
12	13815	30279	S7	constructed wetland 1	16/07/2018	grab	2 * 1000	18/07/2018

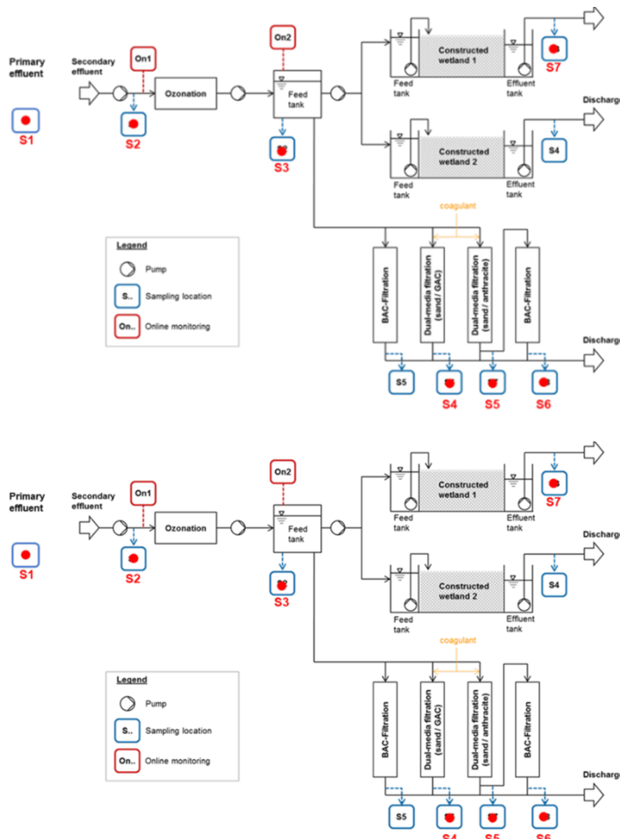


Figure 3-6 Schematic representation of water treatment site 12, indicating sampling points for CALUX bio-analyses

Annex 4 Quantified CALUX bioanalysis results – round 1

Table 4-a CALUX bioanalysis results site 1

Site 1

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<0.89)	ug TBT eq./l water	0.89
28516	2017_11_27_ site 1_permeate NF	LOQ(<0.91)	ug TBT eq./l water	0.91
ERa CALUX				
28515	2017_11_27_ site 1_feed NF	0.22	ng 17b-estradiol eq./l water	0.07
28516	2017_11_27_ site 1_permeate NF	0.15	ng 17b-estradiol eq./l water	0.08
anti-ERa CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<1.2)	ug Tamoxifen eq./l water	1.2
28516	2017_11_27_ site 1_permeate NF	LOQ(<1.2)	ug Tamoxifen eq./l water	1.2
AR CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<0.96)	ng DHT eq./l water	0.96
28516	2017_11_27_ site 1_permeate NF	LOQ(<0.98)	ng DHT eq./l water	0.98
anti-AR CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<6.9)	ug Flutamide eq./l water	6.9
28516	2017_11_27_ site 1_permeate NF	LOQ(<6.9)	ug Flutamide eq./l water	6.9
GR CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<21)	ng Dexamethason eq./l water	21
28516	2017_11_27_ site 1_permeate NF	LOQ(<21)	ng Dexamethason eq./l water	21
anti-GR CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<0.075)	ug Ru486 eq./l water	0.075
28516	2017_11_27_ site 1_permeate NF	LOQ(<0.076)	ug Ru486 eq./l water	0.076
PR CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<2.4)	ng Org2058 eq./l water	2.4
28516	2017_11_27_ site 1_permeate NF	LOQ(<2.5)	ng Org2058 eq./l water	2.5
anti-PR CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<0.88)	ng Ru486 eq./l water	0.88
28516	2017_11_27_ site 1_permeate NF	LOQ(<0.89)	ng Ru486 eq./l water	0.89
PPARa CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<90)	ng GW7647 eq./ll water	90
28516	2017_11_27_ site 1_permeate NF	LOQ(<91)	ng GW7647 eq./ll water	91
PPARd CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<290)	ng L-165,041 eq./l water	290
28516	2017_11_27_ site 1_permeate NF	LOQ(<290)	ng L-165,041 eq./l water	290
PPARg CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<207)	ng Rosiglitazone eq./l water	207
28516	2017_11_27_ site 1_permeate NF	LOQ(<210)	ng Rosiglitazone eq./l water	210
DR CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<9.6)	pg 2,3,7,8 TCDD eq./l water	9.6
28516	2017_11_27_ site 1_permeate NF	LOQ(<9.7)	pg 2,3,7,8 TCDD eq./l water	9.7
PAH CALUX				
28515	2017_11_27_ site 1_feed NF	22	ng Benzo[a]pyrene eq./l water	2.5
28516	2017_11_27_ site 1_permeate NF	4.8	ng Benzo[a]pyrene eq./l water	2.5
PXR CALUX				
28515	2017_11_27_ site 1_feed NF	33	ug Nicardipine eq./l water	8.8
28516	2017_11_27_ site 1_permeate NF	LOQ(<9)	ug Nicardipine eq./l water	9
Nrf2 CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<35)	ug Curcumine/l water	35
28516	2017_11_27_ site 1_permeate NF	LOQ(<36)	ug Curcumine/l water	36
P53 (-S9) CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<0.03)	ug Actinomycin D/l water	0.03
28516	2017_11_27_ site 1_permeate NF	LOQ(<0.03)	ug Actinomycin D/l water	0.03
P53 (+S9) CALUX				
28515	2017_11_27_ site 1_feed NF	LOQ(<1100)	ug Cyclophosphamide/l water	1119.62
28516	2017_11_27_ site 1_permeate NF	LOQ(<1100)	ug Cyclophosphamide/l water	1133.89

Table 4-b Quantified CALUX bioanalysis results site 2

Site 2

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<0.96)	ug TBT eq./l water	0.96
25827	Drewag Netz -B1 - 06/03/2	LOQ (<0.90)	ug TBT eq./l water	0.9
ERa CALUX				
25825	Drewag Netz -A1 - 06/03/2	0.095	ng 17b-estradiol eq./l water	0.073
25827	Drewag Netz -B1 - 06/03/2	LOQ (<0.069)	ng 17b-estradiol eq./l water	0.069
anti-ERa CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<0.80)	ug Tamoxifen eq./l water	0.8
25827	Drewag Netz -B1 - 06/03/2	LOQ (<0.75)	ug Tamoxifen eq./l water	0.75
AR CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<4.4)	ng DHT eq./l water	4.4
25827	Drewag Netz -B1 - 06/03/2	LOQ (<4.1)	ng DHT eq./l water	4.1
anti-AR CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<7.6)	ug Flutamide eq./l water	7.6
25827	Drewag Netz -B1 - 06/03/2	LOQ (<7.1)	ug Flutamide eq./l water	7.1
GR CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<25)	ng Dexamethason eq./l water	25
25827	Drewag Netz -B1 - 06/03/2	LOQ (<23)	ng Dexamethason eq./l water	23
anti-GR CALUX				
25825	Drewag Netz -A1 - 06/03/2	38	ug Ru486 eq./l water	35
25827	Drewag Netz -B1 - 06/03/2	LOQ (<33)	ug Ru486 eq./l water	33
PR CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<1.7)	ng Org2058 eq./l water	1.7
25827	Drewag Netz -B1 - 06/03/2	LOQ (<1.5)	ng Org2058 eq./l water	1.5
anti-PR CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<5.2)	ng Ru486 eq./l water	5.2
25827	Drewag Netz -B1 - 06/03/2	LOQ (<4.9)	ng Ru486 eq./l water	4.9
PPARa CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<33)	ng GW7647 eq./ll water	33
25827	Drewag Netz -B1 - 06/03/2	LOQ (<31)	ng GW7647 eq./ll water	31
PPARd CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<1300)	ng L-165,041 eq./l water	1300
25827	Drewag Netz -B1 - 06/03/2	LOQ (<1200)	ng L-165,041 eq./l water	1200
PPARg CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<440)	ng Rosiglitazone eq./l water	440
25827	Drewag Netz -B1 - 06/03/2	LOQ (<410)	ng Rosiglitazone eq./l water	410
DR CALUX				
25825	Drewag Netz -A1 - 06/03/2	19	pg 2,3,7,8 TCDD eq./l water	11
25827	Drewag Netz -B1 - 06/03/2	LOQ (<10)	pg 2,3,7,8 TCDD eq./l water	10
PAH CALUX				
25825	Drewag Netz -A1 - 06/03/2	52	ng Benzo[a]pyrene eq./l water	1.3
25827	Drewag Netz -B1 - 06/03/2	LOQ (<1.3)	ng Benzo[a]pyrene eq./l water	1.3
PXR CALUX				
25825	Drewag Netz -A1 - 06/03/2	23	ug Nicardipine eq./l water	10
25827	Drewag Netz -B1 - 06/03/2	LOQ (<9.4)	ug Nicardipine eq./l water	9.4
Nrf2 CALUX				
25825	Drewag Netz -A1 - 06/03/2	120	ug Curcumine/l water	36
25827	Drewag Netz -B1 - 06/03/2	92	ug Curcumine/l water	33
P53 (-S9) CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<0.030)	ug Actinomycin D/l water	0.03
25827	Drewag Netz -B1 - 06/03/2	LOQ (<0.030)	ug Actinomycin D/l water	0.03
P53 (+S9) CALUX				
25825	Drewag Netz -A1 - 06/03/2	LOQ (<1000)	ug Cyclophosphamide/l water	1000
25827	Drewag Netz -B1 - 06/03/2	LOQ (<970)	ug Cyclophosphamide/l water	970

Table 4-c Quantified CALUX bioanalysis results site 3

Site 3

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
25835	RW_Q1_20170307	LOQ (<1.2)	ug TBT eq./l water	1.2
25836	RO_Q2_20170307	LOQ (<1.1)	ug TBT eq./l water	1.1
ERa CALUX				
25835	RW_Q1_20170307	LOQ (<0.10)	ng 17b-estradiol eq./l water	0.1
25836	RO_Q2_20170307	LOQ (<0.097)	ng 17b-estradiol eq./l water	0.097
anti-ERa CALUX				
25835	RW_Q1_20170307	LOQ (<1.2)	ug Tamoxifen eq./l water	1.2
25836	RO_Q2_20170307	LOQ (<1.1)	ug Tamoxifen eq./l water	1.1
AR CALUX				
25835	RW_Q1_20170307	LOQ (<5.6)	ng DHT eq./l water	5.6
25836	RO_Q2_20170307	LOQ (<5.2)	ng DHT eq./l water	5.2
anti-AR CALUX				
25835	RW_Q1_20170307	LOQ (<8.2)	ug Flutamide eq./l water	8.2
25836	RO_Q2_20170307	LOQ (<7.6)	ug Flutamide eq./l water	7.6
GR CALUX				
25835	RW_Q1_20170307	LOQ (<29)	ng Dexamethason eq./l water	29
25836	RO_Q2_20170307	LOQ (<27)	ng Dexamethason eq./l water	27
anti-GR CALUX				
25835	RW_Q1_20170307	86	ug Ru486 eq./l water	84
25836	RO_Q2_20170307	LOQ (<79)	ug Ru486 eq./l water	79
PR CALUX				
25835	RW_Q1_20170307	LOQ (<1.7)	ng Org2058 eq./l water	1.7
25836	RO_Q2_20170307	LOQ (<1.6)	ng Org2058 eq./l water	1.6
anti-PR CALUX				
25835	RW_Q1_20170307	LOQ (<4.0)	ng Ru486 eq./l water	4
25836	RO_Q2_20170307	LOQ (<3.7)	ng Ru486 eq./l water	3.7
PPARa CALUX				
25835	RW_Q1_20170307	LOQ (<61)	ng GW7647 eq./ll water	61
25836	RO_Q2_20170307	LOQ (<56)	ng GW7647 eq./ll water	56
PPARd CALUX				
25835	RW_Q1_20170307	LOQ (<1200)	ng L-165,041 eq./l water	1200
25836	RO_Q2_20170307	LOQ (<1100)	ng L-165,041 eq./l water	1100
PPARg CALUX				
25835	RW_Q1_20170307	LOQ (<720)	ng Rosiglitazone eq./l water	720
25836	RO_Q2_20170307	LOQ (<670)	ng Rosiglitazone eq./l water	670
DR CALUX				
25835	RW_Q1_20170307	14	pg 2,3,7,8 TCDD eq./l water	13
25836	RO_Q2_20170307	11	pg 2,3,7,8 TCDD eq./l water	12
PAH CALUX				
25835	RW_Q1_20170307	19	ng Benzo[a]pyrene eq./l water	1.3
25836	RO_Q2_20170307	52	ng Benzo[a]pyrene eq./l water	1.3
PXR CALUX				
25835	RW_Q1_20170307	28	ug Nicardipine eq./l water	17
25836	RO_Q2_20170307	LOQ (<16)	ug Nicardipine eq./l water	16
Nrf2 CALUX				
25835	RW_Q1_20170307	110	ug Curcumine/l water	43
25836	RO_Q2_20170307	120	ug Curcumine/l water	40
P53 (-S9) CALUX				
25835	RW_Q1_20170307	LOQ (<0.040)	ug Actinomycin D/l water	0.04
25836	RO_Q2_20170307	LOQ (<0.040)	ug Actinomycin D/l water	0.04
P53 (+S9) CALUX				
25835	RW_Q1_20170307	LOQ (<1300)	ug Cyclophosphamide/l water	1300
25836	RO_Q2_20170307	LOQ (<1200)	ug Cyclophosphamide/l water	1200

Table 4-d Quantified CALUX bioanalysis results site 4

Site 4

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
25277	Mosina treatment station Mo1	LOQ (<1.1)	ug TBT eq./l water	1.1
25278	Mosina treatment station Mo2	LOQ (<1.1)	ug TBT eq./l water	1.1
ERa CALUX				
25277	Mosina treatment station Mo1	LOQ (<0.070)	ng 17b-estradiol eq./l water	0.07
25278	Mosina treatment station Mo2	LOQ (<0.070)	ng 17b-estradiol eq./l water	0.07
anti-ERa CALUX				
25277	Mosina treatment station Mo1	LOQ (<0.49)	ug Tamoxifen eq./l water	0.49
25278	Mosina treatment station Mo2	LOQ (<0.49)	ug Tamoxifen eq./l water	0.49
AR CALUX				
25277	Mosina treatment station Mo1	LOQ (<0.41)	ng DHT eq./l water	0.41
25278	Mosina treatment station Mo2	LOQ (<0.41)	ng DHT eq./l water	0.41
anti-AR CALUX				
25277	Mosina treatment station Mo1	4	ug Flutamide eq./l water	2.9
25278	Mosina treatment station Mo2	LOQ (<2.9)	ug Flutamide eq./l water	2.9
GR CALUX				
25277	Mosina treatment station Mo1	LOQ (<8.7)	ng Dexamethason eq./l water	8.7
25278	Mosina treatment station Mo2	LOQ (<8.7)	ng Dexamethason eq./l water	8.7
anti-GR CALUX				
25277	Mosina treatment station Mo1	LOQ (<32)	ug Ru486 eq./l water	32
25278	Mosina treatment station Mo2	LOQ (<32)	ug Ru486 eq./l water	32
PR CALUX				
25277	Mosina treatment station Mo1	LOQ (<1.0)	ng Org2058 eq./l water	1
25278	Mosina treatment station Mo2	LOQ (<1.0)	ng Org2058 eq./l water	1
anti-PR CALUX				
25277	Mosina treatment station Mo1	LOQ (<0.37)	ng Ru486 eq./l water	0.37
25278	Mosina treatment station Mo2	LOQ (<0.37)	ng Ru486 eq./l water	0.37
PPARa CALUX				
25277	Mosina treatment station Mo1	LOQ (<38)	ng GW7647 eq./ll water	38
25278	Mosina treatment station Mo2	LOQ (<38)	ng GW7647 eq./ll water	38
PPARd CALUX				
25277	Mosina treatment station Mo1	LOQ (<270)	ng L-165,041 eq./l water	270
25278	Mosina treatment station Mo2	LOQ (<270)	ng L-165,041 eq./l water	270
PPARg CALUX				
25277	Mosina treatment station Mo1	LOQ (<87)	ng Rosiglitazone eq./l water	87
25278	Mosina treatment station Mo2	LOQ (<87)	ng Rosiglitazone eq./l water	87
DR CALUX				
25277	Mosina treatment station Mo1	25	pg 2,3,7,8 TCDD eq./l water	1
25278	Mosina treatment station Mo2	17	pg 2,3,7,8 TCDD eq./l water	1
PAH CALUX				
25277	Mosina treatment station Mo1	7	ng Benzo[a]pyrene eq./l water	1
25278	Mosina treatment station Mo2	LOQ (1.0)	ng Benzo[a]pyrene eq./l water	1
PXR CALUX				
25277	Mosina treatment station Mo1	13	ug Nicardipine eq./l water	1.7
25278	Mosina treatment station Mo2	3.7	ug Nicardipine eq./l water	1.7
Nrf2 CALUX				
25277	Mosina treatment station Mo1	99	ug Curcumine/l water	14
25278	Mosina treatment station Mo2	22	ug Curcumine/l water	14
P53 (-S9) CALUX				
25277	Mosina treatment station Mo1	LOQ (<0.010)	ug Actinomycin D/l water	0.01
25278	Mosina treatment station Mo2	LOQ (<0.010)	ug Actinomycin D/l water	0.01
P53 (+S9) CALUX				
25277	Mosina treatment station Mo1	1700	ug Cyclophosphamide/l water	900
25278	Mosina treatment station Mo2	LOQ (<900)	ug Cyclophosphamide/l water	900

