Water Research 104 (2016) 473–484

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Bioassay battery interlaboratory investigation of emerging contaminants in spiked water extracts – Towards the implementation of bioanalytical monitoring tools in water quality assessment and monitoring

Carolina Di Paolo [a], Richard Ottermanns [a], Steffen Keiter [a, b], Selim Ait-Aissa [c], Kerstin Bluhm [a], Werner Brack [d], Magnus Breitholtz [e], Sebastian Buchinger [f], Mario Carere [g], Carole Chalon [h], Xavier Cousin [i, j], Valeria Dulio [c], Beate I. Escher [d, k, l], Timo Hamers [m], Klára Hilscherová [n], Sergio Jarque [n], Adam Jonas [n], Emmanuelle Maillot-Marechal [c], Yves Marneffe [h], Mai Thao Nguyen [o], Pascal Pandard [c], Andrea Schifferli [p], Tobias Schulze [d], Sven Seidensticker [a, l], Thomas-Benjamin Seiler [a], Janet Tang [k], Ron van der Oost [q], Etienne Vermeirssen [p], Radka Zounková [n], Nick Zwart [m], Henner Hollert [a, *]

[a] Institute for Environmental Research, RWTH Aachen University, Aachen, Germany
[b] Man-Technology-Environment Research Centre, School of Science and Technology, Örebro University, Örebro, Sweden
[c] INERIS, Verneuil-en-Halatte, France
[d] UFZ-Helmholtz Centre for Environmental Research, Leipzig, Germany
[e] Department of Applied Environmental Science - ITM, Stockholm University, Stockholm, Sweden
[f] Department Biochemistry and Ecotoxicology, Federal Institute of Hydrology, Koblenz, Germany
[g] Italian Institute of Health, Rome, Italy
[h] ISSeP (Scientific Institute of Public Service), Liège, Wallonia, Belgium
[i] Laboratoire d'Ecotoxicologie, Ifremer, L'Houmeau, France
[j] Laboratoire de Physiologie et Génétique des Poissons, Inra, Rennes, France
[k] National Research Centre for Environmental Toxicology - Entox, The University of Queensland, Brisbane, Australia
[l] Centre for Applied Geosciences, Eberhard Karls University Tübingen, Germany
[m] Institute for Environmental Studies -IVM, VU University Amsterdam, The Netherlands
[n] Research Centre for Toxic Compounds in the Environment - RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic
[o] Waterproef Laboratory, Edam, The Netherlands
[p] Swiss Centre for Applied Ecotoxicology Eawag-EPFL, Dübendorf, Switzerland
[q] WATERNET Institute for the Urban Water Cycle, Division of Technology Research & Engineering, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form
30 July 2016
Accepted 9 August 2016
Available online 12 August 2016

Keywords:
Triclosan
Acridine

ABSTRACT

Bioassays are particularly useful tools to link the chemical and ecological assessments in water quality monitoring. Different methods cover a broad range of toxicity mechanisms in diverse organisms, and account for risks posed by non-target compounds and mixtures. Many tests are already applied in chemical and waste assessments, and stakeholders from the science-police interface have recommended their integration in regulatory water quality monitoring. Still, there is a need to address bioassay suitability to evaluate water samples containing emerging pollutants, which are a current priority in water quality monitoring. The presented interlaboratory study (ILS) verified whether a battery of miniaturized bioassays, conducted in 11 different laboratories following their own protocols, would produce comparable results when applied to evaluate blinded samples consisting of a pristine water extract spiked

* Corresponding author. Department of Ecosystem Analysis, Institute for Environmental Research (Biology 5), RWTH Aachen University, Worringerweg 1, D-52074, Aachen, Germany.
E-mail address: henner.hollert@bio5.rwth-aachen.de (H. Hollert).

http://dx.doi.org/10.1016/j.watres.2016.08.018

17α-ethinylestradiol
3-Nitrobenzanthrone
Organism-level toxicity
Mechanism-specific toxicity

with four emerging pollutants as single chemicals or mixtures, i.e. triclosan, acridine, 17α-ethinylestradiol (EE2) and 3-nitrobenzanthrone (3-NBA). Assays evaluated effects on aquatic organisms from three different trophic levels (algae, daphnids, zebrafish embryos) and mechanism-specific effects using *in vitro* estrogenicity (ER-Luc, YES) and mutagenicity (Ames fluctuation) assays. The test battery presented complementary sensitivity and specificity to evaluate the different blinded water extract spikes. Aquatic organisms differed in terms of sensitivity to triclosan (algae > daphnids > fish) and acridine (fish > daphnids > algae) spikes, confirming the complementary role of the three taxa for water quality assessment. Estrogenicity and mutagenicity assays identified with high precision the respective mechanism-specific effects of spikes even when non-specific toxicity occurred in mixture. For estrogenicity, although differences were observed between assays and models, EE2 spike relative induction EC_{50} values were comparable to the literature, and E2/EE2 equivalency factors reliably reflected the sample content. In the Ames, strong revertant induction occurred following 3-NBA spike incubation with the TA98 strain, which was of lower magnitude after metabolic transformation and when compared to TA100. Differences in experimental protocols, model organisms, and data analysis can be sources of variation, indicating that respective harmonized standard procedures should be followed when implementing bioassays in water monitoring. Together with other ongoing activities for the validation of a basic bioassay battery, the present study is an important step towards the implementation of bioanalytical monitoring tools in water quality assessment and monitoring.

1. Introduction

Water quality investigation and monitoring in Europe and worldwide is facing a challenge. There is societal, regulatory and scientific consensus on the urgent need to achieve good water quality in national and transboundary river basins. Meanwhile, an immense variety of contaminants is constantly reaching aquatic systems, which complicates the identification of drivers of chemical toxicity to be routinely monitored (von der Ohe et al., 2011). Further, there is a lack of direct indicators on the regulatory level to verify the biological relevance of chemical monitoring in different water bodies. While the ecological status assessment is certainly of high environmental relevance, it is based primarily on biodiversity indices that often do not present consistency with respective chemical monitoring (Wernersson et al., 2015). Therefore, complementary monitoring strategies are required to achieve the Water Framework Directive (WFD) aim to maintain and improve water quality in Europe (EC, 2000).

Effect-based tools such as bioassays and biomarkers are particularly useful to bridge the gap between chemical contamination and ecological status, since they can cover a broad range of toxicity mechanisms in diverse organisms, and account for additional risks posed by non-target compounds and mixtures. Bioassays already provide the regulatory basis to derive environmental quality standards (EQS) (EC, 2011) and to evaluate pelagic toxicity under the REACH authorization process (ECHA 2014). They are also applied to assess effluents from domestic wastewater treatment plants and industrial sectors (OSPAR, 2007; Gartiser et al., 2009). Moreover, the recommendation to integrate bioassays in regulatory water quality monitoring (Hecker and Hollert, 2011; Hamers et al., 2013; Wernersson et al., 2015) is supported by many tests being available as standardized methods (OECD guidelines, ISO standards). However, there are still open questions that prevent their application in effect-based monitoring of water bodies. A major issue is whether reliable results can be achieved when evaluating effects of samples containing diverse aquatic pollutants and chemical mixtures. Particularly, the evaluation of emerging contaminants, such as pharmaceuticals, personal care and disinfection products, is a current priority in regulatory water quality monitoring (Loos et al., 2009; von der Ohe et al., 2012).

In response to that, the present interlaboratory study (ILS) was developed as a collaborative exercise to investigate whether a battery of miniaturized bioassays would produce consistent results for the evaluation of blinded samples containing pristine water extract spiked with representative emerging pollutants as single-chemicals or mixtures. These included:

(i) Triclosan, a chlorinated phenoxy phenol used as biocide in personal care and household products, already suggested as candidate priority substance (von der Ohe et al., 2012);
(ii) 17α-ethinylestradiol (EE2), a synthetic estrogenic human and veterinary pharmaceutical recently included in the European chemical watch list for water quality monitoring (EC, 2013; Kunz et al., 2015);
(iii) Acridine, a heterocyclic aromatic hydrocarbon of industrial origin and a carbamazepine transformation product found in aquatic sediments and groundwater (Hartnik et al., 2007; de Voogt and Laane, 2009);
(iv) 3-Nitrobenzanthrone (3-NBA), a potent mutagenic diesel exhaust component that occurs in aquatic sediments and rainwater (Murahashi et al., 2003; Lübcke-von Varel et al., 2012).

The water extract included a realistic environmental matrix as a sample component, increasing the relevance of the study for water quality assessment. Methods evaluated effects on organisms from three trophic levels (algae, daphnids, fish) and mechanism-specific effects using *in vitro* estrogenicity and mutagenicity assays. The resulting interlaboratory trial brings a novel approach since, with very few exceptions (Carvalho et al., 2014; Escher et al., 2014), previous bioassay ILS focused on only one or few methods, a single mode of action, or single chemical or sample (Hoss et al., 2012; Reifferscheid et al., 2012; Feiler et al., 2014). Finally, a unique aspect of this study that is reflected in the discussion is the clear aim to promote the regulatory use of bioassays for water quality monitoring at the European policy-makers level.

2. Material and methods

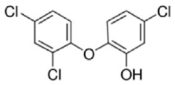
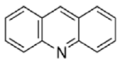
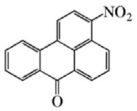
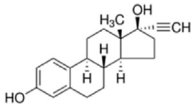
2.1. Chemicals

Information on the test chemicals is provided in Table 1.

2.2. Participant institutes and design of study

The study was coordinated by the Department of Ecosystem

Table 1
Chemical properties of the compounds used for water extract spiking.

Chemical	CAS number	Formula	Supplier	Purity	Structure	Molecular weight (g mol^{-1})	logK_{ow}	Solubility in water (mgL^{-1})
Triclosan	3380-34-5	$C_{12}H_7Cl_3O_2$	Sigma-Aldrich (Germany)	≥97%		289.6	4.76 [a]	10 (20 °C) [a]
Acridine	260-94-6	$C_{13}H_9N$	Merck (Germany)	>98%		179.2	3.40 [a]	38.4 mg/L (24 °C) [a]
3-Nitrobenzanthrone (3-NBA)	17117-34-9	$C_{17}H_9NO_3$	Chiron AS (Norway)	>98%		275.3	4.5 [b]	0.025 [b]
17α-Ethinylestradiol (EE2)	57-63-6	$C_{20}H_{24}O_2$	Sigma-Aldrich (Germany)	≥98%		296.4	3.67 [a]	11.3 (27 °C) [a]

[a] National Center for Biotechnology Information. PubChem Compound Database (September 2015).
[b] Predicted data, US Environmental Protection Agency's EPISuite™, KOWWIN v1.67 estimate.

