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# Effect-based monitoring of chemical hazards in drinking water from source to tap: seasonal trends over 2 years of sampling

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## Abstract

Drinking water producers have a far-reaching responsibility to provide safe, clean and wholesome drinking water, using water resources possibly effected by the thousands of chemicals used in societies' daily life. This study has monitored chemical hazards in drinking water from source to tap, using effect-based methods. The study was conducted at a Swedish drinking water treatment plant sourcing lake water and aimed to investigate potential seasonal variations in both the raw water and water after different treatment steps. Furthermore, a granular activated carbon (GAC) pilot facility was evaluated. Samples were analyzed for estrogenicity, androgenicity, aryl hydrocarbon receptor (AhR) activity, oxidative stress (Nrf2) response and genotoxicity (micronucleus formation). We observed seasonal differences in oxidative stress and genotoxic effects in both raw and drinking water with higher activities during the late fall of each year. The removal efficiency for both oxidative stress and genotoxicity was limited in the full-scale treatment process and occasionally the genotoxicity was also detected in outgoing drinking water from the treatment plant and in samples collected at consumers tap on the distribution network. AhR activity was present in all raw water samples and the removal was limited. Estrogenic activities were observed in most of the raw water samples but in contrast to the other parameters estrogenicity was effectively reduced by the full-scale conventional treatment. The GAC pilot treatment was generally more efficient than the full-scale conventional treatment in removing all observed bioactivities and could be a viable complement to the current treatment to assure drinking water free from genotoxic compounds. Genotoxic activities in drinking water was observed while all currently regulated chemical parameters were fulfilled. This highlights the need for effect-based monitoring in efforts to ensure the chemical safety of drinking water, as target chemical analysis of single compounds will overlook both unknown hazardous compounds as well as potential mixture effects.

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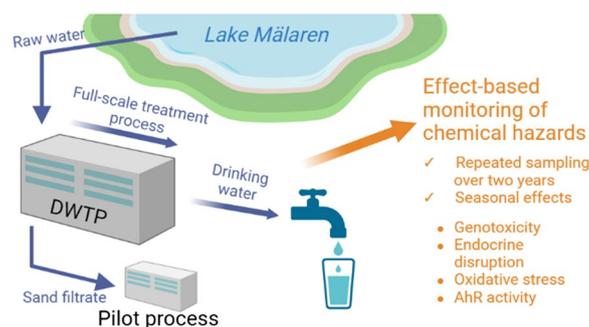
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## Graphical Abstract



## Introduction

Tens of thousands of chemical substances are present in the water environment, both naturally occurring compounds and anthropogenic pollutants. Some of these are hazardous and could pose a risk to human and/or environmental health. A daily human consumption of 2–2.5 L [1], the life-long exposure in all population groups and the fact that we often consume drinking water from the same water source for extended periods of our lives indicate that even low levels of chemical contaminants in drinking water can pose a threat to human health.

Chemical safety in drinking water is a question of high societal relevance and the drinking water producers have a far-reaching responsibility to provide clean and wholesome drinking water. It has been repeatedly reported [2–7] that the regulated as well as the well-known and most often analyzed environmental pollutants only explain a small fraction of the biological effects that can be observed in environmental water samples using effect-based methods. For some toxicity endpoints, as little as a few percent of the observed biological effects can be explained by the substances detected by chemical analysis [4]. The remaining, vast majority, of biological effects are caused by unknown compounds, their metabolites or mixture effects thereof. This has highlighted the need for a new approach for chemical drinking water safety, where chemical analysis needs to be complemented with other analytical tools with the capacity to capture also effects from unknown compounds and mixture effects.

Effect-based methods, often based on cultured mammalian cells and then referred to as *in vitro* bioassays, are designed to respond to compounds with a specific biological effect closely linked to toxic effects, e.g., oxidative

stress, genotoxicity or endocrine disruption. Effect-based methods thereby measure the total biological effects of all the compounds in a sample causing this specific biological effect, including both known and unknown chemicals and mixture effects. Effect-based methods are suggested as a valuable tool to improve drinking water safety assessment [8–10]. Conventional (coagulation–sedimentation–filtration) drinking water treatment processes are generally not designed to target chemical pollutants in the water and we have shown in previous studies limited toxicity-removal for effects detected with *in vitro* bioassays [2, 5, 6].

