Bioanalytical Assessment of Adaptive Stress Responses in Drinking Water: A Predictive Tool to Differentiate between Micropollutants and Disinfection By-Products

Armelle Hebert[a], Cedric Feliers[b], Caroline Lecarpentier[b], Peta A. Neale[c], Rita Schlichting[d], Sylvie Thibert[e], Beate I. Escher[c,d,f*]

[a]Veolia Research & Innovation, 78600 Maisons-Laffitte, France

[b]Veolia Eau d'Ile de France, Le Vermont, 28 Boulevard de Pesaro, TSA 31197, 92739 Nanterre France

[c]Australian Rivers Institute, Griffith School of Environment, Griffith University, Southport QLD 4222, Australia

[d]UFZ – Helmholtz Centre for Environmental Research, 04318 Leipzig, Germany

[e]Syndicat des Eaux D'Ile-de-France (SEDIF), 14 Rue Saint-Benoît, 75006 Paris, France

[f]Eberhard Karls University Tübingen, Environmental Toxicology, Center for Applied Geosciences, 72074 Tübingen, Germany

*corresponding author: beate.escher@ufz.de; Ph: +49 341 235 1244

Abstract

Drinking water can contain low levels of micropollutants, as well as disinfection by-products (DBPs) that form from the reaction of disinfectants with organic and inorganic matter in water. Due to the complex mixture of trace chemicals in drinking water, targeted chemical analysis alone is not sufficient for monitoring. The current study aimed to apply *in vitro* bioassays indicative of adaptive stress responses to monitor the toxicological profiles and the formation of DBPs in drinking water. Water samples from three drinking water distribution networks in France were tested with bioassays indicative of the Nrf2-mediated oxidative stress response, the p53-mediated response to genotoxicity and the NF-κB-mediated response to inflammation. Bioanalysis was complemented with chemical analysis of forty DBPs. All water samples were active in the oxidative stress response assay, but only after considerable sample enrichment, while no effects were detected in the p53 assay and few samples showed low activity in the NF-κB assay. As both micropollutants in source water and DBPs formed during treatment can contribute to the effect, the bioanalytical equivalent concentration (BEQ) approach was applied for the first time to determine the contribution of DBPs, with DBPs found to contribute between 17 to 58% of the oxidative stress response. Further, the BEQ approach was also used to assess the contribution of volatile DBPs to the observed effect, with volatile DBPs found to have only a minor contribution as compared to the measured effects of the non-volatile chemicals enriched by solid-phase extraction. The observed effects in the distribution networks were below any level of concern, quantifiable only at high enrichment and not different from bottled mineral water. Integrating bioanalytical tools and the BEQ mixture model for monitoring drinking water quality is an additional assurance that chemical monitoring is not overlooking any unknown chemicals or transformation products and can help to ensure chemically safe drinking water.

Keywords: bioassay; disinfection by-product; drinking water; oxidative stress, micropollutants

1. Introduction

Access to chemically and microbiologically safe drinking water is essential for human health. In recent years, micropollutants, such as pharmaceuticals, perfluorinated compounds and pesticides, have been detected at very low levels in both source water and treated drinking water (Loos et al. 2007, Mompelat et al. 2011, Glassmeyer et al. 2017). As microbial contamination is a more acute concern, drinking water is commonly treated with disinfectants, such as chlorine, chloramine and chlorine dioxide, to inactive waterborne pathogens (Fawell and Nieuwenhuijsen 2003, WHO 2011). However, common disinfectants can react with natural organic and inorganic matter in water to form disinfection by-products (DBPs), with DBPs associated with chronic adverse health outcomes, including bladder cancer (Villanueva et al. 2007). Residual disinfectants, such as chlorine, may also react further within the distribution network, both by further reactions with dissolved natural organic matter and with biofilms present in the pipes (Rossman et al. 2001, Rodriquez et al. 2004). In addition to being highly influenced by the type and concentration of organic and inorganic matter in the source water, the different species and concentrations of DBPs vary according to the type of disinfectant used, the disinfectant dose, the time since dosing (i.e., water age), temperature and pH of the water (Zhang et al. 2000, Hua and Reckhow 2008, Richardson and Postigo 2015). Disinfectants can also react with micropollutants present in source water, forming transformation products (Postigo and Richardson 2014). Consequently, a complex mixture of DBPs, micropollutants and their transformation products may potentially be present in drinking water.

To date, drinking water monitoring has focused on chemical analysis, but targeted chemical analysis alone is not sufficient given that chemicals are likely to be present in drinking water in mixtures at low concentrations. *In vitro* bioassays can be applied in parallel to chemical analysis to assess the effect of all active (known and unknown) chemicals in a water sample (Escher and Leusch 2012). Despite the fact that chemicals will potentially be present at very low levels, the additive effects of the chemical mixture may still be detected by bioassays (Silva et al. 2002). Previous studies have typically shown no or negligible effects in drinking water using assays

indicative of specific effects, such as activation of the estrogen receptor and activation of the androgen receptor (Brand et al. 2013, Escher et al. 2014). In contrast, bioassays indicative of adaptive stress responses, such as oxidative stress, have been shown to be suitable for the assessment of drinking water (Neale et al. 2012, Farré et al. 2013, Wang et al. 2013).

Adaptive stress responses are well conserved pathways that help restore the cell to homeostasis after damage by stressors (Simmons et al. 2009). Adaptive stress responses tend to be induced at lower concentrations than cell death, meaning that adaptive stress response assays can act as sensitive monitoring tools. In this study, we applied bioassays indicative of the Nrf2-mediated oxidative stress response, the p53-mediated response to genotoxicity and the NF-κB-mediated response to inflammation. Electrophilic chemicals and chemicals that produce reactive oxygen species can induce the oxidative stress response as the presence of these chemicals releases transcription factor Nrf2 from negative regulator Keap1, which in turn activates the antioxidant response element (Zhang 2006). Environmental water samples (Escher et al. 2012), micropollutants (Martin et al. 2010, Escher et al. 2013) and DBPs (Stalter et al. 2016a) have been shown to activate the oxidative stress response, with 23% of analysed chemicals in the US EPA ToxCast database reported to induce oxidative stress (US EPA 2015). The p53 transcription factor responds to DNA damage and will initiate repair proteins, alter the cell cycle or induce apoptosis (Knight et al. 2009). The p53 response assay can detect genotoxic compounds and has previously been applied to individual DBPs (Stalter et al. 2016a) and water samples (Yeh et al. 2014, Neale et al. 2015a). NF-κB is an important transcription factor associated with the inflammation response, as well as cell growth and apoptosis (Simmons et al. 2009). Certain pharmaceuticals have been shown to inhibit the NF-κB pathway (Miller et al. 2010), but the application of assays indicative of the NF-κB response for water quality monitoring has been limited to date, though there has been some promising results for surface water (Neale et al. 2015a).

In this study, bioassays indicative of adaptive stress responses were applied to monitor the toxicological profile and assess DBP formation in three drinking water distribution networks in

France. A preliminary screening study of the three distribution networks found no effect in assays indicative of hormonal activity, including both activation and inhibition of the androgen receptor, the estrogen receptor, the progesterone receptor and the glucocorticoid receptor, and activation of the thyroid receptor beta and the peroxisome proliferator-activated receptor alpha (Besselink 2013). The studied distribution networks were fed by three water treatment plants (WTPs) treating surface water from three different rivers, respectively, and utilizing either conventional treatment or a combination of nanofiltration and conventional treatment (70% and 30% of flow, respectively). Water samples were collected after treatment and at different water ages throughout the three distribution networks, with sampling taking place four times over different seasons.

