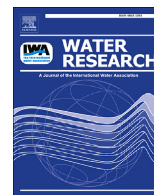
Water Research 155 (2019) 182–192

Contents lists available at ScienceDirect

Water Research

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



Innovative drinking water treatment techniques reduce the disinfection-induced oxidative stress and genotoxic activity

Johan Lundqvist [a, *], Anna Andersson [b], Anders Johannisson [c], Elin Lavonen [d, e], Geeta Mandava [a], Henrik Kylin [b, f], David Bastviken [b], Agneta Oskarsson [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 Thematic Studies-Environmental Change, Linköping University, SE-581 83, Linköping, Sweden
[c] Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, SE-750 07, Uppsala, Sweden
[d] Norrvatten, Box 2093, SE-169 02, Solna, Sweden
[e] Stockholm Vatten och Avfall, 106 36, Stockholm, Sweden
[f] Research Unit: Environmental Sciences and Management, North–West University, Potchefstroom, South Africa

ARTICLE INFO

Article history:
Received 1 January 2019
Received in revised form
20 February 2019
Accepted 23 February 2019
Available online 28 February 2019

Keywords:
Drinking water
Disinfection byproducts
Oxidative stress
Nrf2
Genotoxicity

ABSTRACT

Disinfection of drinking water using chlorine can lead to the formation of genotoxic by-products when chlorine reacts with natural organic matter (NOM). A vast number of such disinfection by-products (DBPs) have been identified, making it almost impossible to routinely monitor all DBPs with chemical analysis. In this study, a bioanalytical approach was used, measuring oxidative stress (Nrf2 activity), genotoxicity (micronucleus test), and aryl hydrocarbon receptor (AhR) activation to evaluate an innovative water treatment process, including suspended ion exchange, ozonation, in-line coagulation, ceramic microfiltration, and granular activated carbon. Chlorination was performed in laboratory scale after each step in the treatment process in order to investigate the effect of each treatment process to the formation of DBPs. Suspended ion exchange had a high capacity to remove dissolved organic carbon (DOC) and to decrease UV absorbance and Nrf2 activity in non-chlorinated water. High-dose chlorination (10 mg Cl_2 L^{-1}) of raw water caused a drastic induction of Nrf2 activity, which was decreased by 70% in water chlorinated after suspended ion exchange. Further reduction of Nrf2 activity following chlorination was achieved by ozonation and the concomitant treatment steps. The ozonation treatment resulted in decreased Nrf2 activity in spite of unchanged DOC levels. However, a strong correlation was found between UV absorbing compounds and Nrf2 activity, demonstrating that Nrf2 inducing DBPs were formed from pre-cursors of a specific NOM fraction, constituted of mainly aromatic compounds. Moreover, high-dose chlorination of raw water induced genotoxicity. In similarity to the DOC levels, UV absorbance and Nrf2 activity, the disinfection-induced genotoxicity was also reduced by each treatment step of the innovative water treatment technique. AhR activity was observed in the water produced by the conventional process and in the raw water, but the activity was clearly decreased by the ozonation step in the innovative water treatment process.

© 2019 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The use of different forms of chlorine for drinking water disinfection was a major public health breakthrough to avoid outbreaks of waterborne diseases. However, using disinfectants in drinking water production can lead to the formation of unwanted disinfection by-products (DBPs), of which some are bioactive and genotoxic (Richardson et al., 2007; Li and Mitch, 2018). In epidemiological studies, exposure to DBPs in chlorinated drinking water has been associated with various human health effects, such as bladder cancer, miscarriage and birth defects (Villanueva et al., 2015; Bove et al., 2002). DBPs are formed when a disinfectant such as chlorine, monochloramine or chlorine dioxide reacts with different fractions of natural organic matter (NOM) (Richardson et al., 2007).

NOM is a complex mixture and its structure and characteristics

* Corresponding author. Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health, Box 7028, SE-750 07, Uppsala, Sweden.

E-mail address: Johan.Lundqvist@slu.se (J. Lundqvist).

https://doi.org/10.1016/j.watres.2019.02.052
0043-1354/© 2019 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

vary with different NOM sources and the hydrographic conditions of the watershed (Barrett et al., 2000; Ledesma et al., 2012). Furthermore, natural occurrence of bromide and iodide in the raw water can lead to the formation of bromo- or iodo- DBPs which are often more toxic than the chloro-DBP equivalents (Sharma et al., 2014; Richardson et al., 1999; Liu et al., 2017; Postigo et al., 2017; Plewa et al., 2008). The DBP formation also depends on additional factors such as disinfectant type, dosage, contact time, pH and temperature (Liang and Singer, 2003).

One way to reduce DBP exposure is to optimize the removal of NOM at the drinking water treatment plants, before the point of disinfection. To increase NOM removal, and thereby decrease DBP formation, ion exchange is a possible alternative (Galjaard et al., 2009), especially for raw waters with lower specific UV absorbance (SUVA) that are not optimal for conventional chemical coagulation. For some waters, ion exchange has a higher NOM removal capacity than coagulation and is particularly useful to remove the large fraction of NOM that is negatively charged at natural pH (Boyer and Singer, 2008; Allpike et al., 2005; Croué et al., 1999). Another potential treatment, ozonation, causes breakage of carbon-carbon double bonds in NOM, which results in the production of more bioavailable organic matter. Therefore, ozonation may enhance the removal of organic material in subsequent biologically active filters, *e.g.* slow sand filters or biological active carbon (BAC) filters (Siddiqui et al., 1997; Camel and Bermond, 1998).

At present, more than 700 different DBPs have been identified (Richardson and Ternes, 2018), highlighting the challenge to routinely monitor DBPs with chemical analysis alone. For that cause, bioanalytical methods provide valuable tools to assess the combined effects of all DBPs present in a sample. Bioanalytical methods, such as *in vitro* bioassays based on human cells designed to respond to specific toxicity mechanisms, have been used for water quality assessments of drinking water in general (Rosenmai et al., 2018; Brand et al., 2013; Conley et al., 2017; Escher et al., 2014; Hebert et al., 2018; Leusch et al., 2018; Macova et al., 2011) and also more specifically to address the issue of DBPs (Hebert et al., 2018; Farré et al., 2013; Neale et al., 2012; Stalter et al. 2013, 2016a; Escher et al. 2012, 2013; Tang et al., 2014). DBPs have been reported to activate oxidative stress response systems, such as the Nrf2 pathway (Farré et al., 2013; Neale et al., 2012; Wang et al., 2013). Oxidative stress can cause genotoxicity, *e.g.* by induction of micronuclei. By measuring the oxidative stress response in water samples before and after disinfection, it is possible to monitor the total bioactivity of formed DBPs, both known and unknown. Another biological pathway that can be activated by a broad range of chemicals is the aryl hydrocarbon receptor (AhR) pathway, which has been associated with regulation of cellular responses to xenobiotic compounds.

In this study, we have used bioanalytical methods to assess the induction of oxidative stress, genotoxic activity and AhR activation in water from a conventional drinking water treatment plant using coagulation, rapid sand filtration and slow sand filtration prior to disinfection and compared it with a pilot plant facility that included suspended ion exchange (SIX®), ozonation, in-line coagulation, ceramic micro-filtration (CeraMac®) and filtration through granular activated carbon (GAC). Further, we have investigated the oxidative stress and genotoxic activities after laboratory scale chlorination to evaluate the efficiency of these new treatment processes to remove precursors forming such bioactive compounds.

2. Materials and methods

2.1. Conventional full scale and novel pilot scale treatment

Lovö drinking water treatment plant (DWTP) is one of three surface DWTPs in Stockholm, Sweden treating raw water from Lake Mälaren, the third largest lake in Sweden. Lovö and Norsborg DWTP belongs to Stockholm Vatten och Avfall (SVOA) and Görvälnverket to the municipal council Norrvatten and together the three DWTPs provide approximately 2 million inhabitants with drinking water. Norrvatten and SVOA share a number of challenges in order to secure a future drinking water production with sufficient quality and quantity of potable water. Therefore, a collaborative one year study of a novel treatment process, which was performed at Lovö DWTP, was initiated in 2016.