Drinking water can be produced from different water sources, including surface water, groundwater and artificially recharged groundwater. In Sweden, as an example, around 50% of the drinking water is produced from surface water, around 25% from groundwater and around 25% from artificially recharged groundwater. Lake Mälaren is the largest drinking water source in Sweden providing drinking water to around 2 million people. Using effect-based methods, we have previously shown [5, 6] adverse biological effects, including oxidative stress and genotoxicity, in the untreated raw water for one of the drinking water treatment plants using this lake as a raw water source. In some cases, these adverse biological effects were also present in the treated drinking water being distributed to the consumers, highlighting the limited removal efficiency of the currently used treatment technologies.

The aim of this study was to use effect-based methods to evaluate potential seasonal differences in the toxic effects of chemicals in drinking water from source to tap in water from Lake Mälaren, covering a range of

toxicity pathways. Furthermore, the aim was to evaluate how efficiently the observed biological effects could be removed by different drinking water treatment technologies. Third, the aim was to demonstrate the applicability of effect-based methods in monitoring programs.

## Materials and methods

### Lake Mälaren and Görvål drinking water facility

Mälaren is the third largest lake in Sweden, providing drinking water to around 2 million people, but is also the receiving water body for the effluent water from several large-scale wastewater treatment plants, landfills and industrial sites. Furthermore, the lake is impacted by urban storm water run-off from multiple cities and is also used for cargo shipping. Görvål drinking water treatment plant (DWTP) is using Lake Mälaren as the water source to produce drinking water to around 700 000 consumers in the Stockholm area. The lake is dimictic meaning that the lake develops a thermocline which is most pronounced in the summer time and a lake turnover occurs in the autumn (around mid-September to beginning of October) and in the spring time (around April). Temperatures in the raw water at 22 m (below the thermocline) vary normally between 0.5 and 15.7 °C. The point of abstraction is in a deep part of the lake, below the thermocline. Thus, the raw water to the Görvål DWTP is protected to some extent, kept cool and avoiding summer algal bloom that occurs mostly in the surface waters above the thermocline.

The full-scale treatment process consists of micro-sieving (250 µm) followed by coagulation treatment using aluminum sulfate, coagulation ( $\text{Al}_2(\text{SO}_4)_3$ ) and sedimentation/flotation, rapid sand filtration, biologically activated carbon (BAC) filtration, UV disinfection, and lastly dosing with monochloramine ( $\text{NH}_2\text{Cl}$ ) for secondary disinfection and lime for alkalization and pH adjustment. The BAC filters have a run time of approximately 10–15 years and a short empty bed contact time (EBCT) of approximately 4–6 min. The treatment plant has been in use for approximately 100 years, originally designed for removal of known raw water macropollutants as algae and particles. Furthermore, measured occurrence of organic micropollutants in the raw water [11], and the drinking water exceeding the PFAS threshold value recently implemented in the Swedish drinking water regulation, together with the earlier identified genotoxic activity in the raw water and drinking water source calls for additional treatment. Therefore, several treatment technologies have been investigated in pilot scale during the last decade including GAC filtration with different types of activated carbon and EBCTs.

