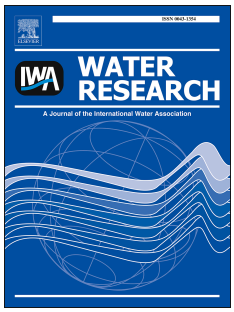
Accepted Manuscript



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, Richard Ottermanns, Steffen Keiter, Selim Ait-Aissa, Kerstin Bluhm, Werner Brack, Magnus Breitholtz, Sebastian Buchinger, Mario Carere, Carole Chalon, Xavier Cousin, Valeria Dulio, Beate I. Escher, Timo Hamers, Klára Hilscherová, Sergio Jarque, Adam Jonas, Emmanuelle Maillot-Marechal, Yves Marneffe, Mai Thao Nguyen, Pascal Pandard, Andrea Schifferli, Tobias Schulze, Sven Seidensticker, Thomas-Benjamin Seiler, Janet Tang, Ron van der Oost, Etienne Vermeirssen, Radka Zounková, Nick Zwart, Henner Hollert

PII: S0043-1354(16)30618-2

DOI: 10.1016/j.watres.2016.08.018

Reference: WR 12289

To appear in: *Water Research*

Received Date: 12 May 2016

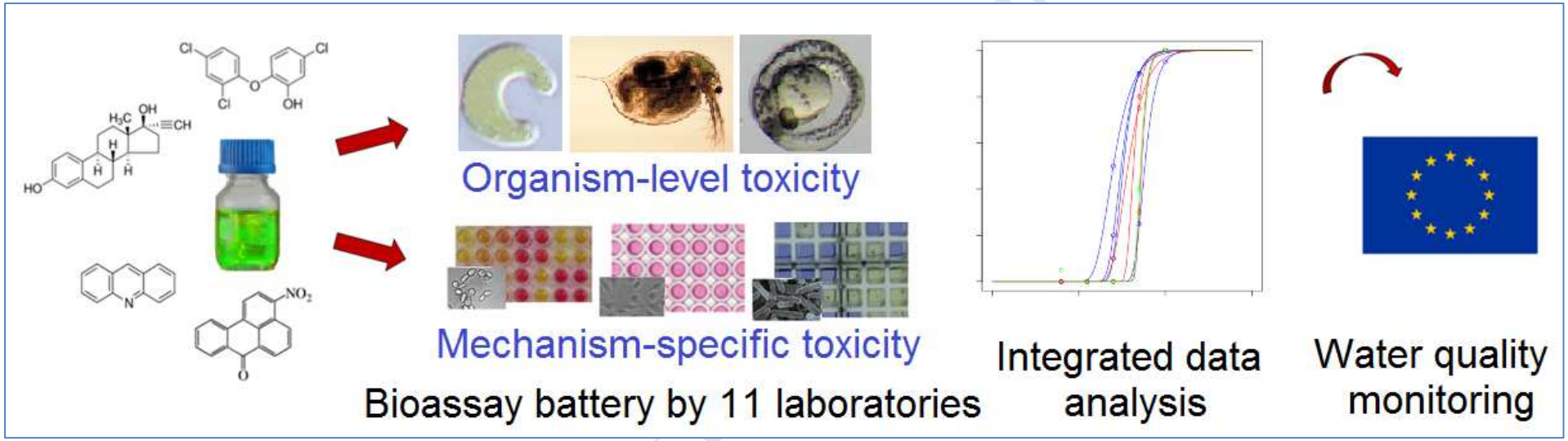
Revised Date: 30 July 2016

Accepted Date: 9 August 2016

Please cite this article as: Di Paolo, C., Ottermanns, R., Keiter, S., Ait-Aissa, S., Bluhm, K., Brack, W., Breitholtz, M., Buchinger, S., Carere, M., Chalon, C., Cousin, X., Dulio, V., Escher, B.I., Hamers, T., Hilscherová, K., Jarque, S., Jonas, A., Maillot-Marechal, E., Marneffe, Y., Nguyen, M.T., Pandard, P., Schifferli, A., Schulze, T., Seidensticker, S., Seiler, T.-B., Tang, J., van der Oost, R., Vermeirssen, E., Zounková, R., Zwart, N., Hollert, H., Bioassay battery interlaboratory investigation of emerging contaminants in spiked water extracts – Towards the implementation of bioanalytical monitoring tools in water quality assessment and monitoring, *Water Research* (2016), doi: 10.1016/j.watres.2016.08.018.

WR35437

Graphical abstract



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 [1], Richard Ottermanns [1], Steffen Keiter [1,2], Selim Ait-Aissa [3], Kerstin Bluhm [1], Werner Brack [4], Magnus Breitholtz [5], Sebastian Buchinger [6], Mario Carere [7], Carole Chalon [8], Xavier Cousin [9,10], Valeria Dulio [3], Beate I. Escher [4,11,12], Timo Hamers [13], Klára Hilscherová [14], Sergio Jarque [14], Adam Jonas [14], Emmanuelle Maillot-Marechal [3], Yves Marneffe [8], Mai Thao Nguyen [15], Pascal Pandard [3], Andrea Schifferli [16], Tobias Schulze [4], Sven Seidensticker [1,12], Thomas-Benjamin Seiler [1], Janet Tang [11], Ron van der Oost [1,7], Etienne Vermeirssen [16], Radka Zounková [14], Nick Zwart [13], Henner Hollert [1,*]