Table 4-e Quantified CALUX bioanalysis results site 5

Site 5

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
29312	Ganga	LOQ (<1.2)	ug TBT eq./l water	1.2
29313	SP1 RBF	LOQ (<1.2)	ug TBT eq./l water	1.2
29314	SP4 A0	LOQ (<1.2)	ug TBT eq./l water	1.2
ERa CALUX				
29312	Ganga	LOQ (<0.12)	ng 17b-estradiol eq./l water	0.12
29313	SP1 RBF	LOQ (<0.12)	ng 17b-estradiol eq./l water	0.12
29314	SP4 A0	LOQ (<0.11)	ng 17b-estradiol eq./l water	0.11
anti-ERa CALUX				
29312	Ganga	LOQ (<0.92)	ug Tamoxifen eq./l water	0.92
29313	SP1 RBF	LOQ (<0.96)	ug Tamoxifen eq./l water	0.96
29314	SP4 A0	LOQ (<1.1)	ug Tamoxifen eq./l water	1.1
AR CALUX				
29312	Ganga	LOQ (<0.81)	ng DHT eq./l water	0.81
29313	SP1 RBF	LOQ (<0.85)	ng DHT eq./l water	0.85
29314	SP4 A0	LOQ (<1.9)	ng DHT eq./l water	1.9
anti-AR CALUX				
29312	Ganga	LOQ (<9.9)	ug Flutamide eq./l water	9.9
29313	SP1 RBF	LOQ (<10)	ug Flutamide eq./l water	10
29314	SP4 A0	LOQ (<14)	ug Flutamide eq./l water	14
GR CALUX				
29312	Ganga	LOQ (<25)	ng Dexamethason eq./l water	25
29313	SP1 RBF	LOQ (<26)	ng Dexamethason eq./l water	26
29314	SP4 A0	LOQ (<32)	ng Dexamethason eq./l water	32
anti-GR CALUX				
29312	Ganga	LOQ (<0.049)	ug Ru486 eq./l water	0.049
29313	SP1 RBF	LOQ (<0.052)	ug Ru486 eq./l water	0.052
29314	SP4 A0	LOQ (<0.059)	ug Ru486 eq./l water	0.059
PR CALUX				
29312	Ganga	LOQ (<2.2)	ng Org2058 eq./l water	2.2
29313	SP1 RBF	LOQ (<2.3)	ng Org2058 eq./l water	2.3
29314	SP4 A0	LOQ (<2.1)	ng Org2058 eq./l water	2.1
anti-PR CALUX				
29312	Ganga	LOQ (<6.2)	ng Ru486 eq./l water	6.2
29313	SP1 RBF	LOQ (<6.5)	ng Ru486 eq./l water	6.5
29314	SP4 A0	LOQ (<6.2)	ng Ru486 eq./l water	6.2
PPARa CALUX				
29312	Ganga	LOQ (<32)	ng GW7647 eq./ll water	32
29313	SP1 RBF	LOQ (<34)	ng GW7647 eq./ll water	34
29314	SP4 A0	LOQ (<40)	ng GW7647 eq./ll water	40
PPARd CALUX				
29312	Ganga	LOQ (<620)	ng L-165,041 eq./l water	620
29313	SP1 RBF	LOQ (<650)	ng L-165,041 eq./l water	650
29314	SP4 A0	LOQ (<580)	ng L-165,041 eq./l water	580
PPARg CALUX				
29312	Ganga	LOQ (<670)	ng Rosiglitazone eq./l water	670
29313	SP1 RBF	LOQ (<700)	ng Rosiglitazone eq./l water	700
29314	SP4 A0	LOQ (<330)	ng Rosiglitazone eq./l water	330
DR CALUX				
29312	Ganga	138	pg 2,3,7,8 TCDD eq./l water	11
29313	SP1 RBF	<LOQ (11)	pg 2,3,7,8 TCDD eq./l water	11
29314	SP4 A0	68	pg 2,3,7,8 TCDD eq./l water	11
PAH CALUX				
29312	Ganga	90	ng Benzo[a]pyrene eq./l water	2.9
29313	SP1 RBF	62	ng Benzo[a]pyrene eq./l water	2.5
29314	SP4 A0	55	ng Benzo[a]pyrene eq./l water	3.1
PXR CALUX				
29312	Ganga	LOQ (<7.0)	ug Nicardipine eq./l water	7
29313	SP1 RBF	LOQ (<7.3)	ug Nicardipine eq./l water	7.3
29314	SP4 A0	9.2	ug Nicardipine eq./l water	7
Nrf2 CALUX				
29312	Ganga	LOQ (<27)	ug Curcumine/l water	27
29313	SP1 RBF	LOQ (<29)	ug Curcumine/l water	29
29314	SP4 A0	33	ug Curcumine/l water	28
P53 (-S9) CALUX				
29312	Ganga	LOQ (<0.020)	ug Actinomycin D/l water	0.02
29313	SP1 RBF	LOQ (<0.020)	ug Actinomycin D/l water	0.02
29314	SP4 A0	LOQ (<0.020)	ug Actinomycin D/l water	0.02
P53 (+S9) CALUX				
29312	Ganga	LOQ (<800)	ug Cyclophosphamide/l water	800
29313	SP1 RBF	LOQ (<840)	ug Cyclophosphamide/l water	840
29314	SP4 A0	LOQ (<810)	ug Cyclophosphamide/l water	810

Table 4-f Quantified CALUX bioanalysis results site 6

Site 6

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
27118	1 - raw river Wiese wate	LOQ (<0.98)	ug TBT eq./l water	0.98
27119	2 - pre-treated river Wie	LOQ (<0.73)	ug TBT eq./l water	0.73
27120	3 - after AOP treatment	LOQ (<0.71)	ug TBT eq./l water	0.71
27121	4 - blank	LOQ (<0.74)	ug TBT eq./l water	0.74
ERa CALUX				
27118	1 - raw river Wiese wate	0.15	ng 17b-estradiol eq./l water	0.096
27119	2 - pre-treated river Wie	LOQ (<0.072)	ng 17b-estradiol eq./l water	0.072
27120	3 - after AOP treatment	LOQ (<0.054)	ng 17b-estradiol eq./l water	0.054
27121	4 - blank	LOQ (<0.057)	ng 17b-estradiol eq./l water	0.057
anti-ERa CALUX				
27118	1 - raw river Wiese wate	LOQ (<2.2)	ug Tamoxifen eq./l water	2.2
27119	2 - pre-treated river Wie	LOQ (<1.6)	ug Tamoxifen eq./l water	1.6
27120	3 - after AOP treatment	LOQ (<0.57)	ug Tamoxifen eq./l water	0.57
27121	4 - blank	LOQ (<0.60)	ug Tamoxifen eq./l water	0.6
AR CALUX				
27118	1 - raw river Wiese wate	LOQ (<0.44)	ng DHT eq./l water	0.44
27119	2 - pre-treated river Wie	LOQ (<0.32)	ng DHT eq./l water	0.32
27120	3 - after AOP treatment	LOQ (<0.26)	ng DHT eq./l water	0.26
27121	4 - blank	LOQ (<0.27)	ng DHT eq./l water	0.27
anti-AR CALUX				
27118	1 - raw river Wiese wate	13	ug Flutamide eq./l water	12
27119	2 - pre-treated river Wie	17	ug Flutamide eq./l water	8.8
27120	3 - after AOP treatment	LOQ (<15)	ug Flutamide eq./l water	15
27121	4 - blank	LOQ (<16)	ug Flutamide eq./l water	16
GR CALUX				
27118	1 - raw river Wiese wate	LOQ (<15)	ng Dexamethason eq./l water	15
27119	2 - pre-treated river Wie	LOQ (<11)	ng Dexamethason eq./l water	11
27120	3 - after AOP treatment	LOQ (<12)	ng Dexamethason eq./l water	12
27121	4 - blank	LOQ (<13)	ng Dexamethason eq./l water	13
anti-GR CALUX				
27118	1 - raw river Wiese wate	LOQ (<0.086)	ug Ru486 eq./l water	0.086
27119	2 - pre-treated river Wie	0.18	ug Ru486 eq./l water	0.064
27120	3 - after AOP treatment	LOQ (<0.044)	ug Ru486 eq./l water	0.044
27121	4 - blank	LOQ (<0.046)	ug Ru486 eq./l water	0.046
PR CALUX				
27118	1 - raw river Wiese wate	LOQ (<1.1)	ng Org2058 eq./l water	1.1
27119	2 - pre-treated river Wie	LOQ (<0.82)	ng Org2058 eq./l water	0.82
27120	3 - after AOP treatment	LOQ (<1.4)	ng Org2058 eq./l water	1.4
27121	4 - blank	LOQ (<1.4)	ng Org2058 eq./l water	1.4
anti-PR CALUX				
27118	1 - raw river Wiese wate	LOQ (<5.2)	ng Ru486 eq./l water	5.2
27119	2 - pre-treated river Wie	LOQ (<3.8)	ng Ru486 eq./l water	3.8
27120	3 - after AOP treatment	LOQ (<2.4)	ng Ru486 eq./l water	2.4
27121	4 - blank	LOQ (<2.6)	ng Ru486 eq./l water	2.6
PPARa CALUX				
27118	1 - raw river Wiese wate	LOQ (<44)	ng GW7647 eq./ll water	44
27119	2 - pre-treated river Wie	LOQ (<33)	ng GW7647 eq./ll water	33
27120	3 - after AOP treatment	LOQ (<17)	ng GW7647 eq./ll water	17
27121	4 - blank	LOQ (<18)	ng GW7647 eq./ll water	18
PPARd CALUX				
27118	1 - raw river Wiese wate	LOQ (<1200)	ng L-165,041 eq./l water	1200
27119	2 - pre-treated river Wie	LOQ (<860)	ng L-165,041 eq./l water	860
27120	3 - after AOP treatment	LOQ (<710)	ng L-165,041 eq./l water	710
27121	4 - blank	LOQ (<750)	ng L-165,041 eq./l water	750
PPARg CALUX				
27118	1 - raw river Wiese wate	LOQ (<190)	ng Rosiglitazone eq./l water	190
27119	2 - pre-treated river Wie	LOQ (<140)	ng Rosiglitazone eq./l water	140
27120	3 - after AOP treatment	LOQ (<120)	ng Rosiglitazone eq./l water	120
27121	4 - blank	LOQ (<120)	ng Rosiglitazone eq./l water	120
DR CALUX				
27118	1 - raw river Wiese wate	51	pg 2,3,7,8 TCDD eq./l water	10
27119	2 - pre-treated river Wie	19	pg 2,3,7,8 TCDD eq./l water	7.6
27120	3 - after AOP treatment	LOQ (<7.6)	pg 2,3,7,8 TCDD eq./l water	7.6
27121	4 - blank	LOQ (<7.6)	pg 2,3,7,8 TCDD eq./l water	7.6
PAH CALUX				
27118	1 - raw river Wiese wate	79	ng Benzo[a]pyrene eq./l water	3.5
27119	2 - pre-treated river Wie	47	ng Benzo[a]pyrene eq./l water	2.6
27120	3 - after AOP treatment	6	ng Benzo[a]pyrene eq./l water	2.7
27121	4 - blank	LOQ (<2.8)	ng Benzo[a]pyrene eq./l water	2.8
PXR CALUX				
27118	1 - raw river Wiese wate	10	ug Nicardipine eq./l water	8
27119	2 - pre-treated river Wie	LOQ (<6.2)	ug Nicardipine eq./l water	6.2
27120	3 - after AOP treatment	6.2	ug Nicardipine eq./l water	5.4
27121	4 - blank	LOQ (<5.6)	ug Nicardipine eq./l water	5.6
Nrf2 CALUX				
27118	1 - raw river Wiese wate	LOQ (<57)	ug Curcumine/l water	57
27119	2 - pre-treated river Wie	LOQ (<42)	ug Curcumine/l water	42
27120	3 - after AOP treatment	LOQ (<42)	ug Curcumine/l water	42
27121	4 - blank	LOQ (<44)	ug Curcumine/l water	44
P53 (-S9) CALUX				
27118	1 - raw river Wiese wate	LOQ (<0.02)	ug Actinomycin D/l water	0.02
27119	2 - pre-treated river Wie	LOQ (<0.01)	ug Actinomycin D/l water	0.01
27120	3 - after AOP treatment	LOQ (<0.01)	ug Actinomycin D/l water	0.01
27121	4 - blank	LOQ (<0.01)	ug Actinomycin D/l water	0.01
P53 (+S9) CALUX				
27118	1 - raw river Wiese wate	LOQ (<820)	ug Cyclophosphamide/l water	820
27119	2 - pre-treated river Wie	LOQ (<620)	ug Cyclophosphamide/l water	610
27120	3 - after AOP treatment	LOQ (<600)	ug Cyclophosphamide/l water	600
27121	4 - blank	LOQ (<630)	ug Cyclophosphamide/l water	630

Table 4-g Quantified CALUX bioanalysis results site 7

Site 7

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
27814	SHAF_R1	120	ug TBT eq./l water	1.1
27815	SHAF_OZA500	LOQ (<1.1)	ug TBT eq./l water	1.1
27816	SHAF_OZOAOZ	LOQ (<1.0)	ug TBT eq./l water	1
ERa CALUX				
27814	SHAF_R1	57	ng 17b-estradiol eq./l water	0.11
27815	SHAF_OZA500	2	ng 17b-estradiol eq./l water	0.11
27816	SHAF_OZOAOZ	LOQ (<0.14)	ng 17b-estradiol eq./l water	0.14
anti-ERa CALUX				
27814	SHAF_R1	LOQ (<2.7)	ug Tamoxifen eq./l water	2.7
27815	SHAF_OZA500	LOQ (<0.87)	ug Tamoxifen eq./l water	0.87
27816	SHAF_OZOAOZ	LOQ (<0.77)	ug Tamoxifen eq./l water	0.77
AR CALUX				
27814	SHAF_R1	100	ng DHT eq./l water	4.5
27815	SHAF_OZA500	LOQ (<4.5)	ng DHT eq./l water	4.5
27816	SHAF_OZOAOZ	LOQ (<2.7)	ng DHT eq./l water	2.7
anti-AR CALUX				
27814	SHAF_R1	130	ug Flutamide eq./l water	10
27815	SHAF_OZA500	13	ug Flutamide eq./l water	9.8
27816	SHAF_OZOAOZ	LOQ (<9.6)	ug Flutamide eq./l water	9.6
GR CALUX				
27814	SHAF_R1	140	ng Dexamethason eq./l water	22
27815	SHAF_OZA500	110	ng Dexamethason eq./l water	22
27816	SHAF_OZOAOZ	LOQ (<25)	ng Dexamethason eq./l water	25
anti-GR CALUX				
27814	SHAF_R1	LOQ (<0.18)	ug Ru486 eq./l water	0.18
27815	SHAF_OZA500	LOQ (<0.059)	ug Ru486 eq./l water	0.059
27816	SHAF_OZOAOZ	LOQ (<0.022)	ug Ru486 eq./l water	0.022
PR CALUX				
27814	SHAF_R1	LOQ (<6.5)	ng Org2058 eq./l water	6.5
27815	SHAF_OZA500	LOQ (<2.1)	ng Org2058 eq./l water	2.1
27816	SHAF_OZOAOZ	LOQ (<2.9)	ng Org2058 eq./l water	2.9
anti-PR CALUX				
27814	SHAF_R1	170	ng Ru486 eq./l water	7.4
27815	SHAF_OZA500	7.4	ng Ru486 eq./l water	7.3
27816	SHAF_OZOAOZ	LOQ (<5.5)	ng Ru486 eq./l water	5.5
PPARa CALUX				
27814	SHAF_R1	540	ng GW7647 eq./ll water	16
27815	SHAF_OZA500	LOQ (<15)	ng GW7647 eq./ll water	15
27816	SHAF_OZOAOZ	LOQ (<23)	ng GW7647 eq./ll water	23
PPARd CALUX				
27814	SHAF_R1	16000	ng L-165,041 eq./l water	3700
27815	SHAF_OZA500	LOQ (<3600)	ng L-165,041 eq./l water	3600
27816	SHAF_OZOAOZ	LOQ (<1400)	ng L-165,041 eq./l water	1400
PPARg CALUX				
27814	SHAF_R1	1800	ng Rosiglitazone eq./l water	180
27815	SHAF_OZA500	LOQ (<170)	ng Rosiglitazone eq./l water	170
27816	SHAF_OZOAOZ	LOQ (<160)	ng Rosiglitazone eq./l water	160
DR CALUX				
27814	SHAF_R1	540	pg 2,3,7,8 TCDD eq./l water	12
27815	SHAF_OZA500	440	pg 2,3,7,8 TCDD eq./l water	12
27816	SHAF_OZOAOZ	120	pg 2,3,7,8 TCDD eq./l water	12
PAH CALUX				
27814	SHAF_R1	1200	ng Benzo[a]pyrene eq./l water	1.5
27815	SHAF_OZA500	170	ng Benzo[a]pyrene eq./l water	1.5
27816	SHAF_OZOAOZ	103	ng Benzo[a]pyrene eq./l water	1.4
PXR CALUX				
27814	SHAF_R1	410	ug Nicardipine eq./l water	10
27815	SHAF_OZA500	48	ug Nicardipine eq./l water	10
27816	SHAF_OZOAOZ	11	ug Nicardipine eq./l water	7.6
Nrf2 CALUX				
27814	SHAF_R1	LOQ (<2000)	ug Curcumine/l water	2000
27815	SHAF_OZA500	160	ug Curcumine/l water	64
27816	SHAF_OZOAOZ	150	ug Curcumine/l water	64
P53 (-S9) CALUX				
27814	SHAF_R1	LOQ (<0.60)	ug Actinomycin D/l water	0.6
27815	SHAF_OZA500	LOQ (<0.02)	ug Actinomycin D/l water	0.02
27816	SHAF_OZOAOZ	LOQ (<0.02)	ug Actinomycin D/l water	0.02
P53 (+S9) CALUX				
27814	SHAF_R1	LOQ (<26000)	ug Cyclophosphamide/l water	26000
27815	SHAF_OZA500	LOQ (<840)	ug Cyclophosphamide/l water	840
27816	SHAF_OZOAOZ	680	ug Cyclophosphamide/l water	840