### Sampling strategy

The main sampling was conducted at nine occasions during the period of January to November, 2021. At all occasions, samples were collected from the raw water (after the micro-sieve), after the sand filtration and of the finished drinking water being distributed to the consumers. During most sampling occasions, two samples were also collected from the distribution network, approximately 3.8 and 6 km from the plant, respectively. Based on previously published findings [5], indicating an increased oxidative stress response and genotoxicity in late fall, the three final sampling occasions were extended to also include a pilot scale water treatment facility, based on granular activated carbon (GAC) filtration or biofiltration on old fullscale used activated carbon (BAC) and biofiltration on filtralite (expanded clay). The aim was to evaluate the efficiency of GAC in comparison with biofiltration (biofilm interaction) BAC, respectively, to remove these activities in the water. Details regarding the activated carbon filter material and empty bed contact times (EBCTs) for each pilot column are presented in Table 1. All pilot scale filters were fed with the sand filtrate water from the full-scale process. Based on the findings in this study, a follow-up sampling for genotoxicity and oxidative stress only was conducted for select months during the period of January 2022 to February 2023, covering both raw and drinking water from the full-scale facility and selected samples from the GAC pilot. The sampling strategy is summarized in Table 1.

Samples were first enriched 5000 times over a solid phase extraction column (HLB eluted with ethanol) and then diluted 100 times (dilution factor 0.01) or more in the cell culture medium used for the effect-based methods (described below). The concentration of the water samples in the cell culture medium is presented as relative enrichment factor (REF) and calculated as the product of the enrichment factor and the dilution factor. In this case, the highest tested REF was 50. Details regarding the sampling procedure and sample preparation are presented in Additional file 1: Sect. "Introduction".

### Effect-based methods

The samples were evaluated with a panel of effect-based methods representing toxicity pathways which have been reported to be responsive to chemical pollutants in water samples [12] and are also of high relevance to human health. Endocrine disruption (e.g., estrogenic, androgenic and antiandrogenic effects) and genotoxicity (e.g., micronucleus formation) can cause serious adverse health effects. Oxidative stress response and aryl hydrocarbon receptor activity are biological markers that are activated by a broad range of pollutants and thereby serves

**Table 1** Overview of sampling strategy, samples collected and for which endpoints the samples were analyzed

	Main sampling events					Extended sampling events					Follow-up sampling for genotoxicity and oxidative stress											
	January 2021	March 2021	April 2021	May 2021	June 2021	August 2021	September 2021	October 2021	November 2021	January 2022	February 2022	March 2022	April 2022	May 2022	September 2022	October 2022	November 2022	December 2022	February 2023			
Raw water	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
After sand filter	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
Drinking water	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
Distribution network, 3.8 km	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
Distribution network, 6 km	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
Full-scale BAC reference (Norit 830, 5-6 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
BF-20 (Filtralite, 20 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
BAC-6 (Norit 830, 6 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
BAC-20 (Norit 830, 20 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
GAC1-20-a (Norit 1240, 20 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
GAC1-20-b (Norit 1240, 20 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
GAC2-20 (Filtrisorb 300, 20 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
GAC3-20 (Filtrisorb 300HA, 20 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
GAC4-20 (Filtrisorb 400, 20 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
GAC5-10 (Filtrisorb TL830, 10 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		
GAC5-20 (Filtrisorb TL830, 20 min)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		

● Indicates that the sample was analyzed for all effect-based parameters except micronucleus assay. ○ Indicates that the sample was analyzed for Nf2 activity, but not the other parameters. MN indicates that the sample was analyzed in the micronucleus assay. The two samples from November 2022 were collected early and late in the month, respectively. All BAC filters were filled with used activated carbon from full-scale filters that had been in use for 10–15 years and expected to have no remaining adsorptive function