1: Institute for Environmental Research, RWTH Aachen University, Aachen, Germany

2: Man-Technology-Environment Research Centre, School of Science and Technology, Örebro University, Örebro, Sweden

3: INERIS, Verneuil-en-Halatte, France

4: UFZ-Helmholtz Centre for Environmental Research, Leipzig, Germany

5: Department of Applied Environmental Science - ITM, Stockholm University, Stockholm, Sweden

6: Department Biochemistry and Ecotoxicology, Federal Institute of Hydrology, Koblenz, Germany

7: Italian Institute of Health, Rome, Italy

8: ISSeP (Scientific Institute of Public Service), Liège-Wallonia, Belgium

9: Laboratoire d'Ecotoxicologie, Ifremer, L'Houmeau, France

10: Laboratoire de Physiologie et Génétique des Poissons, Inra, Rennes, France

11: National Research Centre for Environmental Toxicology - Entox, the University of Queensland, Brisbane, Australia

12: Centre for Applied Geosciences, Eberhard Karls University Tübingen, Germany

13: Institute for Environmental Studies -IVM, VU University Amsterdam, the Netherlands

14: Research Centre for Toxic Compounds in the Environment - RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic

15: Waterproef Laboratory, Edam, the Netherlands

16: Swiss Centre for Applied Ecotoxicology Eawag-EPFL, Dübendorf, Switzerland

17: WATERNET Institute for the Urban Water Cycle, Division of Technology Research & Engineering, Amsterdam, the Netherlands

*: To whom correspondence should be addressed at Department of Ecosystem Analysis, Institute for Environmental Research (Biology 5), RWTH Aachen University, Worringerweg 1, D-52074, Aachen, Germany. E-mail: henner.hollert@rwth-aachen.de

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 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>FET) and acridine (FET>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

harmonised 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.

Keywords: triclosan, acridine, 17α-ethinylestradiol, 3-nitrobenzanthrone, organism-level toxicity, mechanism-specific toxicity

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, an 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 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

146 method is described in the supplementary material. The water extract was evaluated in some
147 bioassays (Table S3) by the coordinator.

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

161 **2.5 Exposure setup and tested concentration ranges**

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

164 **2.6 Integrated data and statistical analysis**

165 Bioassay results (expressed as described in Table 2) were evaluated following the same data
166 preparation and statistical analysis methods. Results from experimental replicates were pooled and
167 EC_{50} values were calculated for grouped experiments either by 2-parameter Weibull function
168 using R language package (*Daphnia*), two parameter log-logistic curve from 0 to 100% with the
169 two adjustable parameters being slope and EC_{50} by GraphPad Prism 6 (algae, FET, Ames), or
170 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 $\mu L_{extract}/mL_{bioassay}$ (S.I.) 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= $EC_{50\text{-E2 or EE2}}/EC_{50\text{-sample}}$. The only exception was the water extract, for which the EEQ was obtained with the PC10 approach (Besselink 2015).

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>FET) and acridine (FET>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 $EC_{50\text{-}meas}$ for *Selenastrum capricornutum* (current *P. subcapitata*) exposed in 100-250 mL 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 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 to 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 to 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, 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 to 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 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 to 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 $EC_{50\text{-single}}$:$EC_{50\text{-mixture}}$, 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} 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 (Figure 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-spikes, 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-spikes (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 $2.5.10^{-7}$ (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 to 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 to 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 $\geq 0.5\ \mu L_{extract}/mL_{medium}$ 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 mix 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, Di Paolo et al. 2015b, Brack et al. 2016). 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 methods 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 towards the validation of low volume, high-throughput bioassay batteries (Brack et al. 2013, Altenburger et al. 2015, Brack 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

393 nematodes (Hoss et al. 2012). Recent studies include also a methodological investigation of a
394 freshwater ostracod sub-chronic test (Casado-Martinez et al. 2016); and a tiered strategy for
395 sediment risk assessment integrating different toxicity tests (Diepens et al. 2016).

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

409

410 **4. Conclusions and outcomes**

411 The battery of miniaturized bioassays presented complementary sensitivity and specificity to
412 the water extract spikes containing four emerging pollutants as single-chemicals or mixtures.
413 Aquatic organism sensitivity varied following exposure to different chemicals, confirming the
414 complementary role of the tests with the three taxa for water quality assessment. Estrogenicity and
415 mutagenicity assays identified with high precision the respective mechanism-specific effects of
416 spikes, even though non-specific toxicity of mixture compounds affected the evaluation of higher
417 test concentrations. Since differences in experimental protocols, model organisms, and data

418 analysis can affect the determination of effect-concentrations, respective standard methods and
419 harmonized procedures should be followed when implementing bioassays in water monitoring.
420 Together with other ongoing activities for the validation of a basic battery of bioassays, the
421 present study is an important step towards the implementation of bioanalytical monitoring tools in
422 water quality assessment and monitoring.