Table 4-h Quantified CALUX bioanalysis results site 8

Site 8

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
27619	raw water WWTP inlet	15	ug TBT eq./l water	1.1
27620	raw water WWTP outlet (before Mare a Sorre)	3.6	ug TBT eq./l water	1.1
27621	raw water WWTP outlet (Mare a Sorre)	3.6	ug TBT eq./l water	1.1
ERa CALUX				
27619	raw water WWTP inlet	71	ng 17b-estradiol eq./l water	0.1
27620	raw water WWTP outlet (before Mare a Sorre)	3.1	ng 17b-estradiol eq./l water	0.12
27621	raw water WWTP outlet (Mare a Sorre)	1.1	ng 17b-estradiol eq./l water	0.12
anti-ERa CALUX				
27619	raw water WWTP inlet	LOQ (<2.5)	ug Tamoxifen eq./l water	2.5
27620	raw water WWTP outlet (before Mare a Sorre)	LOQ (<1.3)	ug Tamoxifen eq./l water	1.3
27621	raw water WWTP outlet (Mare a Sorre)	LOQ (<1.3)	ug Tamoxifen eq./l water	1.3
AR CALUX				
27619	raw water WWTP inlet	190	ng DHT eq./l water	3
27620	raw water WWTP outlet (before Mare a Sorre)	LOQ (<2.0)	ng DHT eq./l water	2
27621	raw water WWTP outlet (Mare a Sorre)	LOQ (<2.1)	ng DHT eq./l water	2.1
anti-AR CALUX				
27619	raw water WWTP inlet	LOQ (<31)	ug Flutamide eq./l water	31
27620	raw water WWTP outlet (before Mare a Sorre)	21	ug Flutamide eq./l water	10
27621	raw water WWTP outlet (Mare a Sorre)	18	ug Flutamide eq./l water	11
GR CALUX				
27619	raw water WWTP inlet	LOQ (<80)	ng Dexamethason eq./l water	80
27620	raw water WWTP outlet (before Mare a Sorre)	39	ng Dexamethason eq./l water	28
27621	raw water WWTP outlet (Mare a Sorre)	32	ng Dexamethason eq./l water	29
anti-GR CALUX				
27619	raw water WWTP inlet	0.3	ug Ru486 eq./l water	0.024
27620	raw water WWTP outlet (before Mare a Sorre)	0.066	ug Ru486 eq./l water	0.029
27621	raw water WWTP outlet (Mare a Sorre)	0.056	ug Ru486 eq./l water	0.03
PR CALUX				
27619	raw water WWTP inlet	LOQ (<9.5)	ng Org2058 eq./l water	9.5
27620	raw water WWTP outlet (before Mare a Sorre)	LOQ (<4.0)	ng Org2058 eq./l water	4
27621	raw water WWTP outlet (Mare a Sorre)	LOQ (<4.1)	ng Org2058 eq./l water	4.1
anti-PR CALUX				
27619	raw water WWTP inlet	44	ng Ru486 eq./l water	6
27620	raw water WWTP outlet (before Mare a Sorre)	19	ng Ru486 eq./l water	5.9
27621	raw water WWTP outlet (Mare a Sorre)	9.7	ng Ru486 eq./l water	6.1
PPARa CALUX				
27619	raw water WWTP inlet	660	ng GW7647 eq./ll water	25
27620	raw water WWTP outlet (before Mare a Sorre)	LOQ (<15)	ng GW7647 eq./ll water	15
27621	raw water WWTP outlet (Mare a Sorre)	LOQ (<15)	ng GW7647 eq./ll water	15
PPARd CALUX				
27619	raw water WWTP inlet	6300	ng L-165,041 eq./l water	1500
27620	raw water WWTP outlet (before Mare a Sorre)	LOQ (<16000)	ng L-165,041 eq./l water	16000
27621	raw water WWTP outlet (Mare a Sorre)	LOQ (<16000)	ng L-165,041 eq./l water	16000
PPARg CALUX				
27619	raw water WWTP inlet	1900	ng Rosiglitazone eq./l water	180
27620	raw water WWTP outlet (before Mare a Sorre)	290	ng Rosiglitazone eq./l water	230
27621	raw water WWTP outlet (Mare a Sorre)	260	ng Rosiglitazone eq./l water	230
DR CALUX				
27619	raw water WWTP inlet	1700	pg 2,3,7,8 TCDD eq./l water	13
27620	raw water WWTP outlet (before Mare a Sorre)	200	pg 2,3,7,8 TCDD eq./l water	12
27621	raw water WWTP outlet (Mare a Sorre)	211	pg 2,3,7,8 TCDD eq./l water	12
PAH CALUX				
27619	raw water WWTP inlet	14000	ng Benzo[a]pyrene eq./l water	1.3
27620	raw water WWTP outlet (before Mare a Sorre)	113	ng Benzo[a]pyrene eq./l water	2.1
27621	raw water WWTP outlet (Mare a Sorre)	33	ng Benzo[a]pyrene eq./l water	2.1
PXR CALUX				
27619	raw water WWTP inlet	130	ug Nicardipine eq./l water	8.6
27620	raw water WWTP outlet (before Mare a Sorre)	76	ug Nicardipine eq./l water	7
27621	raw water WWTP outlet (Mare a Sorre)	40	ug Nicardipine eq./l water	7.2
Nrf2 CALUX				
27619	raw water WWTP inlet	750	ug Curcumine/l water	210
27620	raw water WWTP outlet (before Mare a Sorre)	380	ug Curcumine/l water	67
27621	raw water WWTP outlet (Mare a Sorre)	620	ug Curcumine/l water	200
P53 (-S9) CALUX				
27619	raw water WWTP inlet	LOQ (<0.60)	ug Actinomycin D/l water	0.06
27620	raw water WWTP outlet (before Mare a Sorre)	LOQ (<0.02)	ug Actinomycin D/l water	0.02
27621	raw water WWTP outlet (Mare a Sorre)	LOQ (<0.60)	ug Actinomycin D/l water	0.06
P53 (+S9) CALUX				
27619	raw water WWTP inlet	6800	ug Cyclophosphamide/l water	2700
27620	raw water WWTP outlet (before Mare a Sorre)	LOQ (<870)	ug Cyclophosphamide/l water	870
27621	raw water WWTP outlet (Mare a Sorre)	LOQ (<2700)	ug Cyclophosphamide/l water	2700

Table 4-i Quantified CALUX bioanalysis results site 9

Site 9

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
27751	Nootdorp BASSIN	1.4	ug TBT eq./l water	1
27752	Nootdorp ASR	LOQ (<1.1)	ug TBT eq./l water	1.1
27753	Nootdorp OPPW	2.8	ug TBT eq./l water	1.1
27754	Nootdorp VLOTTER(KIST)	LOQ (<1.0)	ug TBT eq./l water	1
ERa CALUX				
27751	Nootdorp BASSIN	0.2	ng 17b-estradiol eq./l water	0.11
27752	Nootdorp ASR	LOQ (<0.12)	ng 17b-estradiol eq./l water	0.12
27753	Nootdorp OPPW	0.2	ng 17b-estradiol eq./l water	0.16
27754	Nootdorp VLOTTER(KIST)	LOQ (<0.15)	ng 17b-estradiol eq./l water	0.15
anti-ERa CALUX				
27751	Nootdorp BASSIN	LOQ (<1.5)	ug Tamoxifen eq./l water	1.5
27752	Nootdorp ASR	LOQ (<1.7)	ug Tamoxifen eq./l water	1.7
27753	Nootdorp OPPW	LOQ (<1.8)	ug Tamoxifen eq./l water	1.8
27754	Nootdorp VLOTTER(KIST)	LOQ (<1.7)	ug Tamoxifen eq./l water	1.7
AR CALUX				
27751	Nootdorp BASSIN	LOQ (<2.2)	ng DHT eq./l water	2.2
27752	Nootdorp ASR	LOQ (<2.4)	ng DHT eq./l water	2.4
27753	Nootdorp OPPW	LOQ (<2.4)	ng DHT eq./l water	2.4
27754	Nootdorp VLOTTER(KIST)	LOQ (<2.4)	ng DHT eq./l water	2.4
anti-AR CALUX				
27751	Nootdorp BASSIN	LOQ (<11)	ug Flutamide eq./l water	11
27752	Nootdorp ASR	LOQ (<12)	ug Flutamide eq./l water	12
27753	Nootdorp OPPW	32	ug Flutamide eq./l water	13
27754	Nootdorp VLOTTER(KIST)	LOQ (<12)	ug Flutamide eq./l water	12
GR CALUX				
27751	Nootdorp BASSIN	LOQ (<16)	ng Dexamethason eq./l water	16
27752	Nootdorp ASR	LOQ (<18)	ng Dexamethason eq./l water	18
27753	Nootdorp OPPW	LOQ (<17)	ng Dexamethason eq./l water	17
27754	Nootdorp VLOTTER(KIST)	LOQ (<17)	ng Dexamethason eq./l water	17
anti-GR CALUX				
27751	Nootdorp BASSIN	LOQ (<0.028)	ug Ru486 eq./l water	0.028
27752	Nootdorp ASR	LOQ (<0.030)	ug Ru486 eq./l water	0.03
27753	Nootdorp OPPW	0.39	ug Ru486 eq./l water	0.068
27754	Nootdorp VLOTTER(KIST)	LOQ (<0.065)	ug Ru486 eq./l water	0.065
PR CALUX				
27751	Nootdorp BASSIN	LOQ (<4.0)	ng Org2058 eq./l water	4
27752	Nootdorp ASR	LOQ (<4.4)	ng Org2058 eq./l water	4.4
27753	Nootdorp OPPW	LOQ (<5.1)	ng Org2058 eq./l water	5.1
27754	Nootdorp VLOTTER(KIST)	LOQ (<4.9)	ng Org2058 eq./l water	4.9
anti-PR CALUX				
27751	Nootdorp BASSIN	LOQ (<4.6)	ng Ru486 eq./l water	4.6
27752	Nootdorp ASR	LOQ (<5.0)	ng Ru486 eq./l water	5
27753	Nootdorp OPPW	8.4	ng Ru486 eq./l water	5.3
27754	Nootdorp VLOTTER(KIST)	LOQ (<5.1)	ng Ru486 eq./l water	5.1
PPARa CALUX				
27751	Nootdorp BASSIN	LOQ (<18)	ng GW7647 eq./ll water	18
27752	Nootdorp ASR	LOQ (<19)	ng GW7647 eq./ll water	19
27753	Nootdorp OPPW	LOQ (<44)	ng GW7647 eq./ll water	44
27754	Nootdorp VLOTTER(KIST)	LOQ (<42)	ng GW7647 eq./ll water	42
PPARd CALUX				
27751	Nootdorp BASSIN	LOQ (<1100)	ng L-165,041 eq./l water	1100
27752	Nootdorp ASR	LOQ (<1200)	ng L-165,041 eq./l water	1200
27753	Nootdorp OPPW	LOQ (<900)	ng L-165,041 eq./l water	900
27754	Nootdorp VLOTTER(KIST)	LOQ (<870)	ng L-165,041 eq./l water	870
PPARg CALUX				
27751	Nootdorp BASSIN	LOQ (<240)	ng Rosiglitazone eq./l water	240
27752	Nootdorp ASR	LOQ (<260)	ng Rosiglitazone eq./l water	260
27753	Nootdorp OPPW	LOQ (<260)	ng Rosiglitazone eq./l water	260
27754	Nootdorp VLOTTER(KIST)	LOQ (<250)	ng Rosiglitazone eq./l water	250
DR CALUX				
27751	Nootdorp BASSIN	57	pg 2,3,7,8 TCDD eq./l water	12
27752	Nootdorp ASR	62	pg 2,3,7,8 TCDD eq./l water	13
27753	Nootdorp OPPW	120	pg 2,3,7,8 TCDD eq./l water	13
27754	Nootdorp VLOTTER(KIST)	32	pg 2,3,7,8 TCDD eq./l water	12
PAH CALUX				
27751	Nootdorp BASSIN	44	ng Benzo[a]pyrene eq./l water	1.7
27752	Nootdorp ASR	54	ng Benzo[a]pyrene eq./l water	1.9
27753	Nootdorp OPPW	150	ng Benzo[a]pyrene eq./l water	1.3
27754	Nootdorp VLOTTER(KIST)	54	ng Benzo[a]pyrene eq./l water	1.2
PXR CALUX				
27751	Nootdorp BASSIN	23	ug Nicardipine eq./l water	8.9
27752	Nootdorp ASR	15	ug Nicardipine eq./l water	10
27753	Nootdorp OPPW	LOQ (<7.3)	ug Nicardipine eq./l water	7.3
27754	Nootdorp VLOTTER(KIST)	22	ug Nicardipine eq./l water	7.1
Nrf2 CALUX				
27751	Nootdorp BASSIN	610	ug Curcumine/l water	67
27752	Nootdorp ASR	LOQ (<74)	ug Curcumine/l water	74
27753	Nootdorp OPPW	LOQ (<210)	ug Curcumine/l water	210
27754	Nootdorp VLOTTER(KIST)	85	ug Curcumine/l water	67
P53 (-S9) CALUX				
27751	Nootdorp BASSIN	0.09	ug Actinomycin D/l water	0.02
27752	Nootdorp ASR	LOQ (<0.03)	ug Actinomycin D/l water	0.03
27753	Nootdorp OPPW	LOQ (<0.60)	ug Actinomycin D/l water	0.06
27754	Nootdorp VLOTTER(KIST)	LOQ (<0.02)	ug Actinomycin D/l water	0.02
P53 (+S9) CALUX				
27751	Nootdorp BASSIN	3000	ug Cyclophosphamide/l water	890
27752	Nootdorp ASR	LOQ (<960)	ug Cyclophosphamide/l water	960
27753	Nootdorp OPPW	LOQ (<2700)	ug Cyclophosphamide/l water	2700
27754	Nootdorp VLOTTER(KIST)	LOQ (<880)	ug Cyclophosphamide/l water	880

Table 4-j Quantified CALUX bioanalysis results site 10

Site 10

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
27671	No. 1	13	ug TBT eq./l water	0.82
27672	No. 2	35	ug TBT eq./l water	0.79
27673	No. 3	LOQ (<0.89)	ug TBT eq./l water	0.89
ERa CALUX				
27671	No. 1	87	ng 17b-estradiol eq./l water	0.058
27672	No. 2	96	ng 17b-estradiol eq./l water	0.062
27673	No. 3	0.4	ng 17b-estradiol eq./l water	0.093
anti-ERa CALUX				
27671	No. 1	LOQ (<4.9)	ug Tamoxifen eq./l water	4.9
27672	No. 2	LOQ (<1.7)	ug Tamoxifen eq./l water	1.7
27673	No. 3	LOQ (<0.74)	ug Tamoxifen eq./l water	0.74
AR CALUX				
27671	No. 1	70	ng DHT eq./l water	1.7
27672	No. 2	100	ng DHT eq./l water	1.8
27673	No. 3	LOQ (<1.9)	ng DHT eq./l water	1.9
anti-AR CALUX				
27671	No. 1	47	ug Flutamide eq./l water	12
27672	No. 2	460	ug Flutamide eq./l water	13
27673	No. 3	LOQ (<13)	ug Flutamide eq./l water	13
GR CALUX				
27671	No. 1	280	ng Dexamethason eq./l water	27
27672	No. 2	120	ng Dexamethason eq./l water	28
27673	No. 3	52	ng Dexamethason eq./l water	23
anti-GR CALUX				
27671	No. 1	LOQ (<0.24)	ug Ru486 eq./l water	0.24
27672	No. 2	0.42	ug Ru486 eq./l water	0.086
27673	No. 3	LOQ (<0.047)	ug Ru486 eq./l water	0.047
PR CALUX				
27671	No. 1	LOQ (<11)	ng Org2058 eq./l water	11
27672	No. 2	LOQ (<3.2)	ng Org2058 eq./l water	3.2
27673	No. 3	LOQ (<2.7)	ng Org2058 eq./l water	2.7
anti-PR CALUX				
27671	No. 1	LOQ (<4.6)	ng Ru486 eq./l water	4.6
27672	No. 2	120	ng Ru486 eq./l water	1.7
27673	No. 3	LOQ (<4.8)	ng Ru486 eq./l water	4.8
PPARa CALUX				
27671	No. 1	510	ng GW7647 eq./ll water	45
27672	No. 2	180	ng GW7647 eq./ll water	48
27673	No. 3	55	ng GW7647 eq./ll water	19
PPARd CALUX				
27671	No. 1	4900	ng L-165,041 eq./l water	1300
27672	No. 2	4400	ng L-165,041 eq./l water	1300
27673	No. 3	LOQ (<1200)	ng L-165,041 eq./l water	1200
PPARg CALUX				
27671	No. 1	3100	ng Rosiglitazone eq./l water	220
27672	No. 2	930	ng Rosiglitazone eq./l water	230
27673	No. 3	LOQ (<210)	ng Rosiglitazone eq./l water	210
DR CALUX				
27671	No. 1	390	pg 2,3,7,8 TCDD eq./l water	11
27672	No. 2	360	pg 2,3,7,8 TCDD eq./l water	12
27673	No. 3	250	pg 2,3,7,8 TCDD eq./l water	11
PAH CALUX				
27671	No. 1	517.8365691	ng Benzo[a]pyrene eq./l water	1.2
27672	No. 2	57.83656915	ng Benzo[a]pyrene eq./l water	2.2
27673	No. 3	LOQ (<1.9)	ng Benzo[a]pyrene eq./l water	1.9
PXR CALUX				
27671	No. 1	56	ug Nicardipine eq./l water	4.5
27672	No. 2	71	ug Nicardipine eq./l water	4.8
27673	No. 3	42	ug Nicardipine eq./l water	15
Nrf2 CALUX				
27671	No. 1	1500	ug Curcumine/l water	60
27672	No. 2	1200	ug Curcumine/l water	64
27673	No. 3	290	ug Curcumine/l water	60
P53 (-S9) CALUX				
27671	No. 1	LOQ (<0.02)	ug Actinomycin D/l water	0.02
27672	No. 2	LOQ (<0.02)	ug Actinomycin D/l water	0.02
27673	No. 3	LOQ (<0.02)	ug Actinomycin D/l water	0.02
P53 (+S9) CALUX				
27671	No. 1	21000	ug Cyclophosphamide/l water	1300
27672	No. 2	150000	ug Cyclophosphamide/l water	1400
27673	No. 3	1600	ug Cyclophosphamide/l water	1300