Given that chemicals will be present in mixtures in the water samples, the mixture toxicity model of concentration addition was applied for the apportionment of effects between DBPs and micropollutants and to predict the effects of volatile DBPs using the bioanalytical equivalent concentration (BEQ) approach. This is appropriate as it has previously been shown that mixtures of chemicals act in a concentration additive manner in the oxidative stress response assay (Escher et al. 2013). Further, the BEQ approach has recently been applied to assess the contribution of detected chemicals to the biological effect in surface water and wastewater (Neale et al. 2015a, Neale et al. 2017). As water in distribution networks may contain both micropollutants from the source water and formed DBPs, bioanalytical equivalent concentrations from bioanalysis (BEQ_{bio}) were compared before and after chlorination to predict the contribution of DBPs to the biological effect. The BEQ concept was also applied to determine the contribution of volatile DBPs. As volatile DBPs are not captured by typical sample enrichment processes used for bioanalysis and the effects in the volatile fraction are typically well explained by the known volatile DBPs (Stalter et al. 2016b), forty non-volatile and volatile DBPs including haloacetic acids (HAA), haloacetonitriles (HAN) and trihalomethanes (THM) were quantified by gas chromatography with electron capture detector (GC-ECD) and gas chromatography with mass spectrometry (GC-MS). Using the detected concentrations and the relative effect potency of the individual DBPs in the assays, bioanalytical

equivalent concentrations from detected chemical concentrations (BEQ_{chem}) were calculated for volatile and non-volatile DBPs, which were then compared to BEQ_{bio}.

2. Materials and Methods

2.1. Water treatment plants

Three WTPs, Méry-sur-Oise, Choisy-le-Roi and Neuilly-sur-Marne, in the Paris metropolitan area were included in the current study (Table 1). Méry-sur-Oise WTP, which produces 150,000 m^3/day, uses a combination of nanofiltration treatment processes (70%) and conventional treatment processes (clarification, sand filtration, ozonation, granular activated carbon (GAC) filtration and chlorine disinfection) (30%) to treat water from the Oise River, with the treated water from these two processes being mixed before final chlorination and distribution. Choisy-le-Roi WTP, which produces 320,000 m^3/day from the Seine River, applies complete conventional treatment including pre-ozonation, clarification, sand filtration, ozonation, GAC filtration, UV and final chlorine disinfection. Neuilly-sur-Marne WTP also applies the same treatment processes, with the exception of pre-ozonation, and produces approximately 300,000 m^3/day from the Marne River.

2.2. Chemical analysis

Forty DBPs, including nitrosamines, HAAs, HANs, haloketones (HK) and THMs, were analysed in the current study. HAAs and HANs were quantified using GC-ECD based on the standard methods NF EN ISO 23631 (2006) and EPA 551.1 (Munch and Hautman 1995), respectively, while the THMs were analysed using purge & trap GC-MS based on the standard method NF EN ISO 15680 (2003). Nitrosamines were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and HKs were analysed using GC-MS. The properties of the studied chemicals are shown in Table S1 of the Supplementary Information (SI).

2.3. Sample collection and enrichment for bioanalysis

Water samples were collected from the outlet of the WTPs and at different points along the distribution network in November 2015 and March, May and September 2016 (Table 1), with duplicate samples collected at most sampling points. Water quality parameters for the treated water, including temperature, total organic carbon (TOC), pH and residual free chlorine, are provided in Tables S2 and S3 of the SI. Immediately after sampling, 20 mg/L thiosulfate was added to neutralise the free chlorine. Water samples were also collected from Choisy-le-Roi and Neuilly-sur-Marne WTPs prior to chlorination. Evian water with and without 20 mg/L thiosulfate was used as controls. Two litres of water were enriched per sample using 500 mg Oasis HLB solid phase extraction (SPE) cartridges without pH adjustment. After drying, the cartridges were eluted using 20 mL of methanol followed by 20 mL of methyl tert-butyl ether (MTBE). The extracts were blown to dryness and resolubilised in 1 mL of methanol, giving an enrichment factor of 2000 in the final extract.

2.4. Bioanalysis

The water extracts were assessed in three assays indicative of adaptive stress responses for oxidative stress (AREc32), p53 response (p53RE-*bla*) and NF-κB response (NF-κB-*bla*). The studied assays are summarised in Table 2. All sample extracts were blown down to dryness and resolubilised in assay media prior to bioanalysis, with blown down solvent controls also included to ensure there was no effect from the solvent. In addition, Evian water controls with and without thiosulfate were also tested in the assays. Initially, all samples were run in a 12-step serial dilution series as a range finder, with active samples repeated twice independently in a 12-step linear dilution series (see Supplementary Information for more details and all dilution series).

The AREc32 assay, a reporter gene assay based on the MCF7 breast cancer cell line (Wang et al. 2006), was conducted as described by Escher et al. (2012) with some modifications. Briefly, 10 µL of extract serially diluted in DMEM with 10% fetal bovine serum (FBS) were added to a 384 well plate containing 30 µL of cells with a density of 8.33×10^4 cells/mL. The plate was incubated

for 23 h at 37°C, 5% CO_2. Cell viability was assessed using PrestoBlue, with fluorescence measured after 1 h incubation at 37°C, followed by determining luciferase production by measuring luminescence. tert-Butylhydroquinone (tBHQ) served as the positive reference compound.

The CellSensor p53RE-bla assay (Invitrogen, Carlsbad, US) uses the HCT-116 human colon cells, with the assay performed according to Neale et al. (2015b) with some modifications. Briefly, 10 µL of extract serially diluted in Opti-MEM with 2% charcoal-dextran treated FBS were added to a 384 well plate containing 30 µL of cells with a density of 1.33×10^5 cells/mL. The samples were incubated for 48 h, with induction of p53 and cell viability measured in parallel using the ToxBLAzer FRET-B/G CCG4-AM substrate. The positive reference compound was mitomycin.

The CellSensor NF-κB-bla assay (Invitrogen, Carlsbad, US) is based on the human monocytic THP-1 cell line, with the assay conducted based on König et al. (2017). Briefly, 10 µL of extract serially diluted in Opti-MEM with 2% charcoal-dextran treated FBS were added to a 384 well plate with 30 µL of THP-1 cells with a density of 6.67×10^5 cells/mL. The samples were incubated for 22 h at 37°C, with induction and cell viability measured simultaneously using the ToxBLAzer FRET-B/G CCG4-AM substrate. The positive reference compound was tumour necrosis factor alpha (TNFα). The concentration-effect curves for the three reference compounds are provided in the SI, Figure S1.

2.5. Data evaluation

Activation of the transcription factors in the assay was expressed as an induction ratio (IR), which was calculated using the signal of the sample and the signal of the unexposed cells (control; Equation 1). Linear concentration-effect curves up to an IR of 5 (e.g. Figure S1) were applied to determine the effect concentration causing an induction ratio of 1.5 ($EC_{IR1.5}$) (Equation 2). This indicates a 50% increase in IR compared to the unexposed cells (IR =1) and Escher et al. (2012) demonstrated than an IR of 1.5 was consistently higher than the limit of reporting (three times the

standard deviation of the controls), indicating that $EC_{IR1.5}$ is a sensitive benchmark value. All duplicate samples were combined for data analysis.

(1)

(2)

Cell viability, which was measured in parallel to induction, was determined with Equation 3 using a log-sigmoidal concentration-effect curve. The adjustable parameters include slope and the inhibitory concentration causing 50% reduction in cell viability (IC_{50}). As full concentration-effect curves were often not obtained, the concentration causing 10% effect (IC_{10}) was calculated using Equation 4. Further information about data evaluation can be found in Escher et al. (2014).

(3)

(4)

The $EC_{IR1.5}$ and the IC_{10} values were expressed in units of relative enrichment factor (REF), which is calculated based on the sample enrichment factor by SPE and the assay dilution factor (Escher and Leusch 2012). The maximum REF for the p53RE-*bla* and NF-κB-*bla* assays was 500, while a maximum REF of 200 was used for the AREc32 assay. Note that this is a far higher enrichment

than typically used for bioanalytical testing and was necessary due to the low levels of effects observed.

Sample $EC_{IR1.5}$ values were converted to BEQ_{bio} using Equation 5 using the $EC_{IR1.5}$ of the assay reference compound.