Analysis, Institute for Environmental Research, RWTH Aachen University, Germany. The 11 participant laboratories (Table S1, S.I.) are associates of the NORMAN working group (WG) on bioassays and biomarkers. The battery composition was defined during a WG meeting in agreement with the different participants, considering the relevance of different bioassays for water quality assessment. After, the WG participants responded to a query regarding their interest in performing the different tests. Finally, three to four laboratories were selected to perform each bioassay, with inclusion of all interested.

2.3. Battery of bioassays

The bioassay battery (Table 2, Table S2) evaluated effects on organisms from different trophic levels: unicellular green algae growth inhibition (Algae), daphnid immobilization (*Daphnia*), and zebrafish embryo lethality and morphological effects (FET). Mechanism-specific assays evaluated estrogenicity (ER-Luc and YES) and mutagenicity (Ames). Experiments were performed in miniaturized format following static exposure without vessel pre-incubation with test solutions.

2.4. Water sample extract spiking

A 180 L water sample was collected at the pristine creek Wormsgraben (Harz Mountains, Germany), transported to the laboratory in stainless steel drums, extracted using large-volume solid phase extraction (Schulze et al., in preparation), and concentrated in 18 mL methanol. The method is described in the supplementary material. The water extract was evaluated in some bioassays (Table S3) by the coordinator.

Chemicals for spiking (Table 1) were selected due to relevance as emerging pollutants and bioactivity. Effect-data from previous studies and own preliminary tests (Table S3) provided the basis for spiking composition decision. Two or three spikes were designed per assay (Table 3) having either the most active toxicant(s) for each method or a final chemical mixture containing a fixed ratio of respective single chemical(s). Concentrations aimed to produce full dose-response curves considering as maximum test concentration 1 $\mu L_{extract}/mL_{medium}$, corresponding to an enrichment factor of 10 (10 $mL_{water\text{-}equivalent}/mL_{medium}$). Spikes for *Daphnia*, FET, ER-Luc and Ames were prepared by water extract evaporation to dryness, addition of DMSO as carrier, and spiking of chemicals using stock solutions in DMSO followed by separation in aliquots for each participant. For algae and YES, the water extract was spiked with the chemicals in methanol, divided in aliquots, and evaporated to dryness. Aliquots were coded and shipped at room temperature to the laboratories, who were not informed on sample composition during the testing period. DMSO was also provided for solvent control conditions. Samples were then stored at 4 °C.

2.5. Exposure setup and tested concentration ranges

Experiments were repeated mostly three times per bioassay, in each test with 3–4 replicate wells/vessels for each test condition following exposure setups described in Table 3.

2.6. Integrated data and statistical analysis

Bioassay results (expressed as described in Table 2) were evaluated following the same data preparation and statistical analysis methods. Results from experimental replicates were pooled and EC_{50} values were calculated for grouped experiments either by 2-parameter Weibull function using R language package (*Daphnia*), two parameter log-logistic curve from 0 to 100% with the two adjustable parameters being slope and EC_{50} by GraphPad Prism 6 (Algae, FET, Ames), or four-parameter log-logistic function with GraphPad (ER-Luc, YES). Differences between logEC_{50} values from different laboratories were compared by *t*-test or one-way ANOVA followed by Tukey's multiple comparisons test. EC_{50} values obtained in $\mu L_{extract}/mL_{bioassay}$ were converted to nominal concentrations of individual chemicals contained in each sample. For Algae, *Daphnia* and FET, ratios between EC_{50} ($\mu L_{extract}/mL_{bioassay}$) values of single-chemical and mixture spikes ($EC_{50\text{-}single}$:$EC_{50\text{-}mixture}$) were calculated. That allowed comparing single- and mixture-spike effects, since the mixture contained a fixed ratio of triclosan and acridine. For ER-Luc and YES, toxic-equivalent factors to respective standard chemical, 17β-estradiol (E2) or EE2, were obtained. Relative estrogenic potencies are expressed as E2 or EE2 equivalents (EEQ), calculated as a ratio between the EC_{50} of the reference compound and the EC_{50} of the spiked sample: $EEQ = EC_{50\text{-}E2 \text{ or } EE2}/EC_{50\text{-}sample}$. The only exception was the water extract, for which the EEQ was obtained with the PC_{10} approach (Besselink, 2015).

Table 2
Bioassays performed in the ILS, with indication of respective method title, endpoints, model organisms, exposure duration and protocol.

Bioassay	Method title	Endpoints/expressed results	Model organism	Exposure duration (h)	Exposure vessels	Medium per vessel or well (mL)	Protocols followed by laboratories (identified by codes) [a]
Algae test	Freshwater algal growth inhibition test	Growth inhibition/Growth inhibition normalized to solvent control	*Pseudokirchneriella subcapitata*	72	96-well plates	0.2	10, 9, 11: OECD Test No. 201 (OECD, 2011) or ISO 8692:2012 (ISO, 2012b) modified to 96-well plate
	Combined algae assay	Inhibition of microalgae growth and photosynthesis/Growth and photosynthesis inhibition normalized to solvent control	*P. subcapitata*	24	96-well plates	0.3	2, 3: Combined algae assay (Escher et al., 2008)
Daphnia test	*Daphnia* sp. acute immobilization test	Immobilization of daphnids/Immobilization occurrence	*D. magna*	48	96-well plates, glass tubes, glass beakers	0.2 10 20	5, 6, 7, 10 and 11: OECD Test No. 202 (OECD, 2004) or ISO 6341:2012 (ISO, 2012a)
FET test	Fish embryo acute toxicity test	Fish embryo lethality and occurrence of morphological sublethal endpoints/Occurrence of lethality and cumulative occurrence of lethal and sublethal morphological endpoints	*Danio rerio*	96	96-well plates	0.2	4, 9 and 10: OECD Test No. 236 (OECD, 2013b) with observation of sublethal morphological endpoints modified to 96-well plate
YES assay	Yeast estrogen screening assay	Estrogen receptor binding activity/Induction values converted to % of standard maximum response (after subtracting the solvent response from both sample and standard)	Recombinant yeast cells	18–72	96-well plates	0.2	1: β-galactosidase recombinant yeast following ISO/TC 147/SC 5 N 804 (ISO, 2013); 6: β-galactosidase recombinant yeast (Routledge and Sumpter, 1996)
				2.5	96-well plates	0.2	9: Luciferase recombinant yeast (Leskinen et al., 2003, Leskinen et al. 2005)
ER-Luc assay	Estrogen receptor luciferase reporter-gene assays with permanent cell lines	Estrogen receptor binding activity/Induction values converted to % of standard maximum response (after subtracting the solvent response from both sample and standard)	Luciferase reporter gene permanent human cell lines	19–24	96-well plates	0.2	5: T47D-kbLuc breast cancer cells (Wilson et al., 2004); 8: BG1Luc4E2 ovarian cancer cells (Rogers and Denison, 2000; OECD, 2012); 10: osteosarcoma cells (Maletz et al., 2013; Besselink, 2015)
Ames assay	Ames fluctuation assay	Induction of reverse mutations/Revertant numbers converted to % of positive control maximum response (after subtracting solvent revertants from both sample and positive control)	*Salmonella* strains TA100 and TA98	48 h	24-/384-well plates	0.5 (+2.5)/0.05	1, 8, 10: ISO 11350 (ISO, 2012c);3: (Reifferscheid et al., 2012; Escher et al., 2014)

[a] Laboratory code numbers are described in Table S1.

Table 3
Composition of the spiked water samples for each bioassay, consisting of one or two single-chemical spiking and a chemical mixture for each bioassay.

Bioassay	Sample	Composition of spiking of 10,000 times concentrated water extract				Exposure setup		
		Triclosan (mg/mL extract)	Acridine (mg/mL extract)	EE2 (μg/mL extract)	3-NBA (μg/mL extract)	Maximal test concentration (mL extract/ L medium)	Serial dilution steps	Number of tested dilutions
Algae test	Triclosan	0.1	–	–	–	1–3 [a]	1: 2	5–7 [a]
	Acridine	–	10	–	–	50–33 [b]	(2-fold)	16 [b]
	Mixture	0.1	10	100	–			
Daphnia test	Triclosan	1	–	–	–	1	1: 2	4–5
	Acridine	–	15	–	–		(2-fold)	
	Mixture	1	15	100	2			
FET test	Triclosan	3	–	–	–	0.77	1: 1.3	5
	Acridine	–	2	–	–	1	(1.3-fold)	
	Mixture	3	2	100	2	0.58		
YES assay	EE2	–	–	100	–	0.1–2	3: 10 and 1: 3	9–16
	Mixture	1	2	100	–		(3.3 and 3-fold)	
ER-luc assay	EE2	–	–	1	–	0.5–1	1: 10	7
	Mixture	1	2	1	–		(10-fold)	
Ames assay	3-NBA	–	–	–	2	1	1: 2	6
	Mixture	0.1	2	100	2		(2-fold)	

[a] Freshwater algal growth inhibition test with unicellular green algae.
[b] Combined algae assay.

3. Results and discussion

Differences between assay results are indicated either as not significant (n.s.) or according to p values. Effect-concentration values for different tests and laboratories are detailed in S.I.

3.1. Toxic effects on aquatic organisms

Aquatic organisms differed in terms of sensitivity to triclosan (algae > daphnids > fish) and acridine (fish > daphnids > algae) spikes. Present EC_{50} nominal ($EC_{50\text{-nom}}$) for single-chemical spikes (Fig. 1) were in same range as literature data for tests performed in microtiter plates (Table S4) but tended to be higher than literature values based on measured concentrations or for experiments in higher medium volume.

3.1.1. Algae test

The OECD/ISO Algae test was the most sensitive aquatic organism assay to triclosan, in agreement with freshwater algal growth being more sensitive than endpoints in bacteria, protozoa, macrophytes, daphnids, amphibians and fish (Orvos et al., 2002; Tatarazako et al., 2004; Harada et al., 2008; Tamura et al., 2013). Detected 72 h growth-inhibition $EC_{50\text{-nom}}$ (14.7 and 25.7 μg/L, n.s.) are in the same range as previous 72 and 96 h $EC_{50\text{-nom}}$ for *P. subcapitata* determined also in 96-well plates (Harada et al., 2008; Rosal et al., 2010). However, our values are 3–50 times higher than results obtained by incubation in 20–100 mL of medium (i.e. 100–500 times the present volume) (Orvos et al., 2002; Tatarazako et al., 2004; Yang et al., 2008; Tamura et al., 2013). Since triclosan is relatively hydrophobic, adsorption to the plate material could have occurred (Rojíčková et al., 1998). Triclosan is also prone to phototransformation (Tixier et al., 2002), which could be another source of variability. The OECD TG (2011) already discusses the interference of these aspects with single-chemicals, which can provide a basis for investigating the stability of water extracts components during exposure. Finally, the water extract matrix could have decreased triclosan bioavailability due to its high sorption capacity to organic matter (Reiss et al., 2002).

