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Assessment of source and treated water quality in seven drinking water treatment plants by in vitro bioassays – Oxidative stress and antiandrogenic effects after artificial infiltration

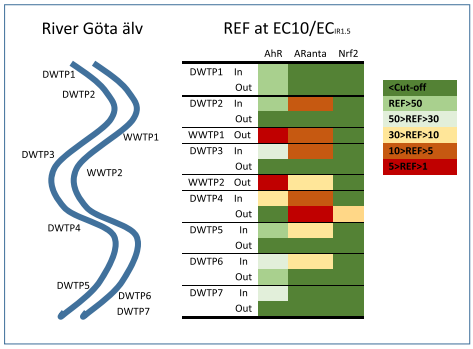
Agneta Oskarsson [a,*], Anna Kjerstine Rosenmai [a], Geeta Mandava [a], Anders Johannisson [b], Andrew Holmes [c], Rikard Tröger [d], Johan Lundqvist [a]

[a] Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Box 7028, SE-750 07 Uppsala, Sweden
[b] Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, SE-750 07 Uppsala, Sweden
[c] Kungälv Drinking Water Treatment Plant, Filaregatan 15, SE-442 81 Kungälv, Sweden
[d] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Box 7050, SE-750 07 Uppsala, Sweden

HIGHLIGHTS

- Source (river) water induced AhR and AR antagonistic activities
- Most DWTPs removed the activities.
- Nrf2 and anti-AR activities were induced after artificial infiltration.
- Target or regulatory chemical analysis did not reveal presence of hazards.

GRAPHICAL ABSTRACT



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ABSTRACT

Drinking water quality and treatment efficacy was investigated in seven drinking water treatment plants (DWTPs), using water from the river Göta Älv, which also is a recipient of treated sewage water. A panel of cell-based bioassays was used, including measurements of receptor activity of aryl hydrocarbon (AhR), estrogen (ER), androgen (AR), peroxisome proliferator-activated receptor alpha (PPARα) as well as induction of oxidative stress (Nrf2) and micronuclei formation. Grab water samples were concentrated by solid phase extraction (SPE) and water samples were analyzed at a relative enrichment factor of 50. High activities of AhR, ER and AR antagonism were present in WWTP outlets along the river. Inlet water from the river exhibited AhR and AR antagonistic activities. AhR activity was removed by DWTPs using granulated activated carbon (GAC) and artificial infiltration. AR antagonistic activity was removed by the treatment plants, except the artificial infiltration plant, which actually increased the activity. Furthermore, treated drinking water from the DWTP using artificial infiltration exhibited high Nrf2 activity, which was not found in any of the other water samples. Nrf2 activity was found in water from eight of the 13 abstraction wells, collecting water from the artificial infiltration. No genotoxic activity was detected at non-cytotoxic concentrations. No Nrf2 or AR antagonistic activities were detected in the inlet or outlet water after the DWTP had been replaced by a new plant, using membrane ultrafiltration and GAC. Neither target chemical analysis, nor chemical analysis according to the drinking water regulation, detected any

* Corresponding author at: Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Box 7028, SE-750 07 Uppsala, Sweden.
E-mail address: agneta.oskarsson@slu.se (A. Oskarsson).

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presence of chemicals, which could be responsible of the prominent effects on oxidative stress and AR antagonistic activity in the drinking water samples. Thus, bioanalysis is a useful tool for detection of unknown hazards in drinking water and for assessment of drinking water treatments.

1. Introduction

Access to safe drinking water is essential to health (WHO, 2011). Chemical contamination of the environment may be a serious threat, especially when surface water is used as a source for drinking water. In Sweden, 50% of public drinking water is produced directly from surface water, 25% from surface water via artificial infiltration and 25% from ground water (Svenskt Vatten, 2016). Detection and removal of contaminating chemicals in the drinking water treatment plants is important and a great challenge. Monitoring by chemical analysis covers only a small part of the occurring chemicals, those that are known or expected to be present, and not the unknown chemicals. Furthermore, chemical analyses do not give information on the potential toxic effects of the complex mixture of natural and anthropogenic chemicals, which may be present in drinking water. However, effect-based methods have been increasingly applied in water quality monitoring, especially of surface and wastewater, to detect biological activities relevant to adverse health effects (Altenburger et al., 2019; Brack et al., 2019; Dopp et al., 2019; Escher et al., 2018; Konig et al., 2017).

Recent studies on drinking water quality have used in vitro bioassays to detect biological effects of hazardous chemicals in raw and finished drinking water (Brand et al., 2013; Brunner et al., 2020; Escher et al., 2013; Jones et al., 2020; Kakaley et al., 2020; Leusch et al., 2018; Macova et al., 2011; Neale and Escher, 2019; Rosenmai et al., 2018). Efficacy of drinking water treatment has been assessed, and reduced as well as increased bioactivity has been demonstrated following drinking water treatment. Chlorination, ozonation and UV-treatment, which is used to eliminate microbiological contamination in drinking water, is known to cause formation of genotoxic disinfection byproducts, which can be detected by bioassays (Neale et al., 2012) Tak and Vellanki, 2018). In order to identify the micropollutants responsible for bioactivity in polluted water samples, effect-directed analysis has been proposed, where in vitro bioassays are integrated with chemical analyses (Altenburger et al., 2019; Brack et al., 2016).

The bioassays used in the present study were selected due to relevance to human health. Aryl hydrocarbon receptor, AhR, has multiple physiological functions, with impact on chemical and microbial defense, energy metabolism, reproduction, development, immunity and inflammation (Bock, 2019). The nuclear factor erythroid 2-related factor, Nrf2, regulates the cellular defense mechanism against oxidative stress by activation of antioxidant genes (Zheng et al., 2020). Oxidative stress is a common mechanism for genotoxicity and different adverse endpoints, such as tissue damage, carcinogenicity and teratogenicity (Ma, 2013). Endotoxins or lipopolysaccharides (LPS) are components embedded in the outer membranes of Gram-negative bacteria and some cyanobacteria, and known to be health hazards in water (Zhang et al., 2019a). Lipopolysaccharides are reported to increase the Nrf2 expression (Menon and Peltier, 2020). Peroxisome proliferator activated receptor alpha, PPARα, plays crucial roles in lipid and glucose metabolism (Mirza et al., 2019). Estrogen receptor, ER, regulates the actions of estrogens, which are essential for reproduction, cardiovascular function, bone strength, cognitive behavior, successful pregnancy and gastrointestinal systems (Adeel et al., 2017). Androgen receptor, AR, regulates the actions of androgens, which have important role in the development and function of the reproductive, musculoskeletal, cardiovascular, immune, and neural systems (Davey and Grossmann, 2016).

The aim of the present study was to assess drinking water quality and treatment efficacy in seven DWTPs using the panel of in vitro bioassays, described above. The DWTPs used water from the same surface water source, the river Göta Älv, which is the second largest source of raw water in Sweden. The river is a recipient of treated wastewater, storm water discharges and effluents from industries. The impact on bioactivity of effluents from upstream wastewater treatment plants was also investigated. As high activity of oxidative stress and antiandrogenic activity was detected in outlet water in one of the DWTPs, this specific DWTP was followed up with multiple resampling in an effort to detect the source of contamination. Genotoxicity was also tested in samples with high activity of oxidative stress. In parallel, the occurrence of micropollutants in the water samples was analyzed and previously reported (Troger et al., 2020).

2. Materials and methods

2.1. Study area and sampling

The river Göta Älv is 93 km long and drains Lake Vänern, the largest lake in Sweden, into Kattegat at the city of Gothenburg on the West Coast of Sweden. Along the river are six municipalities, effluents from more than 20 small and large wastewater treatments facilities, as well as industrial and agricultural activities. The Göta Älv is a surface water source for drinking water, serving a population of around 750,000, including Gothenburg.

Grab samples (12 L) of water (for bioassays and chemical analysis) were collected in September (20th and 21st) 2016 at 11 sampling sites from the source in Vänern and along the Göta Älv: from inlet and outlet of seven DWTPs, surface water samples at two sites along the river, and outlet of two WWTPs (Fig. 1; Table 1).

Five of the DWTPs used raw water directly from the Göta Älv, while Lackarebäck (DWTP7) took raw water from a lake (Delsjön) receiving its water from the Göta Älv, and Mölndal (DWTP6) used water from a separate lake (Rådasjön). Most of the DWTPs used conventional water treatment methods, including lime and CO_2 for pH adjustment, coagulation, sedimentation, sand filtration, and UV irradiation and chlorination for disinfection. One DWTP, Kungälv (DWTP4), used artificial infiltration, four DWTPs (DWTPs 3, 5, 6 and 7) had GAC filters and one (DWTP7) had ultrafiltration (Table 1).

In DWTP4 river water was pumped to a sedimentation basin, from which the water flowed into nine infiltration basins (Fig. S1). From infiltration basins water was percolated through a layer of clay and gravel for 7–30 days (artificial infiltration) and then collected in 13 abstraction wells. Water from three of the wells (9, 11 and 13) were treated by coagulation and sand filtration before mixed with water from the other abstraction wells, and finally treated with mild UV disinfection (400 J/m2). Due to the high Nrf2 and AR antagonistic activities in the outlet water from DWTP4, which were revealed in this study, inlet and outlet water were resampled in May 2017 (three raw water and three treated water, sampled with 5 min intervals); and in February 2018 (one raw water, three treated waters, sampled on three consecutive days and one tap water from the distribution network at 2.5 km from the DWTP). In an effort to localize the source of the contamination, water was sampled from the 13 abstraction wells (numbered 1–3 and 6–15) at DWTP4 in May 2018 and from inlet and outlet water, and tested for Nrf2 and AR antagonistic activities, micronuclei formation and endotoxin concentrations. The artificial infiltration had been in place since the 1950s. In December 2018, DWTP4 was replaced by a plant using the following process: aeration, biofiltration, coagulation upfront of ultrafilter and granulated activated carbon (GAC) filtration plus UV disinfection, instead of artificial