Lovö DWTP currently employ conventional coagulation treatment using aluminium sulfate ($Al_2(SO_4)_3$) followed by sedimentation, rapid sand filtration, slow sand filtration, UV disinfection and dosing of monochloramine (Fig. 1).

The pilot plant facility included novel ion exchange and membrane treatment provided by the Dutch PWNT Water Technology (Fig. 1). Additional information regarding suspended ion exchange (SIX®) and membrane filtration with the ceramic microfilter membrane (CeraMac®) used in this study can be found elsewhere (Galjaard et al., 2009; Metcalfe et al., 2016). Both ozone and in-line coagulation (here the coagulant PIX-311($FeCl_3$) was used) were available as pre-treatments for the CeraMac® membrane. For SIX®, an acrylic quaternary amine, strongly basic, gel-type anionic resin was used (Lewatit S5128, Lanxess, Germany). When the sampling for this study was performed, the same resin had been in continuous use for approximately 10 months. Due to operating issues with the CeraMac® membrane the GAC filter, which constituted the next treatment step, was only running intermittently, it was, however, backwashed regularly. In total 720 m^3 of water was filtered which was equivalent to 2200 bed volumes with the empty bed contact time of 20 min that was used (this can be translated to approximately 30 days of continuous operation). Therefore, the GAC performance was higher than what can be expected in a full-scale application where the activated carbon may be regenerated or replaced after 1–2 years of operation or in some cases not at all.

2.2. Sampling and chlorination experiments

From Lovö DWTP and its connected pilot plant water samples (5 L) were collected and filtered through pre-combusted (450 °C for 5 h) GF/F glass fiber filters (0.7 μm porosity). Hypochlorite and monochloramine were taken from Lovö DWTP and used the same day in laboratory scale chlorination and chloramination experiments. The level of free chlorine (as Cl_2) and total chlorine, the sum of combined and free chlorine (as Cl_2) analyzed upon dosage, were measured using an eXact idip photometer. For the chlorine additions, abbreviated as HOCl in tables and figures, free chlorine was measured and for the monochloramine additions (abbreviated as NH_2Cl in tables and figures) total chlorine was measured. The experimental setup including additional treatments and chlorine residuals are summarized in Table 1.

In experiment 1, samples from the conventional full scale plant (RW, DW) and the novel pilot scale plant (RW, SIX_{out}, $Ozone_{out}$, $CeraMac_{out}$, GAC_{out}) were extracted by solid phase extraction (SPE) (see 2.3.) without further modifications in order to investigate the potential inherent toxicity of the raw water and effects from the different treatments. The exception was $Ozone_{out}$ where 2.5 ml/L 0.01 M $Na_2S_2O_3$ was added to the bottles before sampling to quench the excess ozone (1 mg L^{-1}) present at this sampling point only.

In experiment 2, performed to evaluate the effects on bioactivity from the different treatment processes in the pilot plant due to potential DBP formation, sodium hypochlorite and monochloramine were added to finished GAC_{out} water samples from the pilot process at doses typical for Swedish DWTPs (0.3–0.4 mg L^{-1} residual, measured as free and total chlorine respectively), referred to

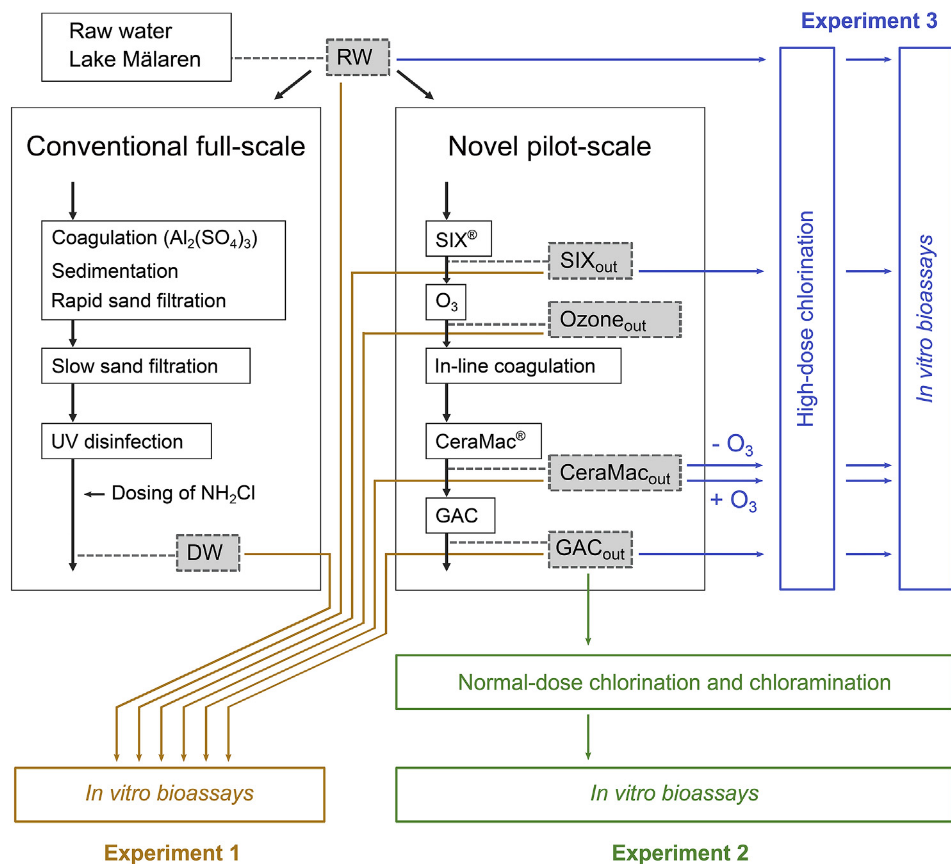


Fig. 1. Treatment steps for the conventional process at Lovö DWTP and the new pilot process investigated. The sampling points RW, DW, SIX_{out}, $Ozone_{out}$, $CeraMac_{out}$ and GAC_{out} are indicated in boxes with dashed lines. Experiments 1, 2 and 3 are described in detail in section 2.2.

Table 1
Water characteristics and various experimental conditions. Abbreviations for the sampling points are explained in Fig. 1.

	Sample collected	DOC (mg L^{-1})	Abs 254 nm (cm^{-1})	SUVA (L mg^{-1} m^{-1})	Additional treatment	Chlorine residual (mg Cl_2 L^{-1})
Conventional full-scale treatment (Experiment 1)	RW	6.9	0.191	2.8	–	–
	DW	3.9	0.077	2.0	–	0.3
Novel pilot scale treatment (Experiment 1)	RW	6.9	0.191	2.8	–	–
	SIX_{out}	2.5	0.048	1.9	–	–
	$Ozone_{out}$	2.6	0.032	1.2	–	–
	$CeraMac_{out}$	2.0	0.013	0.7	–	–
	GAC_{out}	0.4	0.001	0.3	–	–
Normal-dose chlorination and chloramination (Experiment 2)	GAC_{out}	0.4	0.001	0.3	–	–
	GAC_{out}	0.4	0.001	0.3	HOCl	0.3–0.4
	GAC_{out}	0.4	0.001	0.3	NH_2Cl	0.3–0.4
High-dose chlorination (Experiment 3)	RW	6.6	0.187	2.8	HOCl	10.9–11.5
	SIX_{out}	2.6	0.049	1.9	HOCl	10.5–11.6
	$CeraMac_{out}$-O_3	2.0	0.029	1.5	HOCl	9.6–10.3
	$CeraMac_{out}$ + O_3	2.0	0.014	0.7	HOCl	10.0–11.7
	GAC_{out}	0.5	0.002	0.4	HOCl	10.0–11.7

as normal dose in text, tables and figures. pH was adjusted to ~8.5 using 1 M NaOH. The experiment was performed on nine GAC_{out} samples with three replicates for control (without addition), sodium hypochlorite and monochloramine addition respectively. After disinfectant addition, the samples were stored in the dark at 15 °C for 24 h before SPE, conditions designed to mimic a situation for analyzing water at the consumer's tap.