For acridine, even if our $EC_{50\text{-nom}}$ differed (5.9 and 4.1 mg/L, $p < 0.01$), values were in good agreement with previous 72 h $EC_{50\text{-nom}}$ for *Desmodesmus subspicatus* following exposure in 24-well plates (Eisentraeger et al., 2008). However, values were circa one order of magnitude higher than 96 h $EC_{50\text{-measured}}$ for *Selenastrum capricornutum* (current *P. subcapitata*) exposed in 100–250 mL

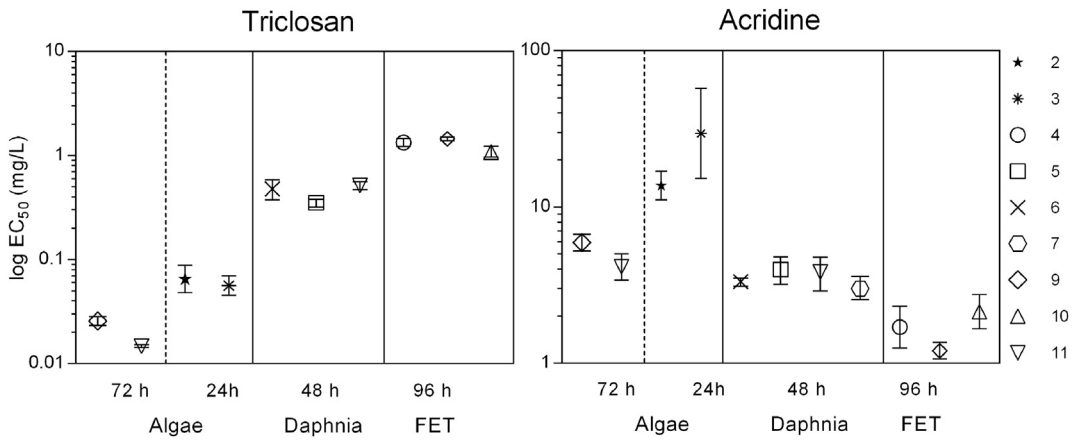


Fig. 1. Effect-concentration values (log EC_{50} and 95% C.I., mg/L) obtained for pooled data from one to three experiments for each assay for the triclosan (left) and acridine (right) spikes in the algae (72 h or 24 h growth inhibition), *Daphnia* (48 h immobilization) and FET (96 h cumulative effects) tests. Results are presented according to laboratory code numbers (Table S1).

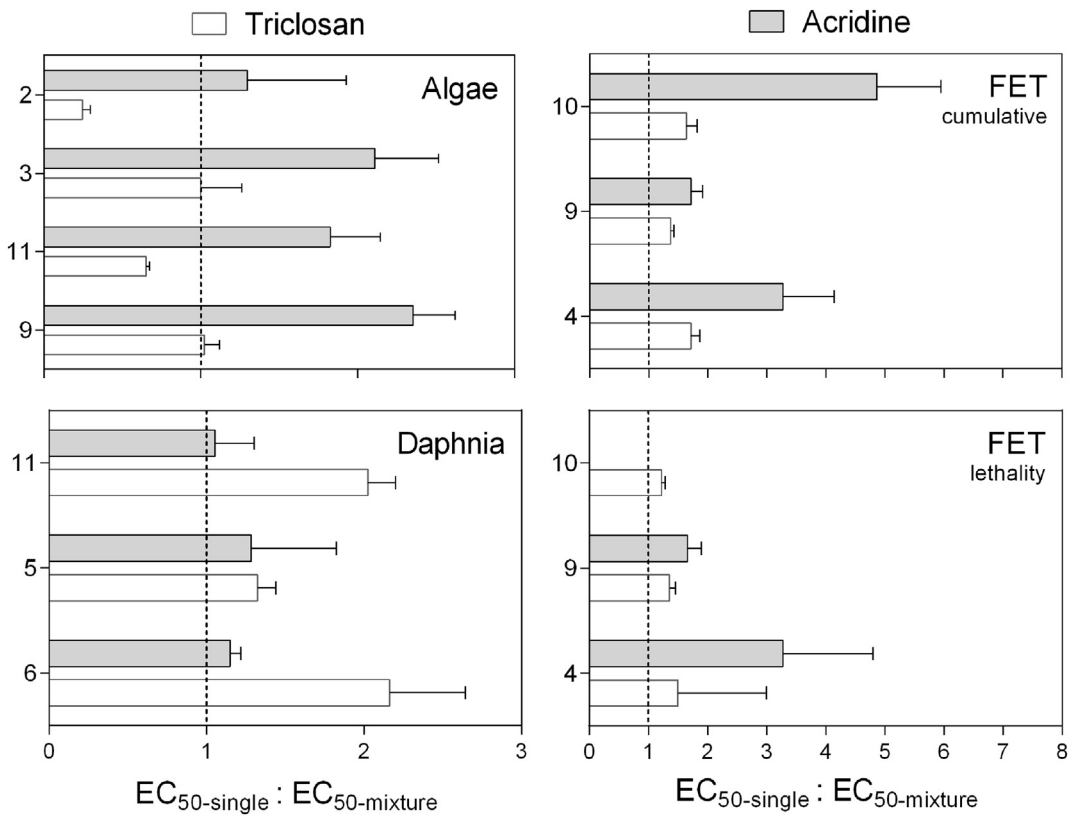


Fig. 2. Ratios between EC_{50} values (μL/mL) for the single-chemical and mixture spikes containing a fixed ratio of respective single compounds ($EC_{50\text{-single}}$: $EC_{50\text{-mixture}}$) for the triclosan (white bars) and acridine (grey bars) spikes in the algae, *Daphnia* and FET (cumulative effects and lethality) tests. Error bars correspond to the ratios between 95% C.I. for single chemicals and the $EC_{50\text{-mixture}}$ value. Y-axis correspond to laboratory code numbers (Table S1).

medium (Blaylock et al., 1985; Dijkman et al., 1997). Sensitivity differences are not known for acridine due to non-specific toxicity mechanism (Dijkman et al., 1997). Decrease in exposure concentration instead may be relevant, since 40–60% losses occurred already prior to exposure start, followed by additional circa 10% decrease during 72 h incubation in 24-well plates (Eisentraeger et al., 2008). Therefore for acridine chemical losses during sample shipping, handling and experiments could have interfered with effective test concentrations.

In the combined algae assay, 24 h growth inhibition $EC_{50\text{-nom}}$ values for triclosan (65.0 and 56.2 μg/L, n.s.) and acridine (13.7 and 29.6, $p < 0.001$) spikes were 2–3 and 2–7 times higher than for the OECD tests, respectively. That indicates time-dependency of effects for both chemicals on algae growth. No tendency for specific photosynthesis inhibition was observed since the photosynthesis endpoint was equally or less sensitive than growth inhibition (results not shown) (Escher et al., 2008; Tang and Escher, 2014). Still, this is a very relevant endpoint since many current WFD priority and emerging compounds present this mode of action.

$EC_{50\text{-single}}$:$EC_{50\text{-mixture}}$ ratios for triclosan (Fig. 2) reached values near or less than 1 and were lower than those for acridine, suggesting its effects were prevalent in the mixture. EE2 is not considered to have caused substantial growth inhibition, since the higher exposure concentration (0.1 mg/L) was seven to ten-fold lower than previous NOEC (0.71 mg/L) or LOEC (1.2 mg/L) (Maes et al., 2014).

3.1.2. *Daphnia* test

The OECD/ISO *Daphnia* immobilization test presented intermediate sensitivity to both triclosan and acridine spikes. Present triclosan 48 h immobilization $EC_{50\text{-nom}}$ (351–516 μg/L, n.s.) are in similar range as previous studies (Orvos et al., 2002; Harada et al., 2008; Peng et al., 2013). The compound was also found to cause effects in *D. magna* reproduction test lasting 21 days, with LOEC values for reduced number of neonates being circa half of respective 48 h immobilization EC_{50} (Orvos et al., 2002; Peng et al., 2013).

Also for acridine the obtained $EC_{50\text{-nom}}$ (3.0–5.1 mg/L, n.s.) agree with previous results (Blaylock et al., 1985; Feldmannová et al., 2006; Eisentraeger et al., 2008). Acridine caused also reduction in offspring number produced per brood in semi-static exposure during 14 d, with the LOEC being less than half of respective acute EC_{50} (Blaylock et al., 1985).

Considering $EC_{50\text{-single}}$:$EC_{50\text{-mixture}}$ ratios (Fig. 2), acridine values were near 1 and lower than for triclosan, indicating that its effects were prevalent in the mixture. EE2 effects are considered to be negligible, since its highest exposure concentration (0.1 mg/L) was 50 times lower than previous NOEC (Goto and Hiromi, 2003). Although no information for 3-NBA was found in the literature, acute effects are not considered relevant due to low concentrations.

3.1.3. FET test

The OECD FET test presented the lowest sensitivity to triclosan and the highest sensitivity to acridine among aquatic organism tests.