(5)

BEQ_{chem} was used to determine the effect of the detected DBPs. Firstly, $EC_{IR1.5}$ values for the detected chemicals i were collected from Stalter et al. (2016a) (SI, Table S1), with relative effect potency (REP_i) calculated using Equation 6 and the assay reference compounds and their $EC_{IR1.5}$ given in Table 2. The detected concentration in molar units (C_i) and REP_i (SI, Table S1) were used to calculate the BEQ_{chem} (Equation 7).

(6)

(7)

Chemicals with a Henry's Law Constant less than 1.00×10^{-6} atm m^3/mol were used to calculate $BEQ_{chem,\ non\text{-}volatile}$, while chemicals with a Henry's Law Constant greater than 1.00×10^{-6} atm m^3/mol were used to calculate $BEQ_{chem,\ volatile}$, with the Henry's Law Constant cut-off for volatile compounds adopted from Stalter et al. (2016a).

3. Results and Discussion

3.1. Chemical analysis

The detected concentrations of DBPs at each sampling site over the four sampling campaigns are provided in Tables S4 to S7, with the sum molar concentration of detected DBPs for each chemical class shown in Figure 1. Samples from the Méry-sur-Oise distribution network had the lowest DBP concentrations, which can be attributed to the low concentrations of TOC in the water (0.38 to 0.74 mg/L) after treatment with a combination of nanofiltration and conventional treatment processes (Table S2). Choisy-le-Roi and Neuilly-sur-Marne, which both use conventional treatment without membrane filtration, had TOC concentrations generally over 1 mg/L, with the highest TOC concentrations of 1.60 and 1.65 mg/L, respectively, in May, which correlated with increased DBP concentrations in the distribution networks.

THMs, including bromoform, chloroform, dibromochloromethane and bromochloromethane, were the dominant type of DBP formed after disinfection for all distribution networks, though the sum THM concentrations were significantly lower than the parameter value of 100 µg/L in the European Union Drinking Water Directive (European Commission 1998). DBP concentrations generally increased with water age in the distribution network, with the concentration noticeably increasing after re-chlorination in May (refer to Table 1 for re-chlorination information). Increasing DBP formation along the distribution network has been observed previously (e.g. Rodriquez et al. 2004, Dominguez-Tello et al. 2015), with greater DBP formation attributed to the longer contact time between the disinfectant and organic and inorganic matter in

276 water. Overall, chemical analysis reveals generally low DBP concentrations, which can be
277 attributed to the low TOC concentration in the treated water.
278
279 *3.2. Bioanalysis*
280 The $EC_{IR1.5}$ and IC_{10} values for the AREc32, p53RE-*bla* and NF-κB-*bla* assays are provided in
281 Tables S8 to S10, with concentration-effect curves for cytotoxicity and induction provided in
282 Figures S2 to S7. AREc32 was the most responsive assay, with all samples showing an effect, but
283 only after at least 15 times enrichment of the water sample. The Evian water samples with and
284 without thiosulfate also induced a response in the AREc32 assay, but only at very high enrichment
285 (REF 56-100). No cytotoxicity was observed at the active concentrations. Water samples from the
286 Méry-sur-Oise distribution network had the lowest effects in all sampling campaigns, which fits
287 with the lower DBP and TOC concentrations (Figure 1). An increased effect was observed in
288 samples from May and September compared to November and March for all distribution networks.
289 While the increased effect in May fits well with increased TOC and detected DBPs, the TOC and
290 DBP concentrations in September were similar to concentrations in November and March.
291 Temperature is a factor in DBP formation (Rodriquez et al. 2004, Hua and Reckhow 2008), thus
292 higher levels of some DBPs may be expected in September compared to November and March due
293 to the increased temperature (Tables S2 and S3). However, this was not observed for the targeted
294 DBPs. Different sample preparation methods were used for chemical analysis and bioanalysis, with
295 sample enrichment for bioanalysis mainly extracting non-volatile and semi-volatile DBPs, while
296 chemical analysis mostly targeted volatile DBPs (Stalter et al. 2016b). Therefore, formation of
297 undetected non-volatile or semi-volatile DBPs may explain the increased effect in September.
298 Alternatively, the increase in observed effect may be related to other existing micropollutants in the
299 source water. The contribution of micropollutants and DBPs to the oxidative stress response will be
300 explored further below.

Cytotoxicity masked induction for all samples in the p53RE-*bla* assay, thus $EC_{IR1.5}$ values could not be derived for this assay. This has also been observed for other water types, including wastewater and surface water (Escher et al. 2014, Neale et al. 2017). While both individual DBPs (Stalter et al. 2016a) and highly chlorinated pool water (Yeh et al. 2014) have been shown to induce the p53 response, the window between cytotoxicity and induction was small. Consequently, the lack of p53 response in the current study supports the high quality of the treated water.

Similar to the p53RE-*bla*, cytotoxicity often masked induction in the NF-κB-*bla* assay. Induction was often highest before chlorination in the Choisy-le-Roi WTP (November and September) and the Neuilly-sur-Marne WTP (November), which suggests that the effect was not due to DBP formation during chlorination, but to other micropollutants in the water. This is consistent with the observation that known DBPs are not active in this assay (Stalter et al. 2016a). The NF-κB-*bla* assay has only recently been applied for water quality monitoring and it is still unclear which environmental chemicals induce a response in this assay (Neale et al. 2015a, Neale et al. 2017).

Overall, bioanalysis indicates low effects in the treated water in the studied distribution networks. As all samples were active in the AREc32 assay, the following discussion will primarily focus on the oxidative stress response.

3.3. Which chemical mixtures can be assessed by the oxidative stress response?

Bioassays alone cannot provide information about the effect of individual chemicals in a sample, but rather the effects of all chemicals in a sample. As indicated by the ToxCast database (US EPA 2015), a wide variety of chemicals, including both micropollutants and DBPs, may activate the oxidative stress response. In the current study, the contribution of DBPs to the oxidative stress response in the Choisy-le-Roi and Neuilly-sur-Marne networks was estimated by considering the effect before and after chlorination using the BEQ approach. No sample before chlorination was measured at the Méry-sur-Oise WTP. The $EC_{IR1.5}$ values were translated to BEQ_{bio}, which relates the

effect in a sample to the concentration of a reference compound, e.g. tBHQ, which would elicit the same effect as the sample. $BEQ_{bio,DBP}$ was calculated using Equation 8 assuming additive effects of micropollutants and DBPs.

(8)

Figure 2 shows that DBPs contributed up to 58% of the oxidative stress burden. DBPs tended to contribute more to the oxidative stress response in the Choisy-le-Roi distribution network, with $BEQ_{bio,DBP}$ typically increasing with longer water ages in the distribution network. In contrast, other micropollutants had a greater contribution to the biological effect in the Neuilly-sur-Marne distribution network. Boucherie et al. (2010) previously showed that the treatment processes at the Neuilly-sur-Marne WTP were effective at removing a range of pharmaceuticals and pesticides. The observed difference may be related to the differences in treatment processes between the two WTPs, with pre-ozonation applied only at the Choisy-le-Roi WTP, as well as the varying natural organic matter properties in the raw waters. Overall, comparing effect before and after chlorination indicates that DBPs did not contribute substantially to the observed effects. It should be noted that it was not possible to calculate $BEQ_{bio,\ DBP}$ for the Choisy-le-Roi distribution network in May as a sample was not collected before disinfection, while the effect before chlorination was higher than after chlorination at Neuilly-sur-Marne in November, so this sampling date was also excluded.

In addition to reacting with natural organic matter, disinfectants may react with micropollutants to form transformation products, which may be more or less toxic than their parent compound (Postigo and Richardson 2014). The applied BEQ approach cannot differentiate between

the effect of DBPs formed from natural organic matter and micropollutant transformation products; however, the contribution of transformation products is expected to be small and the chlorinated transformation products can be considered as DBPs.