In experiment 3, a high-dose chlorination experiment was performed to water samples after each treatment step in the pilot plant (RW, SIX_{out}, $CeraMac_{out}$-O_3, $CeraMac_{out}$ + O_3, GAC_{out}). To distinguish between the effect of ozone and coagulation on the ceramic membrane (direct filtration), samples were collected with ozone turned on and off respectively. The ozone levels were controlled by online-measurements as well as repeated manual sampling and measurements (AccuVac® MR method, HACH) at $Ozone_{out}$ and $CeraMac_{out}$. Sodium hypochlorite was added to the water samples at a residual of ~10 mg Cl_2 L^{-1} measured as free chlorine at pH ~8.5. The chlorinated samples were stored in the dark at room temperature for 3 days. Hypochlorite was added to fifteen water samples in total, three replicate samples for each step in the treatment process. Two controls were included for each step without addition of hypochlorite. One control for each treatment

step was collected at the same day while the other was collected another day, due to practical reasons. After 3 days all samples had levels of free chlorine above 6 mg L^{-1}. To shift the equilibrium towards gaseous chlorine and hence remove reactive chlorine from the water before extraction, pH was lowered to 1.5 using 3 M HCl, prepared by hydrochloric acid 32% (puriss P.A) and ultrapure water. The level of free chlorine was below 1 mg L^{-1} before SPE was initiated.

2.3. Sample preparation

Water samples, except those subject to high-dose chlorination, were adjusted to pH ~2.5 using 3 M HCl, prepared by hydrochloric acid 32% (puriss P.A) and ultrapure water and extracted using SPE with a Agilent Bond Elute PPL resin (1 g, 6 ml) and a vacuum manifold system. The cartridges were conditioned with LC-MS Ultra CHROMASOLV® methanol (MeOH; 10 ml) followed by ultrapure water (10 ml; pH 2.5). The volume used for extraction was based on the amount of dissolved organic carbon (DOC); for raw water 2.5 L and 5 L for other samples. The water samples were connected to the cartridges by Teflon tubing and the flow was kept below 20 ml min^{-1} by a peristaltic pump. After extraction the cartridges were washed with LC-MS Ultra CHROMASOLV® 0.1% formic acid in water to remove remaining ions and dried briefly (15 s) with air using the vacuum manifold. Finally, the cartridges were eluted with LC-MS Ultra CHROMASOLV® MeOH (10 ml) and the extracts stored in freezer at −20 °C. Before bioanalysis, 9.5 ml of the MeOH sample was spiked with 50 μL (2.5 L samples) or 100 μL (5 L samples) dimethyl sulfoxide (DMSO) as a keeper and the MeOH was evaporated using an Automatic Environmental SpeedVac System. Each sample was then re-dissolved in a lower volume of ethanol (total volume of 0.5 mL for the 2.5 L samples and total volume of 1 mL for the 5 L samples), giving an enrichment factor of 5000.

2.4. Analysis of water characteristics

Dissolved organic carbon (DOC) and absorbance at 254 nm (UVA_{254}) was used to assess NOM quantity and quality. The specific absorbance (SUVA) determined at 254 nm have been used to indicate the degree of aromaticity of NOM (Weishaar et al., 2003). Water samples were stored at +8 °C until analysis of DOC (maximum 6 days) and absorbance (maximum 3 days). DOC was measured at the Norrvatten accredited lab using the non-purgeable organic carbon (NPOC) method (Multi N/C 3100, Analytik Jena). Absorbance at 254 nm (4 cm cuvette) was measured using a HACH Lange DR6000. Specific absorbance (SUVA) was determined by normalizing the absorbance at 254 nm with DOC, reported in the unit L mg^{-1} m^{-1} (Weishaar et al., 2003).

2.5. Bioassays

The water samples and vehicle control were tested for AhR and Nrf2 activities in reporter gene assays, and for cytotoxicity by cell viability assays (MTS-assay). In addition, water samples from the high-dose chlorination experiment (experiment 3) were tested for genotoxicity by an *in vitro* micronucleus test. 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. This cell line was purchased from Signosis Inc. (Santa Clara, CA, catalog number SL-0046-NP) and the assay was performed in accordance with the standardized protocol, including recommended positive control, provided by the manufacturer. AhR activity was assayed in transiently transfected HepG2 cells, using a luciferase reporter plasmid under the control of a DNA element responsive to ligand activated AhR (Promega) (Rosenmai et al., 2018). Micronuclei formation was assayed by flow cytometry in human lymphoblast TK6 cells, using a MicroFlow Kit (Litron Laboratories, US). A detailed description of the bioanalytical methods is provided in Supplementary Information. When incubated with the cells, the concentrated water samples were diluted 100-fold with cell medium to get a final concentration of 0.9% ethanol and 0.1% DMSO and a relative enrichment factor (REF) of 50. 0.9% ethanol and 0.1% DMSO was used as solvent control. For Nrf2 activity, selected samples were run in dilution series to enable the calculation of effect concentrations. The enrichment and dilution of the samples constitute the REF, calculated as described by Escher et al., (2014):

$$REF = enrichment\ factor_{SPE} * dilution\ factor_{bioassay}$$

The dilution and enrichment factors are calculated by the following equations:

$$enrichment\ factor_{SPE} = \frac{volume\ water}{volume\ extract}$$

$$dilution\ factor_{bioassay} = \frac{volume\ of\ extract\ added\ to\ bioassay}{total\ volume\ of\ bioassay}$$

Positive controls were analyzed in parallel with the water samples: 2,3,7,8-tetrachlorodibenzodioxin (TCDD) for AhR, tert-butylhydroquinone (tBHQ) for Nrf2, and mitomycin C (MMC) for the micronucleus test. The standard curve for TCDD was used to calculate the TCDD equivalent concentration (TCDDEQ) for observed AhR activities.

2.6. Technical replicates

For each sample site and experimental treatment of water, three technical replicate samples were collected or produced to prove technical reproducibility. All three technical replicates were analyzed in quadruplicate in the bioassays for Nrf2 and AhR activities. All three technical replicates for each sampling site and experimental treatment showed reproducible bioassay result. One technical replicate for each sampling site or experimental treatment was then reanalyzed in an independent experiment for each bioassay, to prove biological reproducibility. The independent bioassays showed reproducible results. The results from one of these representative experiments are presented.

2.7. Data analysis

Initially, all water samples were analyzed for bioactivity at REF 50 in the bioassays for Nrf2 and AhR activities. For Nrf2 activity, an induction ratio of 1.5 compared to vehicle control was used as the cut-off value for bioactivity, as proposed by Escher et al., (2012). Samples showing Nrf2 activity at REF 50 was then analyzed in a 2-fold dilution series from REF 25 to REF 0.78, to investigate concentration-response relationships and enable the calculation of the relative enrichment factor corresponding to an induction ratio of 1.5 ($EC_{IR1.5}$).

For AhR, a 2-fold induction of the activity compared to the vehicle control was used as the cut-off value for bioactivity. This definition of bioactivity for the AhR bioassay was calculated from the limit of detection (LOD) for the assay, which was defined as 1 plus 3 times the standard deviation for the vehicle control. Only minor effects on AhR activity were observed at REF 50, and for that reason no concentration-response experiments were performed for the AhR activity.

Micronucleus test was performed for samples in the high-dose

chlorination experiment (experiment 3). Each sample was analyzed at the highest non-cytotoxic REF value.

3. Results and discussion

3.1. Water characteristics

DOC concentrations, UVA_{254} and SUVA values are presented in Table 1. In the conventional full-scale treatment DOC was reduced from 6.9 to 3.9 mg L^{-1}. In the novel pilot scale treatment DOC was reduced to about 2.5 mg L^{-1} already after SIX®. Ozonation had little effect on the DOC concentration, while in-line coagulation together with the ceramic micro-filtration further reduced DOC to about 2 mg L^{-1}. Finally, the activated carbon filter removed DOC to a final concentration of 0.4–0.5 mg L^{-1} in the finished pilot process water. It should be noted that the GAC filter was not saturated at the time of sampling and the DOC removal capacity might be lower in a full-scale application where filters may be operated as biological filters, *i.e.* as biological active carbon (BAC).