Triclosan 96 h $LC_{50\text{-nom}}$ (1.3–1.9 mg/L, n.s.) and $EC_{50\text{-nom}}$ (Table S5) are circa three times higher than previous 96 h $LC_{50\text{-nom}}$ for zebrafish embryos exposed in 24-well plates (Oliveira et al., 2009) or medaka in petri dishes under semi-static conditions (Ishibashi et al., 2004). This discrepancy could be related to differences in medium volumes and ratios surface area to volume of

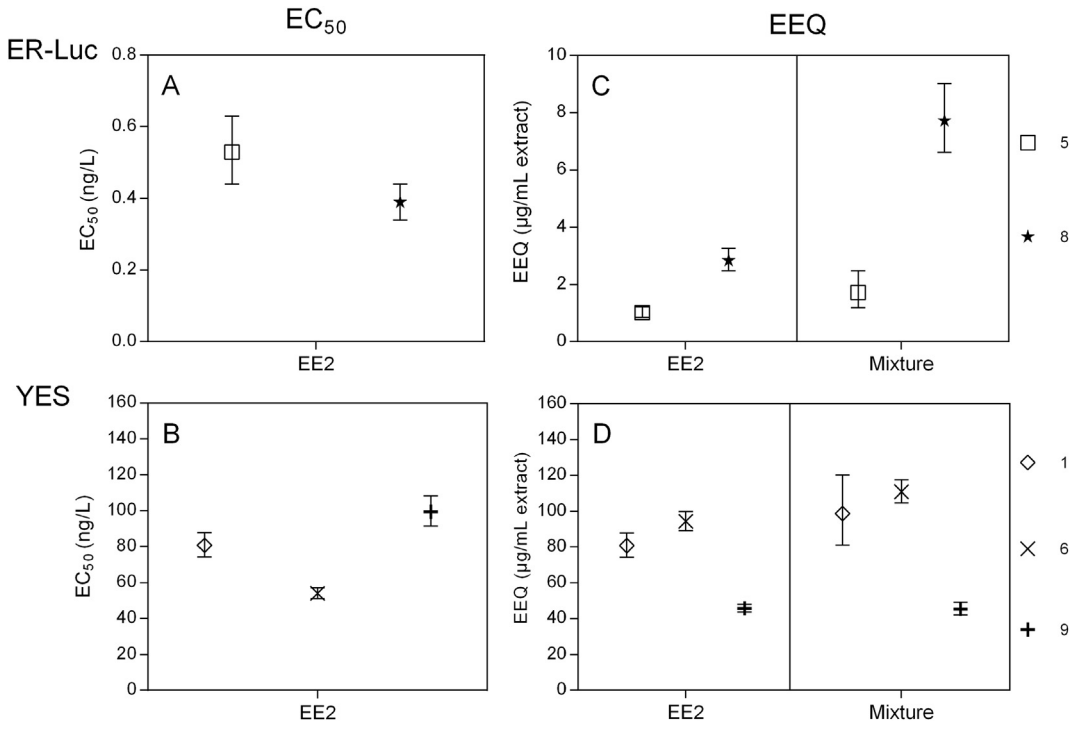


Fig. 3. EC_{50} (ng/L) values for EE2 in the ER-Luc (A) and YES (B) assays, and EEQ values obtained for the EE2 and the mixture spikes in the ER-Luc (C) and the YES (D) assays. EC_{50} values (symbols) and 95% C.I. (error bars) for respective sample. Results are presented according to laboratory code numbers (Table S1). Biological models are: T47D-kbLuc (5) BG1Luc4E2 (8), β-galactosidase recombinant yeast by McDonnell et al. (1991) (1), β-galactosidase recombinant yeast by Routledge and Sumpter 1996 (6), and luciferase recombinant yeast by Leskinen et al., 2003 (9).

exposure vessels. However, triclosan concentrations decreased to circa half even in 1 L of water after 24 h adult medaka exposure (Ishibashi et al., 2004). Therefore other factors could play a role such as phototransformation, which can be minimized by incubation in dark. Among sublethal effects, reduced growth and delayed development were prevalent, similarly to effects in *Xenopus laevis* embryos (Harada et al., 2008). Triclosan was also related to delayed swim-up behaviour initiation and reduced survival in rainbow trout early-life stages (Orvos et al., 2002) and to disrupted swimming and predator avoidance in fathead minnow larvae (Cherednichenko et al., 2012; Fritsch et al., 2013). We observed increased heartbeat rates at 96 h in zebrafish exposed to 1.0 (47.0 beats/20 s, $p < 0.01$) and 1.3 mg/L (48.7 beats/20 s, $p < 0001$) compared to water and solvent controls, concentrations which caused none and circa 10% ($p < 001$) cumulative effects, respectively. Since triclosan can impair the excitation-contraction coupling of cardiac and skeletal muscle (Cherednichenko et al., 2012; Fritsch et al., 2013), increased compensatory heartbeat rate could have occurred. Therefore the assessment of sublethal endpoints can support the identification of toxic effects other than lethality (Di Paolo et al., 2015a; Jonas et al., 2015).

For acridine, FET 96 h $LC_{50\text{-nom}}$ (0.71–1.28 mg/L, n.s.) were circa three times lower than those from *Daphnia* and algae tests. Present values are slightly higher than previous measured 48 h LC_{50} performed in 24-well plates (Peddinghaus et al., 2012). That can be related to possible acridine losses before and during experiments, since concentrations were shown to decrease to less than half of nominal values (Peddinghaus et al., 2012). Performance of semi-static exposure with solution renewal could be a possible solution to maintain exposure concentrations (OECD, 2013b).

Considering the $EC_{50\text{-single}}$:$EC_{50\text{-mixture}}$ (Fig. 2), triclosan tended to present lower values when compared to acridine, indicating it was prevalent in the mixture toxicity. EE2 effects are considered to be negligible, since its highest exposure concentration (0.1 mg/L) was 50 times lower than previous NOEC (5 mg/L) (Goto and Hiromi, 2003). For 3-NBA, although no information was found in the literature, acute effects are considered to be negligible.

3.2. Estrogenicity assessment

Although differences occurred between different estrogenicity assays and models, relative induction $EC_{50\text{-nom}}$ values were comparable to the literature, and obtained EEQ for the EE2 spike are in good agreement with previous values for ER-Luc and YES (Fig. 3).

3.2.1. ER-Luc assay

Among all assays performed by the coordinator (Table S3), the non-spiked water extract was active only in the ER-Luc (ER-CALUX), with an EEQ of 0.17 ± 0.01 ng/L_{water} for the enrichment factor of 1. EE2 spike induction EC_{50} (0.53 and 0.39 ng/L_{medium}, n.s.) were within the range of previously reported values for EE2 (Legler et al., 2002; Murk et al., 2002; Wilson et al., 2004; Bermudez et al., 2012; OECD, 2012). Although EEQ values showed some variation (Fig. 3C), which could be related to differences in assay protocol or model sensitivity (Jarošová et al., 2014), EEQ determination showed to be a reliable measurement for sample content.

Considering the mixture spike, concentrations ≥0.5 $\mu L_{extract}/mL_{medium}$ caused cytotoxicity and were excluded from regression analysis. This effect is considered to be caused by triclosan concentrations (≥0.5 mg/L_{medium}) in the cytotoxic range for human cells (Henry and Fair, 2013); while no acridine cytotoxicity is indicated (Brinkmann et al., 2014). Tendency for higher EEQ values was observed for the mixture spike (Fig. 3C). It could be discussed that such response is related to estrogen receptor binding by other chemicals in mixture, since acridine induction in T47Dluc assay produced an estradiol equivalency factor (EEF) of $2.5.10^{-7}$

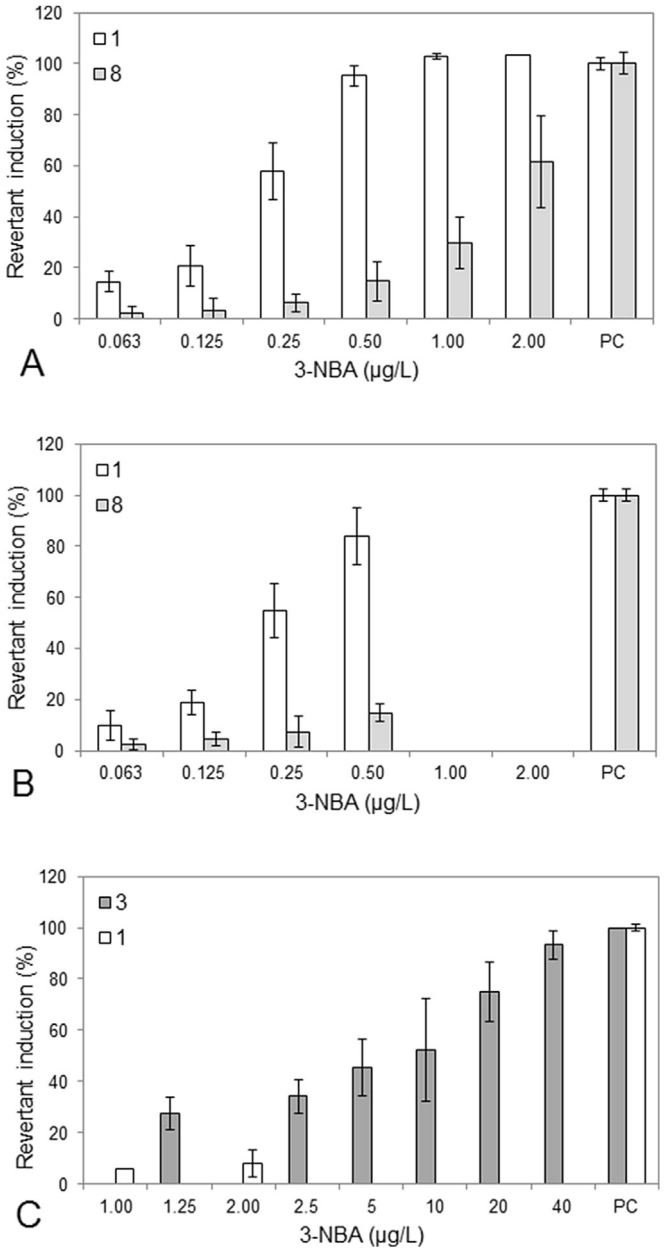


Fig. 4. Revertant induction versus 3-NBA concentrations (µg/L) contained in (A) 3-NBA spike in TA98-S9, (B) mixture spike in TA98 -S9, and (C) 3-NBA spike in TA100 -S9; plus respective positive control (PC) conditions. Average values (bars) and standard deviations (error bars) for two to three experiments. Results are presented using laboratory code numbers (Table S1).

(Brinkmann et al., 2014). However there is no evidence of triclosan agonism in estrogen-receptor reporter gene cell-based assays (own results) (Ahn et al., 2008). More likely, non-specific effects on cellular membranes or metabolism (Ajao et al., 2015) could have interfered with induction.

3.2.2. YES assay

Our induction EC_{50} for the EE2 spike varied up to 2.5-fold (54.1–132.7 ng/L, $p < 0.01$ to 0.0001), in similar range to literature data (Table S4). The lowest EE2 spike EC_{50} was produced by the Routledge/Sumpter strain (1996), in agreement with previous studies (Van den Belt et al., 2004; Balsiger et al., 2010), while the bioluminescent strain (Leskinen et al., 2005) produced the highest value. For the McDowell/ISO assay (ISO, 2013), the EC_{50} of 99.5 ng/L was slightly higher than the EC_{50} obtained for the standard curve (80.4 ng/L), which also uses EE2 in this assay. EEQ values varied circa 2-fold (45.8–94.3 $\mu g/mL_{extract}$), which can be related to the fact that different yeast strains and protocols can produce different EEF values (Svobodová et al., 2009; Jarošová et al., 2014). Therefore for the application of estrogenicity assays in water quality, effect-concentrations for the standard chemical, main estrogens and investigated samples should be determined using the same model and protocol (Jarošová et al., 2014; Kunz et al., 2015).