AquaNES

Table 4-k Quantified CALUX bioanalysis results site 11

Site 11

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<1.0)	ug TBT eq./l water	1
25944	Pilot RSF Rheinbed Outflow	LOQ (<1.0)	ug TBT eq./l water	1
26780	Inflow WWTP	260	ug TBT eq./l water	1.2
ERa CALUX				
25943	Pilot RSF Rheinbed Inflow	0.57	ng 17b-estradiol eq./l water	0.1
25944	Pilot RSF Rheinbed Outflow	LOQ (<0.10)	ng 17b-estradiol eq./l water	0.1
26780	Inflow WWTP	44	ng 17b-estradiol eq./l water	0.11
anti-ERa CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<0.91)	ug Tamoxifen eq./l water	0.91
25944	Pilot RSF Rheinbed Outflow	LOQ (<0.89)	ug Tamoxifen eq./l water	0.89
26780	Inflow WWTP	4.3	ug Tamoxifen eq./l water	0.9
AR CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<5.9)	ng DHT eq./l water	5.9
25944	Pilot RSF Rheinbed Outflow	LOQ (<5.8)	ng DHT eq./l water	5.8
26780	Inflow WWTP	89	ng DHT eq./l water	1.6
anti-AR CALUX				
25943	Pilot RSF Rheinbed Inflow	7.7	ug Flutamide eq./l water	7.6
25944	Pilot RSF Rheinbed Outflow	LOQ (<7.5)	ug Flutamide eq./l water	7.5
26780	Inflow WWTP	440	ug Flutamide eq./l water	12
GR CALUX				
25943	Pilot RSF Rheinbed Inflow	120	ng Dexamethason eq./l water	35
25944	Pilot RSF Rheinbed Outflow	LOQ (<34)	ng Dexamethason eq./l water	34
26780	Inflow WWTP	LOQ (<59)	ng Dexamethason eq./l water	59
anti-GR CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<72)	ug Ru486 eq./l water	72
25944	Pilot RSF Rheinbed Outflow	LOQ (<70)	ug Ru486 eq./l water	70
26780	Inflow WWTP	5200	ug Ru486 eq./l water	110
PR CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<3.6)	ng Org2058 eq./l water	3.6
25944	Pilot RSF Rheinbed Outflow	LOQ (<3.5)	ng Org2058 eq./l water	3.5
26780	Inflow WWTP	LOQ (<5.5)	ng Org2058 eq./l water	5.5
anti-PR CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<5.7)	ng Ru486 eq./l water	5.7
25944	Pilot RSF Rheinbed Outflow	LOQ (<5.6)	ng Ru486 eq./l water	5.6
26780	Inflow WWTP	320	ng Ru486 eq./l water	2.8
PPARa CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<28)	ng GW7647 eq./ll water	28
25944	Pilot RSF Rheinbed Outflow	LOQ (<27)	ng GW7647 eq./ll water	27
26780	Inflow WWTP	720	ng GW7647 eq./ll water	59
PPARd CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<1300)	ng L-165,041 eq./l water	1300
25944	Pilot RSF Rheinbed Outflow	LOQ (<1300)	ng L-165,041 eq./l water	1300
26780	Inflow WWTP	14000	ng L-165,041 eq./l water	1100
PPARg CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<610)	ng Rosiglitazone eq./l water	610
25944	Pilot RSF Rheinbed Outflow	LOQ (<600)	ng Rosiglitazone eq./l water	600
26780	Inflow WWTP	LOQ (<1100)	ng Rosiglitazone eq./l water	1100
DR CALUX				
25943	Pilot RSF Rheinbed Inflow	300	pg 2,3,7,8 TCDD eq./l water	12
25944	Pilot RSF Rheinbed Outflow	11	pg 2,3,7,8 TCDD eq./l water	12
26780	Inflow WWTP	380	pg 2,3,7,8 TCDD eq./l water	12
PAH CALUX				
25943	Pilot RSF Rheinbed Inflow	262	ng Benzo[a]pyrene eq./l water	2.6
25944	Pilot RSF Rheinbed Outflow	41	ng Benzo[a]pyrene eq./l water	1.7
26780	Inflow WWTP	2442	ng Benzo[a]pyrene eq./l water	2.1
PXR CALUX				
25943	Pilot RSF Rheinbed Inflow	130	ug Nicardipine eq./l water	11
25944	Pilot RSF Rheinbed Outflow	LOQ (<11)	ug Nicardipine eq./l water	11
26780	Inflow WWTP	360	ug Nicardipine eq./l water	8
Nrf2 CALUX				
25943	Pilot RSF Rheinbed Inflow	320	ug Curcumine/l water	42
25944	Pilot RSF Rheinbed Outflow	40	ug Curcumine/l water	41
26780	Inflow WWTP	LOQ (<46)	ug Curcumine/l water	46
P53 (-S9) CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<0.040)	ug Actinomycin D/l water	0.04
25944	Pilot RSF Rheinbed Outflow	LOQ (<0.040)	ug Actinomycin D/l water	0.04
26780	Inflow WWTP	LOQ (<0.0016)	ug Actinomycin D/l water	0.0016
P53 (+S9) CALUX				
25943	Pilot RSF Rheinbed Inflow	LOQ (<1200)	ug Cyclophosphamide/l water	1200
25944	Pilot RSF Rheinbed Outflow	LOQ (<1200)	ug Cyclophosphamide/l water	1200
26780	Inflow WWTP	LOQ (<770)	ug Cyclophosphamide/l water	770

Table 4-l Quantified CALUX bioanalysis results site 12

Site 12

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	15	ug TBT eq./l water	1.2
27613	WWTP Schonerlinde. Secondary sedimentation	1.5	ug TBT eq./l water	1.1
27614	WWTP Schonerlinde. Ozonation	LOQ (<1.0)	ug TBT eq./l water	1
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<1.0)	ug TBT eq./l water	1
ERa CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	55	ng 17b-estradiol eq./l water	0.1
27613	WWTP Schonerlinde. Secondary sedimentation	0.61	ng 17b-estradiol eq./l water	0.13
27614	WWTP Schonerlinde. Ozonation	LOQ (<0.15)	ng 17b-estradiol eq./l water	0.15
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<0.15)	ng 17b-estradiol eq./l water	0.15
anti-ERa CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	LOQ (<4.2)	ug Tamoxifen eq./l water	4.2
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<1.4)	ug Tamoxifen eq./l water	1.4
27614	WWTP Schonerlinde. Ozonation	LOQ (<0.98)	ug Tamoxifen eq./l water	0.98
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<1.0)	ug Tamoxifen eq./l water	1
AR CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	240	ng DHT eq./l water	2.2
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<2.3)	ng DHT eq./l water	2.3
27614	WWTP Schonerlinde. Ozonation	LOQ (<2.5)	ng DHT eq./l water	2.5
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<2.5)	ng DHT eq./l water	2.5
anti-AR CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	LOQ (<37)	ug Flutamide eq./l water	37
27613	WWTP Schonerlinde. Secondary sedimentation	15	ug Flutamide eq./l water	12
27614	WWTP Schonerlinde. Ozonation	LOQ (<11)	ug Flutamide eq./l water	11
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<11)	ug Flutamide eq./l water	11
GR CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	180	ng Dexamethason eq./l water	28
27613	WWTP Schonerlinde. Secondary sedimentation	170	ng Dexamethason eq./l water	27
27614	WWTP Schonerlinde. Ozonation	54	ng Dexamethason eq./l water	30
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	54	ng Dexamethason eq./l water	30
anti-GR CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	LOQ (<0.15)	ug Ru486 eq./l water	0.15
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<0.050)	ug Ru486 eq./l water	0.05
27614	WWTP Schonerlinde. Ozonation	LOQ (<0.068)	ug Ru486 eq./l water	0.068
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<0.070)	ug Ru486 eq./l water	0.07
PR CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	LOQ (<12)	ng Org2058 eq./l water	12
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<4.0)	ng Org2058 eq./l water	4
27614	WWTP Schonerlinde. Ozonation	LOQ (<4.1)	ng Org2058 eq./l water	4.1
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<4.2)	ng Org2058 eq./l water	4.2
anti-PR CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	43	ng Ru486 eq./l water	4
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<3.9)	ng Ru486 eq./l water	3.9
27614	WWTP Schonerlinde. Ozonation	LOQ (<4.1)	ng Ru486 eq./l water	4.1
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<4.2)	ng Ru486 eq./l water	4.2
PPARa CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	1000	ng GW7647 eq./ll water	29
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<29)	ng GW7647 eq./ll water	29
27614	WWTP Schonerlinde. Ozonation	LOQ (<40)	ng GW7647 eq./ll water	40
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<40)	ng GW7647 eq./ll water	40
PPARd CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	6700	ng L-165,041 eq./l water	1300
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<1200)	ng L-165,041 eq./l water	1200
27614	WWTP Schonerlinde. Ozonation	LOQ (<980)	ng L-165,041 eq./l water	980
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<1000)	ng L-165,041 eq./l water	1000
PPARg CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	1200	ng Rosiglitazone eq./l water	100
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<98)	ng Rosiglitazone eq./l water	98
27614	WWTP Schonerlinde. Ozonation	LOQ (<170)	ng Rosiglitazone eq./l water	170
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<170)	ng Rosiglitazone eq./l water	170
DR CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	780	pg 2,3,7,8 TCDD eq./l water	12
27613	WWTP Schonerlinde. Secondary sedimentation	340	pg 2,3,7,8 TCDD eq./l water	12
27614	WWTP Schonerlinde. Ozonation	100	pg 2,3,7,8 TCDD eq./l water	12
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	91	pg 2,3,7,8 TCDD eq./l water	12
PAH CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	1400	ng Benzo[a]pyrene eq./l water	0.9
27613	WWTP Schonerlinde. Secondary sedimentation	124	ng Benzo[a]pyrene eq./l water	0.9
27614	WWTP Schonerlinde. Ozonation	LOQ (<0.8)	ng Benzo[a]pyrene eq./l water	0.8
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	12	ng Benzo[a]pyrene eq./l water	0.8
PXR CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	130	ug Nicardipine eq./l water	8.7
27613	WWTP Schonerlinde. Secondary sedimentation	39	ug Nicardipine eq./l water	8.4
27614	WWTP Schonerlinde. Ozonation	13	ug Nicardipine eq./l water	10
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	21	ug Nicardipine eq./l water	10
Nrf2 CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	1700	ug Curcumine/l water	200
27613	WWTP Schonerlinde. Secondary sedimentation	280	ug Curcumine/l water	200
27614	WWTP Schonerlinde. Ozonation	240	ug Curcumine/l water	67
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	210	ug Curcumine/l water	68
P53 (-S9) CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	LOQ (<0.60)	ug Actinomycin D/l water	0.06
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<0.60)	ug Actinomycin D/l water	0.06
27614	WWTP Schonerlinde. Ozonation	LOQ (<0.02)	ug Actinomycin D/l water	0.02
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<0.02)	ug Actinomycin D/l water	0.02
P53 (+S9) CALUX				
27612	WWTP Schonerlinde. Primary sedimentation	7500	ug Cyclophosphamide/l water	2700
27613	WWTP Schonerlinde. Secondary sedimentation	LOQ (<2600)	ug Cyclophosphamide/l water	2600
27614	WWTP Schonerlinde. Ozonation	LOQ (<880)	ug Cyclophosphamide/l water	880
27615	WWTP Schonerlinde. Deeop-bed filter (sand/anthracite)	LOQ (<890)	ug Cyclophosphamide/l water	890

Table 4-m Quantified CALUX bioanalysis results site 13

Site 13

BDS no.	client code	Result	Unit	LOQ
Cytotox CALUX				
28141	S13-1	9.7	ug TBT eq./l water	1.1
28142	S13-2	3.9	ug TBT eq./l water	1.1
28143	S13-3	LOQ (<1.1)	ug TBT eq./l water	1.1
ERa CALUX				
28141	S13-1	19	ng 17b-estradiol eq./l water	0.097
28142	S13-2	0.62	ng 17b-estradiol eq./l water	0.095
28143	S13-3	0.17	ng 17b-estradiol eq./l water	0.11
anti-ERa CALUX				
28141	S13-1	LOQ (<1.8)	ug Tamoxifen eq./l water	1.8
28142	S13-2	LOQ (<1.7)	ug Tamoxifen eq./l water	1.7
28143	S13-3	LOQ (<1.6)	ug Tamoxifen eq./l water	1.6
AR CALUX				
28141	S13-1	200	ng DHT eq./l water	2.0
28142	S13-2	LOQ (<1.9)	ng DHT eq./l water	1.9
28143	S13-3	LOQ (<1.6)	ng DHT eq./l water	1.6
anti-AR CALUX				
28141	S13-1	120	ug Flutamide eq./l water	9.6
28142	S13-2	35	ug Flutamide eq./l water	9.0
28143	S13-3	LOQ (<9.4)	ug Flutamide eq./l water	9.4
GR CALUX				
28141	S13-1	360	ng Dexamethason eq./l water	23
28142	S13-2	LOQ (<22)	ng Dexamethason eq./l water	22
28143	S13-3	LOQ (<21)	ng Dexamethason eq./l water	21
anti-GR CALUX				
28141	S13-1	LOQ (<0.061)	ug Ru486 eq./l water	0.061
28142	S13-2	LOQ (<0.058)	ug Ru486 eq./l water	0.058
28143	S13-3	LOQ (<0.034)	ug Ru486 eq./l water	0.034
PR CALUX				
28141	S13-1	39	ng Org2058 eq./l water	3.5
28142	S13-2	LOQ (<3.3)	ng Org2058 eq./l water	3.3
28143	S13-3	LOQ (<1.3)	ng Org2058 eq./l water	1.3
anti-PR CALUX				
28141	S13-1	20.000	ng Ru486 eq./l water	6.3000
28142	S13-2	6.5	ng Ru486 eq./l water	5.9000
28143	S13-3	LOQ (<3.9)	ng Ru486 eq./l water	3.9
PPARa CALUX				
28141	S13-1	970	ng GW7647 eq./ll water	48
28142	S13-2	LOQ (<46)	ng GW7647 eq./ll water	46
28143	S13-3	LOQ (<9.0)	ng GW7647 eq./ll water	9
PPARd CALUX				
28141	S13-1	LOQ (<1300)	ng L-165,041 eq./l water	1300
28142	S13-2	LOQ (<1200)	ng L-165,041 eq./l water	1200
28143	S13-3	LOQ (<1400)	ng L-165,041 eq./l water	1400
PPARg CALUX				
28141	S13-1	LOQ (<94)	ng Rosiglitazone eq./l water	94
28142	S13-2	LOQ (<90)	ng Rosiglitazone eq./l water	90
28143	S13-3	LOQ (<45)	ng Rosiglitazone eq./l water	45
DR CALUX				
28141	S13-1	420	pg 2,3,7,8 TCDD eq./l water	13
28142	S13-2	41	pg 2,3,7,8 TCDD eq./l water	13
28143	S13-3	110	pg 2,3,7,8 TCDD eq./l water	13
PAH CALUX				
28141	S13-1	397	ng Benzo[a]pyrene eq./l water	2.1
28142	S13-2	54	ng Benzo[a]pyrene eq./l water	1.6
28143	S13-3	48	ng Benzo[a]pyrene eq./l water	2.6
PXR CALUX				
28141	S13-1	110	ug Nicardipine eq./l water	8.4
28142	S13-2	98	ug Nicardipine eq./l water	7.8
28143	S13-3	67	ug Nicardipine eq./l water	10
Nrf2 CALUX				
28141	S13-1	730	ug Curcumine/l water	47
28142	S13-2	LOQ (<45)	ug Curcumine/l water	45
28143	S13-3	130	ug Curcumine/l water	45
P53 (-S9) CALUX				
28141	S13-1	LOQ (<0.030)	ug Actinomycin D/l water	0.030
28142	S13-2	LOQ (<0.030)	ug Actinomycin D/l water	0.030
28143	S13-3	LOQ (<0.030)	ug Actinomycin D/l water	0.030
P53 (+S9) CALUX				
28141	S13-1	21000	ug Cyclophosphamide/l water	2200
28142	S13-2	LOQ (<2100)	ug Cyclophosphamide/l water	2100
28143	S13-3	LOQ (<2100)	ug Cyclophosphamide/l water	2100

Annex 5 Quantified CALUX bioanalysis results – round 2

Table 5-a CALUX bioanalysis results site 4

samplig campaign	date of sampling
1	12/03/2018
2	21/08/2018
3	08/10/2018

12/03/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	1.6	0.5	0.5	0.5	0.5	0.5
AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	2.5	2.4	0.5	0.5	0.5	0.5
ERa CALUX	0.5	0.5	1.8	0.5	0.5	0.5
GR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
anti-PR CALUX	18.8	1.3	0.5	0.5	0.5	0.5
PPARa2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5
PPARg2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5
PXR CALUX	4.6	3.5	4.0	1.9	1.5	1.7
Nrf2 CALUX	66.7	10.0	0.5	0.5	1.1	1.6
P53 CALUX (+S9)	0.5	0.5	0.5	0.5	0.5	0.5

21/08/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	0.5	0.5	0.5	0.5	0.5	0.5
AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
ERa CALUX	14.0	56.0	0.5	0.5	13.0	0.5
GR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
anti-PR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
PPARa2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5
PPARg2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5
PXR CALUX	3.9	3.5	1.9	0.5	1.2	0.5
Nrf2 CALUX	3	1.9	2.3	2.0	1.2	0.5
P53 CALUX (+S9)	3.9	0.5	3.9	0.5	0.5	0.5

08/10/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	0.5	0.5	0.5	0.5	0.5	0.5
AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
ERa CALUX	1.1	4.2	0.5	3.1	0.5	0.5
GR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
anti-PR CALUX	0.5	0.5	0.5	0.5	0.5	0.5
PPARa2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5
PPARg2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5
PXR CALUX	4.2	8.8	2.8	1.7	3.2	1.2
Nrf2 CALUX	1	5.9	0.5	1.2	0.5	1.9
P53 CALUX (+S9)						