The pilot treatment process also affected UVA_{254} and SUVA more dramatically compared to the conventional process (Table 1). The conventional process reduced SUVA from 2.8 to 2.0 L mg^{-1} m^{-1} while the pilot process provided a reduction down to 0.3–0.4 L mg^{-1} m^{-1}. All steps in the pilot process reduced 254 nm absorbance and SUVA. With no DOC removal but a major reduction in UV absorbance, the effect of ozone on SUVA was clear, indicating a modification of the organic material composition resulting from cleavage of aromatic structures. For the other treatments, UVA_{254} and SUVA reductions were likely due to removal of UV absorbing organic structures, *e.g.* aromatic structures.

3.2. Cell viability

To ensure that the bioassays were conducted under conditions where the general cell viability was not compromised, we assayed the effects of all concentrated water samples on the cell viability of HepG2 cells at REF 50. None of the water samples exerted cytotoxic effects, defined as a cell viability <80% compared to the vehicle control, at REF 50 (Fig. S1).

3.3. AhR activity

For the conventional process, a 2-fold induction in the AhR activity was observed in the concentrated samples representing both raw water and the finished drinking water at REF 50 (Fig. 2A), indicating that there are compounds present in the raw water causing this effect and that the current treatment process steps are unable to remove these compounds. We have previously observed similar results regarding AhR activities for drinking water produced from the same water source (Rosenmai et al., 2018). For the pilot plant, the observed 2-fold induction of AhR activity in the raw water was unaltered by suspended ion exchange treatment, but clearly decreased by ozonation (Fig. 2B), possibly due to decreased concentration of aromatic compounds after ozone treatment. Normal-dose disinfection of water collected after GAC with chlorine or monochloramine did not induce AhR activity (Fig. 2D) and furthermore the AhR activity did not differ from the GAC effluent without hypochlorite or monochloramine addition. This demonstrates the benefit of increased NOM removal prior to monochloramine dosing compared to the full scale treatment. In contrast, high-dose chlorination, caused a 2-fold induction of AhR activity in all samples from the pilot treatment process (Fig. 2E). The

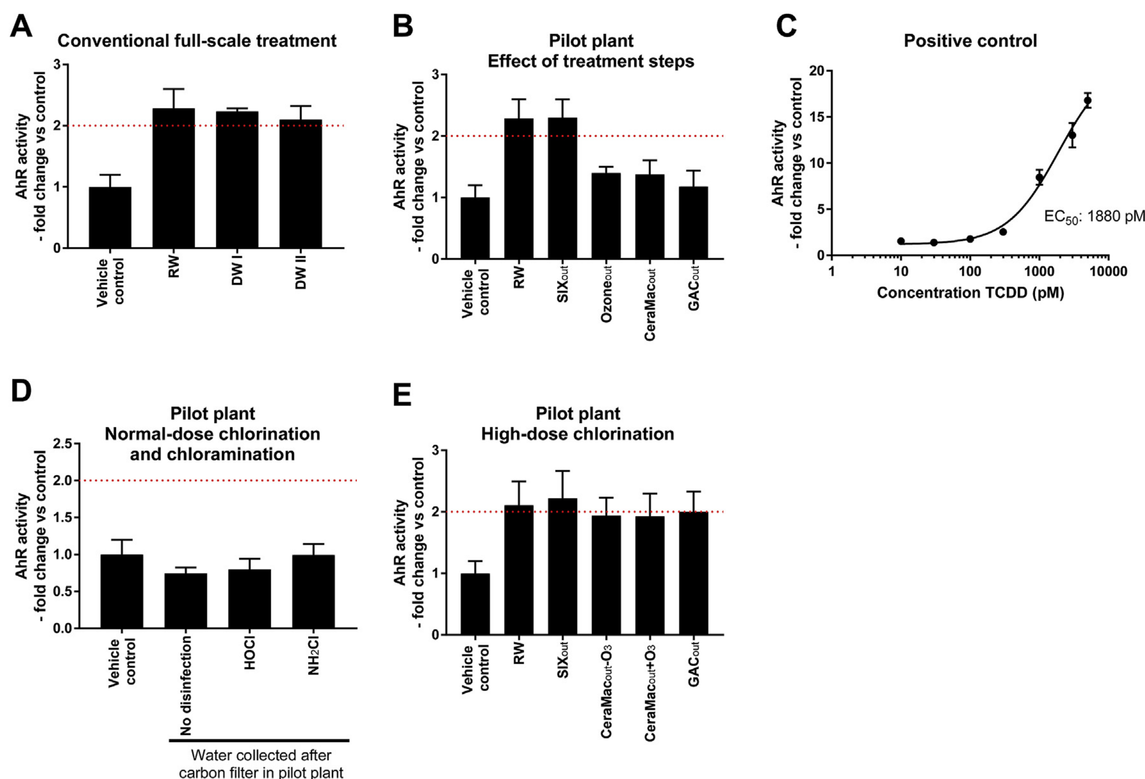


Fig. 2. AhR activities observed at REF 50 for the current drinking water treatment process (A), the pilot treatment process (B), after normal-dose disinfection of finished water from the pilot treatment process (D), and after high-dose chlorination of samples throughout the pilot treatment process (E). TCDD was used as positive control (C). Data presented as mean ± standard deviation, n = 4.

observed effects are equivalent to effects caused by TCDD at concentrations in the range of 125–167 pM (Table S1).

3.4. Nrf2 activity in conventional and pilot treatment process

Experiment 1 showed that the induction for Nrf2 activity in the raw water was below the cut-off level for activity of 1.5 fold at REF 50 (Fig. 3A). The two samples of finished drinking water from the conventional treatment process, showed an induction ratio for Nrf2 of approximately 2, at REF 50 (Fig. 3A). A higher induction ratio in the treated drinking water compared to the raw water indicates that Nrf2 inducing compounds are formed during the conventional water treatment process. The change in induction ratio in this case, however, is marginal and statistically non-significant, and should therefore be interpreted with caution. Raw water and two drinking water samples from the conventional process was also analyzed in a dilution series covering REF 25 to 0.78 (Fig. 4A–C). $EC_{IR1.5}$ was calculated to REF 21.9 for raw water, 21.0 and 24.9 respectively for the two drinking water samples (Fig. 4A–C).

The pilot process (without disinfection), resulted in a decreased induction ratio for Nrf2 after suspended ion exchange, indicating that this treatment removes Nrf2 inducing compounds (Fig. 3B) (experiment 1). All these samples had an $EC_{IR1.5}$ of >50 REF. Disinfection of finished pilot plant water (GAC filtrate) with normal doses of hypochlorite and monochloramine (experiment 2) did not induce Nrf2 activity, indicating that the pilot plant has removed pre-cursors, resulting in decreased formation of toxic DBPs compared to the conventional full scale process (Fig. 3D). The REF values of drinking water from the current process can be compared to the study by Hebert et al., (2018), reporting $EC_{IR1.5}$ in the range from REF 15 to 100 from a study of three drinking water plants in France, with rather big differences in $EC_{IR1.5}$ REF values between plants and sampling seasons. For all three water distribution systems, higher Nrf2 activities were observed in May and September compared to November and March. For May, the authors also observed higher levels of total organic carbon (TOC) and DBPs as compared to November and March, which could be an explanation for the observed increase in Nrf2 activity. However, the TOC and DBP levels in September were similar to those observed in November and March.

In a bioanalytical assessment of an Australian drinking water treatment plant, Neale et al., (2012) found increased toxicity, including Nrf2 activity, after chlorination, which was associated with increased levels of both adsorbable organic halogens (AOXs), which represent the total level of halogenated compounds, and specific DBPs ($CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$). They reported an $EC_{IR1.5}$ of REF 35 for the raw inlet water, an $EC_{IR1.5}$ of REF 3.23 after chlorination (2–2.5 mg L^{-1} free chlorine residual, 2.3 mg L^{-1} DOC) and of REF 5.54 after subsequent chloramination (2.5 mg L^{-1} total chlorine residual, 2.3 mg L^{-1} DOC). In the present study, we observed higher DOC levels in the samples from the conventional full scale process compared to the plants studied by Neale *et al*, but also higher $EC_{IR1.5}$ values for the oxidative stress response. However, the monochloramine residual used in our study was lower, 0.3 mg l^{-1} measured as total chlorine, likely explaining part of the difference in $EC_{IR1.5}$ values.