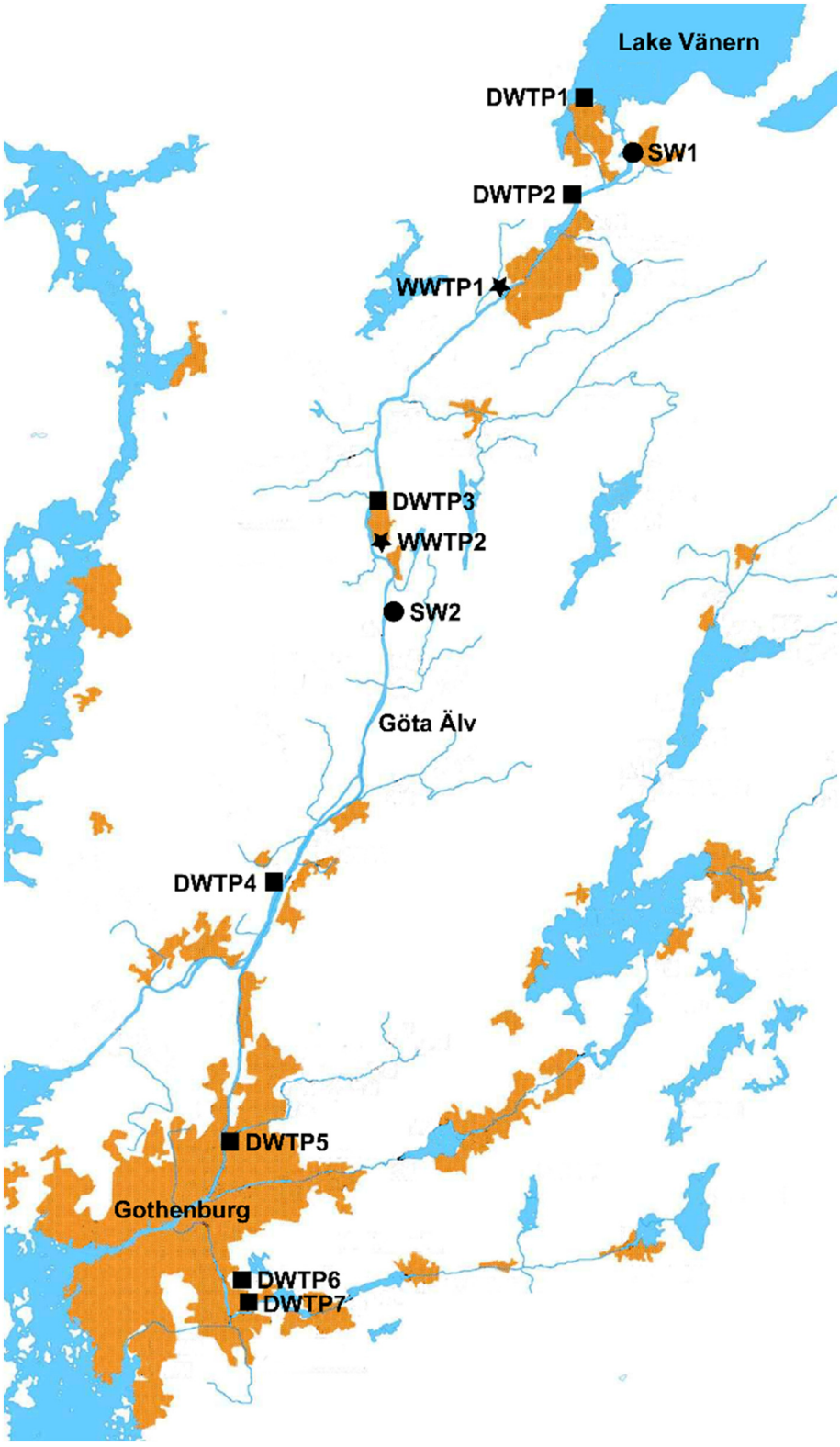

Fig. 1. Sampling sites along the Göta Älv. Squares indicate drinking water treatment plants (DWTP), stars indicate wastewater treatment plants (WWTP), and circles indicate surface waters sampling sites (SW).

infiltration. In October 2019 outlet water from the new DWTP was sampled in addition to samples from three of the abstraction wells at the old DWTP.

All water samples were collected in stainless steel containers (Sharpsville Container Corporation, Sharpsville, PA 16150, USA) washed with ethanol (AnalaR quality) followed by Milli-Q water. The containers were rinsed with the sample water three times prior to water collection. The samples were stored at +4 °C before extraction, which was performed within a week of sampling.

Furthermore, in April 2018, water from three of the abstraction wells at DWTP4 (wells 6, 7 and 8) were sampled for chemical analysis according to the Swedish drinking water regulation.

Table 1
Sample identification (ID), sites and drinking water production – sampling in September 2016.

Sample ID	Sampling site	Volume produced (m^3/day)	Population equivalent	Water treatment after coarse prefiltration, pH adjustment, coagulation and sedimentation (except DWTP4)
DWTP1	Inlet/outlet Vänersborg	7700	28,000	Slow sand filtration, UV irradiation, chlorination
SW1	River water Vargön			
DWTP2	Inlet/outlet Trollhättan	13,200	50,000	Rapid and slow sand filtration, UV irradiation, chlorination
WWTP1	Outlet Trollhättan			
DWTP3	Inlet/outlet Lilla Edet	2000	7500	[a]Rapid sand filtration, GAC, UV irradiation, chlorination
WWTP2	Outlet Lilla Edet			
SW2	River water Garn			
DWTP4	Inlet/outlet Kungälv	5000	36,000	[b]Infiltration; pH adjustment before UV irradiation
DWTP5	Inlet/outlet Alelyckan	91,000	250,000	Chlorination, GAC, UV irradiation, chlorination
DWTP6[c]	Inlet/outlet Mölndal	14,400	63,000	Rapid sand filtration, GAC, UV irradiation, chlorination
DWTP7[d]	Inlet/outlet Lackarebäck	87,000	300,000	GAC, UV irradiation, chlorination, ultrafiltration at 30 kDalton,

[a] Chlorination also before coagulation and sedimentation; pH adjustment only after GAC.
[b] Infiltration (7–30 days); no coarse prefiltration or pH adjustment, coagulation and sand filtration of water from 3 of 13 abstraction wells.
[c] Raw water taken from Lake Rådasjön.
[d] Raw water taken from Göta Älv, via Lake Delsjön.

2.2. Extraction of water samples

Extraction of water samples was performed by solid phase extraction (SPE), using a semi-automated extraction system (4790 SPE-DEX®, Horizon Technology, Salem, New Hampshire, USA) with 47 mm Atlantic HLB-M SPE disks (Horizon Technology). The water samples, 5 L (except WWTP1, which was 4.3 L and DWTP5 outlet, which was 3.3 L), were filtered using an in-line 1 μm glass fiber filter (1 Micron – Fine, Fast Flow Sediment Pre-Filters, Horizon Technology) placed in front of the SPE disk. The system was preconditioned with 2 × 25 mL methanol (LC-MS grade, Merck, Darmstadt, Germany) followed by 2 × 25 mL Milli-Q water. The sample was applied to the disk via the filter, the filter and SPE disk were then washed with 2 × 25 mL 5% methanol and finally air dried for 10 min before elution with 3 × 25 mL methanol.

The three eluates were pooled and reduced to approximately 0.5 mL using a TurboVap Classic II System (Biotage, USA), transferred to a glass vial and diluted with ethanol to 1.0 mL. By this procedure the water samples were enriched by a factor of 5000, except for WWTP1 and DWTP5 outlet, which were enriched by a factor of 4300 and 3300, respectively. The concentrated water samples were stored at −20 °C until bioanalysis.

2.2.1. Control water samples

To assess the performance of the extraction method and any potential contribution to the bioactivity from the SPE, control samples were prepared. Five control samples, prepared in 5 L glass bottles, were concentrated by SPE, as described above for the test water samples. The control samples included two Milli-Q (MQ) samples (un-spiked and spiked before extraction) and three drinking water (DW) samples (from the laboratory) (un-spiked, spiked before extraction and spiked after extraction, but before evaporation). The un-spiked samples were used to assess if the SPE method itself would induce any bioactivity. A mixture of 269 chemicals including pesticides, pharmaceuticals, perfluoroalkyl substances and personal care products was added before or after extraction (Table S1). Each chemical was added to a sample concentration of 20 ng/L (100 ng absolute in each sample of 5 L). The sample identifications (ID) of the control water samples were: MQ non-spiked, MQ spiked, DW non-spiked and DW spiked before SPE and DW spiked after, respectively.

2.3. Bioassays

Detailed description of the bioassays is provided in Section S1 (SI). Water samples and controls were assessed for activities of AhR, Nrf2, PPARα, ER and AR agonistic and antagonistic activities in reporter gene assays and for cytotoxicity in all the used cell lines by cell viability assays (MTS and ATP-assay). In addition, water samples from DWTP4 were tested for genotoxicity by an in vitro micronucleus test and for endotoxin activity by the amebocyte lysate assay.

The activities of AhR and PPARα were studied in transiently transfected human hepatocarcinoma cells, HepG2. For AhR activity, HepG2 cells were transfected with a luciferase reporter plasmid under control of a DNA element responsive to ligand activated AhR (donated by Prof. Michael Denison, University of California, Davis, USA), and for PPARα activity, cells were transfected with a human PPARα ligand binding domain-GAL4-plasmid, together with a GAL4-responsive luciferase reporter plasmid (donated by Dr. Jan Fleckner, Novo Nordic, Denmark) (Rosenmai et al., 2018). Nrf2 activity was assayed in a HepG2 cell line, stably transfected with a luciferase plasmid, where the expression of the luciferase protein is under the control of an Nrf2 responsive promoter element (Signosis Inc., Santa Clara, CA, USA, catalog number SL-0046-NP) (Lundqvist et al., 2019). ER activity was assessed in a variant of the human breast carcinoma MCF7 cell line, VM7Luc4E2 (donated by Professor Michael Denison, University of California, USA), containing a stably integrated ER-responsive luciferase reporter plasmid (Rosenmai et al., 2018). AR agonistic and antagonistic activity was studied in the stably transfected Chinese Hamster Ovary cell line AR-EcoScreen™ (National Institutes of Biomedical Innovation, Health, and Nutrition JCRB cell bank) with a human AR expression construct and a luciferase reporter construct under the control of the androgen response element (Rosenmai et al., 2018). ER and AR activities were analyzed mainly according to OECD guidelines (OECD, 2012; OECD, 2016). Micronuclei formation was assessed by flow cytometry in human lymphoblast TK6 cells, using a MicroFlow Kit (Litron Laboratories, USA) (Lundqvist et al., 2019). To control for non-specific effects due to general cytotoxicity, cells were stained with ethidium monoazide (EMA). Endotoxin concentration was assayed in SPE-extracted water samples by Pierce™ Chromogenic Endotoxin Quant Kit (Thermo Fisher Scientific, USA).

When incubated with the cells, the water SPE extracts (controls and water samples) were diluted 100 fold with cell medium to get a final concentration of 1% ethanol. The resulting relative enrichment factor (REF) in the bioassays was 5000 (enrichment factor at SPE) × 0.01 (dilution factor at bioassay) = 50. REF > 1 denotes an enriched water sample and REF < 1 denotes a diluted water sample. The water samples were tested in cell viability and reporter gene assays in 4 replicates at REF = 50 for water samples, which means that the samples were 50 times enriched in the cellular medium, with a few exceptions due to compromised cell viability in AR-EcoScreen cells (DWTPs 2, 3 and 4 inlet, DWTP4 outlet, and SW1) or due to reduced sample volume

(WWTP1 and DWTP5 outlet). Each bioassay was repeated at least two times. Selected water samples were tested for concentration-effect relationships in two fold dilutions.

Positive controls were analyzed in parallel with the water samples. 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) (SUPELCO, Sigma-Aldrich, USA), tert-butylhydroquinone (tBHQ) (Sigma Aldrich, USA) and pirinixic acid (WY-14643) (Sigma-Aldrich, USA) and 17β-estradiol (E2) (Sigma-Aldrich, USA) were used in the AhR, Nrf2, PPARα and ER reporter gene assays, respectively. In the AR reporter gene assay, the positive control for agonist activity was dihydrotestosterone (DHT) (Sigma-Aldrich, USA) and for AR antagonist activity the positive control was hydroxyflutamide (OHF) (Sigma-Aldrich, USA). The positive controls in the reporter gene assays were analyzed in 6–12 concentrations to obtain a standard curve. Concentrations and EC_{10}/$EC_{IR1.5}$-values of the positive controls are reported in Section S1 and Table S2, respectively. Mitomycin C (MMC) (Sigma-Aldrich, USA) was used as a positive control in the micronuclei formation assay and lyophilized *E. coli* (O111: B4) endotoxin standard in the endotoxin assay. In all bioassays, vehicle controls consisting of 1% ethanol, equivalent to the ethanol concentration in the water samples, were included. This ethanol concentration did not affect cell viability. Vehicle controls were tested in 8 replicates.