The highest mixture spike test concentrations (≥ 0.1 $\mu L_{extract}/mL_{medium}$) caused cytoxicity to the yeast cells and were excluded from regression analysis. This is attributed mostly to triclosan (≥ 0.1 mg/L_{medium}), since acridine concentrations are not expected to be toxic to the yeast cells (Brinkmann et al., 2014). No differences occurred between respective EEQ values for single and mixture spikes (Fig. 3D). Previously, acridine was not identified as estrogenic by the lyticase YES assay (Brinkmann et al., 2014). Although triclosan was active in the Routledge/Sumpter strain, the compound was not identified as estrogenic in the bioluminescent YES (Svobodová et al., 2009).

3.3. Mutagenicity assessment by the Ames fluctuation assay

Strong revertant induction occurred following 3-NBA spike incubation with the TA98 strain in the absence of S9 fraction (-S9) (Fig. 4A), which was of lower magnitude after metabolic transformation and for TA100 -S9 (Fig. 4B–C). 3-NBA spike revertant induction EC_{50} values were 0.21 and 1.56 µg/L ($p < 0.01$) for TA98 -S9; and 5.73 µg/L for TA100 -S9. Therefore the compound was clearly identified as mutagenic, although further improvement might be needed if precise effect-concentration values are required. Such results are in agreement with previous studies describing 3-NBA as a strong direct-acting mutagen in the TA98 strain, and the fact that it is less active in TA100 suggests that it causes frameshift-type mutations (Enya et al., 1997; IARC, 2014). Further, there are indications that 3-NBA is also genotoxic *in vitro* and in *in vivo* (Watanabe et al., 2005b). 3-NBA is a major mutagen in diesel particles, sediments, and surface soils (Enya et al., 1997; Watanabe et al., 2005a; Lübcke-von Varel et al., 2012) and concentrations up to 2.6 ng/L were identified in rainwater (Murahashi et al., 2003).

For the mixture spike, test concentrations ≥ 0.5 $\mu L_{extract}/mL_{medium}$ caused toxic effects in −S9 exposures (attributed to triclosan 50 ng/mL medium), which were excluded from regression analysis (Fig. 4B, Fig. S7). Cytotoxic effects were reduced by the S9 fraction incubation (Fig. S7), suggesting that resulting triclosan metabolites present less toxic effects than the parent compound. Our results showed that neither triclosan nor acridine caused increase in the number of revertants (Table S4), in agreement with previous studies investigating their mutagenicity through the Ames plate incorporation method (Eisentraeger et al., 2008; SCCP, 2009).

3.4. Bioassay battery strategy

Bioassay battery assessment of water quality is based on the consideration that one single bioassay does not provide an overview on potential effects on different organisms and toxicity mechanisms. Since sensitivity to different toxicants varies between organisms, multi-taxa assessment supports the comprehension of toxicant effects on aquatic communities (Guillen et al., 2012). The organism-level assays proposed in the present study investigate population-level effects in freshwater algae as primary producers, acute toxicity to the filter-feeder invertebrate *Daphnia*, and acute toxicity to fish individuals. Multi-taxa toxicity assessment is applied for EQS derivation within the WFD, which requires

evaluation of acute and chronic data for (i) alga/macrophyte, (ii) *Daphnia*/another invertebrate, and (iii) fish (EC, 2011). Similar strategy is applied in REACH to evaluate aquatic pelagic toxicity (ECHA 2014). The suitability of the algae, *Daphnia* and FET assays to compose a basic (eco)toxicity test battery was evaluated for hazard waste, wastewater effluent, freshwater and drinking water assessment (Keddy et al., 1995; Diaz-Baez et al., 2002; Manusadžianas et al., 2003; Pandard et al., 2006; Gartiser et al., 2009; Römbke and Moser, 2009); and for effect-directed analysis (Brack et al., 2013, 2016; Di Paolo et al., 2015b). Therefore the assays are expected to be already established in diverse laboratories worldwide. Finally, the followed miniaturized assay performance has already been investigated in comparison with higher-volume methods and with adult fish for the FET (Eisentraeger et al., 2003; Knobel et al., 2012; Baumann et al., 2014).

Complementary, mechanism-specific bioassays can provide information on modes-of-action that are intrinsically of concern for ecosystems and health. For example, the photosynthesis inhibition endpoint of the performed combined algae test covers many current WFD priority compounds and emerging compounds. Furthermore, endocrine disruption and mutagenicity are of particular relevance for population-level effects and humans (EC, 2000, 2011; ECHA 2014). For estrogens, regulatory strategies involving bioassays are reinforced after the recent inclusion of estrogenic pharmaceuticals in the WFD watch list (Hecker and Hollert, 2011; EC, 2013). In fact, both ER-Luc and YES assays have been recommended for estrogen monitoring in water bodies (Loos, 2012). Regarding mutagenicity, the Ames fluctuation assay round-robin study was the first step towards its regulatory implementation in water legislation (Wolz et al., 2010; Reifferscheid et al., 2012). Moreover, the Ames and umu tests are recommended as mutagenicity and genotoxicity methods for the waste ecotoxicological characterization (Römbke and Moser, 2009). Due to their environmental and health relevance, estrogenicity and mutagenicity assays are also established in many laboratories.

The present results complement previous validation studies of the organism-level and mechanism-specific tests by demonstrating the good performance of methods not only with single chemicals but also to evaluate water extracts spiked with emerging contaminants. Our approach can provide useful information to link chemical testing and field studies with those assays. A relevant aspect to consider is that the assays can be applied to evaluate not only water extracts but raw water samples and effluents. In this sense the proposed bioassay battery presents a flexible setup for diverse applications in the context of water quality monitoring.

3.5. Stepping-stones towards the establishment of bioassays in water quality monitoring

Currently there are diverse European initiatives towards bioassay application in water quality assessment, such as the Technical Report on effect-based tools in the context of the WFD (Wernersson et al., 2015) and activities for the validation of low volume, high-throughput bioassay batteries (Brack et al., 2013, 2015; Altenburger et al., 2015; Neale et al., 2015; Schulze et al., 2015). Such applied studies will be of high relevance for the decision on a basic battery for water monitoring. Similarly to our approach, these initiatives tend to focus on assays that allow relatively fast performance. Consequently, only acute toxicity is evaluated in fish and daphnids, while mechanism-specific methods are investigated in the *in vitro* level. However, after the setup of such basic battery, its composition can certainly be expanded according to regional requirements or specific investigation. For instance, when chronic fish toxicity is suspected, the decision on whether to perform chronic tests can be supported by toxicity assays with fish early-life stages (OECD, 2013a; Villeneuve et al., 2014; Di Paolo et al., 2015a). In cases when freshwater sediments present a concern, whole-sediment toxicity assays with different organisms are available. Ring tests have demonstrated the good performance of tests evaluating macrophyte growth impairment (Feiler et al., 2014); and growth and reproduction effects on interstitial water nematodes (Hoss et al., 2012). Recent studies include also a methodological investigation of a freshwater ostracod sub-chronic test (Casado-Martinez et al., 2016); and a tiered strategy for sediment risk assessment integrating different toxicity tests (Diepens et al., 2016).

Importantly, the investigation of additional mechanism-specific toxicities can rely on diverse reporter-gene assays, for which effect-based trigger values to support decisions on water quality assessment are being established (Loos, 2012; Brand et al., 2013; Escher et al., 2015). In parallel to these tests, it is necessary to investigate the occurrence of non-specific toxicity caused by sample components, which can interfere with the performance of assays and even mask mechanism-specific effects (Brack et al., 2016). That was demonstrated in our study for the ubiquitous contaminant triclosan, which was cytotoxic to human cells, yeast and bacteria at concentrations representative of water samples or extracts (von der Ohe et al., 2012). Finally, further studies can investigate remaining aspects of relevance for bioassays screening of water sample and extracts. For instance, different conditions of sample storage can partially affect chemical composition, including of endocrine disruptors (Aboulfadl et al., 2010). In the future, the influence of sample shipping and storage conditions should be evaluated not only through chemical analysis but also regarding effects on bioassay performance and results.

4. Conclusions and outcomes

The battery of miniaturized bioassays presented complementary sensitivity and specificity to the water extract spikes containing four emerging pollutants as single-chemicals or mixtures. Aquatic organism sensitivity varied following exposure to different chemicals, confirming the complementary role of the tests with the three taxa for water quality assessment. Estrogenicity and mutagenicity assays identified with high precision the respective mechanism-specific effects of spikes, even though non-specific toxicity of mixture compounds affected the evaluation of higher test concentrations. Since differences in experimental protocols, model organisms, and data analysis can affect the determination of effect-concentrations, respective standard methods and harmonized procedures should be followed when implementing bioassays in water monitoring. Together with other ongoing activities for the validation of a basic battery of bioassays, the present study is an important step towards the implementation of bioanalytical monitoring tools in water quality assessment and monitoring.

Acknowledgements

We would like to thank the colleagues at RWTH Aachen Simone Hotz, Monika Lam, Christine Schönlau and Josef Koch for the contribution to the preliminary tests of the water extract and chemicals; and Yvonne Müller and Sarah Johann for their help in organizing the project meeting in Aachen. Thanks to Dr. Brigitte von Danwitz from the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV) in Germany for providing useful literature to support the discussion of results. Thanks to Dr. Robert Kase from the Swiss Centre for Applied Ecotoxicology Eawag-EPFL in Switzerland for his suggestions on the manuscript draft. This project was developed by the Working Group 2 Bioassays and biomarkers in water quality monitoring of

the NORMAN Association (http://www.norman-network.net). It was funded and supported by the European Marie Curie Initial Training Network EDA-EMERGE (grant agreement no. 290100; www.ufz.de/eda-emerge), the European FP7 Collaborative Project SOLUTIONS (grant agreement no. 603437; www.solutions-project.eu), and the NORMAN Association (www.norman-network.net). CDP was supported by the EDA-EMERGE Marie Curie ITN (ESR1). SA and EM (INERIS) were supported by the French Ministry of Ecology (grant P181) and by the ONEMA.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.08.018.

References

Aboulfadl, K., De Potter, C., Prévost, M., Sauvé, S., 2010. Time-dependent integrity during storage of natural surface water samples for the trace analysis of pharmaceutical products, feminizing hormones and pesticides. Chem. Central J. 4 (1), 1–8.

Ahn, K.C., Zhao, B., Chen, J., Cherednichenko, G., Sanmarti, E., Denison, M.S., Lasley, B., Pessah, I.N., Kültz, D., Chang, D.P.Y., Gee, S.J., Hammock, B.D., 2008. Vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens. Environ. Health Perspect. 116 (9), 1203–1210.

Ajao, C., Andersson, M.A., Teplova, V.V., Nagy, S., Gahmberg, C.G., Andersson, L.C., Hautaniemi, M., Kakasi, B., Roivainen, M., Salkinoja-Salonen, M., 2015. Mitochondrial toxicity of triclosan on mammalian cells. Toxicol. Rep. 2, 624–637.