Sample point	Client sample code	Results campaign 1	Results campaign 2	Results campaign 3	LOQ campagign 1	LOQ campagign 2	LOQ campagign 3
Cytotox CALUX (ug TBT eq./l water)							
S1	untreated water	1	<LOQ	<LOQ	0.63	0.66	0.66
S2	before high-rate filters	<LOQ	<LOQ	<LOQ	0.6	0.66	0.66
S3	after high-rate filters	<LOQ	<LOQ	<LOQ	0.6	0.51	0.71
S4	after ozonation	<LOQ	<LOQ	<LOQ	0.64	0.51	0.71
S5	after carbon filters	<LOQ	<LOQ	<LOQ	0.65	0.5	0.69
S6	after disinfection	<LOQ	<LOQ	<LOQ	0.62	0.49	0.69
AR CALUX (ng DHT eq./l water)							
S1	untreated water	<LOQ	<LOQ	<LOQ	0.99	2.4	0.7
S2	before high-rate filters	<LOQ	<LOQ	<LOQ	0.39	2.4	0.7
S3	after high-rate filters	<LOQ	<LOQ	<LOQ	0.39	2.3	1.4
S4	after ozonation	<LOQ	<LOQ	<LOQ	0.52	2.3	1.4
S5	after carbon filters	<LOQ	<LOQ	<LOQ	0.52	1.6	1.3
S6	after disinfection	<LOQ	<LOQ	<LOQ	0.48	1.6	1.3
anti-AR CALUX (ug Flutamide eq./l water)							
S1	untreated water	18	<LOQ	<LOQ	7.3	4.4	8.8
S2	before high-rate filters	13	<LOQ	<LOQ	5.5	4.4	8.8
S3	after high-rate filters	<LOQ	<LOQ	<LOQ	5.6	5.6	7.8
S4	after ozonation	<LOQ	<LOQ	<LOQ	5.6	5.6	7.8
S5	after carbon filters	<LOQ	<LOQ	<LOQ	5.7	5.8	7.6
S6	after disinfection	<LOQ	<LOQ	<LOQ	5.1	5.7	7.6
ERa CALUX (ng 17b Estradiol eq./l water)							
S1	untreated water	<LOQ	0.77	0.073	0.06	0.057	0.065
S2	before high-rate filters	<LOQ	3.2	0.28	0.077	0.057	0.066
S3	after high-rate filters	0.14	<LOQ	<LOQ	0.077	0.057	0.064
S4	after ozonation	<LOQ	<LOQ	0.2	0.072	0.057	0.065
S5	after carbon filters	<LOQ	0.76	<LOQ	0.073	0.057	0.074
S6	after disinfection	<LOQ	<LOQ	<LOQ	0.044	0.057	0.074
GR CALUX (ng Dexamethasone eq./l water)							
S1	untreated water	<LOQ	<LOQ	<LOQ	17	9.1	9.7
S2	before high-rate filters	<LOQ	<LOQ	<LOQ	10	9.2	9.7
S3	after high-rate filters	<LOQ	<LOQ	<LOQ	10	9.2	12
S4	after ozonation	<LOQ	<LOQ	<LOQ	14	9.2	12
S5	after carbon filters	<LOQ	<LOQ	<LOQ	14	9.5	12
S6	after disinfection	<LOQ	<LOQ	<LOQ	10	9.4	12
anti-PR CALUX (ng Ru486 eq./l water)							
S1	untreated water	62	<LOQ	<LOQ	3.3	3.5	2
S2	before high-rate filters	4.8	<LOQ	<LOQ	3.8	3.5	2
S3	after high-rate filters	<LOQ	<LOQ	<LOQ	3.8	3.1	1.2
S4	after ozonation	<LOQ	<LOQ	<LOQ	3.2	3.2	1.2
S5	after carbon filters	<LOQ	<LOQ	<LOQ	3.2	2.6	1.9
S6	after disinfection	<LOQ	<LOQ	<LOQ	3.4	2.5	1.9
PPARa CALUX (ng GW7647 eq./l water)							
S1	untreated water	<LOQ	<LOQ	<LOQ	21	21	15
S2	before high-rate filters	<LOQ	<LOQ	<LOQ	17	21	15
S3	after high-rate filters	<LOQ	<LOQ	<LOQ	17	15	15
S4	after ozonation	<LOQ	<LOQ	<LOQ	13	15	19
S5	after carbon filters	<LOQ	<LOQ	<LOQ	13	23	21
S6	after disinfection	<LOQ	<LOQ	<LOQ	23	23	21
PPARg CALUX (ng Rosiglitazone eq./l water)							
S1	untreated water	<LOQ	<LOQ	<LOQ	170	200	340
S2	before high-rate filters	<LOQ	<LOQ	<LOQ	410	200	340
S3	after high-rate filters	<LOQ	<LOQ	<LOQ	410	300	300
S4	after ozonation	<LOQ	<LOQ	<LOQ	380	300	300
S5	after carbon filters	<LOQ	<LOQ	<LOQ	380	210	260
S6	after disinfection	<LOQ	<LOQ	<LOQ	140	210	260
PXR CALUX (ug Nicardipine eq./l water)							
S1	untreated water	17	24	23	3.7	6.1	5.5
S2	before high-rate filters	35	22	23	10	6.2	2.6
S3	after high-rate filters	40	16	15	10	8.6	5.3
S4	after ozonation	13	<LOQ	10	6.9	8.8	6
S5	after carbon filters	10	6.4	20	6.5	5.4	6.3
S6	after disinfection	8.5	<LOQ	7.2	5.1	8.8	6.1
Nrf2 CALUX (ug Curcumine eq./l water)							
S1	untreated water	1000	74	<LOQ	15	30	22
S2	before high-rate filters	140	59	130	14	31	22
S3	after high-rate filters	<LOQ	70	<LOQ	15	30	22
S4	after ozonation	<LOQ	60	26	14	30	22
S5	after carbon filters	15	37	<LOQ	14	30	22
S6	after disinfection	22	LOQ (<30)	42	14	30	22
P53 (+S9) CALUX (ug Cyclophosphamide/l water)							
S1	untreated water	<LOQ	1900	pending	1300	486.98	pending
S2	before high-rate filters	<LOQ	<LOQ	pending	420	490.27	pending
S3	after high-rate filters	<LOQ	1900	pending	420	482.97	pending
S4	after ozonation	<LOQ	<LOQ	pending	420	485.18	pending
S5	after carbon filters	<LOQ	<LOQ	pending	420	487.8	pending
S6	after disinfection	<LOQ	<LOQ	pending	420	484.08	pending

Table 5-b CALUX bioanalysis results site 6

samplig campaign	date of sampling
1	15/08/2017
2	21/11/2017
3	19/03/2018

19/03/2018	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Cytotox CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	3.1	0.5	0.5	0.5
ERa CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
GR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
anti-PR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	4.4	0.5	0.5	0.5
PPARa2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PPARg2 CALUX	0.5	0.5	0.5	0.5	8.0	0.5	0.5	0.5	0.5	0.5
PXR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nrf2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
P53 CALUX (+S9)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

19/03/2018	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Cytotox CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
ERa CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
GR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
anti-PR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PPARa2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PPARg2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PXR CALUX	0.5	0.5	0.5	0.5	0.5	1.1	1.3	0.5	0.5	0.5
Nrf2 CALUX	0.5	2.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
P53 CALUX (+S9)	0.5	0.5	12	0.5	0.5	0.5	2.7	0.5	0.5	0.5

19/03/2018	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Cytotox CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
ERa CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
GR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
anti-PR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PPARa2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PPARg2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PXR CALUX	0.5	0.5	0.5	0.5	1.1	0.5	1.1	0.5	0.5	0.5
Nrf2 CALUX	19	2.6	0.5	2.6	2.9	1.1	2.4	1.6	0.5	2.7
P53 CALUX (+S9)	0.5	0.5	1	0.5	2.4	0.5	0.5	10	0.5	0.5

Sample point	Client sample code	Results campaign 1	Results campaign 2	Results campaign 3	LOQ campagign 1	LOQ campagign 2	LOQ campagign 3
Cytotox CALUX (ug TBT eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	0.47	0.53	0.59
S2	RF-n	<LOQ	<LOQ	<LOQ	0.48	0.53	0.59
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	0.51	0.59	0.55
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	0.5	0.61	0.56
S5	RF-v-B4	<LOQ	<LOQ	<LOQ	0.48	0.57	0.8
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	0.46	0.56	0.82
S7	WF-v	<LOQ	<LOQ	<LOQ	0.49	0.44	0.49
S8	WF-n	<LOQ	<LOQ	<LOQ	0.48	0.44	0.51
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	0.48	0.45	0.57
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	0.49	0.47	0.59
AR CALUX (ng DHT eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	0.61	1.2	2.5
S2	RF-n	<LOQ	<LOQ	<LOQ	0.62	1.2	2.5
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	0.67	1.8	3.3
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	0.65	1.8	3.3
S5	RF-v-B4	<LOQ	<LOQ	<LOQ	0.62	2.9	2.4
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	0.6	2.9	2.5
S7	WF-v	<LOQ	<LOQ	<LOQ	0.63	1	1.9
S8	WF-n	<LOQ	<LOQ	<LOQ	0.63	1.1	2
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	0.63	1.4	2
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	0.64	1.5	2.1
anti-AR CALUX (ug Flutamide eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	4.4	6.4	1.9
S2	RF-n	<LOQ	<LOQ	<LOQ	4.4	6.5	1.9
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	4.8	5.9	1.4
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	4.6	6.1	1.4
S5	RF-v-B4	<LOQ	<LOQ	<LOQ	4.4	6.4	1.9
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	4.3	6.3	2
S7	WF-v	14	<LOQ	<LOQ	4.5	5.3	2.3
S8	WF-n	<LOQ	<LOQ	<LOQ	4.5	5.4	2.4
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	4.5	7.6	1.6
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	4.6	8	1.7
ERa CALUX (ng 17b Estradiol eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	0.1	0.064	0.11
S2	RF-n	<LOQ	<LOQ	<LOQ	0.1	0.065	0.11
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	0.11	0.049	0.085
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	0.11	0.051	0.087
S5	RF-v-B4	<LOQ	<LOQ	<LOQ	0.11	0.06	0.075
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	0.1	0.059	0.077
S7	WF-v	<LOQ	<LOQ	<LOQ	0.11	0.044	0.094
S8	WF-n	<LOQ	<LOQ	<LOQ	0.11	0.045	0.098
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	0.11	0.073	0.065
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	0.11	0.076	0.067
GR CALUX (ng Dexamethasone eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	13.21	40	27
S2	RF-n	<LOQ	<LOQ	<LOQ	13.43	41	27
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	14.36	22	32
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	14	23	33
S5	RF-v-B4	<LOQ	<LOQ	<LOQ	13.36	50	30
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	12.87	50	31
S7	WF-v	<LOQ	<LOQ	<LOQ	13.59	35	27
S8	WF-n	<LOQ	<LOQ	<LOQ	13.52	35	28
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	13.46	57	36
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	13.85	60	37
anti-PR CALUX (ng Ru486 eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	2.4	3	3.9
S2	RF-n	<LOQ	<LOQ	<LOQ	2.4	3	3.9
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	2.3	3.1	3.9
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	2.2	3.2	4
S5	RF-v-B4	<LOQ	<LOQ	<LOQ	2	2.9	3.6
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	2	2.9	3.7
S7	WF-v	4.8	<LOQ	<LOQ	1.1	3.3	3.5
S8	WF-n	<LOQ	<LOQ	<LOQ	1.1	3.3	3.7
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	5.1	2.8	3.4
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	5.3	2.9	3.5
PPARa CALUX (ng GW7647 eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	57.27	13	10
S2	RF-n	<LOQ	<LOQ	<LOQ	58.24	13	10
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	62.25	18	13
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	60.72	18	13
S5	RF-v-B4	<LOQ	<LOQ	<LOQ	57.93	14	16
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	55.82	14	17
S7	WF-v	<LOQ	<LOQ	<LOQ	58.95	17	10
S8	WF-n	<LOQ	<LOQ	<LOQ	58.62	17	11
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	58.39	23	12
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	60.07	24	12
PPARg CALUX (ng Rosiglitazone eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	134.29	94	460
S2	RF-n	<LOQ	<LOQ	<LOQ	143.55	95	460
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	140	110	350
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	133.59	110	360
S5	RF-v-B4	1030	<LOQ	<LOQ	128.7	110	460
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	135.92	110	480
S7	WF-v	<LOQ	<LOQ	<LOQ	135.18	94	660
S8	WF-n	<LOQ	<LOQ	<LOQ	134.63	95	690
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	138.51	110	780
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	140.24	110	800
PXR CALUX (ug Nicardipine eq./l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	31.5	5.4	5.6
S2	RF-n	<LOQ	<LOQ	<LOQ	32	5.4	5.5
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	34.2	6.3	6.5
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	33.4	6.5	6.6
S5	RF-v-B4	<LOQ	<LOQ	5.7	31.9	5.8	5
S6	RF-n-B4	<LOQ	6.2	<LOQ	30.7	5.7	5.2
S7	WF-v	<LOQ	6.9	4	32.4	5.4	3.8
S8	WF-n	<LOQ	<LOQ	<LOQ	32.2	5.4	4
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	32.1	6	5.4
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	33	6.3	5.5
Nrf2 CALUX (ug Curcumine eq./l water)							
S1	RF-v	<LOQ	<LOQ	335	44	22	18
S2	RF-n	<LOQ	55	46	44	22	18
S3	RF-v-AK3	<LOQ	<LOQ	<LOQ	47	22	19
S4	RF-n-AK3	<LOQ	<LOQ	50	46	22	19
S5	RF-v-B4	<LOQ	<LOQ	52	44	22	18
S6	RF-n-B4	<LOQ	<LOQ	21	43	22	19
S7	WF-v	<LOQ	<LOQ	41	45	21	17
S8	WF-n	<LOQ	<LOQ	29	45	22	18
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	45	22	18
S10	WF-n-AK3	<LOQ	<LOQ	52	46	23	19
P53 (+S9) CALUX (ug Cyclophosphamide/l water)							
S1	RF-v	<LOQ	<LOQ	<LOQ	620	410	750
S2	RF-n	<LOQ	<LOQ	<LOQ	630	410	750
S3	RF-v-AK3	<LOQ	5000	<LOQ	680	410	760
S4	RF-n-AK3	<LOQ	<LOQ	<LOQ	660	420	780
S5	RF-v-B4	<LOQ	<LOQ	1800	630	420	750
S6	RF-n-B4	<LOQ	<LOQ	<LOQ	610	420	770
S7	WF-v	<LOQ	1100	<LOQ	640	410	710
S8	WF-n	<LOQ	<LOQ	7400	640	410	740
S9	WF-v-AK3	<LOQ	<LOQ	<LOQ	630	420	740
S10	WF-n-AK3	<LOQ	<LOQ	<LOQ	650	440	760

AquaNES

Table 5-c CALUX bioanalysis results site 7

Sampling campaign	date of sampling
1	13/03/2018
2	17/12/2018
3	19/03/2019

13/03/2018	S1	S2	S3	S4	S5	S6	S7	S8	S9
Cytotox CALUX	1.3					0.5	0.5	0.5	
AR CALUX	0.5					0.5	0.5	0.5	
anti-AR CALUX	2.1					0.5	0.5	0.5	
ERa CALUX	43.8					0.5	0.5	0.5	
GR CALUX	5.5					3.2	0.5	0.5	
anti-PR CALUX	1.2					0.5	0.5	0.5	
PPARa2 CALUX	0.5					0.5	0.5	0.5	
PPARg2 CALUX	1.2					0.5	0.5	0.5	
PXR CALUX	10.4					3.9	3.4	3.2	
Nrf2 CALUX	6.3					5.6	1.3	0.5	
P53 CALUX (+S9)	0.5					0.5	0.5	0.5	

18/12/2018	S1	S6	S7	S8	S4	S5	S2	S3	S9
Cytotox CALUX	2.1	1.5	1.6	1.1	0.5	0.5	0.5		
AR CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5		
anti-AR CALUX	3.9	4.0	2.6	3.1	1.3	0.5	1.1		
ERa CALUX	46.0	46.2	39.4	19	0.5	0.5	2.7		
GR CALUX	19.8	13.7	15.2	14	1.4	2.7	0.5		
anti-PR CALUX	5.3	4.7	3.6	3.2	0.5	1.3	0.5		
PPARa2 CALUX	0.5	0.5	0.5	0.5	0.5	0.5	0.5		
PPARg2 CALUX	1.6	1.2	1.0	0.5	0.5	0.5	0.5		
PXR CALUX	10.8	13.1	8.8	13	3.4	3.5	2.6		
Nrf2 CALUX	10.0	8.8	8.8	9.6	2.8	5.9	1.2		
P53 CALUX (+S9)									

19/03/2019	S1	S6	S7	S8	S4	S5	S2	S3	S9
Cytotox CALUX	0.5		0.5	0.5	0.5	0.5	0.5	0.5	0.5
AR CALUX	0.5		0.5	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	0.5		1.1	2.1	0.5	0.5	0.5	0.5	0.5
ERa CALUX	32.5		0.5	63	0.5	0.5	0.5	0.5	0.5
GR CALUX	5.6		0.5	5.1	0.5	1.1	0.5	0.5	1.5
anti-PR CALUX	1.7		1.0	3.5	1.2	4.1	0.5	0.5	0.5
PPARa2 CALUX	0.5		0.5	0.5	0.5	2.6	1.4	0.5	0.5
PPARg2 CALUX	1.0		0.5	1.0	0.5	0.5	0.5	0.5	0.5
PXR CALUX	14		1.6	11	2.2	3.1	0.5	1.7	2.7
Nrf2 CALUX	8.0		6.7	9.0	4.5	5.5	2.4	0.5	3.8
P53 CALUX (+S9)									

Sample point	Client sample code	Results campaign 1	Results campaign 2	Results campaign 3	LOQ campaign 1	LOQ campaign 2	LOQ campaign 3
Cytotox CALUX (ug TBT eq./l water)							
S1	OZA500	0.88	1.0	<LOQ	0.34	0.24	0.25
S2	OZFTEP		0.69			0.23	
S3	OZPTEP		0.66	<LOQ		0.21	0.24
S4	OZAFTA		0.5	<LOQ		0.23	0.25
S5	OZBACT		<LOQ	<LOQ		0.21	0.34
S6	OZAITA	<LOQ	<LOQ	<LOQ	0.33	0.23	0.34
S7	OZOOB1	<LOQ	<LOQ	<LOQ	0.33	0.23	0.25
S8	OZOOB3	<LOQ		<LOQ	0.32		0.23
S9	OZOAUF			<LOQ			0.25
AR CALUX (ng DHT eq./l water)							
S1	OZA500	<LOQ	<LOQ	<LOQ	0.26	0.45	0.60
S2	OZFTEP		<LOQ			0.30	
S3	OZPTEP		<LOQ	<LOQ		0.46	0.75
S4	OZAFTA		<LOQ	<LOQ		0.43	0.60
S5	OZBACT		<LOQ	<LOQ		0.46	0.75
S6	OZAITA	<LOQ	<LOQ	<LOQ	0.23	0.50	0.75
S7	OZOOB1	<LOQ	<LOQ	<LOQ	0.23	0.30	0.85
S8	OZOOB3	<LOQ		<LOQ	0.28		0.75
S9	OZOAUF			<LOQ			0.85
anti-AR CALUX (ug Flutamide eq./l water)							
S1	OZA500	11	29	<LOQ	2.6	3.8	3.2
S2	OZFTEP		27			3.4	
S3	OZPTEP		11	6.6		2.2	3.0
S4	OZAFTA		22	14		3.5	3.4
S5	OZBACT		9.4	<LOQ		3.5	2.1
S6	OZAITA	<LOQ	<LOQ	<LOQ	2.9	3.9	2.1
S7	OZOOB1	<LOQ	7	<LOQ	2.9	3.3	1.6
S8	OZOOB3	<LOQ		<LOQ	2.6		2.9
S9	OZOAUF			<LOQ			1.6
ERa CALUX (ng 17b Estradiol eq./l water)							
S1	OZA500	2.1	2.2	2.5	0.024	0.024	0.039
S2	OZFTEP		1.9			0.021	
S3	OZPTEP		1.7	<LOQ		0.022	0.038
S4	OZAFTA		0.83	5		0.022	0.040
S5	OZBACT		<LOQ	<LOQ		0.026	0.037
S6	OZAITA	<LOQ	<LOQ	<LOQ	0.048	0.029	0.037
S7	OZOOB1	<LOQ	0.11	<LOQ	0.048	0.020	0.034
S8	OZOOB3	<LOQ		<LOQ	0.024		0.036
S9	OZOAUF			<LOQ			0.034
GR CALUX (ng Dexamethasone eq./l water)							
S1	OZA500	72	130	73	6.5	3.3	6.5
S2	OZFTEP		93			3.4	
S3	OZPTEP		98	<LOQ		3.2	7.5
S4	OZAFTA		85	66		3.1	6.5
S5	OZBACT		8.8	<LOQ		3.1	14.5
S6	OZAITA	38	18	33	6.0	3.4	14.5
S7	OZOOB1	<LOQ	<LOQ	<LOQ	6.0	3.4	6.5
S8	OZOOB3	<LOQ		<LOQ	6.0		7.0
S9	OZOAUF			19			6.5
anti-PR CALUX (ng Ru486 eq./l water)							
S1	OZA500	4.6	5.9	2.7	1.9	0.56	0.80
S2	OZFTEP		3.5			0.37	
S3	OZPTEP		3.1	1.4		0.42	0.70
S4	OZAFTA		3.4	6		0.53	0.85
S5	OZBACT		<LOQ	1.1		0.65	0.48
S6	OZAITA	<LOQ	1.9	3.9	1.8	0.73	0.48
S7	OZOOB1	<LOQ	<LOQ	<LOQ	1.8	0.38	0.60
S8	OZOOB3	<LOQ		<LOQ	1.6		0.70
S9	OZOAUF			<LOQ			0.60
PPARa CALUX (ng GW7647 eq./l water)							
S1	OZA500	<LOQ	<LOQ	<LOQ	13	4.6	14
S2	OZFTEP		<LOQ			7.5	
S3	OZPTEP		<LOQ	<LOQ		6.5	5.5
S4	OZAFTA		<LOQ	<LOQ		4.3	14
S5	OZBACT		<LOQ	<LOQ		4.2	7.0
S6	OZAITA	<LOQ	<LOQ	37	7.0	4.6	7.0
S7	OZOOB1	<LOQ	<LOQ	31	7.0	7.0	11
S8	OZOOB3	<LOQ		<LOQ	16		5.5
S9	OZOAUF			<LOQ			11
PPARg CALUX (ng Rosiglitazone eq./l water)							
S1	OZA500	180	140	470	75	45	225
S2	OZFTEP		84			35	
S3	OZPTEP		52	<LOQ		26	115
S4	OZAFTA		<LOQ	480		41	235
S5	OZBACT		<LOQ	<LOQ		29	150
S6	OZAITA	<LOQ	<LOQ	<LOQ	60	32	150
S7	OZOOB1	<LOQ	<LOQ	<LOQ	60	35	155
S8	OZOOB3	<LOQ		<LOQ	240		115
S9	OZOAUF			<LOQ			155
PXR CALUX (ug Nicardipine eq./l water)							
S1	OZA500	57	36	87	2.8	1.7	3.1
S2	OZFTEP		44			1.7	
S3	OZPTEP		27	11		1.5	3.5
S4	OZAFTA		39	70		1.6	3.2
S5	OZBACT		13	13		1.9	2.9
S6	OZAITA	36	15	18	4.6	2.2	3.0
S7	OZOOB1	32	8.7	<LOQ	4.7	1.7	3.3
S8	OZOOB3	16		11	2.5		3.3
S9	OZOAUF			17			3.2
Nrf2 CALUX (ug Curcumine eq./l water)							
S1	OZA500	94	260	160	7.5	13	10
S2	OZFTEP		220			13	
S3	OZPTEP		220	140		13	11
S4	OZAFTA		240	190		13	11
S5	OZBACT		70	89		13	10
S6	OZAITA	84	160	110	7.5	14	10
S7	OZOOB1	19	30	50	7.5	13	11
S8	OZOOB3	<LOQ		<LOQ	8.0		11
S9	OZOAUF			76			10
P53 (+S9) CALUX (ug Cyclophosphamide/l water)							
S1	OZA500	<LOQ	pending	pending	650	pending	pending
S2	OZFTEP		pending			pending	
S3	OZPTEP		pending	pending		pending	pending
S4	OZAFTA		pending	pending		pending	pending
S5	OZBACT		pending	pending		pending	pending
S6	OZAITA	<LOQ	pending	pending	225	pending	pending
S7	OZOOB1	<LOQ	pending	pending	225	pending	pending
S8	OZOOB3	<LOQ		pending	225		pending
S9	OZOAUF			pending			pending