2.4. Data processing

Cell-based bioactivities of water samples and positive controls were normalized to vehicle controls on each plate. The bioactivity was expressed as fold change compared to vehicle controls, set as 1. Standard curves for positive controls for the nuclear receptor based assays were obtained by fitting data to a four parameter sigmoidal curve with the lower and upper limit constrained to one and the maximum observed response value of the positive control, respectively. For Nrf2, where no maximum effect can be reached, linear regression was used for derivation of the standard curve. The curves obtained were used to calculate effect concentrations, such as EC_{10} and $EC_{IR1.5}$.

The cut-off for cytotoxicity was set at 0.8 compared to a vehicle control set at 1. Cut-off levels for classification of samples as positive in the bioassays were based on the limit of detection (LOD) for AhR, Nrf2 and PPARα calculated as 1 plus 3 times the standard deviation (SD) of the normalized vehicle control values from all plates within the same experiment. Cut-offs for ER and AR activities were set as suggested in the OECD guidelines (OECD, 2012; OECD, 2016). In the ER agonist mode the cut-off for activity is defined as 20% of E2 maximum response. For AR agonist activity the cut-off is defined as 10% of DHT maximum response, and for AR antagonist activity as 30% reduction of the 500 pM DHT control. In the micronuclei assay cut-off for cytotoxicity was set at percentage EMA-positive cells >4-fold compared to the vehicle control.

Results on concentration-response relationships for AhR and AR antagonistic activities were normalized to the maximum experimental response of TCDD and OHF, respectively. Linear regression was performed on the concentration-response data with y-axis intercept fixed at zero and the estimated slope from the regression was used to determine the concentration causing 10% effect (EC_{10}) expressed as REFs, as described by Escher et al. (2014). Data on concentration-response relationships for Nrf2 were not fixed at zero, as the results showed a threshold in response, above which the effect was first observed. Nrf2

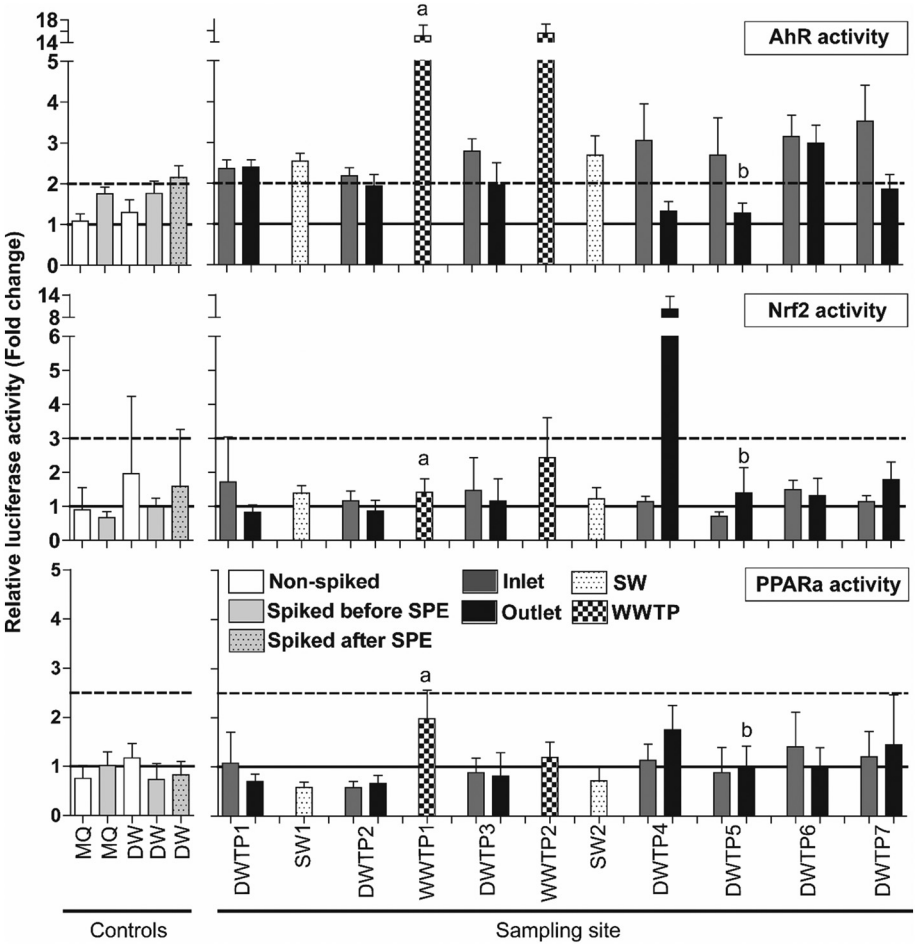

Fig. 2. Bioactivities at 50 REF of water samples in AhR, Nrf2 and PPARα reporter gene assays. Treatment groups ($n = 4$) were normalized to plate vehicle control ($n = 8$) set to 1 (solid line) (mean ± SD). Cut-offs are marked with a dotted line. a) 43 REF and b) 33 REF.

results were fitted to a linear regression model using the highest no observed effect REF and the REFs with effects. The concentration causing a 1.5 fold induction, $EC_{IR1.5}$ was estimated, as described by Escher et al. (2014). Statistical analysis and graphical presentation was performed using GraphPad Prism 5.02. EC_{10} and $EC_{IR1.5}$ values for the positive controls and for the samples were used to calculate bioequivalent concentrations (BEQs) with the following equation (Zhou et al., 2020):

$$BEQ = \frac{(EC_{10}\ or\ EC_{IR1.5})_{positive\ control}}{(EC_{10}\ or\ EC_{IR1.5})_{sample}}$$

3. Results and discussion

3.1. Cell viability

The test water samples did not affect cell viability in HepG2 and VM7Luc4E2 cells, with the threshold set at 80%, while we found a reduced viability in AR-EcoScreen cells after treatment with inlet water samples from DWTPs 2, 3 and 4, outlet water sample from DWTP4, and in surface water from SW1 (Fig. S2). The samples causing decreased cell viability at 50 REF were tested in dilutions to determine the highest non-toxic concentrations, which were found to be 25 REF for inlet water samples from DWTPs 2, 3 and 4, and 12.5 REF for DWTP4 outlet water sample and for SW1. The determined highest non-cytotoxic concentrations were used in the bioassays of AR activity. Control water samples, including the spiked drinking water samples, did not compromise cell viability in any of the cell lines (Fig. S2). Results on cell viability in the resampling studies in DWTP4 are presented in Section 3.6.

3.2. Bioactivity in control water with and without spiking

No activity was observed in non-spiked samples in any of the bioassays (Figs. 2 and 3). Thus, the SPE procedure did not induce any false positive results. Water samples spiked with the mixture of 269 chemicals did not induce Nrf2 or PPARα activity. AhR activity was induced just above the cut-off level in the drinking water control water spiked after SPE, demonstrating a low AhR activity in the spiking mixture.

In the ER reporter gene assay, all spiked samples caused increased activity (Fig. 3). The spiking mixture contained estrone, genistein and bisphenol A, which are known to induce ER activity. The ER activities of these compounds have been compared with 17β-estradiol in luciferase reporter gene assays with MCF-7 cells by Gutendorf and Westendorf (Gutendorf and Westendorf, 2001) and the approximate relative potencies were 1, 0.01, 0.00013 and 0.000025 for 17β-estradiol, estrone, genistein and bisphenol A, respectively. The ER activity in the spiking mixture can be explained by the content of estrone, which would have a concentration of 3.7 nM in the bioassay at 100% recovery in the SPE. Drinking water spiked before SPE had a markedly lower ER activity of approximately 2-fold induction, compared to the 4-fold induction in drinking water spiked after SPE, indicating a loss of ER activity by SPE (Fig. 3). Furthermore, Milli-Q water spiked before SPE also had a 4-fold induction of ER activity (Fig. 3).

The reason for the lower recovery of ER activity in drinking water spiked before SPE compared to spiked after SPE or in Milli-Q water is not known, but it can be speculated that interactions between the SPE sorbent, dissolved organic matter (DOM) in drinking water and estrone may play a role. DOM has been reported to interact with HLB sorbent by

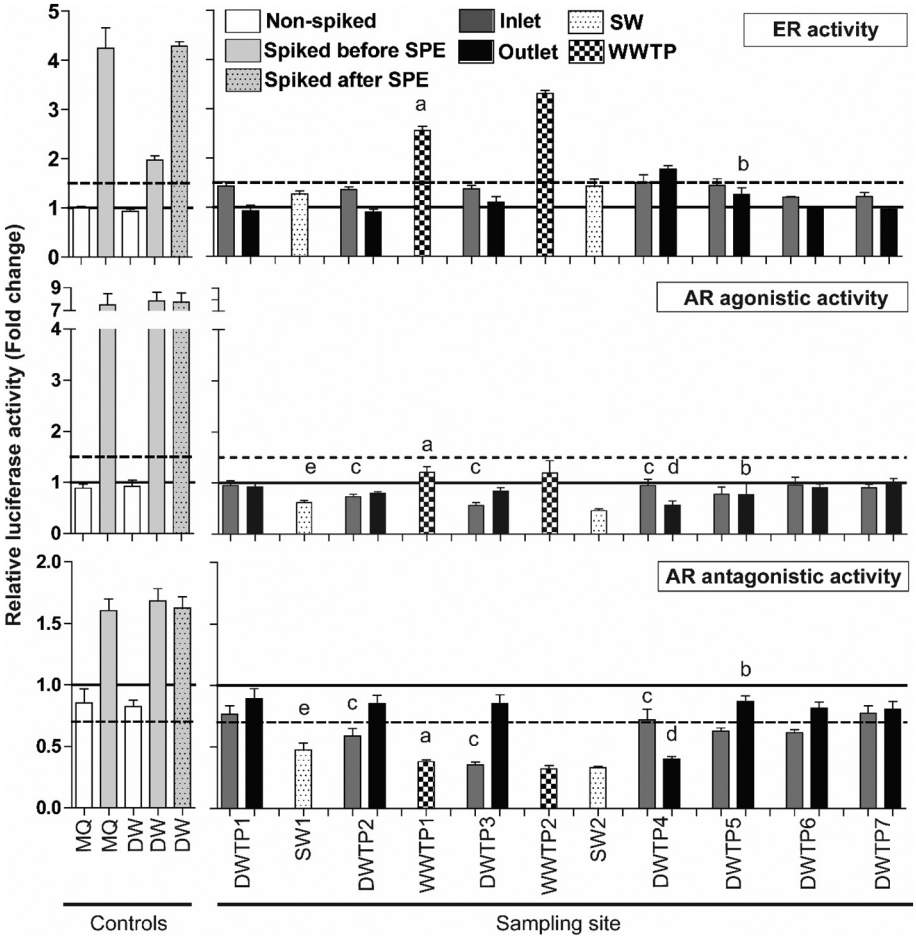

Fig. 3. Bioactivities at 50 REF of water samples in ER, AR agonist, and AR antagonist reporter gene assays. Treatment groups ($n = 3$–4) were normalized to plate vehicle control ($n = 6$–8) set to 1 (solid line) (mean ± SD). Cut-offs are marked with a dotted line. a) 43 REF, b) 33 REF, c) 25 REF, d) 6.25 REF, and e) 12.5 REF.