**Table 2** Effect-based methods applied, reference compounds and effect concentration levels

Endpoint	Cell line	Stimulant treatment	Reference compound	Cytotoxicity test applied	Threshold for cytotoxicity	%-effect level chosen to define BEQ	International guideline document <sup>a</sup>
Androgen receptor agonism (AR ago)	AR-EcoScreen GR-KO M1	–	Dihydrotestosterone (DHT)	MTS	≤ 80% of vehicle control	EC <sub>20</sub>	OECD 458
Androgen receptor antagonism (AR anta)	AR-EcoScreen GR-KO M1	DHT	Hydroxyflutamide (OHF)	MTS	≤ 80% of vehicle control	IC <sub>30</sub>	OECD 458
Estrogen receptor agonism (ER)	T47D-ER	–	17β-estradiol (E2)	MTS	≤ 80% of vehicle control	EC <sub>20</sub>	OECD 455 <sup>b</sup>
Oxidative stress (Nrf2 activity)	MCF7 AREc32	–	tert-Butylhydroquinone (tBHQ)	MTS	≤ 80% of vehicle control	EC <sub>IR1.5</sub>	
Aryl hydrocarbon receptor activation (AhR)	DR-EcoScreen	–	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin (TCDD)	MTS	≤ 80% of vehicle control	EC <sub>10</sub>	
In vitro micronucleus test (MN)	TK-6	–	Mitomycin C	EMA +	≥ fourfold induction vs vehicle control	Not applicable <sup>c</sup>	OECD 487

<sup>a</sup> References to international guidelines given for information purposes. Assays were performed with some minor modifications from these guidelines. A full description of the experimental procedures is given in the Supporting Information

<sup>b</sup> The cell line used is not mentioned in OECD 455, but the assay has generally been performed in accordance with the guideline, with minor modifications

<sup>c</sup> For genotoxic effects, no BEQ is calculated. The samples are instead evaluated as genotoxic or non-genotoxic based on a > threefold induction vs vehicle control

as environmental sensors for the pollution burden. A general description of the applied methods is presented in Table 2. The bioassays used were selected based on the availability of internationally harmonized test guidelines (e.g., OECD test guidelines) or that they are commonly applied methods in this field of science. A detailed methods description, including cell culture conditions, is presented in the Additional file 1: Sect. "Materials and Methods", and has also been previously described [2, 5, 13]. Most assays are based on the reporter gene technology, where the gene expression of a reporter protein is under the regulation of a DNA sequence that is responsive to the class of hazardous chemicals that are to be analyzed. Genotoxicity was evaluated by a flow cytometry-based micronucleus assay. Cell viability was monitored during all experiments, using the MTS assay for reporter gene assays and with EMA + scoring in the cells in the micronucleus assay, to ensure that analyses were conducted under non-cytotoxic conditions. All in vitro bioassays should be performed under non-cytotoxic conditions to ensure that the results obtained are specific and reliable. Further information on the MTS assay and EMA + scoring is available in the Supporting Information. For all reporter gene-based assays, a dilution series of a reference compound was analyzed in parallel with the samples, both as a quality control and to allow the calculation of bioequivalent concentration (BEQ) values for bioactive samples.

### Data evaluation

Initially, any exposure concentration of the concentrated water samples causing cytotoxicity to the cells in question were excluded from further analysis (i.e., these concentrations were not included in the calculation of EC values). The cutoff for cytotoxicity in the respective cell line is presented in Table 2.

For the micronucleus assay, data were normalized to the vehicle control, and if any of the analyzed REF values of a water samples, at non-cytotoxic concentrations, induced the micronucleus formation by threefold or more [14], compared to the vehicle control, the sample was classified as positive for genotoxicity.

Effects observed in reporter gene assays were normalized as the following: (a) agonistic estrogen and androgen responses as well as AhR activity were normalized to vehicle control and assay maximum defined by the mean activity of the highest exposure concentration of the reference compound, (b) antagonistic androgen receptor activity was normalized to the DHT spiked negative control, and (c) oxidative stress response was normalized to the vehicle control. Using these normalized data, effect concentrations (specific for each assay, Table 2) were calculated for each sample and reference compound. The effect concentration (EC) is the concentration (in this case the REF) needed to exert a specific biological effect. For example, the EC<sub>20</sub> is the concentration needed to exert 20% of the maximum effect in the assay. Bioequivalent concentrations (BEQs) were then calculated using

the following Eq. [15, 16], where  $EC_x$  is the effect concentration for that specific sample and assay:

$$BEQ = \frac{(EC_x)_{reference\ compound}}{(EC_x)_{sample}}$$

Results tables for BEQ values have been color-coded with the MS Excel Color Scales tool. The limit of detection (LOD) for each run was calculated based on  $EC_x$  of the reference compound and the highest tested REF. The LODs are presented in Additional file 2: Table S1.