Table 5-d CALUX bioanalysis results site 8

samplig campaign	of sampling
1	11/04/2018
2	30/04/2018
3	22/10/2018
4	19/10-26/11/2018

11/04/2018	S1	S2	S4	S5-1	S5-2	S5-3	S5-4	S5-1	S5-1	S5-1	S5-1	S5-1
Cytotox CALUX	3.8	0.5		0.5	0.5							
AR CALUX	140.0	0.5		0.5	0.5							
anti-AR CALUX	0.5	0.5		0.5	0.5							
ERa CALUX	382.7	12.0		3.4	1.4							
GR CALUX	1.6	1.4		0.5	0.5							
anti-PR CALUX	8.5	0.5		0.5	0.5							
PPARa2 CALUX	1.0	1.2		0.5	0.5							
PPARg2 CALUX	1.4	0.5		0.5	0.5							
PXR CALUX	3.6	4.5		1.9	3.2							
Nrf2 CALUX	14.2	6.1		3.2	2.9							
P53 CALUX (+S9)	1.5	0.5		0.5	0.5							

30/04/2018	S1	S2	S4	S5-1	S5-2	S5-3	S5-4	S5-1	S5-1	S5-1	S5-1	S5-1
Cytotox CALUX			0.5			0.5	0.5					
AR CALUX			0.5			0.5	0.5					
anti-AR CALUX			1.2			0.5	0.5					
ERa CALUX			3.6			1.1	0.5					
GR CALUX			0.5			0.5	0.5					
anti-PR CALUX			0.5			0.5	0.5					
PPARa2 CALUX			0.5			0.5	0.5					
PPARg2 CALUX			0.5			0.5	0.5					
PXR CALUX			4.9			4.0	2.5					
Nrf2 CALUX			5.7			3.7	3.0					
P53 CALUX (+S9)			0.5			0.5	0.5					

22/10/2018	S1	S2	S4	S5-1	S5-2	S5-3	S5-4	S5-1	S5-1	S5-1	S5-1	S5-1
Cytotox CALUX	0.5		0.5	0.5	0.5	0.5	0.5					
AR CALUX	0.5		0.5	0.5	0.5	0.5	0.5					
anti-AR CALUX	0.5		4.5	0.5	0.5	0.5	0.5					
ERa CALUX	16.3		3.5	0.5	1.8	1.5	1.3					
GR CALUX	1.3		0.5	0.5	0.5	0.5	0.5					
anti-PR CALUX	0.5		0.5	0.5	1.2	0.5	0.5					
PPARa2 CALUX	0.5		0.5	0.5	0.5	0.5	0.5					
PPARg2 CALUX	0.5		0.5	0.5	0.5	0.5	0.5					
PXR CALUX	3.8		3.9	1.2	1.2	3.1	3.4					
Nrf2 CALUX	3.9		4	0.5	0.5	1.9	6.0					
P53 CALUX (+S9)												

19/10-26/11/2018	S1	S2	S4	S5-1	S5-2	S5-3	S5-4	S5-1	S5-1	S5-1	S5-1	S5-1
Cytotox CALUX								0.5	0.5	0.5	0.5	0.5
AR CALUX								0.5	0.5	0.5	0.5	0.5
anti-AR CALUX								0.5	0.5	0.5	0.5	0.5
ERa CALUX								0.5	0.5	0.5	1.1	1.9
GR CALUX								0.5	0.5	0.5	0.5	0.5
anti-PR CALUX								0.5	0.5	0.5	0.5	0.5
PPARa2 CALUX								0.5	0.5	0.5	3.4	0.5
PPARg2 CALUX								0.5	0.5	0.5	0.5	0.5
PXR CALUX								0.5	0.5	0.5	2.1	1.2
Nrf2 CALUX								2.0	2.8	2.0	2.2	1.7
P53 CALUX (+S9)												

Sample point	Client sample code	Results campaign				LOQ campaign			
		1	2	3	4	1	2	3	4
Cytotox CALUX (ug TBT eq./l water)									
S1	Inlet WWTP	3				0.78			
S2	Outlet WWTP	<LOQ		<LOQ		0.84		0.63	
S4	Golf pond		<LOQ	<LOQ			0.56	0.93	
S5-1	NP1 Sand Dune Aquifer	<LOQ		<LOQ		0.7		1.9	
S5-2	NP2 Sand Dune Aquifer	<LOQ		<LOQ		0.69		0.76	
S5-3	NP3 Sand Dune Aquifer		<LOQ	<LOQ			0.67	0.67	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	<LOQ			0.52	0.69	
S5-1	NP1-T6				<LOQ				0.84
S5-1	NP1-T13				<LOQ				1.1
S5-1	NP1-T20				<LOQ				1.0
S5-1	NP1-T27				<LOQ				0.97
S5-1	NP1-T34				<LOQ				0.85
AR CALUX (ng DHT eq./l water)									
S1	Inlet WWTP	190				1.4			
S2	Outlet WWTP	<LOQ		<LOQ		1.4		2	
S4	Golf pond		<LOQ	<LOQ			3.8	4.1	
S5-1	NP1 Sand Dune Aquifer	<LOQ		<LOQ		1.4		2	
S5-2	NP2 Sand Dune Aquifer	<LOQ		<LOQ		1.4		3.4	
S5-3	NP3 Sand Dune Aquifer		<LOQ	<LOQ			2.3	2.5	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	<LOQ			3.5	2.5	
S5-1	NP1-T6				<LOQ				0.48
S5-1	NP1-T13				<LOQ				0.67
S5-1	NP1-T20				<LOQ				5.0
S5-1	NP1-T27				<LOQ				4.7
S5-1	NP1-T34				<LOQ				0.41
anti-AR CALUX (ug Flutamide eq./l water)									
S1	Inlet WWTP	<LOQ				3.1			
S2	Outlet WWTP	<LOQ		<LOQ		3.2		1.8	
S4	Golf pond		5.8	9			4.9	2	
S5-1	NP1 Sand Dune Aquifer	<LOQ		<LOQ		2.1		1.8	
S5-2	NP2 Sand Dune Aquifer	<LOQ		<LOQ		2.1		1.6	
S5-3	NP3 Sand Dune Aquifer		<LOQ	<LOQ			3	4.2	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	<LOQ			4.6	4.3	
S5-1	NP1-T6				<LOQ				13
S5-1	NP1-T13				<LOQ				18
S5-1	NP1-T20				<LOQ				16
S5-1	NP1-T27				<LOQ				16
S5-1	NP1-T34				<LOQ				11
ERa CALUX (ng 17b Estradiol eq./l water)									
S1	Inlet WWTP	31				0.081			
S2	Outlet WWTP	1		0.88		0.083		0.054	
S4	Golf pond		0.32	0.25			0.09	0.071	
S5-1	NP1 Sand Dune Aquifer	0.34		<LOQ		0.1		0.054	
S5-2	NP2 Sand Dune Aquifer	0.13		0.11		0.096		0.062	
S5-3	NP3 Sand Dune Aquifer		0.11	0.091			0.1	0.059	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	0.082			0.084	0.061	
S5-1	NP1-T6				<LOQ				0.046
S5-1	NP1-T13				<LOQ				0.064
S5-1	NP1-T20				<LOQ				0.060
S5-1	NP1-T27				0.061				0.058
S5-1	NP1-T34				0.095				0.050
GR CALUX (ng Dexamethasone eq./l water)									
S1	Inlet WWTP	56				34			
S2	Outlet WWTP	53		12		37		9.3	
S4	Golf pond		<LOQ	<LOQ			32	14	
S5-1	NP1 Sand Dune Aquifer	<LOQ		<LOQ		36		9.1	
S5-2	NP2 Sand Dune Aquifer	<LOQ		<LOQ		35		11	
S5-3	NP3 Sand Dune Aquifer		<LOQ	<LOQ			23	13	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	<LOQ			30	14	
S5-1	NP1-T6				<LOQ				7.6
S5-1	NP1-T13				<LOQ				11
S5-1	NP1-T20				<LOQ				12
S5-1	NP1-T27				<LOQ				12
S5-1	NP1-T34				<LOQ				11
anti-PR CALUX (ng Ru486 eq./l water)									
S1	Inlet WWTP	28				3.3			
S2	Outlet WWTP	<LOQ		<LOQ		3.5		1.8	
S4	Golf pond		<LOQ	<LOQ			2.8	3.9	
S5-1	NP1 Sand Dune Aquifer	<LOQ		<LOQ		4		1.8	
S5-2	NP2 Sand Dune Aquifer	<LOQ		3.7		3.9		3.2	
S5-3	NP3 Sand Dune Aquifer		<LOQ	<LOQ			3.3	1.6	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	<LOQ			2.7	1.6	
S5-1	NP1-T6				<LOQ				1.7
S5-1	NP1-T13				<LOQ				2.3
S5-1	NP1-T20				<LOQ				3.1
S5-1	NP1-T27				<LOQ				2.9
S5-1	NP1-T34				<LOQ				1.9
PPARa CALUX (ng GW7647 eq./l water)									
S1	Inlet WWTP	21				21			
S2	Outlet WWTP	27		<LOQ		22		9.4	
S4	Golf pond		<LOQ	<LOQ			31	14	
S5-1	NP1 Sand Dune Aquifer	<LOQ		<LOQ		15		9.4	
S5-2	NP2 Sand Dune Aquifer	<LOQ		<LOQ		15		11	
S5-3	NP3 Sand Dune Aquifer		<LOQ	<LOQ			21	23	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	<LOQ			29	24	
S5-1	NP1-T6				<LOQ				16
S5-1	NP1-T13				<LOQ				22
S5-1	NP1-T20				<LOQ				17
S5-1	NP1-T27				55				16
S5-1	NP1-T34				<LOQ				14
PPARg CALUX (ng Rosiglitazone eq./l water)									
S1	Inlet WWTP	500				360			
S2	Outlet WWTP	<LOQ		<LOQ		380		280	
S4	Golf pond		<LOQ	<LOQ			120	530	
S5-1	NP1 Sand Dune Aquifer	<LOQ		<LOQ		280		280	
S5-2	NP2 Sand Dune Aquifer	<LOQ		<LOQ		270		440	
S5-3	NP3 Sand Dune Aquifer		<LOQ	<LOQ			90	390	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	<LOQ			110	400	
S5-1	NP1-T6				<LOQ				140
S5-1	NP1-T13				<LOQ				190
S5-1	NP1-T20				<LOQ				110
S5-1	NP1-T27				<LOQ				110
S5-1	NP1-T34				<LOQ				120
PXR CALUX (ug Nicardipine eq./l water)									
S1	Inlet WWTP	32				8.8			
S2	Outlet WWTP	42		27		9.3		7.2	
S4	Golf pond		34	36			7	9.3	
S5-1	NP1 Sand Dune Aquifer	15		8.6		7.8		7.3	
S5-2	NP2 Sand Dune Aquifer	24		9.2		7.6		7.6	
S5-3	NP3 Sand Dune Aquifer		27	22			6.7	7	
S5-4	FRE4 Sand Dune Aquifer		16	25			6.5	7.4	
S5-1	NP1-T6				9.8				4.9
S5-1	NP1-T13				19				6.7
S5-1	NP1-T20				13				6.5
S5-1	NP1-T27				14				6.2
S5-1	NP1-T34				12				6.9
Nrf2 CALUX (ug Curcumine eq./l water)									
S1	Inlet WWTP	440				31			
S2	Outlet WWTP	202		200		33		51	
S4	Golf pond		200	256			35	68	
S5-1	NP1 Sand Dune Aquifer	103		<LOQ		32		51	
S5-2	NP2 Sand Dune Aquifer	89		<LOQ		31		56	
S5-3	NP3 Sand Dune Aquifer		110	106			30	55	
S5-4	FRE4 Sand Dune Aquifer		99	335			33	56	
S5-1	NP1-T6				<LOQ				43
S5-1	NP1-T13				<LOQ				59
S5-1	NP1-T20				<LOQ				54
S5-1	NP1-T27				108				51
S5-1	NP1-T34				53				46
P53 (+S9) CALUX (ug Cyclophosphamide/l water)									
S1	Inlet WWTP	1100				710			
S2	Outlet WWTP	<LOQ		pending		760		pending	
S4	Golf pond		<LOQ	pending			500	pending	
S5-1	NP1 Sand Dune Aquifer	<LOQ		pending		740		pending	
S5-2	NP2 Sand Dune Aquifer	<LOQ		pending		720		pending	
S5-3	NP3 Sand Dune Aquifer		<LOQ	pending			420	pending	
S5-4	FRE4 Sand Dune Aquifer		<LOQ	pending			460	pending	
S5-1	NP1-T6				pending				pending
S5-1	NP1-T13				pending				pending
S5-1	NP1-T20				pending				pending
S5-1	NP1-T27				pending				pending
S5-1	NP1-T34				pending				pending

Table 5-e CALUX bioanalysis results site 11

samplig campaign	date of sampling
1	26/03/2018
2	09/05/2018
3	20/06/2018
4	28/08/2018
5	17/10/2018
6	08/11/2018

26/03/2018	S0	S1	S2	S3
Cytotox CALUX		0.5	0.5	0.5
AR CALUX		0.5	0.5	0.5
anti-AR CALUX		0.5	0.5	0.5
ERa CALUX		8.4	2.2	0.5
GR CALUX		1.8	0.5	0.5
anti-PR CALUX		0.5	1.2	0.5
PPARa2 CALUX		0.5	0.5	0.5
PPARg2 CALUX		2.4	0.5	0.5
PXR CALUX		5.3	4.2	3.6
Nrf2 CALUX		5.5	7.4	2.8
P53 CALUX (+S9)		0.5	0.5	0.5

09/05/2018	S0	S1	S2	S3
Cytotox CALUX	57	0.5	0.5	0.5
AR CALUX	150	0.5	0.5	0.5
anti-AR CALUX	4.8	0.5	0.5	0.5
ERa CALUX	1000	6.6	1.9	0.5
GR CALUX	0.5	2.4	1.2	0.5
anti-PR CALUX	28	0.5	0.5	0.5
PPARa2 CALUX	50	0.5	0.5	0.5
PPARg2 CALUX	13	0.5	0.5	0.5
PXR CALUX	11	9.6	5.1	0.5
Nrf2 CALUX	1	4.5	5.8	0.5
P53 CALUX (+S9)	0.5	0.5	0.5	0.5

20/06/2018	S0	S1	S2	S3
Cytotox CALUX		3.7	13.0	16.2
AR CALUX		0.5	0.5	0.5
anti-AR CALUX		3.5	1.7	0.5
ERa CALUX		31.3	7.0	3.4
GR CALUX		6.3	1.3	0.5
anti-PR CALUX		1.4	2.3	0.5
PPARa2 CALUX		0.5	0.5	0.5
PPARg2 CALUX		0.5	0.5	0.5
PXR CALUX		6.9	6.3	4.7
Nrf2 CALUX		11	10.5	10.0
P53 CALUX (+S9)		0.5	0.5	1

28/08/2018	S0	S1	S2	S3
Cytotox CALUX		11.0	4.0	2.4
AR CALUX		0.5	0.5	0.5
anti-AR CALUX		3.4	0.5	0.5
ERa CALUX		4.6	0.5	0.5
GR CALUX		6.4	1.6	0.5
anti-PR CALUX		0.5	0.5	0.5
PPARa2 CALUX		0.5	0.5	0.5
PPARg2 CALUX		0.5	0.5	0.5
PXR CALUX		11.0	5.7	3.9
Nrf2 CALUX		12	10.0	4.1
P53 CALUX (+S9)		0.5	0.5	0.5

17/10/2018	S0	S1	S2	S3
Cytotox CALUX		0.5	0.5	0.5
AR CALUX		0.5	0.5	0.5
anti-AR CALUX		5.0	0.5	0.5
ERa CALUX		8.3	3.5	0.5
GR CALUX		5.4	1.6	0.5
anti-PR CALUX		0.5	0.5	0.5
PPARa2 CALUX		0.5	0.5	0.5
PPARg2 CALUX		0.5	0.5	0.5
PXR CALUX		1.0	1.0	0.5
Nrf2 CALUX				
P53 CALUX (+S9)				