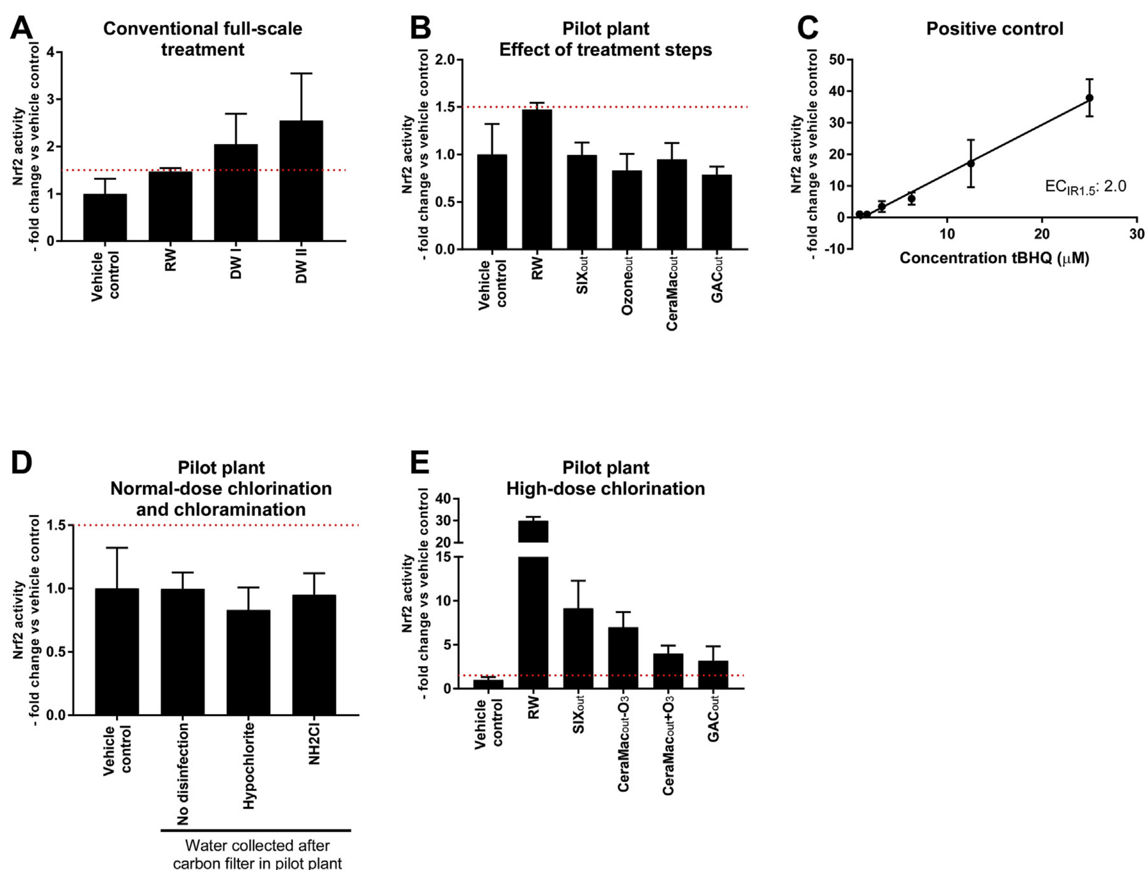


Fig. 3. Nrf2 activities observed at REF 50 for the conventional drinking water production process (A), the pilot treatment process (B), after normal-dose chlorination and chloramination of finished water from the pilot treatment process (D), and after high-dose chlorination of each sample from the pilot treatment process (E). tBHQ was used as positive control (C). Data presented as mean ± standard deviation, n = 4.

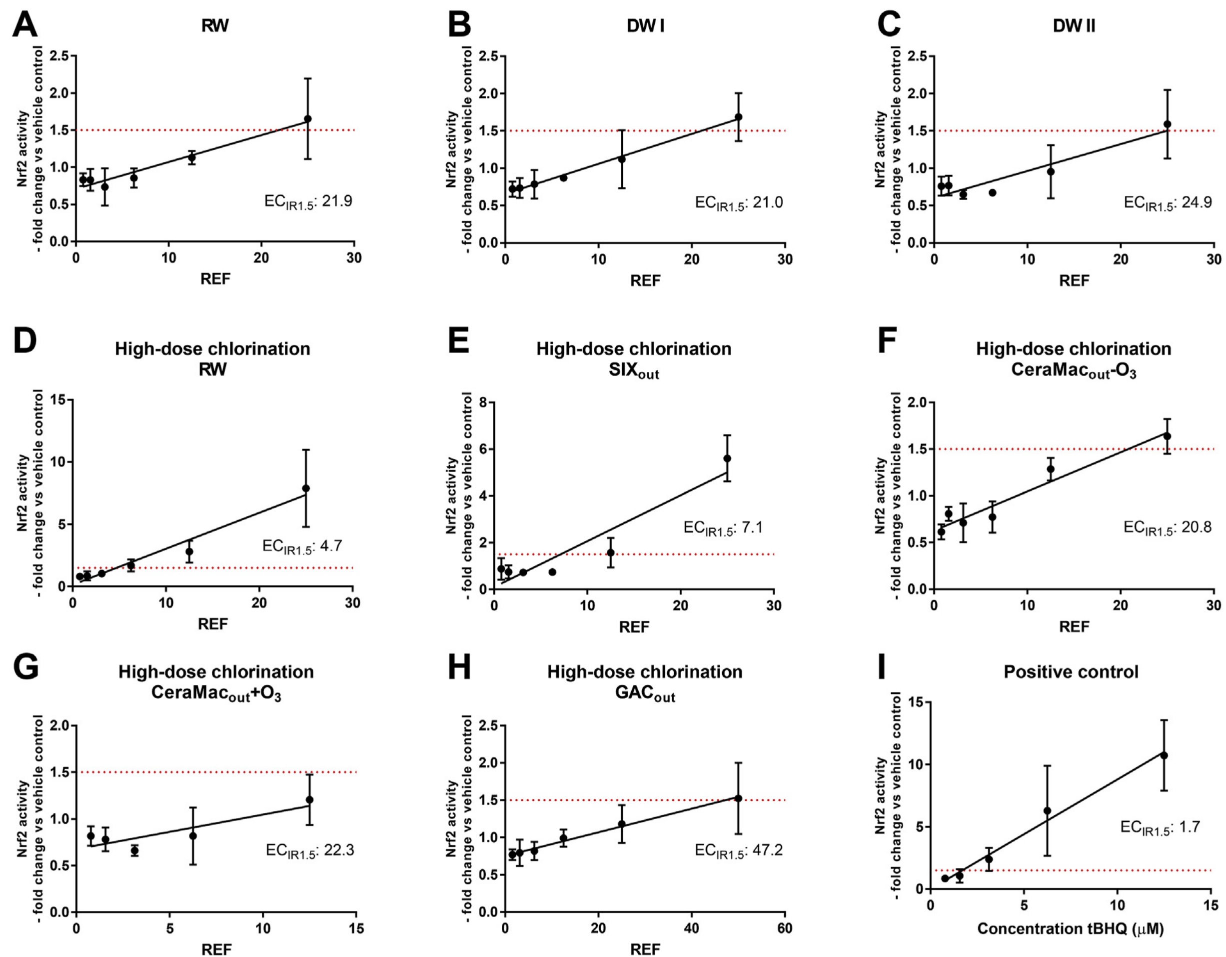


Fig. 4. Nrf2 activities for samples from the current conventional water treatment process (A–C) and the pilot plant process subjected to high-dose chlorination (D–H), analyzed in dilution series to enable the calculation of $EC_{IR1.5}$. tBHQ was used as a positive control. The dotted line represents the induction ratio 1.5 fold change. Data presented as mean ± standard deviation, n = 4.

An effect-based trigger value, based on the Australian Drinking Water guidelines, has been proposed by Escher et al., (2013) as an $EC_{IR1.5}$ of REF 6 for Nrf2 activity. Compared to the proposed effect-trigger value, our study shows a 3.5–4.0-fold margin for the observed effects by water samples from the conventional water treatment process. It should be noted that the effect-based trigger value and our study has been performed with two different assays, although both assay the Nrf2 activity. However, the sensitivity of these two assays are similar, shown by the similarity in $EC_{IR1.5}$ for the positive control. In this study, we report an $EC_{IR1.5}$ of 1.7–2.0 µM for tBHQ while it has been reported to be 1.1 µM (Escher et al., 2013) and 1.9 µM (Hebert et al., 2018) for the AREc32 assay. This indicates that the results obtained with the assay used in this study can be compared with the effect-based trigger value obtained with the AREc32 assay.