Raeke et al. (2016), who showed that 60–80% of DOM was retained on the sorbent and not recovered in the extract. DOM is also known to interact with estrogens via binding or sorption and the sorption coefficient varies with the composition of DOM (Ma and Yates, 2018). The interaction with DOM is supposed to occur via the -OH group at C-3 position in the aromatic ring of estrogens (Ma and Yates, 2018). In a study on removal of estrone and estradiol from water by various adsorbents, Zhang and Zhou (2005) showed a drastic decrease in the adsorption of the estrogens to GAC with increasing water concentrations of the DOM compound, humic acid. They explained the decrease by a strong tendency of humic acid to retain organic compounds, such as estrogens, hence reducing the potential of estrogens to be adsorbed by GAC. Furthermore, they found a higher capacity of GAC to remove estrogens from distilled water than from wastewater, which they explained by the presence of chemicals in wastewater, with the capacity to bind to GAC and reduce the effective surface area in GAC for adsorbing estrogens. In our study, DOM in drinking water may 1) bind to estrone and hence reduce the binding of estrone to HLB, and/or 2) bind to the HLB sorbent and occupy binding sites, reducing the binding of estrone. In both alternatives, estrone will pass through the SPE, which will result in a decreased recovery of estrone in drinking water. Thus, spiking before SPE may lead to a reduced recovery of estrone. The interaction would not occur in Milli-Q water, which does not contain DOM. In conclusion, depending on which estrogenic compounds are present and if they are processed by SPE in a similar way as estrone, as well as the content and composition of DOM, the ER activity in the test water samples may be underestimated. In the present study the SPE recovery of estrone in a sample of tap water was approximately 50%.

In the AR reporter gene assay, all spiked samples showed increased activity in both the antagonist and agonist mode of the assay (Fig. 3). The spiked samples had AR activities in the same range irrespective of water type or when spiking was performed, but with considerably higher responses in the AR agonist mode than the AR antagonist mode (Fig. 3). The spiking mixture contained androstanolone, also known as dihydrotestosterone (DHT). The AR activity in the spiking mixture can be explained by the content of DHT, which would have a concentration of 3.4 nM in the bioassay at 100% recovery in the SPE. There was no difference in AR activity in samples spiked before and after SPE or when added to Milli-Q water. DHT does not have an -OH group at C-3 position in the aromatic ring and would not be affected by DOM in a similar way as estrone. The AR reporter assay can also be activated by the glucocorticoid receptor and the increased activity in the spiked samples could be induced by synthetic glucocorticoids in the spiking mixture, e.g. budesonide. The spiking mixture also contained bicalutamide, which has antiandrogenic properties and would inhibit part of the DHT agonist activity.

3.3. Bioactivity in raw and treated water samples from DWTPs – efficacy of treatment

All DWTP inlet water samples induced AhR activity in the range 2–4 fold of the vehicle control (Fig. 2). Outlet water from four of the DWTPs (DWTPs 3, 4, 5 and 7) had reduced AhR activity compared to the inlet samples, while the remaining three DWTPs had similar activity in inlet and outlet water. The cut-off level for AhR activity of 2-fold the vehicle control corresponds to an AhR activity of 136 pM TCDD as determined from the standard curve (Fig. S3). At REF 50, a concentration of TCDD in the water samples at or above 2.7 pM (0.9 ng/L) would be detected as positive by our bioassay. Our results indicate that GAC (DWTPs 3, 5 and 7) and artificial infiltration (DWTP4) were effective in reducing AhR activity. Presumably, GAC and infiltration soil particles act as sorbents of AhR-inducing chemicals and thereby reduce the activity in the water. However, DWTP6 also had GAC as a treatment step, but did not show reduced AhR activity in the outlet water.

Nrf2 activity was drastically increased to 10-fold compared to vehicle control in outlet water of DWTP4, while no other inlet or outlet water samples exhibited activity above the cut-off level (Fig. 2). As the inlet water sample did not have activity above the cut-off level, contamination of water by Nrf2-inducing compound(s) occurred during the water treatment process. DWTP4 was the only DWTP using artificial infiltration. Nrf2 activity is known to be induced by disinfection by-products (Hebert et al., 2018; Neale et al., 2012). However, at DWTP4 no chlorination and only mild UV disinfection was included in the treatment, and disinfection by-products are not likely to be formed. Hence, Nrf2 activity was most likely induced by other contaminants of anthropogenic or natural origin, possibly released from the infiltration soil environment (further discussed in Section 3.7).

No PPARα activity above the cut-off was observed in any of the water samples (Fig. 2). The absence of PPARα activity indicates that PPARα-inducing compounds were not present in the water in high enough concentrations to be detected by the bioassay, but it cannot be excluded that higher concentrations or a more sensitive bioassay could have revealed PPARα activity.

No ER activities above the cut-off level were detected in the inlet samples (Fig. 3). The ER activities in the outlet samples were lower than in the corresponding inlet samples, except in DWTP4, where there was a slight increase in the outlet water to just above the cut-off level. Resampling of outlet water from DWTP4, did not reveal any ER activity above the cut-off level (see Section 3.6). It should however be noted, that the control samples in our study indicated a recovery after SPE of approximately 50% of estrogenic activity in tap water (as discussed in Section 3.2). Thus, the ER activities may be underestimated, especially in the inlet water with higher levels of DOM. Total dissolved organic carbon (DOC) levels were determined in the studied water samples from the seven DWTPs and varied from 4.16 to 5.54 mg/L in raw water and from 1.43 to 2.79 mg/L in treated water (Troger et al., 2020). Hence, the removal of ER activity in the DWTPs may be more efficient than indicated by the present results. However, no firm conclusions could be drawn on the treatment efficiency in removal of ER activity, as there was no activity above cut-off in the raw water.

The bioassay for ER is highly sensitive and the cut-off level of 1.5 corresponds to 2.8 pM estradiol (0.76 ng/L) as estimated from the standard curve (Fig. S3). At REF 50, a concentration of estradiol in the water samples at or above 0.056 pM (0.012 ng/L) would be detected as positive by our bioassay. The cut-off level of 0.012 ng/L can be compared with the benchmark value of 1 ng/L of β-estradiol in drinking water for assessing occurrence and treatment efficacy, recommended by WHO (WHO, 2017) in the background to the recent revision of the EU drinking water directive (EC, 2015). In the just endorsed EU drinking water directive, β-estradiol is not included with a parametric value, but in the watch list, to be set up by the Commission (EU, 2020).

No AR agonist activity was detected in any of the inlet or outlet water samples (Fig. 3). Due to cytotoxicity in four of the DWTP samples in the AR-EcoScreen cell line, namely DWTPs 2, 3, and 4 inlet, and DWTP4 outlet, the AR activities were tested in diluted water samples to get non-cytotoxic conditions in the bioassay. Water samples from DWTPs 2, 3, and 4 inlet were tested at REFs 25, and DWTP4 outlet at REF 6.25. Cytotoxicity in the AR-EcoScreen cell line in inlet water samples was efficiently reduced in the outlet samples at DWTP 2 and 3, which had UV irradiation and chlorination (Fig. S2), while the cytotoxicity in DWTP4, which had artificial infiltration and UV irradiation, was increased in the outlet water compared to the inlet.

AR antagonistic activity above the cut-off level, identified as ≥30% reduction of the DHT-induced activity, was detected in four of the DWTP inlet samples (DWTPs 2, 3, 5 and 6) at non-cytotoxic concentrations (Fig. 3). The AR antagonistic activity was eliminated by the treatment in all four DWTPs and no AR antagonistic activity was detected in the outlet water samples from the respective DWTPs. All four DWTPs had UV irradiation and chlorination and except for DWTP 2, they also had GAC. In contrast to these four DWTPs, DWTP4 had AR antagonistic activity in the outlet, but not in the inlet water, indicating that a contamination occurred within the DWTP. It should be noted that due to

cytotoxicity, more diluted water samples were tested from the inlet of DWTPs 2, 3 and 4 (REFs 25), and the outlet of DWTP4 (REF 6.25). Hence, the AR antagonistic effects could be expected to be even more pronounced if tested at REF 50.

Purification effects in a drinking water source treated by a constructed wetland were investigated by bioassays and chemical analysis of organic micropollutants (Xu et al., 2019). They found reduced cytotoxicity, levels of reactive oxygen species and antiandrogen activities after treatment by the constructed wetland, while total concentrations of organic pollutants increased. The authors speculated that the increases were due to release of pollutants from artificial media and sediments.

Reduction of bioactivity in DWTPs has been demonstrated in other studies. Efficient removal of ER activity in DWTPs using chlorination or ozone for disinfection, has been reported (Conley et al., 2017; Kakaley et al., 2020). UV combined with hydrogen peroxide treatment was reported to remove 80% of estrogen compounds and activity in wastewater (Cedat et al., 2016). Leusch et al. (2018) did not detect any endocrine activity above detection limit in six samples of drinking water samples, from various sources in six countries and with different treatment techniques. ER, TCDD-like activities and micronuclei formation were detected in source water samples in eastern China, while the bioactivities were removed by drinking water treatment, except for ER activity in one plant, which had only sand filtration and chlorination (Shi et al., 2018). However, detection of bioactivities is dependent both on bioassay used and on the enrichment factor, which is not always reported.

In a study on efficacy of drinking water treatment techniques in pilot plants, Brunner et al. (2020) spiked influent water with 31 industrial chemicals, pharmaceuticals, pesticides and artificial sweeteners in concentrations exceeding 100 times the limit of quantification for most chemicals. Activities of anti-AR, oxidative stress and mutagenicity were induced. Whether it was induced by the spiked chemicals or by compounds already present in the influent water is not known. Ultrafiltration followed by reverse osmosis, and UV/H_2O_2 oxidation followed by GAC removed the activities. Treatment with advanced oxidation by O_3/H_2O_2 plus UV/H_2O_2 did not remove activities of oxidative stress and mutagenicity.

3.4. Bioactivity in surface water and WWTP outlets – impact on DWTP inlet water

Water samples from the two WWTP outlets exhibited drastically increased AhR activity, to the level of the maximum response of the positive control TCDD (Fig. 2; Fig. S3). The activity was reduced in the DWTP3 inlet water (30 km downstream WWTP1) and in the surface water, SW2 (8 km downstream WWTP2) to levels similar to DWTPs upstream of the WWTPs. The reduced AhR activity can be explained by dilution, degradation/deactivation or sequestration to sediment and/or particulate matter of the AhR-inducing compounds from the WWTPs.