08/11/2018	S0	S1	S2	S3
Cytotox CALUX		0.5	0.5	0.5
AR CALUX		0.5	0.5	0.5
anti-AR CALUX		0.5	0.5	0.5
ERa CALUX		4.7	2.6	0.5
GR CALUX		3.1	4.7	0.5
anti-PR CALUX		0.5	0.5	0.5
PPARa2 CALUX		0.5	0.5	0.5
PPARg2 CALUX		0.5	0.5	0.5
PXR CALUX		5.9	5.2	0.5
Nrf2 CALUX		5	6.1	1.0
P53 CALUX (+S9)				

Sample point	Client sample code	Results campaign						LOQ campaign					
		1	2	3	4	5	6	1	2	3	4	5	6
Cytotox CALUX (ug TBT eq./l water)													
S0	WWTP Inflow		39						0.68				
S1	Inflow pilot plant	<LOQ	<LOQ	1.7	5.6	<LOQ	<LOQ	0.87	0.61	0.46	0.49	0.48	0.7
S2	Outflow filter 1	<LOQ	<LOQ	6	2	<LOQ	<LOQ	0.86	0.59	0.46	0.5	0.5	0.7
S3	Outflow filter 3	<LOQ	<LOQ	6.3	1.2	<LOQ	<LOQ	0.76	0.6	0.39	0.49	0.49	0.72
AR CALUX (ng DHT eq./l water)													
S0	WWTP Inflow		260						1.7				
S1	Inflow pilot plant	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.5	1.5	1.9	1.6	3.8	2
S2	Outflow filter 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.5	1.6	1.9	1.6	4	2
S3	Outflow filter 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.9	1.6	1.4	2.7	2.1	2.1
anti-AR CALUX (ug Flutamide eq./l water)													
S0	WWTP Inflow		120						25				
S1	Inflow pilot plant	<LOQ	<LOQ	25	20	11	<LOQ	2.9	10	7.1	5.9	2.2	4.6
S2	Outflow filter 1	<LOQ	<LOQ	13	<LOQ	<LOQ	<LOQ	2.9	5.3	7.5	6	2.2	4.7
S3	Outflow filter 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.6	5.3	5.9	5.2	1.2	3.6
ERa CALUX (ng 17b Estradiol eq./l water)													
S0	WWTP Inflow		61						0.06				
S1	Inflow pilot plant	2.1	0.4	0.97	0.23	0.33	0.23	0.25	0.061	0.031	0.05	0.04	0.049
S2	Outflow filter 1	0.56	0.11	0.23	<LOQ	0.17	0.13	0.25	0.058	0.033	0.051	0.048	0.05
S3	Outflow filter 3	<LOQ	<LOQ	0.11	<LOQ	<LOQ	<LOQ	0.14	0.058	0.032	0.045	0.047	0.066
GR CALUX (ng Dexamethasone eq./l water)													
S0	WWTP Inflow		<LOQ						56				
S1	Inflow pilot plant	140	70	69	48	65	26	76	29	11	7.5	12	8.5
S2	Outflow filter 1	<LOQ	33	14	12	21	40	74	27	11	7.6	13	8.6
S3	Outflow filter 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	31	28	13	8.5	40	9.1
anti-PR CALUX (ng Ru486 eq./l water)													
S0	WWTP Inflow		120						4.3				
S1	Inflow pilot plant	<LOQ	<LOQ	5.7	<LOQ	<LOQ	<LOQ	2.8	3.9	4.2	2	0.73	1.9
S2	Outflow filter 1	3.4	<LOQ	9.7	<LOQ	<LOQ	<LOQ	2.8	2.2	4.3	2	0.76	1.9
S3	Outflow filter 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.5	2.2	5.3	2.9	0.74	2.6
PPARa CALUX (ng GW7647 eq./l water)													
S0	WWTP Inflow		530						11				
S1	Inflow pilot plant	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	16	9.5	25	18	12	18
S2	Outflow filter 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	16	23	26	18	12	18
S3	Outflow filter 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	19	24	7.3	17	21	17
PPARg CALUX (ng Rosiglitazone eq./l water)													
S0	WWTP Inflow		3400						260				
S1	Inflow pilot plant	5100	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2100	230	200	200	290	230
S2	Outflow filter 1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2100	200	200	210	300	230
S3	Outflow filter 3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1700	200	200	220	410	260
PXR CALUX (ug Nicardipine eq./l water)													
S0	WWTP Inflow		28						2.6				
S1	Inflow pilot plant	52	23	31	79	16	24	9.8	2.4	4.5	7.3	16	4.1
S2	Outflow filter 1	42	26	29	42	20	22	9.9	5.1	4.6	7.4	20	4.2
S3	Outflow filter 3	28	<LOQ	26	24	<LOQ	<LOQ	7.8	5.2	5.5	6.2	5.6	4.9
Nrf2 CALUX (ug Curcumine eq./l water)													
S0	WWTP Inflow		<LOQ						23				
S1	Inflow pilot plant	220	95	220	370	pending	102	40	21	20	30	pending	19
S2	Outflow filter 1	290	120	210	310	pending	115	39	21	20	31	pending	19
S3	Outflow filter 3	110	<LOQ	200	130	pending	19	40	22	20	31	pending	19
P53 (+S9) CALUX (ug Cyclophosphamide/l water)													
S0	WWTP Inflow		<LOQ						530				
S1	Inflow pilot plant	<LOQ	<LOQ	<LOQ	<LOQ	pending	pending	910	470	2700	1200	pending	pending
S2	Outflow filter 1	<LOQ	<LOQ	<LOQ	<LOQ	pending	pending	910	480	840	1200	pending	pending
S3	Outflow filter 3	<LOQ	<LOQ	<LOQ	<LOQ	pending	pending	920	490	810	1200	pending	pending

Table 5-f CALUX bioanalysis results site 12

samplig campaign	date of sampling
1	23/01/2018
2	17/04/2018
3	16/07/2018

23/01/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	0.5	3.8	0.5		0.5	0.5	0.5
AR CALUX	120.0	0.5	0.5		0.5	0.5	0.5
anti-AR CALUX	0.5	1.2	0.5		0.5	0.5	0.5
ERa CALUX	5.0	50.0	3.5		16.3	11.9	1.5
GR CALUX	8.5	17.5	6.5		9.3	2.3	4.2
anti-PR CALUX	16.0	0.5	0.5		0.5	0.5	0.5
PPARa2 CALUX	40.0	0.5	0.5		0.5	0.5	0.5
PPARg2 CALUX	4.3	0.5	0.5		0.5	0.5	0.5
PXR CALUX	0.5	4.2	2.2		3.7	0.5	1.2
Nrf2 CALUX	19.0	9.0	5.8		4.1	4.2	2.7
P53 CALUX (+S9)	0.5	21.3	0.5		0.5	0.5	0.5

17/04/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	86.9	0.5	0.5	0.5	0.5	0.5	0.5
AR CALUX	215.0	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	0.5	1.0	1.9	0.5	0.5	1.1	0.5
ERa CALUX	1153.8	3.8	0.5	0.5	0.5	0.5	0.5
GR CALUX	1.4	7.6	0.5	0.5	1.6	0.5	1.4
anti-PR CALUX	18.6	0.5	0.5	0.5	1.5	0.5	0.5
PPARa2 CALUX	12.7	0.5	0.5	0.5	0.5	0.5	0.5
PPARg2 CALUX	0.5	1.2	0.5	0.5	0.5	0.5	0.5
PXR CALUX	14.0	5.9	4.0	0.5	4.9	4.0	2.3
Nrf2 CALUX	20.8	8.6	2.5	0.5	4.3	2.1	3.8
P53 CALUX (+S9)	4.3	1.2	0.5	0.5	0.5	0.5	0.5

16/07/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	35.0	4.0	1.0	0.5	0.5	0.5	0.5
AR CALUX	87.0	0.5	0.5	0.5	0.5	0.5	0.5
anti-AR CALUX	5.1	0.5	0.5	0.5	0.5	0.5	0.5
ERa CALUX	1200.0	22.0	1.5	0.5	0.5	0.5	0.5
GR CALUX	15.0	12.0	4.8	0.5	2.6	2.4	1.8
anti-PR CALUX	15.0	0.5	0.5	0.5	0.5	0.5	0.5
PPARa2 CALUX	26.0	0.5	1.3	0.5	0.5	0.5	0.5
PPARg2 CALUX	3.8	0.5	0.5	0.5	0.5	0.5	0.5
PXR CALUX	20.0	14.0	5.5	1.0	4.5	3.7	3.7
Nrf2 CALUX	20	5.6	4.7	0.5	5.0	2.3	3.1
P53 CALUX (+S9)	30.0	0.5	0.5	0.5	2	0.5	0.5

Sample point	Client sample code	Results campaign			LOQ campaign		
		1	2	3	1	2	3
Cytotox CALUX (ug TBT eq./l water)							
S1	Primary sedimentation effluent	<LOQ	53	25	9.2	0.61	0.72
S2	Ozonation influent	1.8	<LOQ	2.8	0.47	0.65	0.7
S3	Ozonation effluent	<LOQ	<LOQ	0.67	0.47	0.91	0.67
S4	Post-GAC filter		<LOQ	<LOQ		0.79	0.7
S5	Post-sand/anthracite filter	<LOQ	<LOQ	<LOQ	0.47	0.84	0.69
S6	Post- sand/BAC filter	<LOQ	<LOQ	<LOQ	0.46	0.98	0.67
S7	Post-constructed wetland	<LOQ	<LOQ	<LOQ	0.44	0.64	0.7
AR CALUX (ng DHT eq./l water)							
S1	Primary sedimentation effluent	155	430	130	1.3	2	1.5
S2	Ozonation influent	<LOQ	<LOQ	<LOQ	0.81	3	1.4
S3	Ozonation effluent	<LOQ	<LOQ	<LOQ	0.79	4.2	1.8
S4	Post-GAC filter		<LOQ	<LOQ		3.3	1.9
S5	Post-sand/anthracite filter	<LOQ	<LOQ	<LOQ	0.52	3	1.7
S6	Post- sand/BAC filter	<LOQ	<LOQ	<LOQ	0.51	3.5	1.8
S7	Post-constructed wetland	<LOQ	<LOQ	<LOQ	0.62	2.7	2
anti-AR CALUX (ug Flutamide eq./l water)							
S1	Primary sedimentation effluent	<LOQ	<LOQ	21	28	37	4.1
S2	Ozonation influent	4.6	1.1	<LOQ	3.9	1.1	4
S3	Ozonation effluent	<LOQ	2.6	<LOQ	3.8	1.4	5.1
S4	Post-GAC filter		<LOQ	<LOQ		5.7	5.6
S5	Post-sand/anthracite filter	<LOQ	<LOQ	<LOQ	3.8	1.5	5.6
S6	Post- sand/BAC filter	<LOQ	1.8	<LOQ	3.7	1.7	5.2
S7	Post-constructed wetland	<LOQ	<LOQ	<LOQ	4.5	4.7	5.7
ERa CALUX (ng 17b Estradiol eq./l water)							
S1	Primary sedimentation effluent	0.36	1	51	0.072	0.26	0.042
S2	Ozonation influent	1.8	45	0.96	0.036	0.039	0.043
S3	Ozonation effluent	0.13	<LOQ	0.069	0.037	0.36	0.046
S4	Post-GAC filter		<LOQ	<LOQ		0.092	0.063
S5	Post-sand/anthracite filter	0.44	<LOQ	<LOQ	0.027	0.086	0.041
S6	Post- sand/BAC filter	0.32	<LOQ	<LOQ	0.027	0.1	0.046
S7	Post-constructed wetland	0.052	<LOQ	<LOQ	0.034	0.076	0.064
GR CALUX (ng Dexamethasone eq./l water)							
S1	Primary sedimentation effluent	110	160	130	13	21	8.7
S2	Ozonation influent	210	15	110	12	11	9
S3	Ozonation effluent	71	<LOQ	48	11	29	10
S4	Post-GAC filter		<LOQ	<LOQ		43	12
S5	Post-sand/anthracite filter	87	42	41	9.4	27	16
S6	Post- sand/BAC filter	21	<LOQ	24	9.2	31	10
S7	Post-constructed wetland	25	50	22	5.9	36	12
anti-PR CALUX (ng Ru486 eq./l water)							
S1	Primary sedimentation effluent	40	54	40	2.5	2.9	2.6
S2	Ozonation influent	<LOQ	<LOQ	<LOQ	1.9	2.2	2.6
S3	Ozonation effluent	<LOQ	<LOQ	<LOQ	1.9	3.1	2
S4	Post-GAC filter		<LOQ	<LOQ		3.5	1.2
S5	Post-sand/anthracite filter	<LOQ	4.1	<LOQ	2	2.7	1.2
S6	Post- sand/BAC filter	<LOQ	<LOQ	<LOQ	2	3.1	2
S7	Post-constructed wetland	<LOQ	<LOQ	<LOQ	1.2	2.9	1.2
PPARa CALUX (ng GW7647 eq./l water)							
S1	Primary sedimentation effluent	400	140	420	10	11	16
S2	Ozonation influent	<LOQ	<LOQ	<LOQ	8.2	16	15
S3	Ozonation effluent	<LOQ	<LOQ	20	8	23	15
S4	Post-GAC filter		<LOQ	<LOQ		26	21
S5	Post-sand/anthracite filter	<LOQ	<LOQ	<LOQ	29	15	25
S6	Post- sand/BAC filter	<LOQ	<LOQ	<LOQ	28	18	15
S7	Post-constructed wetland	<LOQ	<LOQ	<LOQ	4.9	22	21
PPARg CALUX (ng Rosiglitazone eq./l water)							
S1	Primary sedimentation effluent	1300	<LOQ	1100	300	100	290
S2	Ozonation influent	<LOQ	81	<LOQ	120	65	270
S3	Ozonation effluent	<LOQ	<LOQ	<LOQ	120	91	260
S4	Post-GAC filter		<LOQ	<LOQ		110	310
S5	Post-sand/anthracite filter	<LOQ	<LOQ	<LOQ	130	130	320
S6	Post- sand/BAC filter	<LOQ	<LOQ	<LOQ	120	160	260
S7	Post-constructed wetland	<LOQ	<LOQ	<LOQ	150	91	310
PXR CALUX (ug Nicardipine eq./l water)							
S1	Primary sedimentation effluent	<LOQ	80	100	41	5.7	5.1
S2	Ozonation influent	25	100	72	5.9	17	5.2
S3	Ozonation effluent	12	93	48	5.5	23	8.8
S4	Post-GAC filter		<LOQ	8.3		5.9	8.3
S5	Post-sand/anthracite filter	19	37	30	5.2	7.5	6.7
S6	Post- sand/BAC filter	<LOQ	35	33	5.1	8.7	8.9
S7	Post-constructed wetland	8.1	11	31	6.6	4.8	8.3
Nrf2 CALUX (ug Curcumine eq./l water)							
S1	Primary sedimentation effluent	760	810	740	40	39	37
S2	Ozonation influent	180	320	200	20	37	36
S3	Ozonation effluent	110	130	170	19	52	36
S4	Post-GAC filter		<LOQ	<LOQ		45	36
S5	Post-sand/anthracite filter	77	190	190	19	44	38
S6	Post- sand/BAC filter	79	110	82	19	52	36
S7	Post-constructed wetland	51	140	110	19	37	36
P53 (+S9) CALUX (ug Cyclophosphamide/l water)							
S1	Primary sedimentation effluent	<LOQ	570	25000	940	490	830
S2	Ozonation influent	10000	2200	<LOQ	470	510	800
S3	Ozonation effluent	<LOQ	<LOQ	<LOQ	450	680	800
S4	Post-GAC filter		<LOQ	<LOQ		600	800
S5	Post-sand/anthracite filter	<LOQ	<LOQ	1500	460	590	850
S6	Post- sand/BAC filter	<LOQ	<LOQ	<LOQ	440	690	800
S7	Post-constructed wetland	<LOQ	<LOQ	<LOQ	450	490	810

Annex 6 Heat-map of quantified CALUX bioanalysis results – round 2

Table 6-a CALUX bioanalysis results site 4 and 6

Site 4

12/03/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	1	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	18	13	LOQ	LOQ	LOQ	LOQ
ERa CALUX	LOQ	LOQ	0.1	LOQ	LOQ	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	62	4.8	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	17	35	40	13	10	8.5
Nrf2 CALUX	1000	140	LOQ	LOQ	15	22
P53 CALUX (+S9)	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ

21/08/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	0.8	3.2	LOQ	LOQ	0.8	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	24	22	16	LOQ	6.4	LOQ
Nrf2 CALUX	74	59	70	60	37	LOQ
P53 CALUX (+S9)	1900	LOQ	1900	LOQ	LOQ	LOQ

08/10/2018	S1	S2	S3	S4	S5	S6
Cytotox CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	0.1	0.3	LOQ	0.2	LOQ	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	23.0	23.0	15.0	10.0	20.0	7.2
Nrf2 CALUX	LOQ	130	LOQ	26	LOQ	42
P53 CALUX (+S9)						

Site 6

15/08/2017	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Cytotox CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	14	LOQ	LOQ	LOQ
ERa CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	4.8	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	1030	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Nrf2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
P53 CALUX (+S9)	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ

21/11/2017	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Cytotox CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	6.2	6.9	LOQ	LOQ	LOQ
Nrf2 CALUX	LOQ	55	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
P53 CALUX (+S9)	LOQ	LOQ	5000	LOQ	LOQ	LOQ	1100	LOQ	LOQ	LOQ

19/03/2018	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Cytotox CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
GR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	LOQ	LOQ	LOQ	LOQ	5.7	LOQ	4.0	LOQ	LOQ	LOQ
Nrf2 CALUX	335	46	LOQ	50	52	21	41	29	LOQ	52.0
P53 CALUX (+S9)	LOQ	LOQ	LOQ	LOQ	1800	LOQ	LOQ	7400	LOQ	LOQ

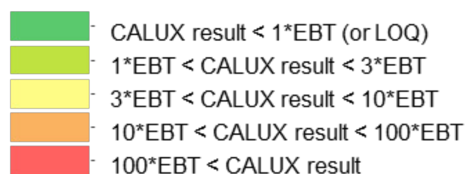


Table 6-b CALUX bioanalysis results site 7 and 8

Site 7

Bioassay	S1	S6	S7	S8	S4	S5	S2	S3	S9
Cytotox CALUX	0.88					LOQ	LOQ	LOQ	
AR CALUX	LOQ					LOQ	LOQ	LOQ	
anti-AR CALUX	11					LOQ	LOQ	LOQ	
ERa CALUX	2.1					LOQ	LOQ	LOQ	
GR CALUX	72					38	LOQ	LOQ	
anti-PR CALUX	4.6					LOQ	LOQ	LOQ	
PPARa2 CALUX	LOQ					LOQ	LOQ	LOQ	
PPARg2 CALUX	180					LOQ	LOQ	LOQ	
PXR CALUX	57					36	32	16	
Nrf2 CALUX	94					84	19	LOQ	
P53 CALUX (+S9)	LOQ					LOQ	LOQ	LOQ	