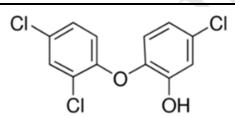
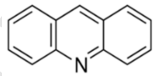
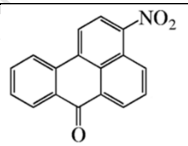
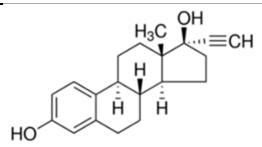
423

424 **Acknowledgements**

425 We would like to thank the colleagues at RWTH Aachen Simone Hotz, Monika Lam, Christine
426 Schönlau and Josef Koch for the contribution to the preliminary tests of the water extract and
427 chemicals. This project was developed by the Working Group 2 Bioassays and biomarkers in
428 water quality monitoring of the NORMAN Association (http://www.norman-network.net). It was
429 funded and supported by the European Marie Curie Initial Training Network EDA-EMERGE
430 (grant agreement no. 290100; www.ufz.de/eda-emerge), the European FP7 Collaborative Project
431 SOLUTIONS (grant agreement no. 603437; www.solutions-project.eu), and the NORMAN
432 Association (www.norman-network.net). CDP was supported by the EDA-EMERGE Marie Curie
433 ITN (ESR1). SA and EM (INERIS) were supported by the French Ministry of Ecology (grant
434 P181) and by the ONEMA.

435

436 **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]

437 a: National Center for Biotechnology Information. PubChem Compound Database (September 2015)

438 b: Predicted data, US Environmental Protection Agency's EPISuite™, KOWWIN v1.67 estimate.

439

440

441 **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 immobilisation 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 survival 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- / 384well plates	0.5 (+2.5) / 0.05	1, 8, 10: ISO 11350 (ISO 2012c) or 3: (Reifferscheid et al. 2012, Escher et al. 2014)

442 a: Laboratory code numbers are described in Table S1.

443

444 **Table 3:** Composition of the spiked water samples for each bioassay, consisting of one or two
445 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] 50-33 [b]	1 : 2 (2-fold)	5-7 [a] 16 [b]
	Acridine	-	10	-	-			
	Mixture	0.1	10	100	-			
***Daphnia* test**	Triclosan	1	-	-	-	1	1 : 2 (2-fold)	4-5
	Acridine	-	15	-	-			
	Mixture	1	15	100	2			
FET test	Triclosan	3	-	-	-	0.77	1: 1.3 (1.3-fold)	5
	Acridine	-	2	-	-	1		
	Mixture	3	2	100	2	0.58		
YES assay	EE2	-	-	100	-	0.1-2	3 : 10 and 1 : 3 (3.3 and 3-fold)	9-16
	Mixture	1	2	100	-			
ER-luc assay	EE2	-	-	1	-	0.5-1	1 : 10 (10-fold)	7
	Mixture	1	2	1	-			
Ames assay	3-NBA	-	-	-	2	1	1 : 2 (2-fold)	6
	Mixture	0.1	2	100	2			

446 a: Freshwater algal growth inhibition test with unicellular green algae

447 b: Combined algae assay

448

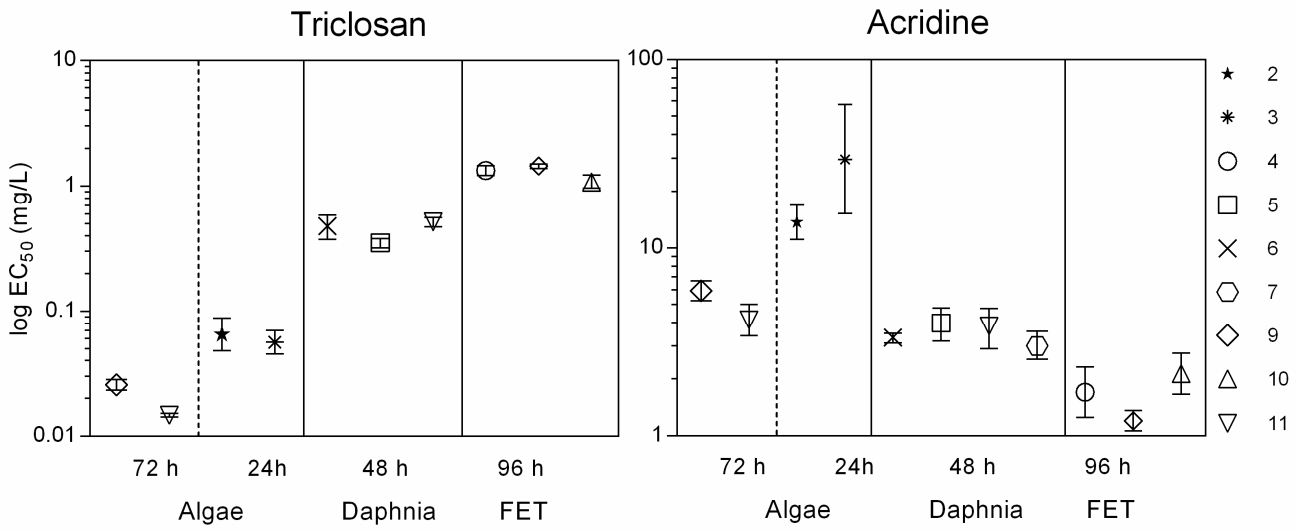
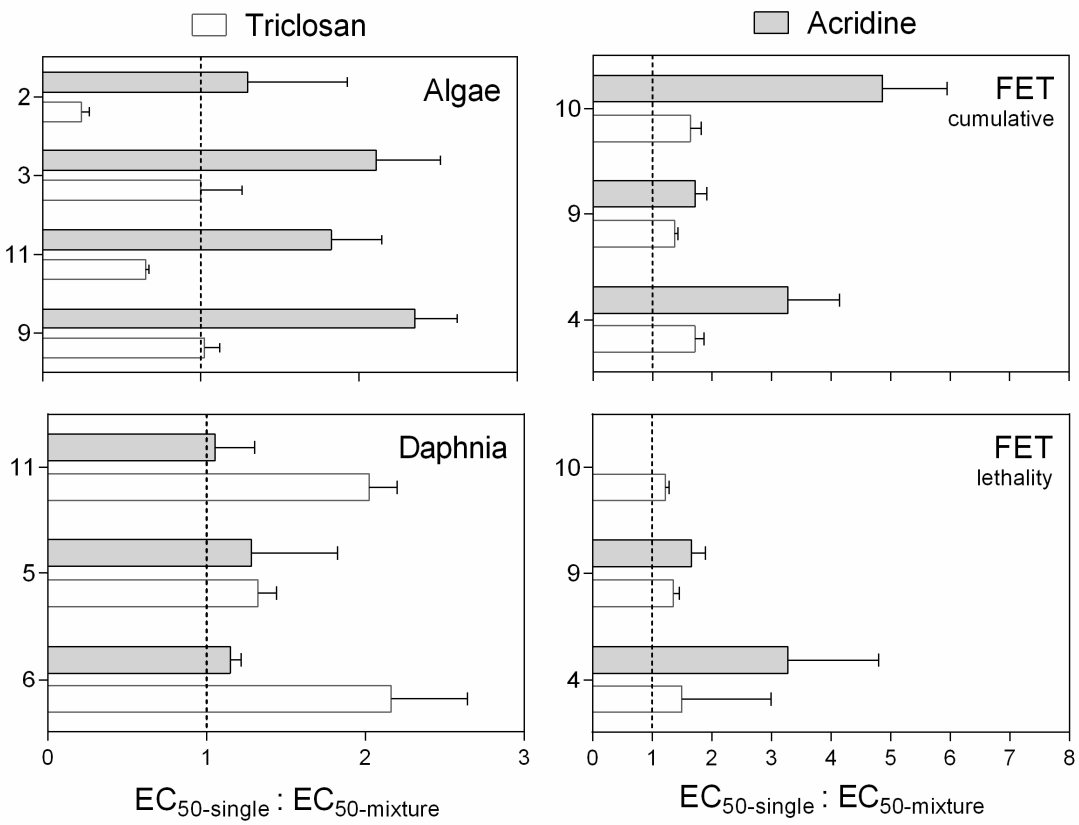