Altenburger, R., Ait-Aissa, S., Antczak, P., Backhaus, T., Barceló, D., Seiler, T.-B., Brion, F., Busch, W., Chipman, K., de Alda, M.L., de Aragão Umbuzeiro, G., Escher, B.I., Falciani, F., Faust, M., Focks, A., Hilscherova, K., Hollender, J., Hollert, H., Jäger, F., Jahnke, A., Kortenkamp, A., Krauss, M., Lemkine, G.F., Munthe, J., Neumann, S., Schymanski, E.L., Scrimshaw, M., Segner, H., Slobodnik, J., Smedes, F., Kughathas, S., Teodorovic, I., Tindall, A.J., Tollefsen, K.E., Walz, K.-H., Williams, T.D., Van den Brink, P.J., van Gils, J., Vrana, B., Zhang, X., Brack, W., 2015. Future water quality monitoring - adapting tools to deal with mixtures of pollutants in water resource management. Sci. Total Environ. 512–513 (0), 540–551.

Balsiger, H.A., de la Torre, R., Lee, W.-Y., Cox, M.B., 2010. A four-hour yeast bioassay for the direct measure of estrogenic activity in wastewater without sample extraction. Conc. Steriliz. Sci. Total Environ. 408 (6), 1422–1429.

Baumann, J., Sakka, Y., Bertrand, C., Koser, J., Filser, J., 2014. Adaptation of the Daphnia sp. acute toxicity test: miniaturization and prolongation for the testing of nanomaterials. Environ. Sci. Pollut. Res. Int. 21 (3), 2201–2213.

Bermudez, D.S., Gray, L.E., Wilson, V.S., 2012. Modelling defined mixtures of environmental oestrogens found in domestic animal and sewage treatment effluents using an in vitro oestrogen-mediated transcriptional activation assay (T47D-KBluc). Int. J. Androl. 35 (3), 397–406.

Besselink, H.T., 2015. Validation of the (Anti-)ERα CALUX Bioassay U2-OS Cells Transcriptional ERalpha CALUX Assay for the Detection of Estrogenic and Anti-estrogenic Chemicals for Inclusion in the OECD TG 455.

Blaylock, B.G., Frank, M.L., McCarthy, J.F., 1985. Comparative toxicity of copper and acridine to fish, Daphnia and algae. Environ. Toxicol. Chem. 4 (1), 63–71.

Brack, W., Ait-Aissa, S., Burgess, R.M., Busch, W., Creusot, N., Di Paolo, C., Escher, B.I., Mark Hewitt, L., Hilscherova, K., Hollender, J., Hollert, H., Jonker, W., Kool, J., Lamoree, M., Muschket, M., Neumann, S., Rostkowski, P., Ruttkies, C., Schollee, J., Schymanski, E.L., Schulze, T., Seiler, T.B., Tindall, A.J., De Aragao Umbuzeiro, G., Vrana, B., Krauss, M., 2016. Effect-directed analysis supporting monitoring of aquatic environments - an in-depth overview. Sci. Total Environ. 544, 1073–1118.

Brack, W., Altenburger, R., Schuurmann, G., Krauss, M., Lopez Herraez, D., van Gils, J., Slobodnik, J., Munthe, J., Gawlik, B.M., van Wezel, A., Schriks, M., Hollender, J., Tollefsen, K.E., Mekenyan, O., Dimitrov, S., Bunke, D., Cousins, I., Posthuma, L., van den Brink, P.J., Lopez de Alda, M., Barcelo, D., Faust, M., Kortenkamp, A., Scrimshaw, M., Ignatova, S., Engelen, G., Massmann, G., Lemkine, G., Teodorovic, I., Walz, K.H., Dulio, V., Jonker, M.T., Jager, F., Chipman, K., Falciani, F., Liska, I., Rooke, D., Zhang, X., Hollert, H., Vrana, B., Hilscherova, K., Kramer, K., Neumann, S., Hammerbacher, R., Backhaus, T., Mack, J., Segner, H., Escher, B., de Aragao Umbuzeiro, G., 2015. The SOLUTIONS project: challenges and responses for present and future emerging pollutants in land and water resources management. Sci. Total Environ. 503–504, 22–31.

Brack, W., Govender, S., Schulze, T., Krauss, M., Hu, M., Muz, M., Hollender, J., Schirmer, K., Schollee, J., Hidasi, A., Slobodnik, J., Rabova, Z., Ait-Aissa, S., Sonavane, M., Carere, M., Lamoree, M., Leonards, P., Tufi, S., Ouyang, X., Schriks, M., Thomas, K., de Almeida, A., Froment, J., Hammers-Wirtz, M., Ahel, M., Koprivica, S., Hollert, H., Seiler, T.-B., Paolo, C., Tindall, A., Spirhanzlova, P., 2013. EDA-EMERGE: an FP7 initial training network to equip the next generation of young scientists with the skills to address the complexity of environmental contamination with emerging pollutants. Environ. Sci. Eur. 25 (1), 18.

Brand, W., de Jongh, C.M., van der Linden, S.C., Mennes, W., Puijker, L.M., van Leeuwen, C.J., van Wezel, A.P., Schriks, M., Heringa, M.B., 2013. Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays. Environ. Int. 55, 109–118.

Brinkmann, M., Maletz, S., Krauss, M., Bluhm, K., Schiwy, S., Kuckelkorn, J., Tiehm, A., Brack, W., Hollert, H., 2014. Heterocyclic aromatic hydrocarbons show estrogenic activity upon metabolization in a recombinant transactivation assay. Environ. Sci. Technol. 48 (10), 5892–5901.

Carvalho, R.N., Arukwe, A., Ait-Aissa, S., Bado-Nilles, A., Balzamo, S., Baun, A., Belkin, S., Blaha, L., Brion, F., Conti, D., Creusot, N., Essig, Y., Ferrero, V.E.V., Flander-Putrle, V., Fürhacker, M., Grillari-Voglauer, R., Hogstrand, C., Jonáš, A., Kharlyngdoh, Joubert B., Loos, R., Lundebye, A.-K., Modig, C., Olsson, P.-E., Pillai, S., Polak, N., Potalivo, M., Sanchez, W., Schifferli, A., Schirmer, K., Sforzini, S., Stürzenbaum, S.R., Søfteland, L., Turk, V., Viarengo, A., Werner, I., Yagur-Kroll, S., Zounková, R., Lettieri, T., 2014. Mixtures of chemical pollutants at european legislation safety concentrations: how safe are they? Toxicol. Sci. 141 (1), 218–233.

Casado-Martinez, M.C., Burga-Pérez, K.F., Bebon, R., Férard, J.-F., Vermeirssen, E.L.M., Werner, I., 2016. The sediment-contact test using the ostracod Heterocypris incongruens: effect of fine sediments and determination of toxicity thresholds. Chemosphere 151, 220–224.

Cherednichenko, G., Zhang, R., Bannister, R.A., Timofeyev, V., Li, N., Fritsch, E.B., Feng, W., Barrientos, G.C., Schebb, N.H., Hammock, B.D., Beam, K.G., Chiamvimonvat, N., Pessah, I.N., 2012. Triclosan impairs excitation–contraction coupling and Ca2+ dynamics in striated muscle. Proc. Natl. Acad. Sci. 109 (35), 14158–14163.

de Voogt, P., Laane, R.W.P.M., 2009. Assessment of azaarenes and azaarones (oxidized azaarene derivatives) in the Dutch coastal zone of the North Sea. Chemosphere 76 (8), 1067–1074.

Di Paolo, C., Groh, K.J., Zennegg, M., Vermeirssen, E.L.M., Murk, A.J., Eggen, R.I.L., Hollert, H., Werner, I., Schirmer, K., 2015a. Early life exposure to PCB126 results in delayed mortality and growth impairment in the zebrafish larvae. Aquat. Toxicol. 169, 168–178.

Di Paolo, C., Seiler, T.-B., Keiter, S., Hu, M., Muz, M., Brack, W., Hollert, H., 2015b. The value of zebrafish as an integrative model in effect-directed analysis - a review. Environ. Sci. Eur. 27 (1), 1–11.

Diaz-Baez, M.C., Sanchez, W.A., Dutka, B.J., Ronco, A., Castillo, G., Pica-Granados, Y., Castillo, L.E., Ridal, J., Arkhipchuk, V., Srivastava, R.C., 2002. Overview of results from the WaterTox intercalibration and environmental testing phase II program: part 2, ecotoxicological evaluation of drinking water supplies. Environ. Toxicol. 17 (3), 241–249.

Diepens, N.J., Koelmans, A.A., Baveco, H., Brink, P.J., Heuvel-Greve, M.J. and Brock, T.C.M. (2016), pp. 1–77, Springer New York, New York, NY.

Dijkman, N.A., van Vlaardingen, P.L.A., Admiraal, W.A., 1997. Biological variation in sensitivity to N-heterocyclic PAHs; effects of acridine on seven species of microalgae. Environ. Pollut. 95 (1), 121–126.

EC, 2000. Directive 2000/60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L 327, 1–73, 22.12.2000.

EC, 2011. European Commission WFD-CIS Guidance Document No. 27 Technical Guidance on Deriving Environmental Quality Standards. Technical report 2011–055.

EC, 2013. Official J. Eur. Union L 266, 1–17.

ECHA, E.C.A, 2014. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint Specific Guidance.

Eisentraeger, A., Brinkmann, C., Hollert, H., Sagner, A., Tiehm, A., Neuwoehner, J., 2008. Heterocyclic compounds: toxic effects using algae, daphnids, and the Salmonella/microsome test taking methodical quantitative aspects into account. Environ. Toxicol. Chem. 27 (7), 1590–1596.

Eisentraeger, A., Dott, W., Klein, J., Hahn, S., 2003. Comparative studies on algal toxicity testing using fluorometric microplate and Erlenmeyer flask growth-inhibition assays. Ecotoxicol. Environ. Saf. 54 (3), 346–354.

Enya, T., Suzuki, H., Watanabe, T., Hirayama, T., Hisamatsu, Y., 1997. 3-Nitrobenzanthrone, a powerful bacterial mutagen and suspected human carcinogen found in diesel exhaust and airborne particulates. Environ. Sci. Technol. 31 (10), 2772–2776.

Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P., Busch, W., Crago, J., Denslow, N.D., Dopp, E., Hilscherova, K., Humpage, A.R., Kumar, A., Grimaldi, M., Jayasinghe, B.S., Jarosova, B., Jia, A., Makarov, S., Maruya, K.A., Medvedev, A., Mehinto, A.C., Mendez, J.E., Poulsen, A., Prochazka, E., Richard, J., Schifferli, A., Schlenk, D., Scholz, S., Shiraishi, F., Snyder, S., Su, G., Tang, J.Y.M., Burg, B.v.d., Linden, S.C.v.d., Werner, I., Westerheide, S.D., Wong, C.K.C., Yang, M., Yeung, B.H.Y., Zhang, X., Leusch, F.D.L., 2014. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. Environ. Sci. Technol. 48 (3), 1940–1956.

Escher, B.I., Bramaz, N., Mueller, J.F., Quayle, P., Rutishauser, S., Vermeirssen, E.L.M., 2008. Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples. J. Environ. Monit. 10 (5), 612–621.

Escher, B.I., Neale, P.A., Leusch, F.D.L., 2015. Effect-based trigger values for in vitro bioassays: reading across from existing water quality guideline values. Water Res. 81, 137–148.

Feiler, U., Ratte, M., Arts, G., Bazin, C., Brauer, F., Casado, C., Doren, L., Eklund, B., Gilberg, D., Grote, M., Gonsior, G., Hafner, C., Kopf, W., Lemnitzer, B., Liedtke, A.,

Matthias, U., Okos, E., Pandard, P., Scheerbaum, D., Schmitt-Jansen, M., Stewart, K., Teodorovic, I., Wenzel, A., Pluta, H.J., 2014. Inter-laboratory trial of a standardized sediment contact test with the aquatic plant Myriophyllum aquaticum (ISO 16191). Environ. Toxicol. Chem. 33 (3), 662–670.
Feldmannová, M., Hilscherová, K., Maršálek, B., Bláha, L., 2006. Effects of N-heterocyclic polyaromatic hydrocarbons on survival, reproduction, and biochemical parameters in Daphnia magna. Environ. Toxicol. 21 (4), 425–431.
Fritsch, E.B., Connon, R.E., Werner, I., Davies, R., Beggel, S., Feng, W., Pessah, I.N., 2013. Triclosan impairs swimming behavior and alters expression of excitation contraction coupling proteins in fathead minnow (Pimephales promelas). Environ. Sci. Technol. 47 (4), 2008–2017.
Gartiser, S., Hafner, C., Oeking, S., Paschke, A., 2009. Results of a "Whole Effluent Assessment" study from different industrial sectors in Germany according to OSPAR's WEA strategy. JEM 11 (2), 359–369.
Goto, T., Hiromi, J., 2003. Toxicity of 17alpha-ethynylestradiol and norethindrone, constituents of an oral contraceptive pill to the swimming and reproduction of cladoceran Daphnia magna, with special reference to their synergetic effect. Mar. Pollut. Bull. 47 (1–6), 139–142.
Guillen, D., Ginebreda, A., Farre, M., Darbra, R.M., Petrovic, M., Gros, M., Barcelo, D., 2012. Prioritization of chemicals in the aquatic environment based on risk assessment: analytical, modeling and regulatory perspective. Sci. Total Environ. 440, 236–252.
Hamers, T., Legler, J., Blaha, L., Hylland, K., Marigomez, I., Schipper, C.A., Segner, H., Vethaak, A.D., Witters, H., de Zwart, D., Leonards, P.E.G., 2013. Expert opinion on toxicity profiling—report from a NORMAN expert group meeting. Integr. Environ. Assess. Manag. 9 (2), 185–191.
Harada, A., Komori, K., Nakada, N., Kitamura, K., Suzuki, Y., 2008. Biological effects of PPCPs on aquatic lives and evaluation of river waters affected by different wastewater treatment levels. Water science and technology. J. Int. Assoc. Water Pollut. Res. 58 (8), 1541–1546.
Hartnik, T., Norli, H.R., Eggen, T., Breedveld, G.D., 2007. Bioassay-directed identification of toxic organic compounds in creosote-contaminated groundwater. Chemosphere 66 (3), 435–443.
Hecker, M., Hollert, H., 2011. Endocrine disruptor screening: regulatory perspectives and needs. Environ. Sci. Eur. 23 (1), 15.
Henry, N.D., Fair, P.A., 2013. Comparison of in vitro cytotoxicity, estrogenicity and anti-estrogenicity of triclosan, perfluorooctane sulfonate and perfluorooctanoic acid. J. Appl. Toxicol. 33 (4), 265–272.
Hoss, S., Ahlf, W., Bergtold, M., Bluebaum-Gronau, E., Brinke, M., Donnevert, G., Menzel, R., Mohlenkamp, C., Ratte, H.T., Traunspurger, W., von Danwitz, B., Pluta, H.J., 2012. Interlaboratory comparison of a standardized toxicity test using the nematode Caenorhabditis elegans (ISO 10872). Environ. Toxicol. Chem. 31 (7), 1525–1535.
IARC, I.A.f.R.o.C, 2014. IARC Working Group on the Evaluation of Carcinogenic Risk to Humans. Diesel and Gasoline Engine Exhausts and Some Nitroarenes.
Ishibashi, H., Matsumura, N., Hirano, M., Matsuoka, M., Shiratsuchi, H., Ishibashi, Y., Takao, Y., Arizono, K., 2004. Effects of triclosan on the early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin. Aquat. Toxicol. 67 (2), 167–179.
ISO, 2012a. ISO 6341:2012 Water Quality – Determination of the Inhibition of the Mobility of Daphnia Magna Straus (Cladocera, Crustacea) – Acute Toxicity Test.
ISO, 2012b. ISO 8692:2012 Water Quality – Fresh Water Algal Growth Inhibition Test with Unicellular Green Algae.
ISO, 2012c. ISO 11350:2012 Water Quality – Determination of the Genotoxicity of Water and Waste Water – Salmonella/microsome Fluctuation Test (Ames Fluctuation Test).
ISO, 2013. ISO/TC 147/SC 5 N 804 Water Quality — Determination of the Estrogenic Potential of Water and Waste Water — Part 1: Yeast Estrogen Screen (Saccharomyces cerevisiae).
Jarošová, B., Bláha, L., Giesy, J.P., Hilscherová, K., 2014. What level of estrogenic activity determined by in vitro assays in municipal waste waters can be considered as safe? Environ. Int. 64, 98–109.
Jonas, A., Scholz, S., Fetter, E., Sychrova, E., Novakova, K., Ortmann, J., Benisek, M., Adamovsky, O., Giesy, J.P., Hilscherova, K., 2015. Endocrine, teratogenic and neurotoxic effects of cyanobacteria detected by cellular in vitro and zebrafish embryos assays. Chemosphere 120, 321–327.
Keddy, C.J., Greene, J.C., Bonnell, M.A., 1995. Review of whole-organism bioassays: soil, freshwater sediment, and freshwater assessment in Canada. Ecotoxicol. Environ. Saf. 30 (3), 221–251.
Knobel, M., Busser, F.J., Rico-Rico, A., Kramer, N.I., Hermens, J.L., Hafner, C., Tanneberger, K., Schirmer, K., Scholz, S., 2012. Predicting adult fish acute lethality with the zebrafish embryo: relevance of test duration, endpoints, compound properties, and exposure concentration analysis. Environ. Sci. Technol. 46 (17), 9690–9700.
Kunz, P.Y., Kienle, C., Carere, M., Homazava, N., Kase, R., 2015. In vitro bioassays to screen for endocrine active pharmaceuticals in surface and waste waters. J. Pharm. Biomed. Analysis 106 (0), 107–115.
Legler, J., Zeinstra, L.M., Schuitemaker, F., Lanser, P.H., Bogerd, J., Brouwer, A., Vethaak, A.D., de Voogt, P., Murk, A.J., van der Burg, B., 2002. Comparison of in vivo and in vitro reporter gene assays for short-term screening of estrogenic activity. Environ. Sci. Technol. 36 (20), 4410–4415.
Leskinen, P., Michelini, E., Picard, D., Karp, M., Virta, M., 2005. Bioluminescent yeast assays for detecting estrogenic and androgenic activity in different matrices. Chemosphere 61 (2), 259–266.
Leskinen, P., Virta, M., Karp, M., 2003. One-step measurement of firefly luciferase activity in yeast. Yeast Chichester Engl. 20 (13), 1109–1113.
Loos, R., 2012. EU JRC Report. 2012: "Analytical Methods Relevant to the European Commission's 2012 Proposal on Priority Substances under the Water Framework Directive. ISBN 978-92-79-26642-3.
Loos, R., Gawlik, B.M., Locoro, G., Rimaviciute, E., Contini, S., Bidoglio, G., 2009. EU-wide survey of polar organic persistent pollutants in European river waters. Environ. Pollut. 157 (2), 561–568.
Lübcke-von Varel, U., Bataineh, M., Lohrmann, S., Löffler, I., Schulze, T., Flückiger-Isler, S., Neca, J., Machala, M., Brack, W., 2012. Identification and quantitative confirmation of dinitropyrenes and 3-nitrobenzanthrone as major mutagens in contaminated sediments. Environ. Int. 44 (0), 31–39.
Maes, H.M., Maletz, S.X., Ratte, H.T., Hollender, J., Schaeffer, A., 2014. Uptake, elimination, and biotransformation of 17α-Ethinylestradiol by the freshwater alga Desmodesmus subspicatus. Environ. Sci. Technol. 48 (20), 12354–12361.
Maletz, S., Floehr, T., Beier, S., Klumper, C., Brouwer, A., Behnisch, P., Higley, E., Giesy, J.P., Hecker, M., Gebhardt, W., Linnemann, V., Pinnekamp, J., Hollert, H., 2013. In vitro characterization of the effectiveness of enhanced sewage treatment processes to eliminate endocrine activity of hospital effluents. Water Res. 47 (4), 1545–1557.
Manusadžianas, L., Balkelyt≐, L., Sadauskas, K., Blinova, I., Põllumaa, L., Kahru, A., 2003. Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests, and correspondence between toxicity and chemical-based indices. Aquat. Toxicol. 63 (1), 27–41.
McDonnell, D.P., Nawaz, Z., O'Malley, B.W., 1991. In situ distinction between steroid receptor binding and transactivation at a target gene. Mol. Cell. Biol. 11 (9), 4350–4355.
Murahashi, T., Iwanaga, E., Watanabe, T., Hirayama, T., 2003. Determination of the mutagen 3-Nitrobenzanthrone in rainwater collected in Kyoto, Japan. J. Health Sci. 49 (5), 386–390.
Murk, A.J., Legler, J., van Lipzig, M.M.H., Meerman, J.H.N., Belfroid, A.C., Spenkelink, A., van der Burg, B., Rijs, G.B.J., Vethaak, D., 2002. Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environ. Toxicol. Chem. 21 (1), 16–23.
Neale, P.A., Ait-Aissa, S., Brack, W., Creusot, N., Denison, M.S., Deutschmann, B., Hilscherova, K., Hollert, H., Krauss, M., Novák, J., Schulze, T., Seiler, T.B., Serra, H., Shao, Y., Escher, B.I., 2015. Linking in vitro effects and detected organic micropollutants in surface water using mixture toxicity modeling. Environ. Sci. Technol. 49 (24), 14614–14624.
OECD, 2004. Test No. 202: Daphnia sp. Acute Immobilisation Test. OECD Publishing.
OECD, 2011. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. OECD Publishing.
OECD, 2012. Test No. 457: BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists. OECD Publishing.
OECD, 2013a. Test No. 210: Fish, Early-life Stage Toxicity Test. OECD Publishing.
OECD, 2013b. Test No. 236: Fish Embryo Acute Toxicity (FET) Test.
Oliveira, R., Domingues, I., Koppe Grisolia, C., Soares, A.M., 2009. Effects of triclosan on zebrafish early-life stages and adults. Environ. Sci. Pollut. Res. 16 (6), 679–688.
Orvos, D.R., Versteeg, D.J., Inauen, J., Capdevielle, M., Rothenstein, A., Cunningham, V., 2002. Aquatic toxicity of triclosan. Environ. Toxicol. Chem. 21 (7), 1338–1349.
OSPAR, 2007. OSPAR Hazardous Substances Committee. OSPAR Practical Study 2005 on Whole Effluent Assessment.
Pandard, P., Devillers, J., Charissou, A.-M., Poulsen, V., Jourdain, M.-J., Férard, J.-F., Grand, C., Bispo, A., 2006. Selecting a battery of bioassays for ecotoxicological characterization of wastes. Sci. Total Environ. 363 (1–3), 114–125.
Peddinghaus, S., Brinkmann, M., Bluhm, K., Sagner, A., Hinger, G., Braunbeck, T., Eisentrager, A., Tiehm, A., Hollert, H., Keiter, S.H., 2012. Quantitative assessment of the embryotoxic potential of NSO-heterocyclic compounds using zebrafish (Danio rerio). Reprod. Toxicol. Elmsford, N.Y. 33 (2), 224–232.
Peng, Y., Luo, Y., Nie, X.P., Liao, W., Yang, Y.F., Ying, G.G., 2013. Toxic effects of triclosan on the detoxification system and breeding of Daphnia magna. Ecotoxicol. Lond. Engl. 22 (9), 1384–1394.
Reifferscheid, G., Maes, H.M., Allner, B., Badurova, J., Belkin, S., Bluhm, K., Brauer, F., Bressling, J., Domeneghetti, S., Elad, T., Fluckiger-Isler, S., Grummt, H.J., Gurtler, R., Hecht, A., Heringa, M.B., Hollert, H., Huber, S., Kramer, M., Magdeburg, A., Ratte, H.T., Sauerborn-Klobucar, R., Sokolowski, A., Soldan, P., Smital, T., Stalter, D., Venier, P., Ziemann, C., Zipperle, J., Buchinger, S., 2012. International round-robin study on the Ames fluctuation test. Environ. Mol. Mutagen. 53 (3), 185–197.
Reiss, R., Mackay, N., Habig, C., Griffin, J., 2002. An ecological risk assessment for triclosan in lotic systems following discharge from wastewater treatment plants in the United States. Environ. Toxicol. Chem. 21 (11), 2483–2492.
Rogers, J.M., Denison, M.S., 2000. Recombinant cell bioassays for endocrine disruptors: development of a stably transfected human ovarian cell line for the detection of estrogenic and anti-estrogenic chemicals. In vitro Mol. Toxicol. 13 (1), 67–82.
Rojíčková, R., Dvořáková, D., Maršálek, B., 1998. The use of miniaturized algal bioassays in comparison to the standard flask assay. Environ. Toxicol. Water Qual. 13 (3), 235–241.
Römbke, J., Moser, H., 2009. Ecotoxicological Characterization of Waste - Results and Experiences of an International Ring Test. Springer Science + Business Media Verlag, New York.
Rosal, R., Rodea-Palomares, I., Boltes, K., Fernández-Piñas, F., Leganés, F., Petre, A., 2010. Ecotoxicological assessment of surfactants in the aquatic environment:

combined toxicity of docusate sodium with chlorinated pollutants. Chemosphere 81 (2), 288–293.
Routledge, E.J., Sumpter, J.P., 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environ. Toxicol. Chem. 15 (3), 241–248.
SCCP, S.C.o.C.P., 2009. Scientific Committee on Consumer Products SCCP Opinion on Triclosan COLIPA n° P32.
Schulze, T., Ahel, M., Ahlheim, J., Aït-Aïssa, S., Brion, F., Di Paolo, C., Hollender, J., Hollert, H., Kloß, A., Koprivica, S., Krauss, M., Oswald, P., Schollée, J., Seiler, T., Shao, Y., Slobodnik, J., Sonavane, M., Tousova, Z., Walz, K., Brack, W., 2016. A Novel Device for Onsite Integrative Large-volume Solid Phase Extraction for the Chemical and Effect-based Screening Analyses of Water Resources in preparation.
Schulze, T., Krauss, M., Novak, J., Hilscherova, K., Ait-Aissa, S., Creusot, N., Macova, M., Neale, P., Escher, B.I., Gomes, T., Tollefsen, K.E., Tarcai, Z., Shao, Y., Deutschmann, B., Seiler, T.B., Hollert, H., Tarabek, P., Tousova, Z., Slobodník, J., Walz, K.-H., Brack, W., 2015. Joint Danube Survey 3-A comprehensive analysis of Danube water quality. In: Liska, I., Wagner, F., Sengl, M., Deutsch, K., Slobodník, J. (Eds.), ICDPR - International Commission for the Protection of the Danube River, Vienna, pp. 284–295.
Svobodová, K., Plačková, M., Novotná, V., Cajthaml, T., 2009. Estrogenic and androgenic activity of PCBs, their chlorinated metabolites and other endocrine disruptors estimated with two in vitro yeast assays. Sci. Total Environ. 407 (22), 5921–5925.
Tamura, I., Kagota, K.-i., Yasuda, Y., Yoneda, S., Morita, J., Nakada, N., Kameda, Y., Kimura, K., Tatarazako, N., Yamamoto, H., 2013. Ecotoxicity and screening level ecotoxicological risk assessment of five antimicrobial agents: triclosan, triclocarban, resorcinol, phenoxyethanol and p-thymol. J. Appl. Toxicol. 33 (11), 1222–1229.
Tang, J.Y.M., Escher, B.I., 2014. Realistic environmental mixtures of micropollutants in surface, drinking, and recycled water: herbicides dominate the mixture toxicity toward algae. Environ. Toxicol. Chem. 33 (6), 1427–1436.
Tatarazako, N., Ishibashi, H., Teshima, K., Kishi, K., Arizono, K., 2004. Effects of triclosan on various aquatic organisms. Environmental sciences. Int. J. Environ. Physiol. Toxicol. 11 (2), 133–140.
Tixier, C., Singer, H.P., Canonica, S., Müller, S.R., 2002. Phototransformation of triclosan in surface waters: a relevant elimination process for this widely used BiocideLaboratory studies, field measurements, and modeling. Environ. Sci. Technol. 36 (16), 3482–3489.
Van den Belt, K., Berckmans, P., Vangenechten, C., Verheyen, R., Witters, H., 2004. Comparative study on the in vitro/in vivo estrogenic potencies of 17β-estradiol, estrone, 17α-ethynylestradiol and nonylphenol. Aquat. Toxicol. 66 (2), 183–195.
Villeneuve, D., Volz, D.C., Embry, M.R., Ankley, G.T., Belanger, S.E., Leonard, M., Schirmer, K., Tanguay, R., Truong, L., Wehmas, L., 2014. Investigating alternatives to the fish early-life stage test: a strategy for discovering and annotating adverse outcome pathways for early fish development. Environ. Toxicol. Chem. 33 (1), 158–169.
von der Ohe, P., Schmitt-Jansen, M., Slobodnik, J., Brack, W., 2012. Triclosan—the forgotten priority substance? Environ. Sci. Pollut. Res. 19 (2), 585–591.
von der Ohe, P.C., Dulio, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R.-U., Ginebreda, A., De Cooman, W., Schüürmann, G., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. Sci. Total Environ. 409 (11), 2064–2077.
Watanabe, T., Ohe, T., Hirayama, T., 2005a. Occurrence and origin of mutagenicity in soil and water environment. Environmental sciences. Int. J. Environ. Physiol. Toxicol. 12 (6), 325–346.
Watanabe, T., Tomiyama, T., Nishijima, S., Kanda, Y., Murahashi, T., Hirayama, T., 2005b. Evaluation of genotoxicity of 3-Amino-, 3-Acetylamino- and 3-Nitrobenzanthrone using the ames Salmonella assay and the comet assay. J. Health Sci. 51 (5), 569–575.
Wernersson, A.-S., Carere, M., Maggi, C., Tusil, P., Soldan, P., James, A., Sanchez, W., Dulio, V., Broeg, K., Reifferscheid, G., Buchinger, S., Maas, H., Van Der Grinten, E., O'Toole, S., Ausili, A., Manfra, L., Marziali, L., Polesello, S., Lacchetti, I., Mancini, L., Lilja, K., Linderoth, M., Lundeberg, T., Fjallborg, B., Porsbring, T., Larsson, D., Bengtsson-Palme, J., Forlin, L., Kienle, C., Kunz, P., Vermeirssen, E., Werner, I., Robinson, C.D., Lyons, B., Katsiadaki, I., Whalley, C., den Haan, K., Messiaen, M., Clayton, H., Lettieri, T., Carvalho, R.N., Gawlik, B.M., Hollert, H., Di Paolo, C., Brack, W., Kammann, U., Kase, R., 2015. The European technical report on aquatic effect-based monitoring tools under the water framework directive. Environ. Sci. Eur. 27 (1), 7.
Wilson, V.S., Bobseine, K., Gray, L.E., 2004. Development and characterization of a cell line that stably expresses an estrogen-responsive luciferase reporter for the detection of estrogen receptor agonist and antagonists. Toxicol. Sci. 81 (1), 69–77.
Wolz, J., Fleig, M., Schulze, T., Maletz, S., Lubcke-von Varel, U., Reifferscheid, G., Kuhlers, D., Braunbeck, T., Brack, W., Hollert, H., 2010. Impact of contaminants bound to suspended particulate matter in the context of flood events. J. Soils Sediments 10, 1174–1185.
Yang, L.H., Ying, G.G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella subcapitata. Environ. Toxicol. Chem. 27 (5), 1201–1208.