Previous studies have shown that disinfection of source waters results in an increased oxidative stress response, which also corresponded with increased DBP concentrations (Neale et al. 2012, Farré et al. 2013). In contrast, there is often little difference in effect before and after chlorination in receptor-mediated assays (Escher et al. 2014) and preliminary screening revealed no hormonal activity in the studied distribution networks (Besselink 2013). Therefore, while they cannot be excluded, micropollutant transformation products are not expected to contribution significantly to $BEQ_{bio,\ after\ chlorination}$.

3.4. Contribution of volatile and non-volatile DBPs to the observed effect

Volatile DBPs are not captured during SPE and consequently will not be present in sample extracts tested in the bioassays. To overcome this limitation, $BEQ_{chem,\ volatile}$ was calculated using Equation 7, with $EC_{IR1.5}$ values for the individual detected DBPs collected from Stalter et al. (2016a) (provided in Table S1). This approach is justified because Stalter et al. (2016b) demonstrated that the $BEQ_{chem,\ volatile}$ stems mainly from known DBPs. Figure 3 indicates that the detected volatile DBPs only had a minor contribution to the oxidative stress response, with the contribution greatest in May and often later in the distribution network. This fits with previous findings of increased volatile THMs concentrations along distribution networks (Dominguez-Tello et al. 2015).

BEQ_{bio} was also compared with $BEQ_{chem,\ non\text{-}volatile}$ for chemicals with a Henry's Law Constant less than 1.00×10^{-6} atm m^3/mol (Table 3) to determine how much the detected non-volatile DBPs contributed to the observed effect. The detected chemicals contributed between 0.16 to 204% of the effect (Table S11), with the effect dominated by the HAN dibromoacetonitrile, particularly in November and March, when it was present at higher concentrations. Smaller contributions stemmed from the haloacetic acids, mainly bromochloroacetic acid and dibromoacetic acid.

Previous studies on surface water and chlorinated pool water have found that detected chemicals typically contribute less than 2% of the oxidative stress response (Yeh et al. 2014, Neale et al. 2017). The good mass balance in the present study might partially be an artefact because the extraction methods differed between bioassays and chemical analysis. Hence the comparison has some limitation. HAAs are fully charged compounds and are poorly extracted by SPE at pH > 2 when they are fully ionised (Stalter et al. 2016b). However, the contribution of HAAs to BEQ_{bio} would be less than 1.3%. Thus the HAAs cannot be the reason for the discrepancy. Stalter et al. (2016b) also found less than 20% recovery of dibromoacetonitrile by TELOS ENV SPE cartridges at pH 1. If we assume a 20% recovery of dibromoacetonitrile, this compound would only contribute 23 to 40% to the overall observed effect, which appears more realistic. No single method will extract all contaminants from a water sample, but the mixture toxicity modelling of volatile compounds in the current study and the work of Stalter et al. (2016b) suggest that we are capturing the majority of the toxicological relevant DBPs with SPE.

3.5. Comparison of effects in current study with other water samples

To gain an understanding of how the oxidative stress response in samples collected from the three distribution networks compares to other water types, the $EC_{IR1.5}$ values were compared to published $EC_{IR1.5}$ values for wastewater, surface water and drinking water (Figure 4). The effect was considerably higher for wastewater effluent than the current study (Escher et al. 2014, Neale et al. 2017), while effects in some surface waters were within the same $EC_{IR1.5}$ range (Escher et al. 2014, Neale et al. 2015a, Neale et al. 2017). When considering disinfected drinking water, the effect of chlorinated water from an Australian WTP was considerably higher (Neale et al. 2012), while formation potential experiments with Australian WTP source water using sodium hypochlorite (HOCl) and monochloramine (NH_2Cl) also yielded greater effects than the current study (Farré et al. 2013). In both examples from the literature, the difference to our findings can be partially attributed to the TOC concentration, which was generally around 2 to 3 mg/L in the Australian

studies, compared to 0.4 to 1.7 mg/L in the current study due to the more advanced treatment processes. Only the DBP formation potential experiments using desalinated seawater, which had a TOC concentration of less than 0.1 mg/L, yielded a similar effect as in the current study.

The NF-κB response in the current study was also compared to other water types (Figure S8). While drinking water samples have not previously been tested in the NF-κB-*bla* assay, the response in the current study is lower than wastewater and surface water (Escher et al. 2014, Neale et al. 2015a, Neale et al. 2017).

3.6. Comparison of observed effects with effect-based trigger values

The likely presence of a complex mixture of micropollutants and DBPs in drinking water emphasises the need for a bioanalytical health-related approach to evaluate drinking water safety (Grummt et al. 2013). At the same time, many bioassays are very sensitive, with effects detected in highly enriched mineral water in the AREc32 assay in the current study. Therefore, there is a need for effect-based trigger values to differentiate between what is an acceptable or unacceptable response (Escher et al. 2015). There have been a number of different approaches proposed in the literature to derive effect-based trigger values. For example, Brand et al. (2013) proposed trigger values based on the acceptable daily intake of the assay reference compound using equivalent concentrations and accounting for some *in vitro* to *in vivo* toxicokinetic differences. Escher et al. (2015) used an alternative approach by reading across from existing drinking water guidelines to derive bioanalytical trigger values. Using the latter approach, the proposed effect-based trigger value for drinking water in the AREc32 value is an $EC_{IR1.5}$ of 6 (Escher et al. 2013). This is based on the Australian Drinking Water Guidelines, but suggests that there is a margin of safety of 2.5 to 16 between the proposed effect-based trigger value and the observed effects in French drinking water (Figure 4). While further work is required to derive a French specific effect-based trigger value, this comparison can be used to further illustrate the high quality of the treated water.

4. Conclusions

Three bioassays indicative of adaptive stress responses were applied in the current study to assess the toxicological profile and monitor DBP formation in drinking water distribution networks. While effects in the p53 response and the NF-κB response assays were generally masked by cytotoxicity, the oxidative stress response assay proved to be a senstive tool to monitor the sum of all bioactive chemicals in water. Not only did the observed effects generally correlate well with the detected DBPs, but by comparing the effect before and after chlorination using the BEQ approach, it was possible to assess the contribution of both formed DBPs and micropollutants in source water to the overall effects in drinking water for the first time. This approach may provide guidance to WTP operators by enabling them to target treatment processes that either reduce the micropollutant concentration in the source water or limit DBP formation during disinfection. While routine sample enrichment for bioanalysis will exclude volatile DBPs, mixture toxicity modelling demonstrated that the volatile DBPs generally did not contribute significantly to the oxidative stress response. This supports the use of current extraction methods to target the majority of the toxicologically relevant DBPs. Overall, the effect of the water samples throughout the distribution networks was low, as confirmed by the proposed effect-based trigger value, which reflects the high quality of the treated water. This study demonstrates the suitability of a combined chemical analysis and bioanalysis approach to monitor micropollutants and DBPs in drinking water.

Acknowledgements

Maria König and Christin Kühnert (both UFZ) are acknowledged for experimental assistance. Gaela Leroy, Delphine Brillant and Valérie Ingrand (all Veolia R&I Environment & Health Department, Chemical Analysis and Mechanisms Unit) are acknowledged for water sample extractions and cartridge experimental preparation and management. ALPA/CAE laboratory and IANESCO laboratory are acknowledged for chemical analysis.