### Correlation analysis

Data were statistically analyzed with Spearman's rank correlation test. Initially, correlations were investigated between AhR, Nrf2, AR, ER and MN for all data from the full-scale treatment process. Then, correlations were evaluated specifically for raw water and drinking water, both separately and combined, both for the entire data set and for defined time periods. Correlations were only evaluated if more than five observations were available. For samples that were below the LOD for a specific assay, 10% of the LOD for that specific assay run has been used as the metric in the correlation analyses. For all analyses, correlations were defined as statistically significant if the  $p$  value was  $< 0.05$ .

## Results and discussion

### Seasonal differences in oxidative stress and genotoxic effects in raw and drinking water

No detected or only low levels of oxidative stress were observed in raw samples collected during the time January to May 2021 (Table 3). During the second half of the year, higher oxidative stress activity was observed in all raw water samples. The highest activity was observed in the raw water collected in November (77 tBHQeq/L) and the full-scale treatment process had a removal efficiency for the oxidative stress of only around 45%. No further decrease was seen during transport in the distribution network and consequently, the finished drinking water and the two samples collected at consumers' taps exhibited comparable oxidative stress activities, in the range of 41–43 tBHQeq/L.

A similar pattern was observed for the genotoxic effects, where almost all samples collected during August to November of 2021 were classified as genotoxic (Table 4). However, genotoxicity was also observed in a few samples collected during the spring of 2021, which were not causing oxidative stress. Notably, the genotoxicity was not removed by the full-scale treatment process in the occasions, where we observed higher genotoxic potency in the raw water ( $> 10$ -fold increase in micronucleus formation compared to vehicle control) (Table 4),

while the full-scale process could remove the activity in the cases, where the genotoxicity in the raw water was lower (April and August, 2021).

Based on these findings and the potential severity of genotoxic effects in finished drinking water, we decided to perform a follow-up sampling campaign focusing only on oxidative stress and genotoxicity for an additional year of monthly sampling (January 2022 to February 2023, except for the months June–August 2022 and January 2023). During this year, a similar trend was observed with absence of genotoxicity in both the raw water and drinking water during the first half of the year and genotoxicity being observed in all raw water samples collected in September 2022 to February 2023. The full-scale treatment process was able to remove the genotoxic effects at the two sampling occasions, where the observed genotoxicity in the raw water had the lowest potency (first sampling occasion of November 2022 and December 2022), as well as in February 2023 (Table 4). Oxidative stress was analyzed for selected raw water and drinking water samples from this follow-up sampling campaign. Unlike during the main sampling campaign, we did not observe a clear pattern of high oxidative stress response in samples with a high genotoxic potential. Raw water samples exhibited oxidative stress activity in the range of 9.8–19.8  $\mu\text{g tBHQeq/L}$  with only one sample having an activity  $< \text{LOD}$ . Activities in the higher end of this range were observed both in samples with a high genotoxic potential (e.g., second sampling occasion in November 2022) and samples with no genotoxic activity (e.g., March 2022).

Oxidative stress is one of multiple mechanisms that can cause genotoxicity. To evaluate if the genotoxicity observed in these samples could be linked to oxidative stress, we performed correlation analyses comparing the oxidative stress (expressed as tBHQ equivalent concentrations) with the micronucleus formation rate (fold change vs vehicle control) in the samples collected in the full-scale process. The micronucleus formation rate was found to be statistically significantly correlated to the oxidative stress response in this entire data set ( $r=0.42$ ,  $p=0.004$ ,  $n=44$ ) (Additional file 2: Table S2 Correlation analyses). When analyzing only raw water and drinking water, both separately and combined, for the entire study period, there were no statistically significant correlation between micronucleus formation rate and oxidative stress response (Additional file 2: Table S2). However, there was a strong correlation in the raw and drinking water samples from September to December in 2021 and 2022 ( $r=0.57$ ,  $p=0.02$ ,  $n=16$ ), indicating a high seasonal impact on the correlation (Additional file 2: Table S2). These results are indicating either that micronucleus formation rate and oxidative stress response were partly caused by the same compounds or by compounds that