The two WWTP outlet water samples induced ER activity to approximately 3 fold the activity of the vehicle control, while the downstream DWTP inlet water (DWTP3) and downstream surface water (SW2) samples had similar activities as the samples from the other DWTPs (Fig. 3).

AR antagonistic activities were induced by the two WWTP outlet and the two surface water samples (Fig. 3). The samples collected downstream from these affected sites also had AR antagonistic activities (DWTPs 2 and 3 inlet, and SW2), indicating that either the compounds causing the antiandrogenic activities were not diluted or degraded efficiently in the water or there were other sources of contamination in the downstream water. The antiandrogenic activity in the outlet water of DWTP4 may be explained by long-term accumulation in the infiltration soil of antiandrogenic compounds originating from WWTP2 and SW2. The compounds may be continuously released from the infiltration soil and contaminate the outlet water.

No activities of Nrf2, PPARα or AR agonism were induced by the WWTP or surface water samples (Figs. 2 and 3).

Due to increased demand of drinking water there is a need in many parts of the world for direct water reuse from WWTPs, and bioassays have been proposed as a useful tool to evaluate the safety of reclaimed water (Pal et al., 2014; Xu et al., 2019). Leusch et al. (2014) studied the treatment efficiency at nine Australian water reclamation plants with high levels of bioactive compounds in the source water. Ultrafiltration or dissolved air flotation/filtration had marginal effects on bioactivity, while reverse osmosis greatly reduced bioactivity. Bioassays were used by Escher et al. (2014) to assess two water reclamation plants in Australia. Reverse osmosis and ozone combined with GAC filtration were both efficient in removing bioactivity, while chlorination and chloramination increased the activity of oxidative stress and induction of xenobiotic metabolism, which according to the authors is consistent with formation of disinfectant byproducts. Jia et al. (2015) used bioassays to evaluate treatment efficiency of WWTP effluents and found that ozone significantly removed oxidative stress, UV removed glucocorticoid activity, chlorination partly removed genotoxicity and infiltration using soil-aquifer treatment efficiently removed all bioactivity except genotoxicity. None of the studies detected any AR antagonistic activities in drinking water samples.

3.5. Effect concentrations of water samples

Water samples showing activity above the cut-off in the AhR, AR antagonist and Nrf2 reporter gene assays were subject to concentration-response assessment, which formed the basis for calculation of EC_{10} values for AhR and AR antagonistic effects and $EC_{IR1.5}$ for Nrf2 (Figs. S4, S5 and S6 and Table 2). Four water samples had EC10 values between REF 1 and 5, namely WWTP1 and WWTP2 in the AhR bioassay and DWTP4 outlet and SW1 in the AR antagonist bioassay. Five water samples had EC10 values between REF 5–10, all in the AR antagonist reporter gene assay, namely DWTP2, DWTP3 and DWTP4 inlets, SW2, and WWTP1. Three samples had EC10 values between REF 10–30 in the AR antagonist reporter gene assay, one sample in the Nrf2 and one sample in the AhR reporter gene assays. Water samples with EC10 values ranging from REF 30–50 and REF >50 were all exhibiting activities in the AhR reporter gene assay (Table 2).

It should be noted that due to reduced sample volumes two water samples could not be tested at REF 50 and thus had lower REFs than 50: DWTP5 outlet with REF 43 and WWTP1 with REF 33. This means that we would not detect activities occurring at higher concentrations than these REFs. However, when these samples caused bioactivity, they were diluted and REFs for EC10 could be estimated as for the other samples.

3.6. Resampling of water – activities of Nrf2 and AR antagonism, and micronuclei formation

To investigate if the Nrf2 and AR antagonistic activities in outlet water from DWTP4 were an occasional effect, we resampled the inlet (river) water and outlet water after 8 months, with three samples of each. In addition, ER activity was assessed and no activity was detected in any of the samples. The inlet water did not exhibit Nrf2 or AR antagonistic activities, while two of the three samples of outlet water (outlet 1 and 2) at REF 50 had a 2.2- and 12.8-fold increase in Nrf2 activity, respectively, compared to vehicle control. The same samples were cytotoxic at REF 12.5 in the AR-EcoScreen cell line, used for AR activity analysis. No cytotoxicity was detected in inlet water or in the third sample of outlet water. AR antagonistic activity, corresponding to 62% of the DHT-induced control, was demonstrated in outlet sample 2 at REF 6.25. Outlet sample 3 had AR antagonistic activities at REFs 50, 25 and 12.5, corresponding to 57, 67 and 69% of the DHT-induced control. Thus, the highest Nrf2 and anti-AR activities were found in the same outlet

Table 2
Heatmap of effect concentrations (EC10 and for Nrf2 $EC_{IR1.5}$) in units of REF (relative enrichment factor) in bold and BEQs as TCDDEQ, OHFEQ and tBHQEQ in parenthesis for water samples tested in the AhR, AR antagonist, and Nrf2 reporter gene assays for concentration-effect relationships. Activities below the cut-off level in the bioassays are marked with (-). No concentration-effect assessments were performed on PPARα, AR agonistic and ER activities due to activities below or at the cut-off level in drinking water samples.

Sample ID		Activity AhR	AR anta	Nrf2	
DWTP1	Inlet		-	-	
	Outlet		-	-	<Cut-off
SW1			**3.1** (1.8 nM)	-	**REF>50**
DWTP2	Inlet		**8.8** (0.7 nM)	-	**50>REF>30**
	Outlet	-	-	-	**30>REF>10**
WWTP1		**3.5** (46 pM)	**8.2** (0.7 nM)	-	**10>REF>5**
DWTP3	Inlet	**31** (5.2 pM)	**8.6** (0.7 nM)	-	5>REF>1
	Outlet	-	-	-	
WWTP2		**2.8** (57 pM)	**11** (0.5 nM)	-	
SW2		**36** (4.5 pM)	**5.9** 1.0 nM)	-	
DWTP4	Inlet	**27** (6.0 pM)	**5.7** (1.0 nM)	-	
	Outlet	-	**2.0** (2.9 nM)	**26** (0.1 μM)	
DWTP5	Inlet		**13** (0.4 nM)	-	
	Outlet	-	-	-	
DWTP6	Inlet	**34** (4.8 pM)	**14** (0.4 nM)	-	
	Outlet		-	-	
DWTP7	Inlet	**45** (6.7 pM)	-	-	
	Outlet	-	-	-	

sample, and the other two outlet samples had lower effects: either elevated Nrf2 or anti-AR activity.

Induced Nrf2 activity indicates oxidative stress, which may be associated with genotoxicity. Hence, analysis of genotoxicity by a micronucleus assay, was performed in a third sampling of water from DWTP4. The sampling included inlet water and domestic water from the distribution network 2.5 km from the DWTP, and three samples of outlet water, collected at three consecutive days. Nrf2 activity and micronuclei formation were analyzed at REFs 50, 25 and 12.5 in the three outlet water samples. Results on Nrf2 activity, percent micronucleus events and percent EMA-positive cells are compiled in Fig. 4. At REF 50, outlet water samples 1 and 2 had induced Nrf2 activity to 5- and 6-fold the vehicle control, respectively, while outlet sample 3 had no Nrf2 activity. No Nrf2 activity was induced in the diluted samples of REFs 25 and 12.5. Micronuclei formation was increased in outlet water samples 1 and 2, however at EMA-positive values far exceeding the cut-off for cytotoxicity of >4-fold of the vehicle control. Two of the dilutions, outlet water sample 1 at REF 25 and outlet water sample 2 at REF 12.5, had EMA-positive values approximately at 4-fold the vehicle control, and slightly elevated micronuclei, 2.1 and 1.7 times the vehicle control, respectively. However, the elevation was not statistically different from

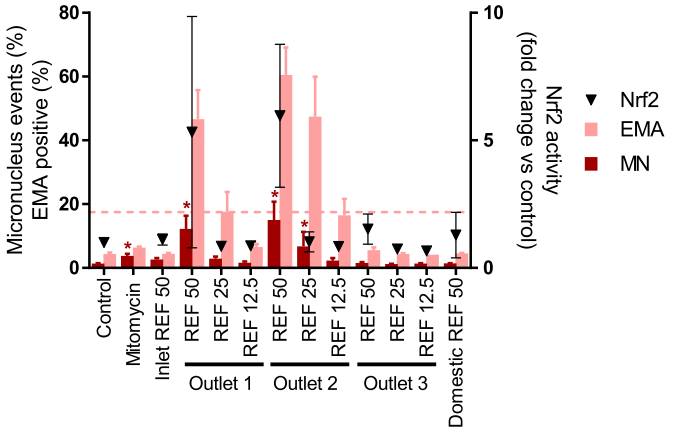

Fig. 4. Micronuclei formation, cytotoxicity (EMA-positive cells) and Nrf2 activity in water samples from DWTP4: inlet (river), outlet (three samples taken on consecutive days) and domestic water (sampled 2.5 km from the DWTP). Mitomycin is the positive control for micronuclei formation. Dotted line denotes cut-off for cytotoxicity based on EMA. Mean ± SD; $n = 4$ for samples and 8 for controls; *statistically significant difference compared to control ($P < 0.05$).

the vehicle control. The third outlet sample, inlet water and water from the distribution network induced neither Nrf2 activity, nor micronuclei formation. The results from the two resampling occasions demonstrated that Nrf2 and AR antagonistic activities were still present in the outlet water from DWTP4, although it seemed to fluctuate as it was not detected in all samples. Genotoxic activity was not detected at non-cytotoxic concentrations of the water samples.

In an effort to localize the source of the compound(s) inducing Nrf2 and AR antagonistic activities, water was sampled from the 13 abstraction wells, which collect water from nine infiltration basins. In addition, inlet and outlet water from DWTP4 was sampled. No cytotoxicity in HepG2 cells, used in the Nrf2 bioassay, was detected in any of the samples. Nrf2 activity was analyzed at REF 50 and 25 (Fig. 5). No Nrf2 activity was detected in water from wells 1, 6, 7, 8 and 15. At REF 50, eight of the 13 abstraction wells had Nrf2 activities above the cut-off level, which in this study was 2.2-fold above the vehicle control, namely wells 2, 3, 9, 10, 11, 12, 13 and 14, in addition to the samples from inlet and outlet water. Nrf2 activities were also detected at REF 25 in wells 3, 12, 13 and 14. Water samples from two wells with high Nrf2 activity (wells 2 and 14 at REFs 50, 25 and 6.25) and from two with no

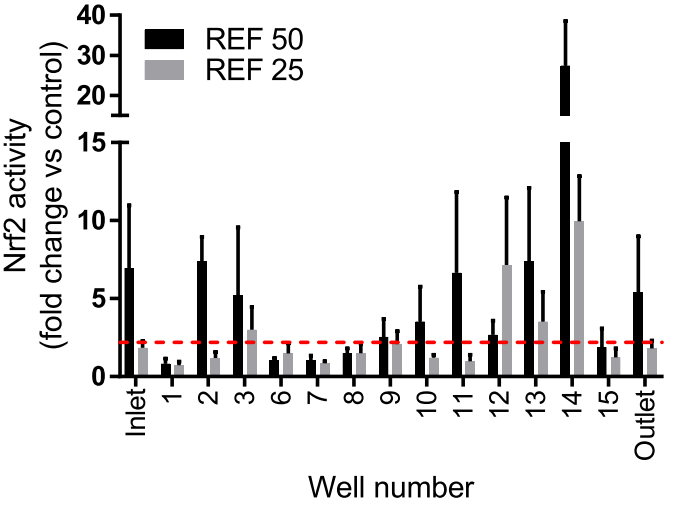

Fig. 5. Nrf2 activity in samples at REFs 50 and 25 from abstraction wells at DWTP4, and from inlet (river) and outlet water. Values normalized to vehicle control, set to 1. Cut-off level is marked with a dotted line. Mean ± SD; $n = 4$ for samples and 8 for controls.