454 **References**

455 Besselink, H., 2013. Determination of (anti)ERα, (anti-)AR, (anti-)GR, (anti-)PR, TRβ, PPARα,
456 P53, Nrf2 and cytotoxicity CALUX activity in water samples, BioDetection Systems B.V. (BDS),
457 Amsterdam, The Netherlands.
458 Brand, W., de Jongh, C.M., van der Linden, S.C., Mennes, W., Puijker, L.M., van Leeuwen, C.J.,
459 van Wezel, A.P., Schriks, M., Heringa, M.B., 2013. Trigger values for investigation of hormonal
460 activity in drinking water and its sources using CALUX bioassays. Environ. Int. 55, 109-118.
461 Dominguez-Tello, A., Arias-Borrego, A., Garcia-Barrera, T., Gomez-Ariza, J.L., 2015. Seasonal
462 and spatial evolution of trihalomethanes in a drinking water distribution system according to the
463 treatment process. Environ. Monit. Assess. 187(11), 662.
464 Escher, B.I., Dutt, M., Maylin, E., Tang, J.Y.M., Toze, S., Wolf, C.R., Lang, M., 2012. Water
465 quality assessment using the AREc32 reporter gene assay indicative of the oxidative stress response
466 pathway. J. Environ. Monit. 14(11), 2877-2885.
467 Escher, B.I., Leusch, F.D.L., 2012. Bioanalytical tools in water quality assessment, IWA
468 Publishing, London.
469 Escher, B.I., van Daele, C., Dutt, M., Tang, J.Y.M., Altenburger, R., 2013. Most oxidative stress
470 response in water samples comes from unknown chemicals: The need for effect-based water quality
471 trigger values. Environ. Sci. Technol. 47(13), 7002-7011.
472 Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P., Busch, W., Crago, J.,
473 Denslow, N.D., Dopp, E., Hilscherova, K., Humpage, A.R., Kumar, A., Grimaldi, M., Jayasinghe,
474 B.S., Jarosova, B., Jia, A., Makarov, S., Maruya, K.A., Medvedev, A., Mehinto, A.C., Mendez, J.E.,
475 Poulsen, A., Prochazka, E., Richard, J., Schifferli, A., Schlenk, D., Scholz, S., Shiraish, F., Snyder,
476 S., Su, G.Y., Tang, J.Y.M., van der Burg, B., van der Linden, S.C., Werner, I., Westerheide, S.D.,
477 Wong, C.K.C., Yang, M., Yeung, B.H.Y., Zhang, X.W., Leusch, F.D.L., 2014. Benchmarking
478 organic micropollutants in wastewater, recycled water and drinking water with *in vitro* bioassays.
479 Environ. Sci. Technol. 48(3), 1940-1956.

480 Escher, B.I., Neale, P.A., Leusch, F.D.L., 2015. Effect-based trigger values for *in vitro* bioassays:
481 Reading across from existing water quality guideline values. Water Res. 81, 137-148.
482 European Commission, 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of
483 water intended for human consumption. Off. J. Eur. Union, L 330/332.
484 Farré, M.J., Day, S., Neale, P.A., Stalter, D., Tang, J.Y.M., Escher, B.I., 2013. Bioanalytical and
485 chemical assessment of the disinfection by-product formation potential: Role of organic matter.
486 Water Res. 47(14), 5409-5421.
487 Fawell, J., Nieuwenhuijsen, M.J., 2003. Contaminants in drinking water: Environmental pollution
488 and health. Br. Med. Bull. 68(1), 199-208.
489 Glassmeyer, S.T., Furlong, E.T., Kolpin, D.W., Batt, A.L., Benson, R., Boone, J.S., Conerly, O.,
490 Donohue, M.J., King, D.N., Kostich, M.S., Mash, H.E., Pfaller, S.L., Schenck, K.M., Simmons,
491 J.E., Varughese, E.A., Vesper, S.J., Villegas, E.N., Wilson, V.S., 2017. Nationwide reconnaissance
492 of contaminants of emerging concern in source and treated drinking waters of the United States.
493 Sci. Total Environ. 581–582(1), 909–922.
494 Grummt, T., Kuckelkorn, J., Bahlmann, A., Baumstark-Khan, C., Brack, W., Braunbeck, T., Feles,
495 S., Gartiser, S., Glatt, H., Heinze, R., Hellweg, C.E., Hollert, H., Junek, R., Knauer, M., Kneib-
496 Kissinger, B., Kramer, M., Krauss, M., Küster, E., Maletz, S., Meinl, W., Noman, A., Prantl, E.-M.,
497 Rabbow, E., Redelstein, R., Rettberg, P., Schadenboeck, W., Schmidt, C., Schulze, T., Seiler, T.-B.,
498 Spitta, L., Stengel, D., Waldmann, P., Eckhardt, A., 2013. Tox-Box: securing drops of life - an
499 enhanced health-related approach for risk assessment of drinking water in Germany. Env. Sci. Eur.
500 25(1), 27.
501 Hua, G., Reckhow, D.A., 2008. DBP formation during chlorination and chloramination: Effect of
502 reaction time, pH, dosage, and temperature. J. Am. Water Works Assoc. 100, 82-95.
503 ISO 15680, 2003. Water quality -- Gas-chromatographic determination of a number of monocyclic
504 aromatic hydrocarbons, naphthalene and several chlorinated compounds using purge-and-trap and
505 thermal desorption.

ISO 23631, 2006. Water quality -- Determination of dalapon, trichloroacetic acid and selected haloacetic acids -- Method using gas chromatography (GC-ECD and/or GC-MS detection) after liquid-liquid extraction and derivatization.

Knight, A.W., Little, S., Houck, K., Dix, D., Judson, R., Richard, A., McCarroll, N., Akerman, G., Yang, C.H., Birrell, L., Walmsley, R.M., 2009. Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCast™ chemicals. Regul. Toxicol. Pharmacol. 55(2), 188-199.

König, M., Escher, B.I., Neale, P.A., Krauss, M., Hilscherová, K., Novák, J., Teodorović, I., Schulze, T., Seidensticker, S., Kamal Hashmi, M.A., Ahlheim, J., Brack, W., 2017. Impact of untreated wastewater on a major European river evaluated with a combination of *in vitro* bioassays and chemical analysis. Environmental Pollution 220, 1220-1230.

Loos, R., Wollgast, J., Huber, T., Hanke, G., 2007. Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy. Anal. Bioanal. Chem. 387(4), 1469-1478.

Martin, M.T., Dix, D.J., Judson, R.S., Kavlock, R.J., Reif, D.M., Richard, A.M., Rotroff, D.M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S.S., Houck, K.A., 2010. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem. Res. Toxicol. 23(3), 578-590.

Miller, S.C., Huang, R.L., Sakamuru, S., Shukla, S.J., Attene-Ramos, M.S., Shinn, P., Van Leer, D., Leister, W., Austin, C.P., Xia, M.H., 2010. Identification of known drugs that act as inhibitors of NF-κB signaling and their mechanism of action. Biochem. Pharmacol. 79(9), 1272-1280.

Mompelat, S., Thomas, O., Le Bot, B., 2011. Contamination levels of human pharmaceutical compounds in French surface and drinking water. J. Environ. Monit. 13(10), 2929-2939.

Munch, D.J., Hautman, D.P., 1995. EPA Method 551. 1 Determination of chlorination disinfection by products, chlorinated solvents, and halogenated pesticides/herbicides in drinking water by liquid-

liquid extraction and gas chromatography with electron-capture detection, Environmental Protection Agency, Cincinnati, OH.

Neale, P.A., Antony, A., Bartkow, M.E., Farré, M.J., Heitz, A., Kristiana, I., Tang, J.Y.M., Escher, B.I., 2012. Bioanalytical assessment of the formation of disinfection byproducts in a drinking water treatment plant. Environ. Sci. Technol. 46(18), 10317-10325.

Neale, P.A., Ait-Aissa, S., Brack, W., Creusot, N., Denison, M.S., Deutschmann, B., Hilscherova, K., Hollert, H., Krauss, M., Novak, J., Schulze, T., Seiler, T.B., Serra, H., Shao, Y., Escher, B.I., 2015a. Linking *in vitro* effects and detected organic micropollutants in surface water using mixture-toxicity modeling. Environ. Sci. Technol. 49(24), 14614-14624.

Neale, P.A., Stalter, D., Tang, J.Y.M., Escher, B.I., 2015b. Bioanalytical evidence that chemicals in tattoo ink can induce adaptive stress responses. J. Hazard. Mater. 296, 192-200.

Neale, P.A., Munz, N.A., Ait-Aissa, S., Altenburger, R., Brion, F., Busch, W., Escher, B.I., Hilscherova, K., Kienle, C., Novak, J., Seiler, T.B., Shao, Y., Stamm, C., Hollender, J., 2017. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. Sci. Total Environ. 576, 785-795.