3.5. Formation potential of Nrf2 inducing and genotoxic disinfection by-products

In the experiment investigating the effect of each pilot treatment step on disinfection-induced toxicity (experiment 3), water samples from each step were disinfected with a high-dose of hypochlorite (10 mg Cl_2 L^{-1}) with a contact time of 3 days. We observed a drastic increase, by 30-fold compared to the vehicle control, in Nrf2 activity when raw water was chlorinated (Fig. 3E). For water that had been treated with suspended ion exchange before chlorination, the Nrf2 induction was reduced by 70% as compared to the raw water, indicating that SIX® removes compounds from the water that can form Nrf2 activating compounds. Additional treatment steps further reduced the potential for Nrf2 induction after chlorination.

All samples from the high-dose chlorination experiment were analyzed in dilution series, to enable the calculation of $EC_{IR1.5}$ for the Nrf2 inducing effect (Fig. 4D–H). The $EC_{IR1.5}$ for raw water exposed to high-dose chlorination was REF 4.7, after SIX® REF 7.1, after ceramic microfiltration (including in-line coagulation and ozonation) REF 22.3 and after activated carbon filter REF 47.2. This increase in the $EC_{IR1.5}$ value for each step in the pilot treatment process indicates that the treatment steps remove compounds from the water that have the potential to form Nrf2 activating compounds in the disinfection process. The $EC_{IR1.5}$ values for each sample is presented in Table 2.

As described above, Escher et al., (2013) have proposed an effect-based trigger value for the Nrf2 activity of an $EC_{IR1.5}$ value of REF 6. In this study, the raw water used for drinking water production had an $EC_{IR1.5}$ below the proposed effect-based trigger value when disinfected with 10 mg L^{-1} chlorine and would thus indicate a potential health hazard if it was drinking water. The treatment techniques in the studied pilot plant increased the $EC_{IR1.5}$ drastically and when water collected after the pilot plant process

Table 2
Color-coded table showing the $EC_{IR1.5}$ for Nrf2 activity, expressed as REF.2

		REF
Conventional full scale treatment (Experiment 1)	Raw water (RW)	21.9
	Drinking water sample 1 (DW)	21.0
	Drinking water sample 2 (DW)	24.9
Novel pilot scale treatment (Experiment 1)	Raw water (RW)	21.9
	Suspended Ion Exchange (SIX_{out})	>50
	$Ozone_{out}$	>50
	Ceramic filter with ozonation ($CeraMac_{out}$)	>50
	Carbon filter (GAC_{out})	>50
Normal-dose chlorination and chloramination (Experiment 2)	Carbon filter (GAC_{out}), no disinfection	>50
	Carbon filter (GAC_{out}), HOCl	>50
	Carbon filter (GAC_{out}), NH_2Cl	>50
High-dose chlorination (Experiment 3)	Raw water (RW)	4.7
	Suspended Ion Exchange (SIX_{out})	7.1
	Ceramic filter without ozonation ($CeraMac_{out}$-O_3)	20.8
	Ceramic filter with ozonation ($CeraMac_{out}$+O_3)	22.3
	Carbon filter (GAC_{out})	47.2

REF>50
50≥REF>30
30>REF>10
10>REF>5
REF<5

was chlorinated with 10 mg L^{-1} chlorine, the $EC_{IR1.5}$ was 47.2, meaning that the treated water has a margin of more than 7-fold for the observed effects as compared to the proposed effect-trigger value.

There is a strong correlation between the DOC concentrations, associated with each stage in the pilot treatment process, and the Nrf2 activity at REF 50 (Fig. 5A), lending support to the hypothesis that the formation of oxidative stress inducing compounds in the high-dose disinfection process depends to a high extent on the DOC level. Nrf2 activities at REF 50 were also plotted against UVA_{254} (Fig. 5B) producing a better fit where UVA_{254} could be used to explain Nrf2 activities associated with DBPs formed, also for the point after ozonation. This indicates that the abundance of UVA_{254} absorbing NOM compounds and their associated structural characteristics, *i.e.* aromatic moieties, could best describe the formation of oxidative stress inducing compounds upon high-dose chlorination. UVA_{254} shows a very high correlation with Nrf2 activity even if the raw water sample is excluded from the analysis ($r^2 = 0.972$, data not shown), which is not the case for the correlation between DOC concentration and Nrf2 activity with the raw water sample excluded ($r^2 = 0.649$, data not shown).

Farré et al., (2013) have reported $EC_{IR1.5}$ of REF 1.8–5.6 following formation potential experiments (chlorine and monochloramine residual: ~2 mg Cl_2 L^{-1}, reaction time: 3 days, pH 7) of water sampled after the coagulation stage for three conventional drinking water treatment plants with $EC_{IR1.5}$ of REF 7.8–9.9 before chlorination. We observed an $EC_{IR1.5}$ of REF 4.7 and 7.1 after chlorination (10 mg L^{-1}, 3 days, pH 8.5) of raw water and after SIX® respectively. One of the DWTP evaluated by Farré *et al* with similar DOC levels (2.8 mg L^{-1}) and SUVA (2.05 L mg^{-1} m^{-1}), after coagulation compared to SIX_{out}, had an $EC_{IR1.5}$ of REF 2.8. The higher Nrf2 activity reported by Farré *et al*, despite lower chlorination, could be due to a higher level of bromide in that water source (0.16 mg L^{-1}) compared to Lake Mälaren (0.03–0.05 mg L^{-1} at the point after SIX®), resulting in elevated levels of more toxic Br-DBPs.

As DBPs may also be genotoxic, we analyzed the samples from the high-dose chlorination experiments in an *in vitro* micronucleus test. As a cytotoxicity control, cells were stained with ethidium monoazide (EMA). In accordance with the instructions from the manufacturer, a cut-off for cytotoxicity was set at 55% EMA positive cells. Samples were analyzed at the highest possible REF, still having an EMA positive score <55%. Raw water disinfected with high-dose chlorination, had an EMA positive score of 77%. This sample was therefore analyzed at REF 25. We found (Fig. 6) that raw water subjected to high-dose chlorination for 3 days exerted a significant genotoxicity with a micronuclei percentage above 20% at REF 25. For each step in the water treatment process, subject to high-dose chlorination, we observed a decreased micronuclei formation, indicating that the water treatment process removes compounds that can form genotoxic products in the disinfection process.

Conflicting results have previously been reported regarding the genotoxicity of DBPs. For example, the three haloacetic acids iodoacetic acid (IAA), bromoacetic acid (BAA) and chloroacetic acid (CAA) were reported to induce formation of micronuclei by Ali et al., (2014), while Liviac et al., (2010) reported that the same

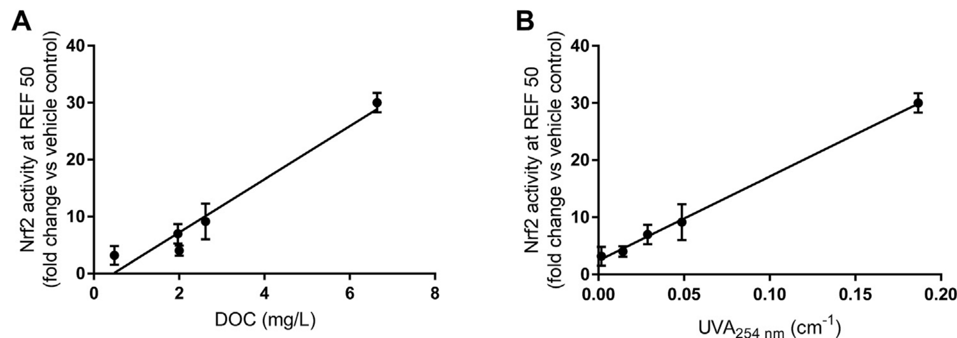


Fig. 5. Correlation between Nrf2 activity at REF50 and DOC concentration (A) and UVA_{254} (B), respectively, for water samples from the high-dose chlorination experiment. The correlation between Nrf2 activity and DOC concentration is described by the equation $y = 4.7x-2.1$, $r^2 = 0.955$; $p = 0.0041$, and for the correlation between Nrf2 activity and UVA_{254} by the equation $y = 147x+2.4$, $r^2 = 0.998$; $p < 0.0001$. Nrf2 activity presented as mean ± standard deviation, n = 4.