Figure 1: Effect-concentration values (log EC_{50} and 95% C.I., mg/L) obtained for pooled data from one to three experiments for each assay for the triclosan (left) and acridine (right) spikes in the algae (72 h or 24 h growth inhibition), *Daphnia* (48 h immobilization) and FET (96 h cumulative effects)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



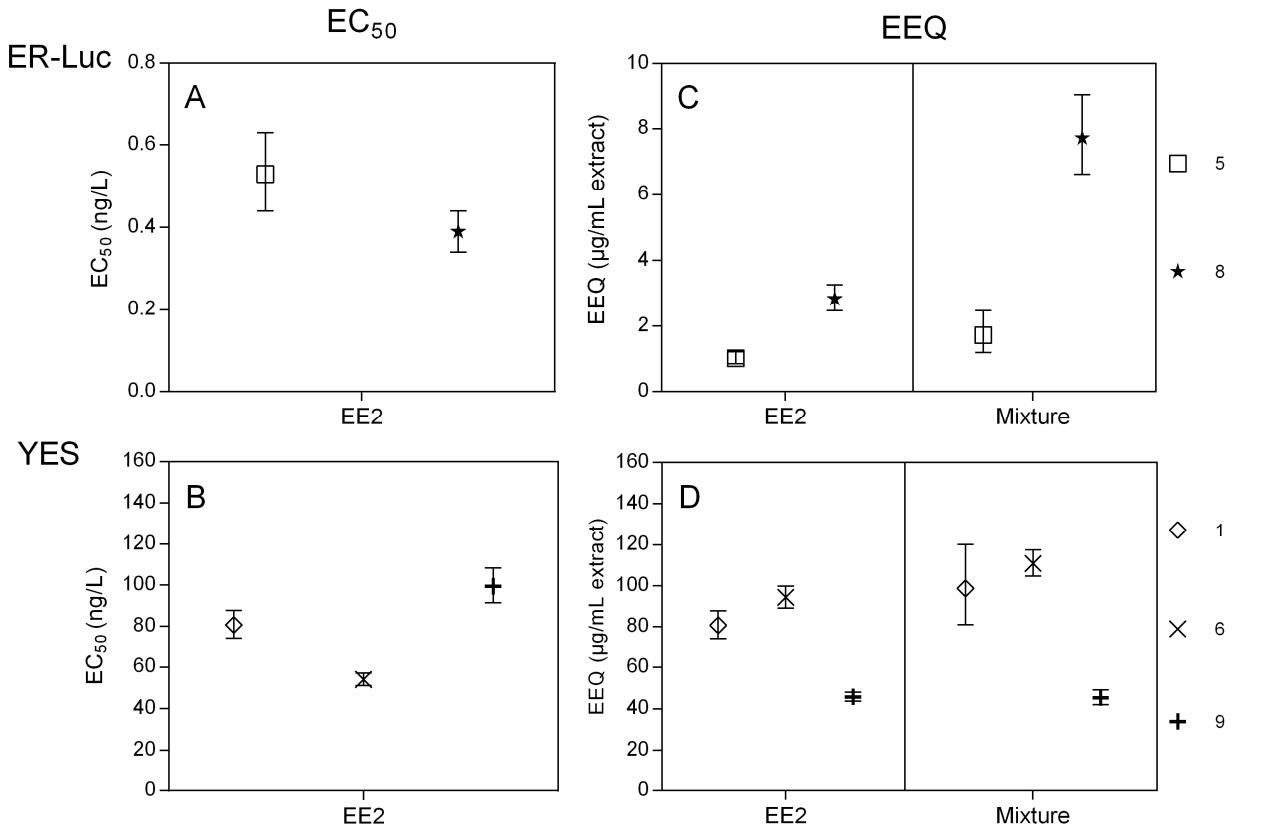
458

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

463

464

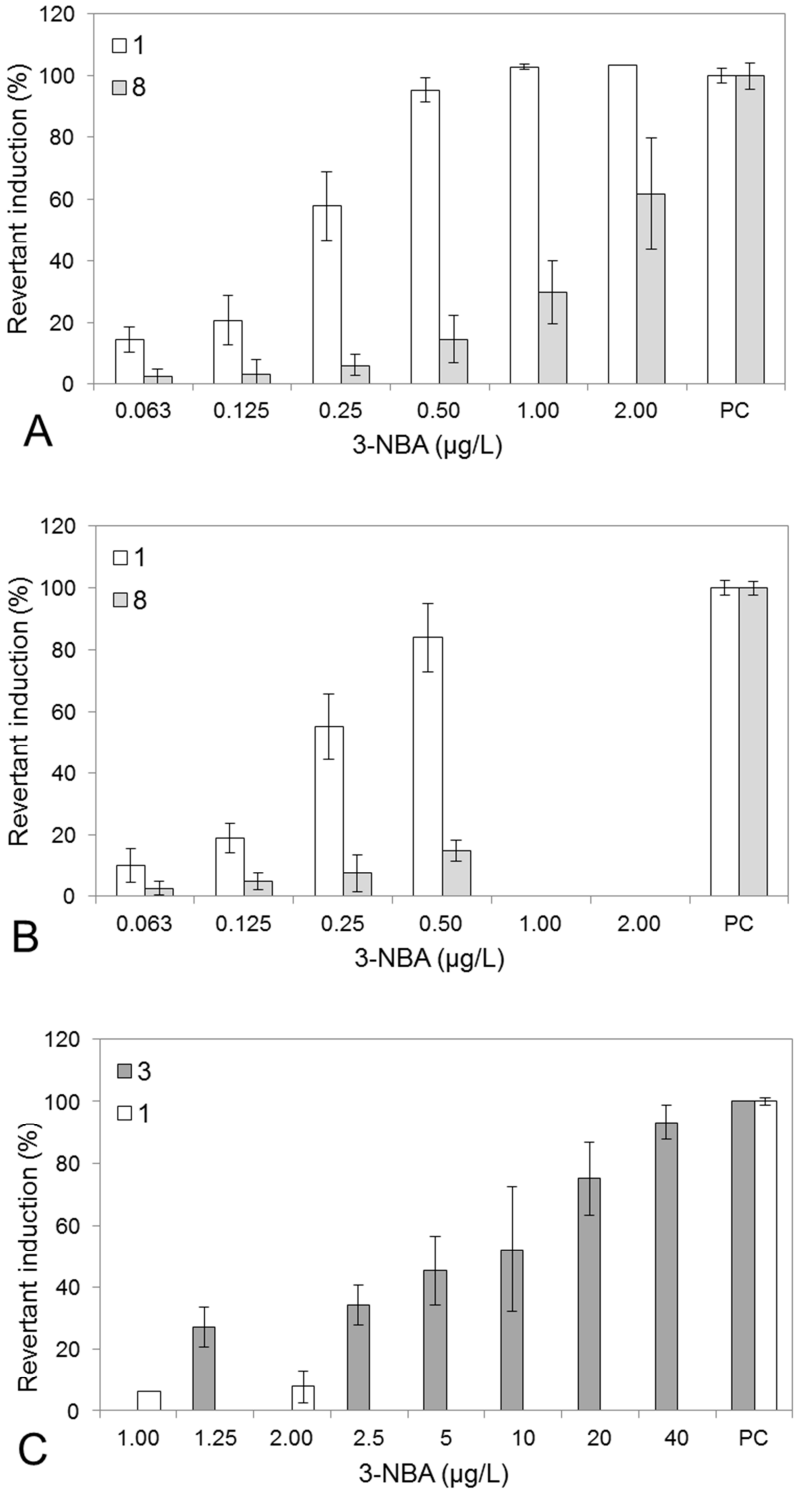
465



466

467 **Figure 3:** EC_{50} (ng/L) values for EE2 in the ER-Luc (A) and YES (B) assays, and EEQ values
468 obtained for the EE2 and the mixture spikes in the ER-Luc (C) and the YES (D) assays. EC_{50}
469 values (symbols) and 95% C.I. (error bars) for respective sample. Results are presented according
470 to laboratory code numbers (Table S1). Biological models are: T47D-kbLuc (5) BG1Luc4E2 (8),
471 β-galactosidase recombinant yeast by McDonnell et al. 1991 (1), β-galactosidase recombinant
472 yeast by Routledge and Sumpter 1996 (6), and luciferase recombinant yeast by Leskinen et al.
473 2003 (9).