Postigo, C., Richardson, S.D., 2014. Transformation of pharmaceuticals during oxidation/disinfection processes in drinking water treatment. J. Hazard. Mater. 279, 461-475.

Richardson, S.D., Postigo, C., 2015. Recent Advances in Disinfection by-Products. Karanfil, T., Mitch, B., Westerhoff, P. and Xie, Y. (eds), pp. 189-214.

Rodriquez, M.J., Serodes, J.B., Levallois, P., 2004. Behavior of trihalomethanes and haloacetic acids in a drinking water distribution system. Water Res. 38(20), 4367-4382.

Rossman, L.A., Brown, R.A., Singer, P.C., Nuckols, J.R., 2001. DBP formation kinetics in a simulated distribution system. Water Res. 35(14), 3483-3489.

Silva, E., Rajapakse, N., Kortenkamp, A., 2002. Something from "nothing" - Eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. 36(8), 1751-1756.

558 Simmons, S.O., Fan, C.Y., Ramabhadran, R., 2009. Cellular stress response pathway system as a
559 sentinel ensemble in toxicological screening. Toxicol. Sci. 111(2), 202-225.
560 Stalter, D., O'Malley, E., von Gunten, U., Escher, B.I., 2016a. Fingerprinting the reactive toxicity
561 pathways of 50 drinking water disinfection by-products. Water Res. 91, 19-30.
562 Stalter, D., Peters, L.I., O'Malley, E., Tang, J.Y.M., Revalor, M., Farre, M.J., Watson, K., von
563 Gunten, U., Escher, B.I., 2016b. Sample enrichment for bioanalytical assessment of disinfected
564 drinking water: Concentrating the polar, the volatiles, and the unknowns. Environ. Sci. Technol.
565 50(12), 6495-6505.
566 US EPA, 2015. Interactive Chemical Safety for Sustainability (iCSS) Dashboard v2,
567 http://actor.epa.gov/dashboard/, Accessed 5th Jan 2016.
568 Villanueva, C.M., Cantor, K.P., Grimalt, J.O., Malats, N., Silverman, D., Tardon, A., Garcia-
569 Closas, R., Serra, C., Carrato, A., Castano-Vinyals, G., Marcos, R., Rothman, N., Real, F.X.,
570 Dosemeci, M., Kogevinas, M., 2007. Bladder cancer and exposure to water disinfection by-products
571 through ingestion, bathing, showering, and swimming in pools. Am. J. Epidemiol. 165(2), 148-156.
572 Wang, S., Zhang, H., Zheng, W.W., Wang, X., Andersen, M.E., Pi, J.B., He, G.S., Qu, W.D., 2013.
573 Organic extract contaminants from drinking water activate Nrf2-mediated antioxidant response in a
574 human cell line. Environ. Sci. Technol. 47(9), 4768-4777.
575 Wang, X.J., Hayes, J.D., Wolf, C.R., 2006. Generation of a stable antioxidant response element-
576 driven reporter gene cell line and its use to show redox-dependent activation of Nrf2 by cancer
577 chemotherapeutic agents. Cancer Res. 66(22), 10983-10994.
578 WHO, 2011. Guidelines for Drinking-Water Quality, 4th Edition, World Health Organization,
579 Geneva, Switzerland.
580 Yeh, R.Y.L., Farre, M.J., Stalter, D., Tang, J.Y.M., Molendijk, J., Escher, B.I., 2014. Bioanalytical
581 and chemical evaluation of disinfection by-products in swimming pool water. Water Res. 59, 172-
582 184.

583 Zhang, D.D., 2006. Mechanistic studies of the Nrf2-Keap1 signaling pathway. Drug Metab. Rev.
584 38(4), 769-789.
585 Zhang, X., Echigo, S., Minear, R.A., Plewa, M.J., 2000. Natural Organic Matter and Disinfection
586 By-Products. Barrett, S.E., Krasner, S.W. and Amy, G.L. (eds), pp. 299-314, American Chemical
587 Society.

588 **Table 1**: Overview of studied water treatment plants (WTP), treatment processes and sampling
589 sites.

WTP location	Treatment processes	WTP capacity (m^3/d)	Sampling site		
			Water age (h)	*Site name*	*Disinfection*
Méry-sur-Oise	1) Clarification, rapid sand filtration, ozonation, anthracite filtration, nanofiltration (70%) and 2) clarification, sand filtration, ozonation, granular activated carbon filtration (30%)	340,000	0	Outlet of WTP	-
			10	Ermont	Before re-chlorination
			50	Bezons	After re-chlorination
Choisy-le-Roi	Pre-ozonation, clarification, sand filtration, ozonation, granular activated carbon filtration, UV	600,000	0	Before chlorination	-
			0	Outlet of WTP	-
			8	Fresnes	Before re-chlorination
			30	Cachan	After re-chlorination
			50	Les-Loges-en-Josas	After re-chlorination
Neuilly-sur-Marne	Clarification, sand filtration, ozonation,granular activated carbon filtration, UV	600,000	0	Before chlorination	-
			0	Outlet of WTP	-
			25	Noisy-le-Sec	Before re-chlorination
			50	Clichy-la-Garenne	After re-chlorination

590

591 **Table 2**: Overview of studied bioassays.

Endpoint	Assay	Method reference	Reference compound	Reference compound $EC_{IR1.5}$ (M)
Oxidative stress response	AREc32	Wang et al. (2006), Escher et al. (2012)	tert-Butylhydroquinone (tBHQ)	1.93×10^{-6}
p53 response	p53RE-*bla*	Neale et al. (2015b)	Mitomycin	1.16×10^{-7}
NF-κB response	NF-κB-*bla*	König et al. (2017)	Tumour necrosis factor alpha (TNFα)	6.65×10^{-3}*

*units of µg/L

592 **Table 3**: BEQ_{bio}, $BEQ_{chem,\ non\text{-}volatile}$ and $BEQ_{chem,volatile}$ (M) for the different samples in the AREc32 assay.