**Table 3** Oxidative stress response in the samples, analyzed with the AREc32 cell line, expressed as bioequivalent concentrations of tBHQ ( $\mu\text{g tBHQeq/L}$ )

Main sampling events	Main sampling events					Extended sampling events					Follow-up sampling for genotoxicity and oxidative stress								
	January 2021	March 2021	April 2021	May 2021	June 2021	August 2021	September 2021	October 2021	November 2021	January 2022	February 2022	March 2022	April 2022	May 2022	September 2022	October 2022	November 2022	December 2022	February 2023
Raw water	4.5	<LOD	<LOD	5.4	15.2	12.0	34.5	25.6	77.4	9.8	n.t.	18.4	9.7	n.t.	<LOD	10.7	19.8	18.2	n.t.
After sand filter	2.6	<LOD	<LOD	<LOD	21.4	5.3	13.6	27.0	48.0	n.t.	n.t.	18.3	14.2	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Drinking water	<LOD	<LOD	<LOD	4.2	34.2	12.4	16.4	23.2	42.8	9.1	n.t.	18.3	14.2	n.t.	<LOD	12.9	13.2	22.7	12.5
Distribution network, 3.8 km	3.2	<LOD	<LOD	<LOD	20.9	4.9	19.6	23.8	42.4	n.t.	n.t.	18.3	14.2	n.t.	<LOD	12.9	13.2	22.7	n.t.
Distribution network, 6 km	<LOD	11.6	<LOD	<LOD	10.5	<LOD	<LOD	23.5	41.4	n.t.	n.t.	18.3	14.2	n.t.	<LOD	12.9	13.2	22.7	n.t.
After sand filter (same as above, presented for reference)							13.6	27.0	48.0										
Full-scale BAC reference (Norit 830, 5-6 min)							<LOD	23.5	43.6										
BR-20 (Filtrisorb TL830, 20 min)							10.2	20.9	42.8										
BAC-e (Norit 830, 6 min)							<LOD	12.6	39.6										
GACI-20-a (Norit 1240, 20 min)							<LOD	<LOD	41.3										
GACI-20-b (Norit 1240, 20 min)							<LOD	<LOD	36.7										
GAC2-20 (Filtrisorb 300, 20 min)							<LOD	<LOD	18.6										
GAC3-20 (Filtrisorb 300HA, 20 min)							<LOD	<LOD	<LOD										
GAC4-20 (Filtrisorb 400, 20 min)							<LOD	<LOD	<LOD										
GAC5-10 (Filtrisorb TL830, 10 min)							<LOD	<LOD	<LOD										
GAC5-20 (Filtrisorb TL830, 20 min)							<LOD	<LOD	<LOD										

Cells that are left blank represent samples that were not analyzed for oxidative stress response. The results have been color-coded with the MS Excel Color Scales tool, with green colors representing lower oxidative stress response and red colors representing higher oxidative stress responses. Samples that were collected but not analyzed for oxidative stress response is marked with n.t. not tested