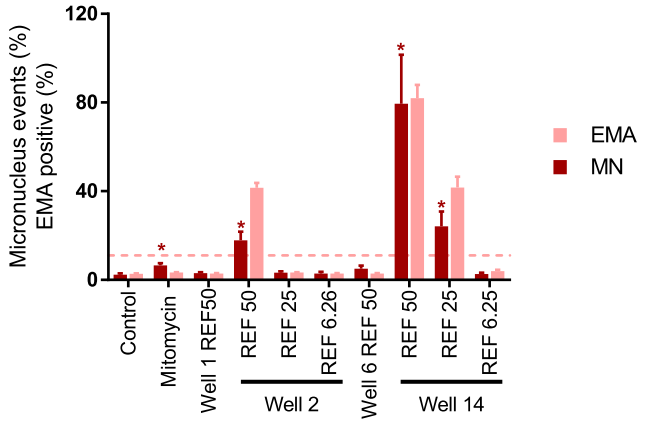

Fig. 6. Micronuclei formation and cytotoxicity (EMA-positive cells) in water samples from two abstraction wells with no Nrf2 activity (wells 1 and 6) and two wells with high Nrf2 activity (wells 2 and 14) at REFs 50, 25 and 6.25 from DWTP4. For Nrf2 activities see Fig. 5. Dotted line denotes cut-off for cytotoxicity based on EMA. Mean ± SD; n = 4 for samples and 8 for controls; * Statistically significant difference compared to control ($P < 0.05$).

Nrf2 activity (well 1 and well 6 at REF 50) were analyzed for micronuclei formation. The samples with high Nrf2 activities also had high values of EMA-positive cells (wells 2 and 14), far above 4-fold of the vehicle control and were thus cytotoxic at the concentrations inducing micronuclei (Fig. 6).

AR antagonistic activity was tested in samples from the 13 abstraction wells and from the inlet and outlet of DWTP4 after cytotoxicity had been determined in AR-EcoScreen cells. At REF 50 only two samples were non-cytotoxic, namely from wells 6 and 7. Thus, all samples were tested for cytotoxicity at REFs 25, 12.5, 6.25 and 3.12 (Fig. 7A). The highest non-cytotoxic concentration was REF 6.25 for wells 10 and 11 and inlet water, and REF 3.12 for wells, 2, 3, 8, 9, 13, 15 and outlet water, while wells 12 and 14 were cytotoxic also at REF 3.12. AR antagonistic activity was not detected at any non-cytotoxic concentrations (Fig. 7B).

The results from sampling of abstraction wells showed a high variation in bioactivities between wells. Two wells did not induce activity of Nrf2 or AR antagonism or cytotoxicity in AR-EcoScreen cells (wells 6 and 7). No AR antagonistic activities were detected in the other wells at non-cytotoxic concentrations. Four wells had Nrf2 activity at REF 25 and cytotoxicity at REFs 3.12 or 6.25 (wells 3, 12, 13 and 14). Thus, the Nrf2 and cytotoxicity in AR-EcoScreen cells seemed to be associated, which can be explained by either the same compound(s) causing the two effects or by co-occurrence of compounds causing either Nrf2 or cytotoxic effects. The fluctuating bioactivity in outlet water may depend on which wells were used for the outlet water at different time-points.

Endotoxin levels, as a possible cause of Nrf2 induction, were determined in water samples from the 13 abstraction wells at DWTP4 (Fig. S1) and in inlet and outlet water. The concentration in wells varied between 0.1 and 0.4 EU/mL. Endotoxin levels in inlet and outlet water were 0.4 EU/mL, which is low compared to literature data (0.5–205 EU/mL) (Zhang et al., 2019b). The low endotoxin levels may partly be explained by a low recovery in the SPE extraction (Neale et al., 2018). There was no correlation between Nrf2 activity and endotoxin levels, as tested by Pearson correlation coefficient (−0.159) (Fig. S7).

Finally, inlet and outlet water was sampled after the drinking water treatment plant had been replaced by a new plant using a membrane ultrafilter in front of a GAC filter. In addition, three of the abstraction wells from the old treatment plant (wells 7, 11 and 12) were sampled (Fig. S1). None of the samples induced any activity of Nrf2. Cytotoxicity,

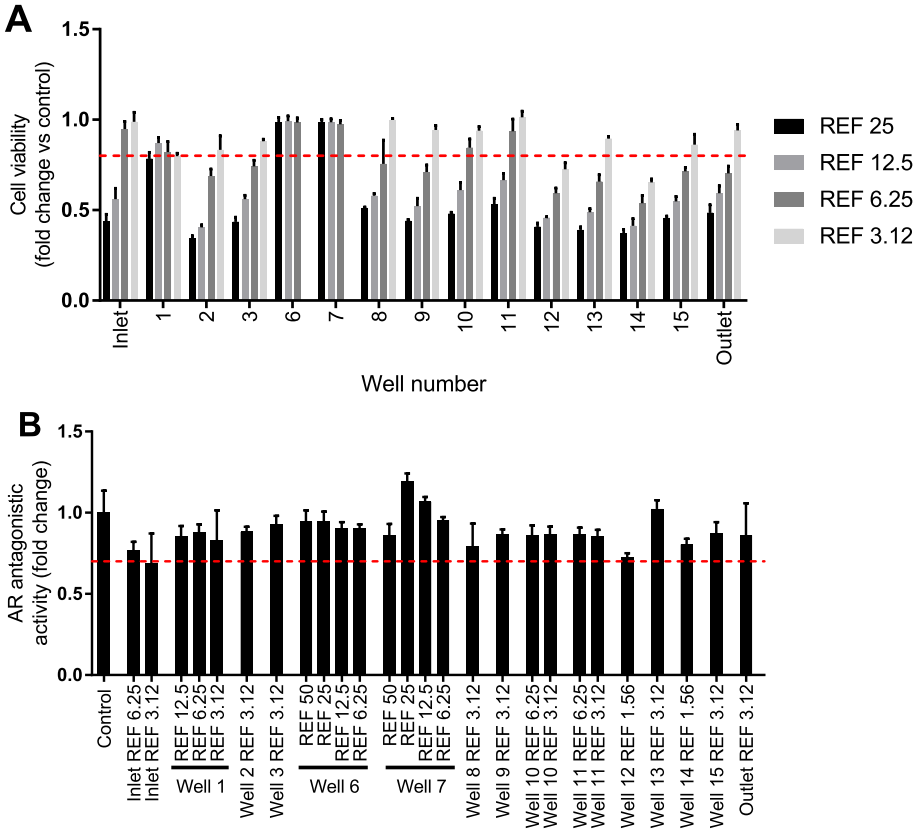

Fig. 7. A) Cell viability and B) AR antagonistic activity, in AR-EcoScreen cells at different REFs of water samples from abstraction wells, inlet (river) and outlet water at DWTP4. Results on cell viability were normalized to vehicle control, set to 1. Cut-off for compromised cell viability was set at 0.8 (dotted line). Result on AR antagonistic activity were normalized to the control induced with 500 pM DHT. Cut-off for AR antagonistic activity was set at 30% reduction of the DHT-induction (dotted line). Mean ± SD; n = 4 for samples and 8 for controls.

corresponding to 71% of the control value, in AR-EcoScreen cells was detected in water from one of the abstraction wells (well 12) at REF 50 and the other two wells had AR antagonistic activity at REF 50 (corresponding to 56% of the DHT induced control). No cytotoxicity or AR antagonistic activity was detected in the inlet or outlet water from the new DWTP.

3.7. Chemicals driving the bioactivity

Parallel to the monitoring of bioactivity, chemical analyses were performed on the same water samples from the seven DWTPs. A complete presentation of the chemical analysis has been reported elsewhere (Troger et al., 2020). Basically, 27 of 163 analyzed micropollutants, were detected, in individual concentrations ranging from 0.01 to 54 ng/L. The analyzed 163 chemicals were included among the 269 chemicals in the spiking mixture, which showed activity of ER and AR in the present study, presumably originating from the natural hormones estrone and dihydrotestosterone in the spiking mixture, as discussed in Section 3.2. Natural hormones were not included in the 163 analyzed micropollutants. The spiking mixture contained pesticides, pharmaceuticals, perfluoroalkyl substances, natural hormones and personal care products, with a final individual concentration of 1 μg/L in the bioassays. The spiking mixture exhibited AhR activity just above the cut-off level when spiked after SPE. The highest total concentration of chemicals determined in any water sample was 117 ng/L, which corresponds to subnanomolar concentration, and far too low to induce any AhR activity. No activity of Nrf2 or PPARα was detected. Hence, it is not likely, that any of the bioactivity detected in the water samples, i.e. AhR, Nrf2, ER or anti-AR, was induced by the low concentrations of the 27 detected micropollutants.

Integrating chemical analysis and bioanalysis is often recommended. Usually it involves target analysis of hundreds of organic micropollutants, selected due to occurrence in water and/or toxicity. However, it has repeatedly been demonstrated, as in the present study, that the targeted chemicals do not contribute, or only marginally, to effects detected in AhR and Nrf2 bioassays (Blackwell et al., 2019; Escher et al., 2013; Neale et al., 2017a; Neale et al., 2017b; Tang et al., 2014; Tousova et al., 2017). In contrast, the main ER activity can usually by explained by the presence of natural estrogens (Conley et al., 2017; Hashmi et al., 2018; Valitalo et al., 2017).

By using effect-directed analysis (EDA) with fractionation of samples and non-target chemical analysis, it has been possible in a few cases to identify chemicals responsible for bioactivities. Thus, Hashmi et al. identified the natural estrogens responsible for the ER activity and the natural androgens responsible for the AR activity in water samples downstream of a WWTP, while the oxidative stress activity was found to be a cumulative effect of the mixture of many compounds present in the sample (Hashmi et al., 2018). Muschket et al. (2018) identified a highly potent coumarin derivative, 4-methyl-7-diethylaminocoumarin, as the main antiandrogenic compound in a river water sample. Muz et al. (2017) used EDA to identify the mutagenic compounds norharman and β-carboline alkaloids in River Rhine, a main drinking water source. However, these compounds could only explain a fraction of the measured mutagenicity in the water samples. Zwart et al. (2020) identified the mutagen 1,2,3-benzotriazole and the androgen androstenedione in fractionated wastewater and surface water samples by EDA. Using data on quantitative structure activity relationships and in silico predictions together with bioassay data on mutagenicity, Shao et al. (2019) could explain almost 50% of the observed mutagenicity by presence of 18 chemicals in surface water samples.