WTP	Water age (h)	November			March			May			September		
		BEQ_{bio}	$BEQ_{chem,\ non\text{-}volatile}$	$BEQ_{chem,\ volatile}$	BEQ_{bio}	$BEQ_{chem,\ non\text{-}volatile}$	$BEQ_{chem,\ volatile}$	BEQ_{bio}	$BEQ_{chem,\ non\text{-}volatile}$	$BEQ_{chem,\ volatile}$	BEQ_{bio}	$BEQ_{chem,\ non\text{-}volatile}$	$BEQ_{chem,\ volatile}$
Méry-sur-Oise	0	$(2.63\pm0.14)\times10^{-8}$	-	1.43×10^{-11}	$(2.79\pm0.15)\times10^{-8}$	5.31×10^{-8}	2.36×10^{-11}	$(4.25\pm0.18)\times10^{-8}$	1.71×10^{-10}	3.05×10^{-11}	$(4.74\pm0.22)\times10^{-8}$	8.12×10^{-11}	2.35×10^{-11}
	10	$(3.11\pm0.19)\times10^{-8}$	4.54×10^{-8}	2.67×10^{-11}	$(2.60\pm0.12)\times10^{-8}$	1.95×10^{-8}	3.83×10^{-11}	$(4.39\pm0.20)\times10^{-8}$	2.54×10^{-8}	4.40×10^{-11}	$(5.10\pm0.19)\times10^{-8}$	8.12×10^{-11}	2.81×10^{-11}
	50	$(2.51\pm0.14)\times10^{-8}$	5.20×10^{-8}	4.90×10^{-11}	$(2.10\pm0.10)\times10^{-8}$	2.27×10^{-8}	3.19×10^{-9}	$(4.85\pm0.20)\times10^{-8}$	2.29×10^{-8}	3.27×10^{-9}	$(4.19\pm0.18)\times10^{-8}$	3.00×10^{-8}	6.40×10^{-11}
Choisy-le-Roi	Before Cl_2	$(1.98\pm0.10)\times10^{-8}$	-	-	$(3.08\pm0.19)\times10^{-8}$	-	-	-	-	-	$(6.67\pm0.41)\times10^{-8}$	-	-
	0	$(3.76\pm0.23)\times10^{-8}$	4.43×10^{-8}	4.10×10^{-9}	$(3.71\pm0.14)\times10^{-8}$	2.73×10^{-8}	4.06×10^{-11}	$(4.72\pm0.14)\times10^{-8}$	3.00×10^{-8}	5.50×10^{-9}	$(8.04\pm0.26)\times10^{-8}$	2.00×10^{-10}	4.95×10^{-11}
	8	$(3.54\pm0.16)\times10^{-8}$	7.15×10^{-8}	5.19×10^{-9}	$(4.60\pm0.13)\times10^{-8}$	3.70×10^{-8}	7.46×10^{-10}	$(8.23\pm0.22)\times10^{-8}$	3.95×10^{-8}	8.78×10^{-9}	$(1.25\pm0.04)\times10^{-7}$	2.07×10^{-10}	6.15×10^{-11}
	30	$(4.13\pm0.15)\times10^{-8}$	8.43×10^{-8}	5.73×10^{-9}	$(3.60\pm0.12)\times10^{-8}$	3.64×10^{-8}	8.73×10^{-10}	$(7.42\pm0.34)\times10^{-8}$	3.39×10^{-8}	8.61×10^{-9}	$(1.06\pm0.03)\times10^{-7}$	2.80×10^{-8}	9.68×10^{-11}
	50	$(4.70\pm0.18)\times10^{-8}$	7.15×10^{-8}	8.99×10^{-9}	$(6.04\pm0.28)\times10^{-8}$	7.79×10^{-8}	9.14×10^{-9}	$(7.86\pm0.21)\times10^{-8}$	3.70×10^{-8}	1.06×10^{-8}	$(1.14\pm0.04)\times10^{-7}$	2.80×10^{-8}	4.55×10^{-9}
Neuilly-sur-Marne	Before Cl_2	$(3.10\pm0.13)\times10^{-8}$	-	-	$(2.54\pm0.07)\times10^{-8}$	-	-	$(4.05\pm0.16)\times10^{-8}$	-	-	$(5.33\pm0.17)\times10^{-8}$	-	-
	0	$(2.72\pm0.12)\times10^{-8}$	4.76×10^{-8}	6.77×10^{-9}	$(4.41\pm0.13)\times10^{-8}$	4.41×10^{-8}	3.47×10^{-11}	$(5.22\pm0.22)\times10^{-8}$	3.95×10^{-8}	7.92×10^{-9}	$(7.06\pm0.17)\times10^{-8}$	3.11×10^{-10}	5.94×10^{-11}
	25	$(3.73\pm0.19)\times10^{-8}$	4.36×10^{-8}	5.57×10^{-9}	$(3.42\pm0.19)\times10^{-8}$	4.49×10^{-8}	5.96×10^{-11}	$(5.97\pm0.17)\times10^{-8}$	3.40×10^{-8}	2.06×10^{-8}	$(7.78\pm0.33)\times10^{-8}$	-	1.01×10^{-10}
	50	$(3.30\pm0.13)\times10^{-8}$	4.62×10^{-8}	6.35×10^{-9}	$(3.93\pm0.15)\times10^{-8}$	1.32×10^{-10}	1.69×10^{-9}	$(5.63\pm0.19)\times10^{-8}$	4.25×10^{-8}	9.65×10^{-9}	$(7.67\pm2.10)\times10^{-8}$	1.91×10^{-10}	3.88×10^{-9}

Figure 1: Sum concentration of detected DBPs and $EC_{IR1.5}$ values for the oxidative stress response and NF-κB response for A) Méry-sur-Oise, B) Choisy-le-Roi and C) Neuilly-sur-Marne. Note the inverse axis for $EC_{IR1.5}$ that a higher effect is further to the top.