Shipment 2 18/12/2018

Bioassay	S1	S6	S7	S8	S4	S5	S2	S3	S9
Cytotox CALUX	1	0.69	0.66	0.5	LOQ	LOQ	LOQ		
AR CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ		
anti-AR CALUX	29	27	11	22	9.4	LOQ	7.0		
ERa CALUX	2.2	1.9	1.7	0.8	LOQ	LOQ	0.1		
GR CALUX	130	93	98	85	8.8	18	LOQ		
anti-PR CALUX	5.9	3.5	3.1	3.4	LOQ	1.9	LOQ		
PPARa2 CALUX	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ		
PPARg2 CALUX	140	84	52	LOQ	LOQ	LOQ	LOQ		
PXR CALUX	36	44	27	39	13	15	8.7		
Nrf2 CALUX	260	220	220	240	70	160	30		
P53 CALUX (+S9)									

Shipment 3 19/03/2019

Bioassay	S1	S6	S7	S8	S4	S5	S2	S3	S9
Cytotox CALUX	LOQ		LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	LOQ		LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ		6.6	14	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	2.5		LOQ	5.0	LOQ	LOQ	LOQ	LOQ	LOQ
GR CALUX	73		LOQ	66	LOQ	33.0	LOQ	LOQ	19.0
anti-PR CALUX	2.7		1.4	6.0	1.1	3.9	LOQ	LOQ	LOQ
PPARa2 CALUX	LOQ		LOQ	LOQ	LOQ	37	31	LOQ	LOQ
PPARg2 CALUX	470		LOQ	480	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	87		11	70	13	18	LOQ	11	17.0
Nrf2 CALUX	160		140	190	89	110	50.0	LOQ	76
P53 CALUX (+S9)									

Site 8

11/04/2018	S1	S2	S4	S5-1	S5-2	S5-3	S5-4	S5-1	S5-1	S5-1	S5-1	S5-1
Cytotox CALUX	3	LOQ		LOQ	LOQ							
AR CALUX	190.0	LOQ		LOQ	LOQ							
anti-AR CALUX	LOQ	LOQ		LOQ	LOQ							
ERa CALUX	31.0	1.0		0.3	0.1							
GR CALUX	56	53.0		LOQ	LOQ							
anti-PR CALUX	28.0	LOQ		LOQ	LOQ							
PPARa2 CALUX	21.0	27.0		LOQ	LOQ							
PPARg2 CALUX	500	LOQ		LOQ	LOQ							
PXR CALUX	32	42		15	24							
Nrf2 CALUX	440	202		103	89							
P53 CALUX (+S9)	1100.0	LOQ		LOQ	LOQ							

30/04/2018	S1	S2	S4	S5-1	S5-2	S5-3	S5-4	S5-1	S5-1	S5-1	S5-1	S5-1
Cytotox CALUX			LOQ			LOQ	LOQ					
AR CALUX			LOQ			LOQ	LOQ					
anti-AR CALUX			5.8			LOQ	LOQ					
ERa CALUX			0.3			0.1	LOQ					
GR CALUX			LOQ			LOQ	LOQ					
anti-PR CALUX			LOQ			LOQ	LOQ					
PPARa2 CALUX			LOQ			LOQ	LOQ					
PPARg2 CALUX			LOQ			LOQ	LOQ					
PXR CALUX			34			27	16					
Nrf2 CALUX			200			110	99					
P53 CALUX (+S9)			LOQ			LOQ	LOQ					

22/10/2018	S1	S2	S4	S5-1	S5-2	S5-3	S5-4	S5-1	S5-1	S5-1	S5-1	S5-1
Cytotox CALUX		LOQ	LOQ	LOQ	LOQ	LOQ	LOQ					
AR CALUX		LOQ	LOQ	LOQ	LOQ	LOQ	LOQ					
anti-AR CALUX		LOQ	9.0	LOQ	LOQ	LOQ	LOQ					
ERa CALUX		0.9	0.3	LOQ	0.1	0.1	0.1					
GR CALUX		12.0	LOQ	LOQ	LOQ	LOQ	LOQ					
anti-PR CALUX		LOQ	LOQ	LOQ	3.7	LOQ	LOQ					
PPARa2 CALUX		LOQ	LOQ	LOQ	LOQ	LOQ	LOQ					
PPARg2 CALUX		LOQ	LOQ	LOQ	LOQ	LOQ	LOQ					
PXR CALUX		27	36	8.6	9.2	22	25					
Nrf2 CALUX		200	256	LOQ	LOQ	106	335					
P53 CALUX (+S9)												

19/10-26/11/2018	S1	S2	S4	S5-1	S5-2	S5-3	S5-4	S5-1	S5-1	S5-1	S5-1	S5-1
Cytotox CALUX								<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
AR CALUX								LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX								LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX								LOQ	LOQ	LOQ	0.1	0.1
GR CALUX								LOQ	LOQ	LOQ	LOQ	LOQ
anti-PR CALUX								LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX								LOQ	LOQ	LOQ	55	LOQ
PPARg2 CALUX								LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX								9.8	19	13	14	12
Nrf2 CALUX								LOQ	LOQ	LOQ	108	53
P53 CALUX (+S9)												

- CALUX result < 1*EBT (or LOQ)
- 1*EBT < CALUX result < 3*EBT
- 3*EBT < CALUX result < 10*EBT
- 10*EBT < CALUX result < 100*EBT
- 100*EBT < CALUX result

Table 6-c CALUX bioanalysis results site 11 and 12

Site 11

26/03/2018	S0	S1	S2	S3
Cytotox CALUX		LOQ	LOQ	LOQ
AR CALUX		LOQ	LOQ	LOQ
anti-AR CALUX		LOQ	LOQ	LOQ
ERa CALUX		2.1	0.6	LOQ
GR CALUX		140	LOQ	LOQ
anti-PR CALUX		LOQ	3	LOQ
PPARa2 CALUX		LOQ	LOQ	LOQ
PPARg2 CALUX		5100	LOQ	LOQ
PXR CALUX		52	42	28
Nrf2 CALUX		220	290	110
P53 CALUX (+S9)		LOQ	LOQ	LOQ

09/05/2018	S0	S1	S2	S3
Cytotox CALUX	39	LOQ	LOQ	LOQ
AR CALUX	260	LOQ	LOQ	LOQ
anti-AR CALUX	120	LOQ	LOQ	LOQ
ERa CALUX	61	0.4	0.1	LOQ
GR CALUX	LOQ	70	33	LOQ
anti-PR CALUX	120	LOQ	LOQ	LOQ
PPARa2 CALUX	530	LOQ	LOQ	LOQ
PPARg2 CALUX	3400	LOQ	LOQ	LOQ
PXR CALUX	28	23.0	26.0	LOQ
Nrf2 CALUX	LOQ	95	120	LOQ
P53 CALUX (+S9)	LOQ	LOQ	LOQ	LOQ

20/06/2018	S0	S1	S2	S3
Cytotox CALUX		1.7	6.0	6.3
AR CALUX		LOQ	LOQ	LOQ
anti-AR CALUX		25	13	LOQ
ERa CALUX		1.0	0.2	0.1
GR CALUX		69	14	LOQ
anti-PR CALUX		5.7	9.7	LOQ
PPARa2 CALUX		LOQ	LOQ	LOQ
PPARg2 CALUX		LOQ	LOQ	LOQ
PXR CALUX		31.0	29.0	26.0
Nrf2 CALUX		220	210	200
P53 CALUX (+S9)		LOQ	LOQ	LOQ

28/08/2018	S0	S1	S2	S3
Cytotox CALUX		5.6	2.0	1.2
AR CALUX		LOQ	LOQ	LOQ
anti-AR CALUX		20	LOQ	LOQ
ERa CALUX		0.2	LOQ	LOQ
GR CALUX		48	12	LOQ
anti-PR CALUX		LOQ	LOQ	LOQ
PPARa2 CALUX		LOQ	LOQ	LOQ
PPARg2 CALUX		LOQ	LOQ	LOQ
PXR CALUX		79.0	42.0	24.0
Nrf2 CALUX		370	310	130
P53 CALUX (+S9)		LOQ	LOQ	LOQ

17/10/2018	S0	S1	S2	S3
Cytotox CALUX		LOQ	LOQ	LOQ
AR CALUX		LOQ	LOQ	LOQ
anti-AR CALUX		11.0	LOQ	LOQ
ERa CALUX		0.3	0.2	LOQ
GR CALUX		65	21	LOQ
anti-PR CALUX		LOQ	LOQ	LOQ
PPARa2 CALUX		LOQ	LOQ	LOQ
PPARg2 CALUX		LOQ	LOQ	LOQ
PXR CALUX		16	20	LOQ
Nrf2 CALUX				
P53 CALUX (+S9)				

08/11/2018	S0	S1	S2	S3
Cytotox CALUX		LOQ	LOQ	LOQ
AR CALUX		LOQ	LOQ	LOQ
anti-AR CALUX		LOQ	LOQ	LOQ
ERa CALUX		0.2	0.1	LOQ
GR CALUX		26	40	LOQ
anti-PR CALUX		LOQ	LOQ	LOQ
PPARa2 CALUX		LOQ	LOQ	LOQ
PPARg2 CALUX		LOQ	LOQ	LOQ
PXR CALUX		24	22	LOQ
Nrf2 CALUX		102	115	19
P53 CALUX (+S9)				

Site 12

23/01/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	LOQ	1.8	LOQ		LOQ	LOQ	LOQ
AR CALUX	155	LOQ	LOQ		LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	4.6	LOQ		LOQ	LOQ	LOQ
ERa CALUX	0.4	1.8	0.1		0.4	0.3	0.1
GR CALUX	110	210	71		87	21	25
anti-PR CALUX	40.0	LOQ	LOQ		LOQ	LOQ	LOQ
PPARa2 CALUX	400.0	LOQ	LOQ		LOQ	LOQ	LOQ
PPARg2 CALUX	1300	LOQ	LOQ		LOQ	LOQ	LOQ
PXR CALUX	LOQ	25	12		19	LOQ	8
Nrf2 CALUX	760	180	110		77	79	51
P53 CALUX (+S9)	LOQ	######	LOQ		LOQ	LOQ	LOQ

17/04/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	53.0	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	430	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	1.1	2.6	LOQ	LOQ	1.8	LOQ
ERa CALUX	1.0	45	LOQ	LOQ	LOQ	LOQ	LOQ
GR CALUX	160	15.0	LOQ	LOQ	42.0	LOQ	50.0
anti-PR CALUX	54	LOQ	LOQ	LOQ	4.1	LOQ	LOQ
PPARa2 CALUX	140	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	81	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	80	100	93	LOQ	37	35	11
Nrf2 CALUX	810	320	130	LOQ	190	110	140
P53 CALUX (+S9)	570	2200	LOQ	LOQ	LOQ	LOQ	LOQ

16/07/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	25	2.8	0.7	LOQ	LOQ	LOQ	LOQ
AR CALUX	130	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	21	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	51	1.0	0.1	LOQ	LOQ	LOQ	LOQ
GR CALUX	130	110	48	LOQ	41	24	22
anti-PR CALUX	40	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	420	LOQ	20	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	1100	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	100	72	48	8.3	30.0	33.0	31.0
Nrf2 CALUX	740	200	170	LOQ	190	82	110
P53 CALUX (+S9)	25000	LOQ	LOQ	LOQ	1500	LOQ	LOQ

- CALUX result < 1*EBT (or LOQ)
- 1*EBT < CALUX result < 3*EBT
- 3*EBT < CALUX result < 10*EBT
- 10*EBT < CALUX result < 100*EBT
- 100*EBT < CALUX result

Annex 7. LC-HRMS based non-target screening: Material, methods and results

Chemicals

Acetonitrile (ACN, HPLC grade) was purchased from Avantor Performance Materials B.V. (Deventer, NL), formic acid (FA) from Fluka Analytical (Sigma-Aldrich, Steinheim, D), the internal standards atrazine-d5 and bentazon-d6 from CDN isotopes (Pointe-Claire, Canada) and LGC Standards (Wesen, Germany), respectively. The ultrapure water used as a blank reference was produced with an Elga Purelab Chorus ultrapure water system through purification of demineralized water in (High Wycombe, UK).

7.1.1 LC-HRMS experiments

LC-HRMS/MS experiments were performed using a Vanquish HPLC system (ThermoFisher Scientific) coupled to a Tribrid Orbitrap Fusion mass spectrometer (ThermoFisher Scientific, Bremen, Germany) with an electrospray ionization source. Chromatographic separation was performed using an XBridge BEH C18 XP column (150 mm × 2.1 mm I.D., particle size 2.5 µm) (Waters, Etten-Leur, The Netherlands) preceded by a 2.0 mm × 2.1 mm I.D. Phenomenex SecurityGuard Ultra column (Phenomenex, Torrance, USA) maintained at a temperature of 25 °C. The LC gradient went from 5% acetonitrile, 95% water and 0.05% formic acid (v/v/v) to 100% acetonitrile with 0.05% formic acid in 25 min, after which it was held constant for 4 min at a flow rate of 0.25 mL/min.

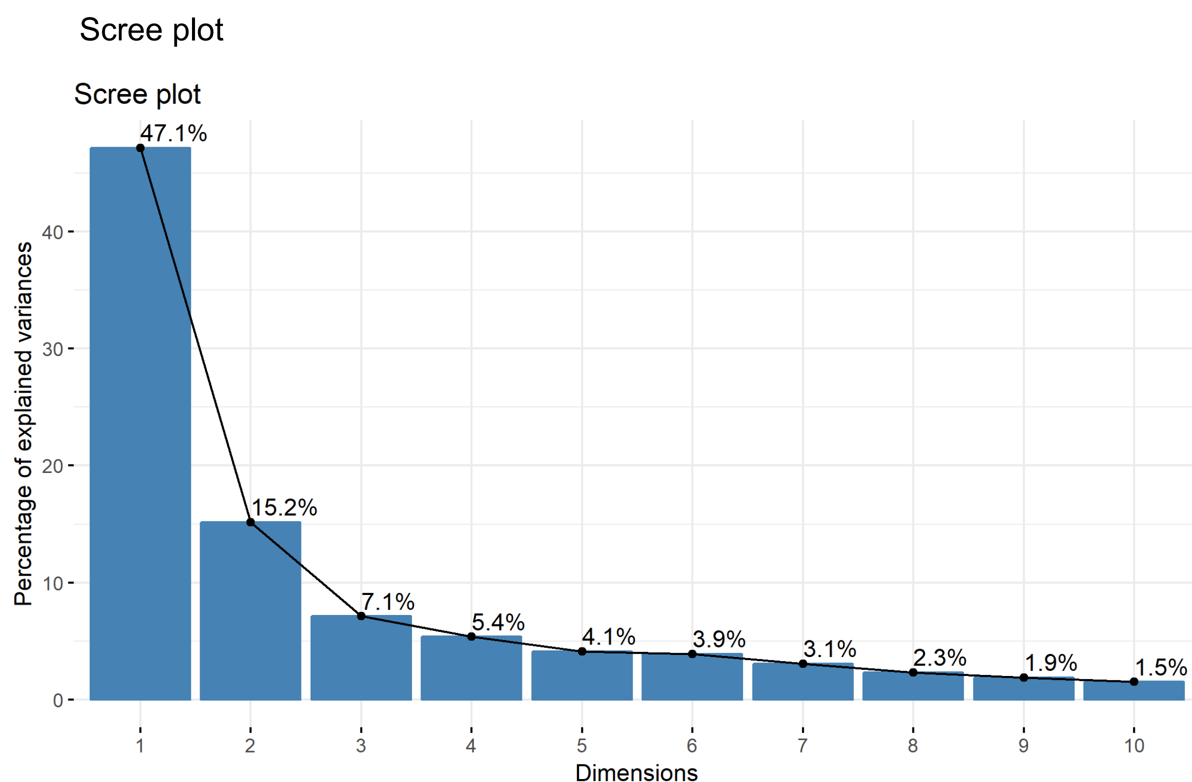
Prior to LC-HRMS analysis, the SPE extracted water samples (6667x concentrated compared to the not extracted original water samples) were diluted 100x, resulting in 66.7x concentrated samples. The internal standards bentazone-d6, atrazine-d5 and benzotriazole-d4 were added to the water samples to a final concentration of 1ug/L. Subsequently, samples were filtered using Phenex™-RC 15mm Syringe Filters 0.2u (Phenomenex, Torrance, USA). 100 µL of each filtered sample was analysed in triplicate. Mass calibration was performed using Pierce ESI positive and negative ion calibration solution. The vaporizer and capillary temperature were set to 300 °C, sheath, auxiliary and sweep gas to arbitrary units of 40, 10 and 5, respectively. The source voltage was 3.0 kV in the positive mode, and -2.5kV in the negative mode respectively. The RF lens was set to 50 %. Full scan high accuracy mass spectra were acquired in the range of 50-1000 m/z with 120,000 FWHM resolution. Quadruple isolation was used for acquisition. Data dependent MS/MS acquisition was performed for the eight most intense ions detected in the full scan, using a High Collision Dissociation (HCD) energy at 35% and 15,000 FWHM resolution.

7.1.2 Data analysis

LC-HRMS raw data files were processed using Compound Discoverer 3.0 (Thermo Scientific, San Jose, USA) for peak picking and suspect screening. Suspect screening was performed using the SusDat database of the European Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances (NORMAN, https://www.norman-network.com/?q=node/236) consists of more than 40000 chemicals relevant for environmental monitoring, as well as the Water Framework Directive (WFD) list of priority substances (http://ec.europa.eu/environment/water/water-dangersub/pri_substances.htm). Searches were performed with 5 ppm mass tolerance. The processed data was exported and imported into R Studio as a .csv file for further data analysis and visualisation (R Core Team 2017). To group and characterize samples and

features, the two multivariate analysis techniques principal component analysis (PCA) and hierarchical clustering (HC) were applied. PCA was performed using the R package FactoMineR, and results visualized in graph of individuals plots using the R package factoextra. Prior to HC, data was normalized through division of feature intensities across samples by the maximum intensity of the respective feature. Both samples and features were clustered based on Euclidean distances and visualized in a heat map using the pheat-map package in R. To show differences in features induced by treatment steps, features were clustered based on their Pearson correlation using the ward D2 method. In addition, changes in features between two corresponding before and after treatment samples were illustrated in so called Volcano plots displaying the change in intensity as the log 2 fold change (log2FC) and its significance, i.e. the negative log 10-transformed p-values of features (Cui and Churchill 2003).

Results LC-HRMS NTS



Two thirds of the variance in the NTS data could be explained by the first two principal components as shown in the Scree plot.