The chemicals responsible for the detected AhR, Nrf2, ER and antiandrogenic activities in our study are not known. AhR activities were most prominent in the WWTP downstream samples and are likely caused by additive effects of a vast number of micropollutants. Examples of known inducers of AhR are polyhalogenated organic compounds (*e.g.* 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD), polyaromatic hydrocarbons (PAHs), some pesticides, numerous polyphenols present in fruits and vegetables, and endogenous tryptophan derivatives (*e.g.* 6-formylindolo[3,2-*b*]carbazole, FICZ). The more hydrophobic compounds would not be extracted by the SPE and not contribute to the bioactivity in the present study. ER activities were induced in the WWTP downstream samples and are likely due to presence of natural estrogens or synthetic ethinyl estradiol from contraceptives.

Nrf2 and AR antagonistic activities as well as cytotoxicity in AR-EcoScreen cells were induced in samples of outlet drinking water and it is therefore of high importance for public health to localize the source of contamination to prevent such exposure. We were able to localize the source to certain abstraction wells, located downstream from the artificial infiltration. It can be speculated that either contaminating compounds have been accumulated in the infiltration soil from earlier high contamination in the raw water from the river, or toxic natural compounds are continuously formed from e.g. bacteria and fungi in water and soil during the infiltration process. Why the activities were not present in all abstraction wells is not known, but could be due to more or less firm binding to soil particles, local occurrence or formation of the contaminants in infiltration soil and abstraction wells. We analyzed endotoxin as a cause of bioactivity, but found no association to Nrf2 activity.

Nrf2 activation can be induced by both environmental toxicants, such as pesticides and toxic metals (Zheng et al., 2020) and by medical drugs and natural compounds, such as curcumin, sulphoraphane, resveratrol and genistein (Robledinos-Anton et al., 2019). Metals are not extracted by the SPE and would not contribute to any bioactivity in the present study. AR antagonistic activity is known to be induced by various chemicals, which may be present in water, such as prostate cancer drugs, pesticides, parabens and bisphenol A (Kortenkamp, 2020). Xu et al. (2019) predicted anti-AR activity in samples from a drinking water source, and found that 3–16% of the observed anti-AR activity could be explained by the chemically detected micropollutants, of which phthalates and PAHs were the main contributors to the predicted activity. Natural compounds may be a more likely source of activity. In fact, high anti-androgenic potential of mycotoxins have been demonstrated (Demaegdt et al., 2016). Of 13 tested mycotoxins, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and α-zearalenone had similar anti-androgenic potencies as flutamide, when tested in a reporter gene assay. These mycotoxins are produced by certain *Penicillium* and *Aspergillus* species, which have frequently been detected in water (Al-Gabr et al., 2014; Hageskal et al., 2009). Furthermore, oxidative stress has been proposed to mediate the toxicity of the mycotoxins fumonisins (Wang et al., 2016) and deoxynivalenol (Mishra et al., 2014).

3.8. Bioassays as a suitable method for monitoring of presence of emerging pollutants and efficacy of drinking water treatment

It is increasingly recognized that targeted chemical monitoring cannot detect the tens of thousands of micropollutants, which may occur in drinking water. Furthermore, the detected micropollutants contribute usually only to a few percent of observed biological activity in water samples. Parametric values for individual contaminants in drinking water are used for regulatory and control purposes, but covers only around ten single or groups of organic compounds, and does not give any indication of mixture effects. In the present study, analyses of water from three of the abstraction wells (6, 7 and 8) were performed according to the drinking water regulation (Table S3). It can be noted that water from well 8, which had a higher cytotoxicity, but no Nrf2 activity, had a weak odour and higher turbidity (1.2 FNU - Formazin Nephelometric Units) than water from wells 6 and 7 (0.5 FNU). Turbidity in water is known to be caused by soil particles, microbiological contamination or chemical precipitates. The results from the analyses of regulated chemical parameters did not indicate any hazard present in the water, and the drinking water was therefore recognized as safe, which highlights the need for bioanalytical methods to obtain a better

understanding of the presence of unknown hazardous compounds in the water.

Interestingly, the chemical analysis of the water samples in the present study showed that removal efficiency of detected chemicals was highest in DWTP4, with an average removal of 65% of the concentration of the 27 detected micropollutants (Troger et al., 2020). In addition, DWTP4 also had the lowest total concentration of detected micropollutants of the seven DWTPs (11.1 ng/L). This is in contrast to the results from bioanalysis, which showed that DWTP4 was the only DWTP with induced bioactivities of Nrf2 and AR antagonistic activity in the outlet water. The present results further support the advantage of bioanalysis for assessment of water quality and treatment efficiency.

Effect-based monitoring provides an understanding of the total toxic potential of the water and detects hazards from both known and unknown contaminants. The just adopted revision of the European Union Drinking Water Directive (EU, 2020) includes a risk-based approach with three components of risk assessment and management: 1. catchment area of the abstraction point, 2. supply system, including treatment, storage and distribution, and 3. domestic distribution system. Bioassays seem to be a valuable tool in quality assessment of drinking water according to the risk-based approach, as has been proposed by Dingemans et al. (2019).

3.9. Conclusions

Raw (river) water exhibited AhR activity, which was removed by DWTPs using GAC or artificial infiltration as a treatment, and AR antagonistic activity, which was removed by all DWTPs, except the one using artificial infiltration, where in contrast, the bioactivity was increased. Artificial infiltration as a source of Nrf2 and AR antagonistic activities were revealed in one DWTP. There was no genotoxic activity at non-cytotoxic concentrations. Nrf2 activity was induced by water from eight of the 13 abstraction wells, collecting water from the artificial infiltration. The responsible chemicals were not identified but may be old contaminants accumulated in the infiltration soil and continuously released into the water, or natural bioactive compounds (toxins) formed by microorganisms present in the infiltration environment. The bioactivities were no longer present in the outlet water after the DWTP had been replaced by a new plant, using membrane ultrafiltration and GAC. High activities of AhR, ER and anti-AR were present in WWTP outlets along the river and may have contaminated the inlet water in one DWTP with anti-AR activity. The recovery of estrone in a control sample of tap water extracted by SPE was approximately 50%, possibly due to interaction between the SPE sorbent, DOM and estrone. Thus, the ER activity may have been underestimated, especially in the inlet samples with high DOM levels.

The benefits of using bioanalysis in monitoring of drinking water quality are highlighted by the present results. As far as we know, this is the first demonstration of artificial infiltration as a cause of contamination of drinking water. Neither target chemical analysis, nor chemical analysis according to the drinking water regulation, demonstrated any presence of hazards in drinking water samples, which were demonstrated by bioassays to have prominent effects on oxidative stress, AR antagonistic activity and cytotoxicity. Thus, bioanalysis is a useful tool for detection of unknown hazards in drinking water and for assessment of drinking water treatments.

CRediT authorship contribution statement

Agneta Oskarsson: Conceptualization, Data curation, Writing - original draft. **Anna Kjerstine Rosenmai:** Conceptualization, Data curation, Writing - original draft. **Geeta Mandava:** Investigation. **Anders Johannisson:** Investigation, Data curation. **Andrew Holmes:** Data curation. **Rikard Tröger:** Investigation. **Johan Lundqvist:** Conceptualization, Data curation, Writing - original draft.

Declaration of competing interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144001.

References

Adeel, M., Song, X.M., Wang, Y.Y., Francis, D., Yang, Y.S., 2017. Environmental impact of estrogens on human, animal and plant life: a critical review. Environ. Int. 99, 107–119.
Al-Gabr HM, Zheng TL, Yu X. Fungi Contamination of Drinking Water. Reviews of Environmental Contamination and Toxicology, Vol 228 2014; 228: 121–139.
Altenburger, R., Brack, W., Burgess, R.M., Busch, W., Escher, B.I., Focks, A., et al., 2019. Future water quality monitoring: improving the balance between exposure and toxicity assessments of real-world pollutant mixtures. Environ. Sci. Eur. 31.
Blackwell, B.R., Ankley, G.T., Bradley, P.M., Houck, K.A., Makarov, S.S., Medvedev, A.V., et al., 2019. Potential toxicity of complex mixtures in surface waters from a nationwide survey of United States streams: identifying in vitro bioactivities and causative chemicals. Environmental Science & Technology 53, 973–983.
Bock, K.W., 2019. Aryl hydrocarbon receptor (AHR): from selected human target genes and crosstalk with transcription factors to multiple AHR functions. Biochem. Pharmacol. 168, 65–70.
Brack, W., Ait-Aissa, S., Burgess, R.M., Busch, W., Creusot, N., Di Paolo, C., et al., 2016. Effect-directed analysis supporting monitoring of aquatic environments - an in-depth overview. Sci. Total Environ. 544, 1073–1118.
Brack W, Ait Aissa S, Backhaus T, Dulio V, Escher BI, Faust M, et al. Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. Environ. Sci. Eur. 2019; 31.
Brand, W., de Jongh, C.M., van der Linden, S.C., Mennes, W., Puijker, L.M., van Leeuwen, C.J., et al., 2013. Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays. Environ. Int. 55, 109–118.
Brunner, A.M., Bertelkamp, C., Dingemans, M.M.L., Kolkman, A., Wols, B., Harmsen, D., et al., 2020. Integration of target analyses, non-target screening and effect-based monitoring to assess OMP related water quality changes in drinking water treatment. Sci. Total Environ. 705.
Cedat, B., de Brauer, C., Metivier, H., Dumont, N., Tutundjan, R., 2016. Are UV photolysis and UV/H2O2 process efficient to treat estrogens in waters? Chemical and biological assessment at pilot scale. Water Res. 100, 357–366.
Conley, J.M., Evans, N., Mash, H., Rosenblum, L., Schenck, K., Glassmeyer, S., et al., 2017. Comparison of in vitro estrogenic activity and estrogen concentrations in source and treated waters from 25 US drinking water treatment plants. Sci. Total Environ. 579, 1610–1617.
Davey, R.A., Grossmann, M., 2016. Androgen receptor structure, function and biology: from bench to bedside. Clin. Biochem. Rev. 37, 3–15.
Demaegdt, H., Daminet, B., Evrard, A., Scippo, M.L., Muller, M., Pussemier, L., et al., 2016. Endocrine activity of mycotoxins and mycotoxin mixtures. Food Chem. Toxicol. 96, 107–116.
Dingemans, M.M.L., Baken, K.A., van der Oost, R., Schriks, M., van Wezel, A.P., 2019. Risk-based approach in the revised European Union drinking water legislation: opportunities for bioanalytical tools. Integr. Environ. Assess. Manag. 15, 126–134.
Dopp, E., Pannekens, H., Itzel, F., Tuerk, J., 2019. Effect-based methods in combination with state-of-the-art chemical analysis for assessment of water quality as integrated approach. Int. J. Hyg. Environ. Health 222, 607–614.
EC. European Commission, EC. Council Directive 98/83/EC on the quality of water intended for human consumption. Consolidated legislation 2015. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:01998L0083-20151027&from=EN, 2015.
Escher, B.I., van Daele, C., Dutt, M., Tang, J.Y.M., Altenburger, R., 2013. Most oxidative stress response in water samples comes from unknown chemicals: the need for effect-based water quality trigger values. Environmental Science & Technology 47, 7002–7011.
Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P., Busch, W., et al., 2014. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. Environ Sci Technol 48, 1940–1956.
Escher, B.I., Ait-Aissa, S., Behnisch, P.A., Brack, W., Brion, F., Brouwer, A., et al., 2018. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. Sci. Total Environ. 628-629, 748–765.
EU. Council of the European Union. Proposal for a Directive of the European Parliament and of the Council on the quality of water intended for human consumption.