474



475

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

481

References

Aboulfadl, K., De Potter, C., Prévost, M. and Sauvé, S. (2010) Time-dependent integrity during storage of natural surface water samples for the trace analysis of pharmaceutical products, feminizing hormones and pesticides. Chemistry Central Journal 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. and Hammock, B.D. (2008) In Vitro Biologic Activities of the Antimicrobials Triclocarban, Its Analogs, and Triclosan in Bioassay Screens: Receptor-Based Bioassay Screens. Environmental Health Perspectives 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. and Salkinoja-Salonen, M. (2015) Mitochondrial toxicity of triclosan on mammalian cells. Toxicology Reports 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. and 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. and Cox, M.B. (2010) A Four-Hour Yeast Bioassay for the Direct Measure of Estrogenic Activity in Wastewater without Sample Extraction, Concentration, or Sterilization. Sci. Total Environ. 408(6), 1422-1429.

Baumann, J., Sakka, Y., Bertrand, C., Koser, J. and Filser, J. (2014) Adaptation of the Daphnia sp. acute toxicity test: miniaturization and prolongation for the testing of nanomaterials. Environmental science and pollution research international 21(3), 2201-2213.

Bermudez, D.S., Gray, L.E. and 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). International journal of andrology 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. and McCarthy, J.F. (1985) Comparative toxicity of copper and acridine to fish, Daphnia and algae. Environmental Toxicology and Chemistry 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. and 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. and 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. and 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 Europe 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. and 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. and 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. and Lettieri, T. (2014) Mixtures of Chemical Pollutants at European Legislation Safety Concentrations: How Safe Are They? Toxicological Sciences 141(1), 218-233.

Casado-Martinez, M.C., Burga-Pérez, K.F., Bebon, R., Férard, J.-F., Vermeirssen, E.L.M. and 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. and Pessah, I.N. (2012) Triclosan impairs excitation–contraction coupling and Ca2+ dynamics in striated muscle. P Natl Acad Sci 109(35), 14158-14163.

de Voogt, P. and 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. and 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. and Hollert, H. (2015b) The value of zebrafish as an integrative model in effect-directed analysis - a review. Environmental Sciences Europe 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. and 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. and Admiraal, W.A. (1997) Biological variation in sensitivity to N-heterocyclic PAHs; effects of acridine on seven species of micro-algae. 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, 22.12.2000, p. 1–73.

EC (2011) European Commission WFD-CIS Guidance document No. 27 Technical guidance on deriving Environmental Quality Standards, Technical report 2011-055.

EC (2013) Official Journal of the European 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. and Neuwoehner, J. (2008) Heterocyclic compounds: toxic effects using algae, daphnids, and the Salmonella/microsome test taking methodical quantitative aspects into account. Environmental Toxicology and Chemistry 27(7), 1590-1596.

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

584 Enya, T., Suzuki, H., Watanabe, T., Hirayama, T. and Hisamatsu, Y. (1997) 3-Nitrobenzanthrone, a Powerful
585 Bacterial Mutagen and Suspected Human Carcinogen Found in Diesel Exhaust and Airborne Particulates.
586 Environ Sci Technol 31(10), 2772-2776.
587 Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P., Busch, W., Crago, J., Denslow, N.D.,
588 Dopp, E., Hilscherova, K., Humpage, A.R., Kumar, A., Grimaldi, M., Jayasinghe, B.S., Jarosova, B., Jia, A.,
589 Makarov, S., Maruya, K.A., Medvedev, A., Mehinto, A.C., Mendez, J.E., Poulsen, A., Prochazka, E., Richard,