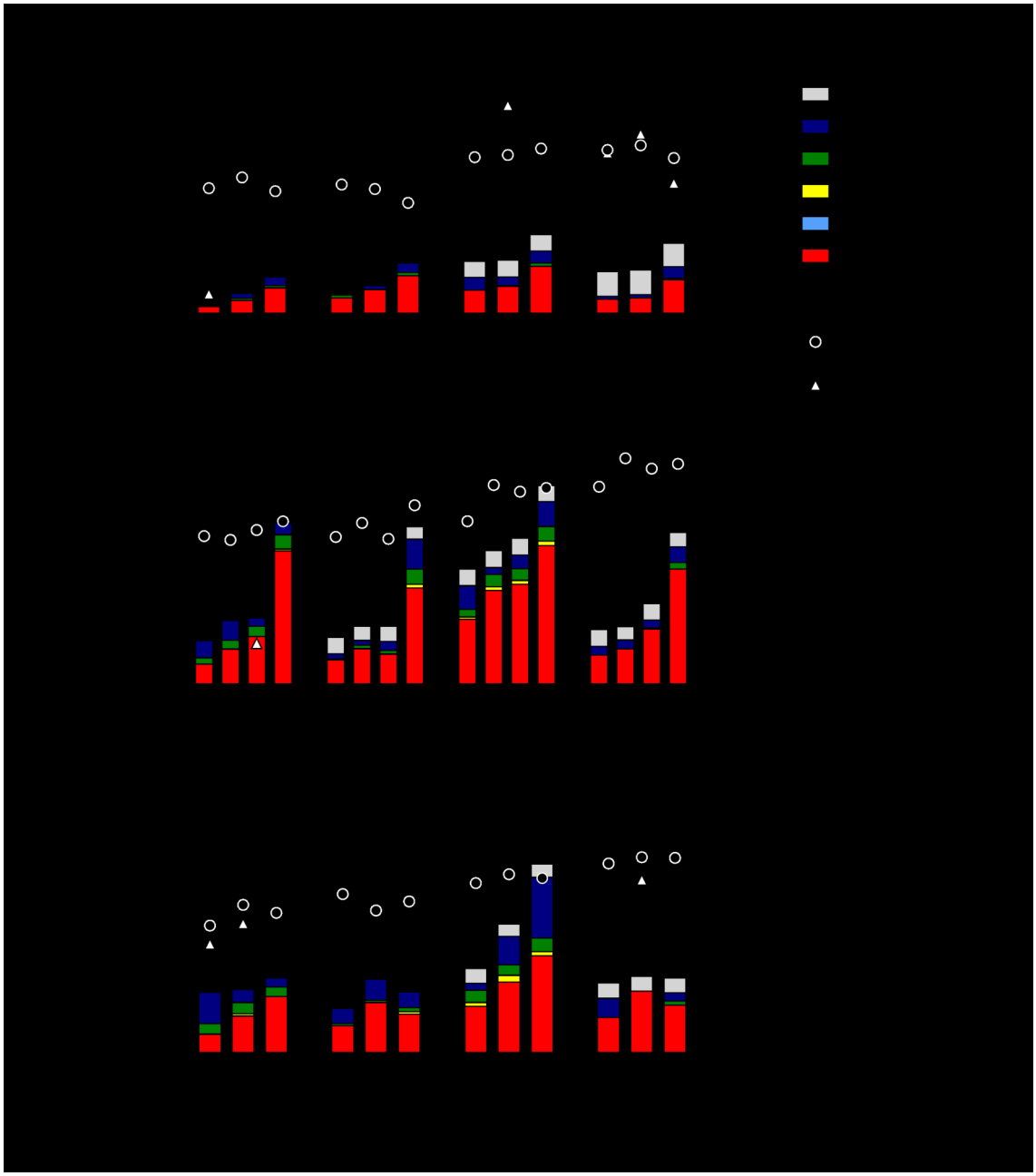
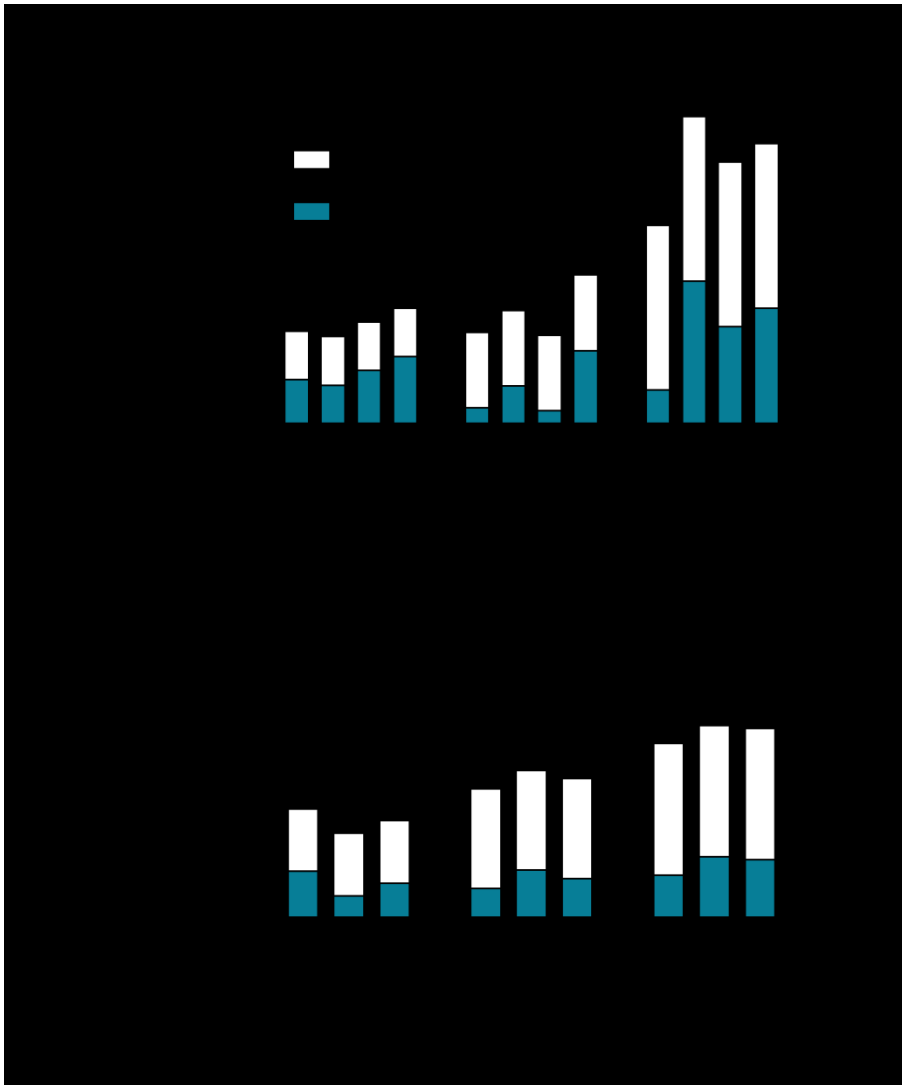
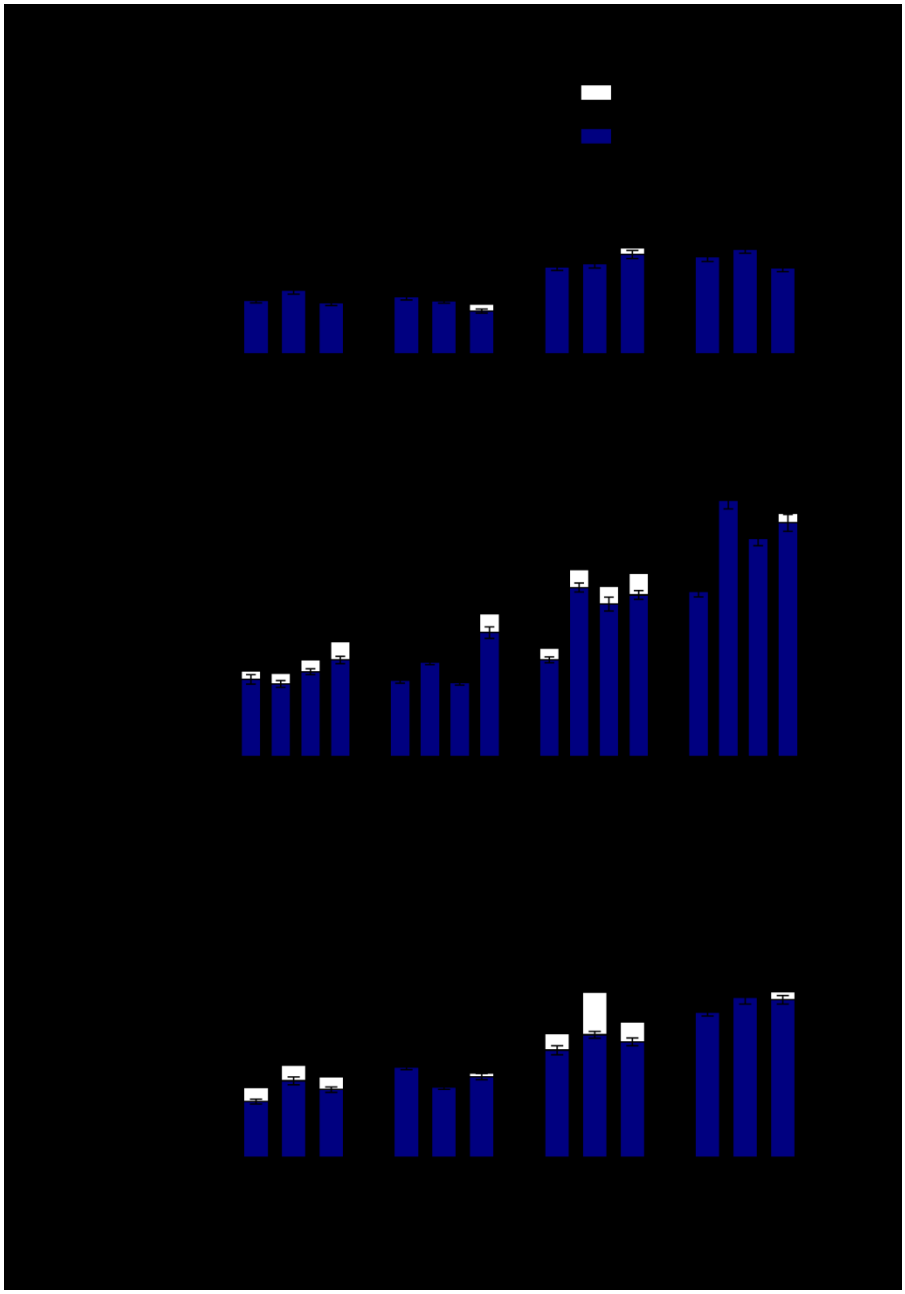


Figure 2: Contribution of DBPs and other micropollutants to the oxidative stress response in A) Choisy-le-Roi and B) Neuilly-sur-Marne.



606 **Figure 3**: Bioanalytical equivalent concentration from bioanalysis (BEQ_{bio}) and bioanalytical
607 equivalent concentration from chemical analysis for volatile chemicals ($BEQ_{chem,\ volatile}$) for A) Méry-
608 sur-Oise, B) Choisy-le-Roi and C) Neuilly-sur-Marne.



609

610

Figure 4: Comparison of AREc32 $EC_{IR1.5}$ values from current study with $EC_{IR1.5}$ values for wastewater, surface water and drinking water from the literature in units of relative enrichment factor (REF). The proposed effect based trigger value for drinking water (REF 6) by Escher et al. (2013) is shown by the red dashed line.

[a]Escher et al. (2014), [b]Neale et al. (2017), [c]Neale et al. (2015a), [d]Neale et al. (2012), [e]Farré et al. (2013).

NB: ARE GeneBLAzer used instead of AREc32 for Neale et al. (2017) and Neale et al. (2015a).