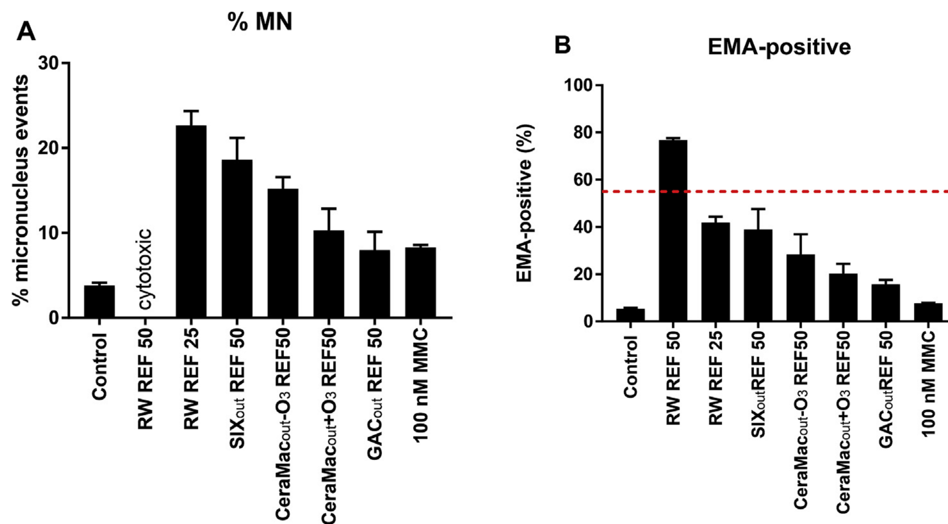


Fig. 6. Micronuclei formation rate (A) and control for cytotoxicity (B) for water samples from the pilot plant process subjected to high-dose disinfection. Micronuclei formation rate was analyzed at REF 50 for all samples except for the raw water subjected to high-dose disinfection, due to cytotoxicity at REF 50 for that sample. The red dotted line in panel B represent the threshold for cytotoxicity as defined in the kit protocol. Data presented as mean ± standard deviation, n = 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

three compounds did not induce formation of micronuclei. For drinking water samples, Chen et al., (2016) reported induced micronuclei formation after chlorine disinfection. Our results lend support to the suggestion that drinking water disinfection may form compounds that can induce micronuclei formation.

Several Swedish drinking water facilities face challenges due to increasing levels of NOM in the source waters. This could partly be due to climate change, but inadequate catchment management including urbanization, construction and development, and agricultural practices can also affect the NOM level. In Eastern Lake Mälaren, which provides drinking water for more than two million inhabitants, the level of total organic carbon (TOC) (yearly average) has increased from approximately 7 mg C L^{-1} to 8 mg C L^{-1} from the 1990's to the 2010's. During the same period, the TOC level in the produced drinking water has also increased; the currently used conventional treatment that includes coagulation has limited ability to remove NOM and the amount of DBPs could be expected to rise. This study provides important knowledge on the design of future drinking water treatment processes, aiming to decrease the risk associated with DBP formation. Specifically, suspended ion exchange showed promising potential with a three-fold reduction of the Nrf2 activity along with decreased micronuclei formation for Lake Mälaren water. Furthermore, ozonation showed to reduce (almost two-fold, Fig. 3E) the formation of Nrf2 inducing compounds in spite of limited DOC removal, highlighting that the organic carbon concentration might not accurately predict the formation of Nrf2 inducing DBPs after this step. The decrease in Nrf2 inducing compounds during ozonation could, however, be explained by a change in structural characteristics of the organic carbon, measured as UVA_{254}, which indicates a strong correlation between aromatic NOM and formation of DBPs causing oxidative stress during chlorination. The combination of in-line coagulation with ozonation and CeraMac® also reduced the Nrf2 activity and micronuclei formation. However, the effect of GAC needs to be interpreted with caution since the filter was rather new and not saturated (which is the normal situation at many drinking water treatment plants).

The findings from these type of experiments reflect the toxicity of the extract from the SPE cartridge used. Alterations in Nrf2 activity or micronuclei formation could be due to changes in the amount of DBPs formed as well as changes in DBP speciation. Importantly, both situations are accounted for providing a relevant bioanalytical assessment of human DBP exposure.

When performing sample preparation with SPE according to the current protocol, it can be expected that only non-volatile and semi-volatile DBPs, but not volatile DBPs, are retained in the

sample. It has been reported that the bioactivity from volatile DBPs to a large extent is explained by known DBPs (Stalter et al., 2016b) and mixture toxicity modeling has shown that the volatile fraction of the DBPs only had a minor contribution to the total bioactivity of DBPs (Hebert et al., 2018). Therefore, the method used is expected to capture a highly relevant fraction of DBPs formed. It should be noted that SPE extraction have a DOC extraction efficiency in the order of 60% for these types of samples and that more hydrophilic compounds are extracted to a lower degree than more hydrophobic compounds (Dittmar et al., 2008). However, SPE extraction following the same protocol as here and analyzed with Fourier-Transform-Ion-Cyclotron-Resonance-Mass-Spectrometry revealed >800 novel DBPs from one single DWTP (Gonsior et al., 2014), confirming that SPE extracts capture a high number and complex mixture of DBPs.

4. Conclusions

In conclusion, we have used a bioanalytical assessment approach to show that a water treatment process, including suspended ion exchange (SIX®) followed by ozonation, in-line coagulation, ceramic microfiltration (CeraMac®) and granular activated carbon (GAC), efficiently decreased the oxidative stress and genotoxic activity, which most probably can be connected to their removal or influence on precursors leading to alterations in DBP formation and toxicity. Further, we have shown that UVA_{254} absorbing NOM compounds show a very high correlation with the risk of disinfection-induced toxicity in the form of Nrf2 activity.

Conflicts of interest

The authors report no conflicts of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Acknowledgement

This work was financially supported by the Swedish Research Council Formas, Sweden by grants to JL and AO (grants 2014-1435, 2012-2124 and 2018-02191) and to DB (grant 2013-01077). Further, DB received financial support from Linköping University and JL from the SLU environmental monitoring programme on a Non-toxic environment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2019.02.052.