590 J., Schifferli, A., Schlenk, D., Scholz, S., Shiraishi, F., Snyder, S., Su, G., Tang, J.Y.M., Burg, B.v.d., Linden,
591 S.C.v.d., Werner, I., Westerheide, S.D., Wong, C.K.C., Yang, M., Yeung, B.H.Y., Zhang, X. and Leusch, F.D.L.
592 (2014) Benchmarking Organic Micropollutants in Wastewater, Recycled Water and Drinking Water with In
593 Vitro Bioassays. Environ Sci Technol 48(3), 1940-1956.
594 Escher, B.I., Bramaz, N., Mueller, J.F., Quayle, P., Rutishauser, S. and Vermeirssen, E.L.M. (2008) Toxic
595 equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve
596 interpretation of ecotoxicity testing of environmental samples. Journal of Environmental Monitoring
597 10(5), 612-621.
598 Escher, B.I., Neale, P.A. and Leusch, F.D.L. (2015) Effect-based trigger values for in vitro bioassays: Reading
599 across from existing water quality guideline values. Water Research 81, 137-148.
600 Feiler, U., Ratte, M., Arts, G., Bazin, C., Brauer, F., Casado, C., Doren, L., Eklund, B., Gilberg, D., Grote, M.,
601 Gonsior, G., Hafner, C., Kopf, W., Lemnitzer, B., Liedtke, A., Matthias, U., Okos, E., Pandard, P.,
602 Scheerbaum, D., Schmitt-Jansen, M., Stewart, K., Teodorovic, I., Wenzel, A. and Pluta, H.J. (2014) Inter-
603 laboratory trial of a standardized sediment contact test with the aquatic plant Myriophyllum aquaticum
604 (ISO 16191). Environmental Toxicology and Chemistry 33(3), 662-670.
605 Feldmannová, M., Hilscherová, K., Maršálek, B. and Bláha, L. (2006) Effects of N-heterocyclic polyaromatic
606 hydrocarbons on survival, reproduction, and biochemical parameters in Daphnia magna. Environ Toxicol
607 21(4), 425-431.
608 Fritsch, E.B., Connon, R.E., Werner, I., Davies, R., Beggel, S., Feng, W. and Pessah, I.N. (2013) Triclosan
609 impairs swimming behavior and alters expression of excitation contraction coupling proteins in fathead
610 minnow (Pimephales promelas). Environmental science & technology 47(4), 2008-2017.
611 Gartiser, S., Hafner, C., Oeking, S. and Paschke, A. (2009) Results of a "Whole Effluent Assessment" study
612 from different industrial sectors in Germany according to OSPAR's WEA strategy. JEM 11(2), 359-369.
613 Goto, T. and Hiromi, J. (2003) Toxicity of 17alpha-ethynylestradiol and norethindrone, constituents of an
614 oral contraceptive pill to the swimming and reproduction of cladoceran Daphnia magna, with special
615 reference to their synergetic effect. Marine pollution bulletin 47(1-6), 139-142.
616 Guillen, D., Ginebreda, A., Farre, M., Darbra, R.M., Petrovic, M., Gros, M. and Barcelo, D. (2012)
617 Prioritization of chemicals in the aquatic environment based on risk assessment: analytical, modeling and
618 regulatory perspective. Sci Total Environ 440, 236-252.
619 Hamers, T., Legler, J., Blaha, L., Hylland, K., Marigomez, I., Schipper, C.A., Segner, H., Vethaak, A.D.,
620 Witters, H., de Zwart, D. and Leonards, P.E.G. (2013) Expert opinion on toxicity profiling—report from a
621 NORMAN expert group meeting. Integrated Environmental Assessment and Management 9(2), 185-191.
622 Harada, A., Komori, K., Nakada, N., Kitamura, K. and Suzuki, Y. (2008) Biological effects of PPCPs on
623 aquatic lives and evaluation of river waters affected by different wastewater treatment levels. Water
624 science and technology : a journal of the International Association on Water Pollution Research 58(8),
625 1541-1546.
626 Hartnik, T., Norli, H.R., Eggen, T. and Breedveld, G.D. (2007) Bioassay-directed identification of toxic
627 organic compounds in creosote-contaminated groundwater. Chemosphere 66(3), 435-443.
628 Hecker, M. and Hollert, H. (2011) Endocrine disruptor screening: regulatory perspectives and needs.
629 Environ Sci Europe 23(1), 15.
630 Henry, N.D. and Fair, P.A. (2013) Comparison of in vitro cytotoxicity, estrogenicity and anti-estrogenicity
631 of triclosan, perfluorooctane sulfonate and perfluorooctanoic acid. Journal of Applied Toxicology 33(4),
632 265-272.
633 Hoss, S., Ahlf, W., Bergtold, M., Bluebaum-Gronau, E., Brinke, M., Donnevert, G., Menzel, R.,
634 Mohlenkamp, C., Ratte, H.T., Traunspurger, W., von Danwitz, B. and Pluta, H.J. (2012) Interlaboratory