https://op.europa.eu/en/publication-detail/-/publication/13def1fc-5711-11ea-8b81-01aa75ed71a1/language-en. 2020.
Gutendorf, B., Westendorf, J., 2001. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. Toxicology 166, 79–89.
Hageskal, G., Lima, N., Skaar, I., 2009. The study of fungi in drinking water. Mycol. Res. 113, 165–172.
Hashmi, M.A.K., Escher, B.I., Krauss, M., Teodorovic, I., Brack, W., 2018. Effect-directed analysis (EDA) of Danube River water sample receiving untreated municipal wastewater from Novi Sad, Serbia. Sci. Total Environ. 624, 1072–1081.
Hebert, A., Feliers, C., Lecarpentier, C., Neale, P.A., Schlichting, R., Thibert, S., et al., 2018. Bioanalytical assessment of adaptive stress responses in drinking water: a predictive tool to differentiate between micropollutants and disinfection by-products. Water Res. 132, 340–349.
Jia, A., Escher, B.I., Leusch, F.D.L., Tang, J.Y.M., Prochazka, E., Dong, B.F., et al., 2015. In vitro bioassays to evaluate complex chemical mixtures in recycled water. Water Res. 80, 1–11.
Jones, R.R., Stavreva, D.A., Weyer, P.J., Varticovski, L., Inoue-Choi, M., Medgyesi, D.N., et al., 2020. Pilot study of global endocrine disrupting activity in Iowa public drinking water utilities using cell-based assays. Sci. Total Environ. 714.
Kakaley, E.K.M., Blackwell, B.R., Cardon, M.C., Conley, J.M., Evans, N., Feifarek, D.J., et al., 2020. De facto water reuse: bioassay suite approach delivers depth and breadth in endocrine active compound detection. Sci. Total Environ. 699.
Konig, M., Escher, B.I., Neale, P.A., Krauss, M., Hilscherova, K., Novak, J., et al., 2017. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. Environ. Pollut. 220, 1220–1230.
Kortenkamp, A., 2020. Which chemicals should be grouped together for mixture risk assessments of male reproductive disorders? Mol. Cell. Endocrinol. 499.
Leusch, F.D.L., Khan, S.J., Laingam, S., Prochazka, E., Froscio, S., Trinh, T., et al., 2014. Assessment of the application of bioanalytical tools as surrogate measure of chemical contaminants in recycled water. Water Res. 49, 300–315.
Leusch, F.D.L., Neale, P.A., Arnal, C., Aneck-Hahn, N.H., Balaguer, P., Bruchet, A., et al., 2018. Analysis of endocrine activity in drinking water, surface water and treated wastewater from six countries. Water Res. 139, 10–18.
Lundqvist, J., Andersson, A., Johannisson, A., Lavonen, E., Mandava, G., Kylin, H., et al., 2019. Innovative drinking water treatment techniques reduce the disinfection-induced oxidative stress and genotoxic activity. Water Res. 155, 182–192.
Ma, Q., 2013. Role of nrf2 in oxidative stress and toxicity. Annu. Rev. Pharmacol. Toxicol. 53, 401–426.
Ma, L., Yates, S.R., 2018. Dissolved organic matter and estrogen interactions regulate estrogen removal in the aqueous environment: a review. Sci. Total Environ. 640, 529–542.
Macova, M., Toze, S., Hodgers, L., Mueller, J.F., Bartkow, M., Escher, B.I., 2011. Bioanalytical tools for the evaluation of organic micropollutants during sewage treatment, water recycling and drinking water generation. Water Res. 45, 4238–4247.
Menon, R., Peltier, M.R., 2020. Novel insights into the regulatory role of nuclear factor (erythroid-derived 2)-like 2 in oxidative stress and inflammation of human fetal membranes. Int. J. Mol. Sci. 21.
Mirza, A.Z., Althagafi, I.I., Shamshad, H., 2019. Role of PPAR receptor in different diseases and their ligands: physiological importance and clinical implications. Eur. J. Med. Chem. 166, 502–513.
Mishra, S., Dwivedi, P.D., Pandey, H.P., Das, M., 2014. Role of oxidative stress in deoxynivalenol induced toxicity. Food Chem. Toxicol. 72, 20–29.
Muschket, M., Di Paolo, C., Tindall, A.J., Touak, G., Phan, A., Krauss, M., et al., 2018. Identification of unknown antiandrogenic compounds in surface waters by effect-directed analysis (EDA) using a parallel fractionation approach. Environmental Science & Technology 52, 288–297.
Muz, M., Krauss, M., Kutsarova, S., Schulze, T., Brack, W., 2017. Mutagenicity in surface waters: synergistic effects of carboline alkaloids and aromatic amines. Environ Sci Technol 51, 1830–1839.
Neale, P.A., Escher, Beate I., 2019. In vitro bioassays to assess drinking water quality. Current Opinion in Environmental Science and Health 7, 1–7.
Neale, P.A., Antony, A., Bartkow, M.E., Farre, M.J., Heitz, A., Kristiana, I., et al., 2012. Bioanalytical assessment of the formation of disinfection byproducts in a drinking water treatment plant. Environmental Science & Technology 46, 10317–10325.
Neale, P.A., Altenburger, R., Ait-Aissa, S., Brion, F., Busch, W., Umbuzeiro, G.D., et al., 2017a. Development of a bioanalytical test battery for water quality monitoring: fingerprinting identified micropollutants and their contribution to effects in surface water. Water Res. 123, 734–750.
Neale, P.A., Munz, N.A., Ait-Aissa, S., Altenburger, R., Brion, F., Busch, W., et al., 2017b. 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.
Neale, P.A., Leusch, F.D.L., Escher, B.I., 2018. What is driving the NF-kappa B response in environmental water extracts? Chemosphere 210, 645–652.
OECD. OECD Guideline for the Testing of Chemicals - Test No. 457: BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists. 2012.
OECD. OECD Guideline for the Testing of Chemicals - Test No. 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals. 2016.
Pal, A., He, Y.L., Jekel, M., Reinhard, M., Gin, K.Y.H., 2014. Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. Environ. Int. 71, 46–62.
Raeke, J., Lechtenfeld, O.J., Wagner, M., Herzsprung, P., Reemtsma, T., 2016. Selectivity of solid phase extraction of freshwater dissolved organic matter and its effect on ultrahigh resolution mass spectra. Environmental Science-Processes & Impacts 18, 918–927.
Robledinos-Anton, N., Fernandez-Gines, R., Manda, G., Cuadrado, A., 2019. Activators and inhibitors of NRF2: a review of their potential for clinical development. Oxidative Med. Cell. Longev. 2019.
Rosenmai, A.K., Lundqvist, J., le Godec, T., Ohisson, A., Troger, R., Hellman, B., et al., 2018. In vitro bioanalysis of drinking water from source to tap. Water Res. 139, 272–280.
Shao, Y., Hollert, H., Tarcai, Z., Deutschmann, B., Seiler, T.B., 2019. Integrating bioassays, chemical analysis and in silico techniques to identify genotoxicants in surface water. Sci. Total Environ. 650, 3084–3092.
Shi, P., Zhou, S.C., Xiao, H.X., Qiu, J.F., Li, A.M., Zhou, Q., et al., 2018. Toxicological and chemical insights into representative source and drinking water in eastern China. Environ. Pollut. 233, 35–44.
Svenskt Vatten. Production of drinking water (In Swedish). https://www.svensktvatten.se/fakta-om-vatten/dricksvattenfakta/produktion-av-dricksvatten/. 2016.
Tak, S., Vellanki, B.P., 2018. Natural organic matter as precursor to disinfection byproducts and its removal using conventional and advanced processes: state of the art review. J. Water Health 16, 681–703.
Tang, J.Y.M., Busetti, F., Charrois, J.W.A., Escher, B.I., 2014. Which chemicals drive biological effects in wastewater and recycled water? Water Res. 60, 289–299.
Tousova, Z., Oswald, P., Slobodnik, J., Blaha, L., Muz, M., Hu, M., et al., 2017. European demonstration program on the effect-based and chemical identification and monitoring of organic pollutants in European surface waters. Sci. Total Environ. 601, 1849–1868.
Troger, R., Kohler, S.J., Franke, V., Bergstedt, O., Wiberg, K., 2020. A case study of organic micropollutants in a major Swedish water source - removal efficiency in seven drinking water treatment plants and influence of operational age of granulated active carbon filters. Sci. Total Environ. 706.
Valitalo, P., Massei, R., Heiskanen, I., Behnisch, P., Brack, W., Tindall, A.J., et al., 2017. Effect-based assessment of toxicity removal during wastewater treatment. Water Res. 126, 153–163.
Wang, X., Wu, Q.H., Wan, D., Liu, Q.Y., Chen, D.M., Liu, Z.L., et al., 2016. Fumonisins: oxidative stress-mediated toxicity and metabolism in vivo and in vitro. Arch. Toxicol. 90, 81–101.
WHO. Guidelines for Drinking-water Quality, 4th edition. http://www.who.int 2011.
WHO. Drinking water Parameter Cooperation Project. Support to the revision of Annex I Council Directive 98/83/EC on the Quality of Water Intended for Human Consumption (Drinking Water Directive). https://ec.europa.eu/environment/water/water-drink/pdf/WHO_parameter_report.pdf. 2017.
Xu, S.H., Zhou, S.C., Xing, L.Q., Shi, P., Shi, W., Zhou, Q., et al., 2019. Fate of organic micropollutants and their biological effects in a drinking water source treated by a field-scale constructed wetland. Sci. Total Environ. 682, 756–764.
Zhang, Y.P., Zhou, J.L., 2005. Removal of estrone and 17 beta-estradiol from water by adsorption. Water Res. 39, 3991–4003.
Zhang, C., Tian, F., Zhang, M.L., Zhang, Z.Q., Bai, M., Guo, G., et al., 2019a. Endotoxin contamination, a potentially important inflammation factor in water and wastewater: a review. Sci. Total Environ. 681, 365–378.
Zhang, Y.T., Li, H., Zhang, J., Shao, B., 2019b. Determination of Alternaria toxins in drinking water by ultra-performance liquid chromatography tandem mass spectrometry. Environ. Sci. Pollut. Res. 26, 22485–22493.
Zheng, F., Goncalves, F.M., Abiko, Y., Li, H., Kumagai, Y., Aschner, M., 2020. Redox toxicology of environmental chemicals causing oxidative stress. Redox Biol. 101475.
Zhou, S.P., Brack, W., Doering, J.A., Seiler, T.-B., Hollert, H., 2020. Bioanalytical equivalents and relative potencies for predicting the biological effects of mixtures. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.143030.
Zwart, N., Jonker, W., Broek, R.T., de Boer, J., Somsen, G., Kool, J., et al., 2020. Identification of mutagenic and endocrine disrupting compounds in surface water and wastewater treatment plant effluents using high-resolution effect-directed analysis. Water Res. 168, 115204.