References

Ali, A., Kurzawa-Zegota, M., Najafzadeh, M., Gopalan, R.C., Plewa, M.J., Anderson, D., 2014. Effect of drinking water disinfection by-products in human peripheral blood lymphocytes and sperm. Mutat. Res. Fund Mol. Mech. Mutagen 770, 136–143.
Allpike, B.P., Heitz, A., Joll, C.A., Kagi, R.I., Abbt-Braun, G., Frimmel, F.H., Brinkmann, T., Her, N., Amy, G., 2005. Size exclusion chromatography to characterize DOC removal in drinking water treatment. Environ. Sci. Technol. 39 (7), 2334–2342.
Barrett, S.E., Krasner, S.W., Amy, G.L., 2000. Natural Organic Matter and Disinfection By-Products. American Chemical Society, pp. 2–14.
Bove, F., Shim, Y., Zeitz, P., 2002. Drinking water contaminants and adverse pregnancy outcomes: a review. Environ. Health Perspect. 110 (Suppl. 1), 61–74.
Boyer, T.H., Singer, P.C., 2008. Stoichiometry of removal of natural organic matter by ion exchange. Environ. Sci. Technol. 42 (2), 608–613.
Brand, W., de Jongh, C.M., van der Linden, S.C., Mennes, W., Puijker, L.M., van Leeuwen, C.J., van Wezel, A.P., Schriks, M., Heringa, M.B., 2013. Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays. Environ. Int. 55, 109–118.
Camel, V., Bermond, A., 1998. The use of ozone and associated oxidation processes in drinking water treatment. Water Res. 32 (11), 3208–3222.
Chen, T., Xu, Y., Liu, Z., Zhu, S., Shi, W., Cui, F., 2016. Evaluation of drinking water treatment combined filter backwash water recycling technology based on comet and micronucleus assay. J. Environ. Sci. 42, 61–70.
Conley, J.M., Evans, N., Mash, H., Rosenblum, L., Schenck, K., Glassmeyer, S., Furlong, E.T., Kolpin, D.W., Wilson, V.S., 2017. Comparison of in vitro estrogenic activity and estrogen concentrations in source and treated waters from 25 U.S. drinking water treatment plants. Sci. Total Environ. 579, 1610–1617.
Croué, J.P., Violleau, D., Bodaire, C., Legube, B., 1999. Removal of hydrophobic and hydrophilic constituents by anion exchange resin. Water Sci. Technol. 40 (9), 207–214.
Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol Oceanogr. Methods 6 (6), 230–235.
Escher, B.I., Dutt, M., Maylin, E., Tang, J.Y., Toze, S., Wolf, C.R., Lang, M., 2012. Water quality assessment using the AREc32 reporter gene assay indicative of the oxidative stress response pathway. J. Environ. Monit. 14 (11), 2877–2885.
Escher, B.I., van Daele, C., Dutt, M., Tang, J.Y., Altenburger, R., 2013. Most oxidative stress response in water samples comes from unknown chemicals: the need for effect-based water quality trigger values. Environ. Sci. Technol. 47 (13), 7002–7011.
Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P., Busch, W., Crago, J., Denslow, N.D., Dopp, E., Hilscherova, K., Humpage, A.R., Kumar, A., Grimaldi, M., Jayasinghe, B.S., Jarosova, B., Jia, A., Makarov, S., Maruya, K.A., Medvedev, A., Mehinto, A.C., Mendez, J.E., Poulsen, A., Prochazka, E., Richard, J., Schifferli, A., Schlenk, D., Scholz, S., Shiraishi, F., Snyder, S., Su, G., Tang, J.Y., van der Burg, B., van der Linden, S.C., Werner, I., Westerheide, S.D., Wong, C.K., Yang, M., Yeung, B.H., Zhang, X., Leusch, F.D., 2014. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. Environ. Sci. Technol. 48 (3), 1940–1956.
Farré, M.J., Day, S., Neale, P.A., Stalter, D., Tang, J.Y.M., Escher, B.I., 2013. Bioanalytical and chemical assessment of the disinfection by-product formation potential: role of organic matter. Water Res. 47 (14), 5409–5421.
Galjaard, G., Kamp, P.C., Koreman, E., 2009. SIX®: a new resin treatment technology for drinking water. Water Pract. Technol. 4 (4) wpt2009076-wpt2009076.
Gonsior, M., Schmitt-Kopplin, P., Stavklint, H., Richardson, S.D., Hertkorn, N., Bastviken, D., 2014. Changes in dissolved organic matter during the treatment processes of a drinking water plant in Sweden and formation of previously unknown disinfection byproducts. Environ. Sci. Technol. 48 (21), 12714–12722.
Hebert, A., Feliers, C., Lecarpentier, C., Neale, P.A., Schlichting, R., Thibert, S., Escher, B.I., 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.
Ledesma, J.L.J., Köhler, S.J., Futter, M.N., 2012. Long-term dynamics of dissolved organic carbon: implications for drinking water supply. Sci. Total Environ. 432, 1–11.
Leusch, F.D.L., Neale, P.A., Arnal, C., Aneck-Hahn, N.H., Balaguer, P., Bruchet, A., Escher, B.I., Esperanza, M., Grimaldi, M., Leroy, G., Scheurer, M., Schlichting, R., Schriks, M., Hebert, A., 2018. Analysis of endocrine activity in drinking water, surface water and treated wastewater from six countries. Water Res. 139, 10–18.
Li, X.-F., Mitch, W.A., 2018. Drinking water disinfection byproducts (DBPs) and human health effects: multidisciplinary challenges and opportunities. Environ. Sci. Technol. 52 (4), 1681–1689.
Liang, L., Singer, P.C., 2003. Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water. Environ. Sci. Technol. 37 (13), 2920–2928.
Liu, S., Li, Z., Dong, H., Goodman, B.A., Qiang, Z., 2017. Formation of iodo-trihalomethanes, iodo-acetic acids, and iodo-acetamides during chloramination of iodide-containing waters: factors influencing formation and reaction pathways. J. Hazard Mater. 321, 28–36.
Liviac, D., Creus, A., Marcos, R., 2010. Genotoxicity testing of three monohaloacetic acids in TK6 cells using the cytokinesis-block micronucleus assay. Mutagenesis 25 (5), 505–509.
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 (14), 4238–4247.
Metcalfe, D., Jarvis, P., Rockey, C., Judd, S., 2016. Pre-treatment of surface waters for ceramic microfiltration. Separ. Purif. Technol. 163, 173–180.
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.
Plewa, M.J., Wagner, E.D., Muellner, M.G., Hsu, K.-M., Richardson, S.D., 2008. Disinfection By-Products in Drinking Water. American Chemical Society,

pp. 36–50.
Postigo, C., Richardson, S.D., Barceló, D., 2017. Formation of iodo-trihalomethanes, iodo-haloacetic acids, and haloacetaldehydes during chlorination and chloramination of iodine containing waters in laboratory controlled reactions. J. Environ. Sci. 58, 127–134.
Richardson, S.D., Ternes, T.A., 2018. Water analysis: emerging contaminants and current issues. Anal. Chem. 90 (1), 398–428.
Richardson, S.D., Thruston, A.D., Caughran, T.V., Chen, P.H., Collette, T.W., Floyd, T.L., Schenck, K.M., Lykins, B.W., Sun, G.-r., Majetich, G., 1999. Identification of new drinking water disinfection byproducts formed in the presence of bromide. Environ. Sci. Technol. 33 (19), 3378–3383.
Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., DeMarini, D.M., 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. Mutat. Res. Rev. Mutat. Res. 636 (1), 178–242.
Rosenmai, A.K., Lundqvist, J., Le Godec, T., Ohlsson, A., Tröger, R., Hellman, B., Oskarsson, A., 2018a. In vitro bioanalysis of drinking water from source to tap. Water Res. 139, 272–280.
Sharma, V.K., Zboril, R., McDonald, T.J., 2014. Formation and toxicity of brominated disinfection byproducts during chlorination and chloramination of water: a review. Journal of Environmental Science and Health, Part B 49 (3), 212–228.
Siddiqui, M.S., Amy, G.L., Murphy, B.D., 1997. Ozone enhanced removal of natural organic matter from drinking water sources. Water Res. 31 (12), 3098–3106.
Stalter, D., Dutt, M., Escher, B.I., 2013. Headspace-free setup of in vitro bioassays for the evaluation of volatile disinfection by-products. Chem. Res. Toxicol. 26 (11), 1605–1614.
Stalter, D., O'Malley, E., von Gunten, U., Escher, B.I., 2016a. Fingerprinting the reactive toxicity pathways of 50 drinking water disinfection by-products. Water Res. 91, 19–30.
Stalter, D., Peters, L.I., O'Malley, E., Tang, J.Y.-M., Revalor, M., Farré, M.J., Watson, K., von Gunten, U., Escher, B.I., 2016b. Sample enrichment for bioanalytical assessment of disinfected drinking water: concentrating the polar, the volatiles, and the unknowns. Environ. Sci. Technol. 50 (12), 6495–6505.
Tang, J.Y., Busetti, F., Charrois, J.W., Escher, B.I., 2014. Which chemicals drive biological effects in wastewater and recycled water? Water Res. 60, 289–299.
Villanueva, C.M., Cordier, S., Font-Ribera, L., Salas, L.A., Levallois, P., 2015. Overview of disinfection by-products and associated health effects. Current Environmental Health Reports 2 (1), 107–115.
Wang, S., Zhang, H., Zheng, W., Wang, X., Andersen, M.E., Pi, J., He, G., Qu, W., 2013. Organic extract contaminants from drinking water activate nrf2-mediated antioxidant response in a human cell line. Environ. Sci. Technol. 47 (9), 4768–4777.
Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ. Sci. Technol. 37 (20), 4702–4708.