comparison of a standardized toxicity test using the nematode Caenorhabditis elegans (ISO 10872). Environmental Toxicology and Chemistry 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. and Arizono, K. (2004) Effects of triclosan on the early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin. Aquatic Toxicology 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. and 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. and 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. and Bonnell, M.A. (1995) Review of whole-organism bioassays: soil, freshwater sediment, and freshwater assessment in Canada. Ecotoxicology and environmental safety 30(3), 221-251.
Knobel, M., Busser, F.J., Rico-Rico, A., Kramer, N.I., Hermens, J.L., Hafner, C., Tanneberger, K., Schirmer, K. and 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. and Kase, R. (2015) In vitro bioassays to screen for endocrine active pharmaceuticals in surface and waste waters. Journal of Pharmaceutical and Biomedical 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. and 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. and Virta, M. (2005) Bioluminescent yeast assays for detecting estrogenic and androgenic activity in different matrices. Chemosphere 61(2), 259-266.
Leskinen, P., Virta, M. and Karp, M. (2003) One-step measurement of firefly luciferase activity in yeast. Yeast (Chichester, England) 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. and 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. and 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. and 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. and 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. and Kahru, A. (2003) Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests, and correspondence between toxicity and chemical-based indices. Aquatic Toxicology 63(1), 27-41.
Murahashi, T., Iwanaga, E., Watanabe, T. and Hirayama, T. (2003) Determination of the Mutagen 3-Nitrobenzanthrone in Rainwater Collected in Kyoto, Japan. Journal of Health Science 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. and Vethaak, D. (2002) Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environmental Toxicology and Chemistry 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. and Escher, B.I. (2015) Linking in vitro effects and detected organic micropollutants in surface water using mixture toxicity modeling. Environmental Science & Technology 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. and 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. and Cunningham, V. (2002) Aquatic toxicity of triclosan. Environmental Toxicology and Chemistry 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. and Bispo, A. (2006) Selecting a battery of bioassays for ecotoxicological characterization of wastes. Science of The Total Environment 363(1–3), 114-125.
Peddinghaus, S., Brinkmann, M., Bluhm, K., Sagner, A., Hinger, G., Braunbeck, T., Eisentrager, A., Tiehm, A., Hollert, H. and Keiter, S.H. (2012) Quantitative assessment of the embryotoxic potential of NSO-heterocyclic compounds using zebrafish (Danio rerio). Reproductive toxicology (Elmsford, N.Y.) 33(2), 224-232.
Peng, Y., Luo, Y., Nie, X.P., Liao, W., Yang, Y.F. and Ying, G.G. (2013) Toxic effects of triclosan on the detoxification system and breeding of Daphnia magna. Ecotoxicology (London, England) 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. and Buchinger, S. (2012) International round-robin study on the Ames fluctuation test. Environmental and molecular mutagenesis 53(3), 185-197.
Reiss, R., Mackay, N., Habig, C. and Griffin, J. (2002) An ecological risk assessment for triclosan in lotic systems following discharge from wastewater treatment plants in the United States. Environmental Toxicology and Chemistry 21(11), 2483-2492.
Rogers, J.M. and 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 & molecular toxicology 13(1), 67-82.
Rojíčková, R., Dvořáková, D. and Maršálek, B. (1998) The use of miniaturized algal bioassays in comparison to the standard flask assay. Environmental Toxicology and Water Quality 13(3), 235-241.
Römbke, J. and 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. and 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. and Sumpter, J.P. (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry 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. and Brack, W. (in preparation) A novel device for onsite integrative large-volume solid phase extraction for the chemical and effect-based screening analyses of water resources.

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. and Brack, W. (2015) Joint Danube Survey 3 - A comprehensive analysis of Danube water quality. Liska, I., Wagner, F., Sengl, M., Deutsch, K. and Slobodník, J. (eds), pp. 284-295, ICDPR - International Commission for the Protection of the Danube River, Vienna.

Svobodová, K., Plačková, M., Novotná, V. and 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. and Yamamoto, H. (2013) Ecotoxicity and screening level ecotoxicological risk assessment of five antimicrobial agents: triclosan, triclocarban, resorcinol, phenoxyethanol and p-thymol. Journal of Applied Toxicology 33(11), 1222-1229.

Tang, J.Y.M. and Escher, B.I. (2014) Realistic environmental mixtures of micropollutants in surface, drinking, and recycled water: Herbicides dominate the mixture toxicity toward algae. Environmental Toxicology and Chemistry 33(6), 1427-1436.

Tatarazako, N., Ishibashi, H., Teshima, K., Kishi, K. and Arizono, K. (2004) Effects of triclosan on various aquatic organisms. Environmental sciences : an international journal of environmental physiology and toxicology 11(2), 133-140.

Tixier, C., Singer, H.P., Canonica, S. and Müller, S.R. (2002) Phototransformation of Triclosan in Surface Waters: A Relevant Elimination Process for This Widely Used BiocideLaboratory Studies, Field Measurements, and Modeling. Environmental Science & Technology 36(16), 3482-3489.

Van den Belt, K., Berckmans, P., Vangenechten, C., Verheyen, R. and Witters, H. (2004) Comparative study on the in vitro/in vivo estrogenic potencies of 17β-estradiol, estrone, 17α-ethynylestradiol and nonylphenol. Aquatic Toxicology 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. and 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. Environmental Toxicology and Chemistry 33(1), 158-169.

von der Ohe, P., Schmitt-Jansen, M., Slobodnik, J. and 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. and 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. and Hirayama, T. (2005a) Occurrence and origin of mutagenicity in soil and water environment. Environmental sciences : an international journal of environmental physiology and toxicology 12(6), 325-346.

Watanabe, T., Tomiyama, T., Nishijima, S., Kanda, Y., Murahashi, T. and Hirayama, T. (2005b) Evaluation of Genotoxicity of 3-Amino-, 3-Acetylamino- and 3-Nitrobenzanthrone Using the Ames Salmonella Assay and the Comet Assay. Journal of Health Science 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.,

788 Larsson, D., Bengtsson-Palme, J., Forlin, L., Kienle, C., Kunz, P., Vermeirssen, E., Werner, I., Robinson, C.D.,
789 Lyons, B., Katsiadaki, I., Whalley, C., den Haan, K., Messiaen, M., Clayton, H., Lettieri, T., Carvalho, R.N.,
790 Gawlik, B.M., Hollert, H., Di Paolo, C., Brack, W., Kammann, U. and Kase, R. (2015) The European technical
791 report on aquatic effect-based monitoring tools under the water framework directive. Environ Sci Europe
792 27(1), 7.
793 Wilson, V.S., Bobseine, K. and Gray, L.E. (2004) Development and Characterization of a Cell Line That
794 Stably Expresses an Estrogen-Responsive Luciferase Reporter for the Detection of Estrogen Receptor
795 Agonist and Antagonists. Toxicol Sci 81(1), 69-77.
796 Wolz, J., Fleig, M., Schulze, T., Maletz, S., Lubcke-von Varel, U., Reifferscheid, G., Kuhlers, D., Braunbeck,
797 T., Brack, W. and Hollert, H. (2010) Impact of contaminants bound to suspended particulate matter in the
798 context of flood events. Journal of Soils and Sediments 10, 1174 - 1185.
799 Yang, L.H., Ying, G.G., Su, H.C., Stauber, J.L., Adams, M.S. and Binet, M.T. (2008) Growth-inhibiting effects
800 of 12 antibacterial agents and their mixtures on the freshwater microalga Pseudokirchneriella
801 subcapitata. Environmental Toxicology and Chemistry 27(5), 1201-1208.

802

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

Highlights

- Bioassay suitability to evaluate emerging aquatic pollutants is a research need
- 11 laboratories evaluated blinded spiked water extracts with a bioassay battery
- Spiked extracts contained 4 emerging pollutants as single chemicals or mixtures
- Tests presented complementary organism-sensitivity and mechanism-specificity
- Standard harmonized procedures are needed for regulatory water quality monitoring