**Table 4** Genotoxic effects in the samples, analyzed with an in vitro micronucleus test in Tk-6 cells

Main sampling events	Extended sampling events					Follow-up sampling for genotoxicity and oxidative stress														
	January 2021	March 2021	April 2021	May 2021	June 2021	August 2021	September 2021	October 2021	November 2021	January 2022	February 2022	March 2022	April 2022	May 2022	September 2022	October 2022	November 2022	December 2022	February 2023	
Raw water	2.36	10.27	3.29	2.55	1.74	6.20	12.13	16.36	11.95	2.41	1.48	2.82	1.51	2.84	3.68	6.95	3.20	7.49	3.10	6.65
After sand filter								6.51	5.62	1.41	1.41			1.92	14.46	3.61	1.18	14.56	1.88	2.86
Drinking water	2.29	3.59	1.55	1.92	1.29	1.55	4.67	3.66	9.88	1.49	1.11	2.41	1.23	1.90	3.73	3.99	1.88	5.98	1.91	2.11
Distribution network, 3.8 km								7.57	11.00								0.96			
Distribution network, 6 km							2.43	6.80												
After sand filter (same as above, presented for reference)								6.51	5.62	1.41	1.41			1.92	14.46	3.61	1.18	14.56	1.88	2.86
Full-scale BAC-reference (Norit 830, 5-6 min)							12.05	4.29	16.23	1.50				1.50	2.62			6.66	2.60	3.98
BF-20 (Filtralite, 20 min)							1.80	2.44	5.27											
BAC-6 (Norit 830, 6 min)									3.88											
BAC-20 (Norit 830, 20 min)								1.28	4.18	1.65				1.41	1.77	1.00				1.79
GAC1-20-a (Norit 1240, 20 min)								1.20	1.34	1.30				1.66	1.25	0.99		1.52		1.87
GAC1-20-b (Norit 1240, 20 min)								1.02	0.87	1.35				1.35	1.89	0.81		1.12		1.55
GAC2-20 (Filtrisorb 300, 20 min)									0.74	1.49				1.64	1.67	1.09		1.47		1.87
GAC3-20 (Filtrisorb 300HA, 20 min)								1.67	0.92									1.25		1.38
GAC4-20 (Filtrisorb 400, 20 min)																		1.13		0.96
GAC5-10 (Filtrisorb T1830, 10 min)																				
GAC5-20 (Filtrisorb T1830, 20 min)																				

Data is presented as fold change compared to vehicle control, at REF 50. Samples with more than three times increase in the number of micronucleus events, compared to the vehicle control, were classified as genotoxic and are highlighted in red. Cells that are left blank represent samples that were not analyzed for genotoxicity

were co-varying during these sampling occasions. Using Spearman correlation analysis on the entire data set from the full-scale process, we also observed statistically significant correlations between the micronucleus formation rate and androgenic activity ( $r=0.59$ ,  $p=0.001$ ,  $n=27$ ). This correlation was also statistically significant when evaluating only the raw and drinking water, both separately and combined, for the entire study period (Additional file 2: Table S2). In the entire data set, there was also a statistically significant correlation between aryl hydrocarbon receptor activity and androgenic activity ( $r=0.55$ ,  $p=0.00008$ ,  $n=46$ ). It should, however, be noted that androgenic activity above LOD was only observed in 7 samples, and the rest of the samples were set at 10% of LOD for the correlation analyses.

As described above, this study reports genotoxic activity in the raw water used for drinking water production. It is noteworthy that the removal efficiency for the genotoxic effects in the full-scale process is limited and that in more than one-third of the sampling occasions, the drinking water being distributed to the consumers was genotoxic in vitro. Genotoxicity is an adverse effect with potential serious health effects. Genotoxic effects can be caused both via a direct effect on the DNA and via indirect mechanisms. If the genotoxicity is caused by compounds with a direct effect on the DNA, it is generally assumed that there is no safe exposure level [17], while genotoxic compounds with an indirect mechanism might have a threshold for the activity and it would thereby be possible to calculate an acceptable level of exposure. The correlation between genotoxic effect and oxidative stress indicates an indirect mechanism, but there may also be a direct mechanism involved. In this case, where it still remains unclear which compound(s) that are causing the activity, it is not possible to determine if this effect has a safe exposure level or not. It is worth mentioning that all chemical parameters regulated in the Swedish drinking water legislation (which is based on the European Drinking Water Directive [18]) have been routinely monitored during this study time (data not shown) with no exceedances of the parametric values. Hence, the compound(s) causing this observed oxidative stress effect and genotoxicity in the water does not seem to be covered by the current drinking water regulation.

We have previously reported both relatively high oxidative stress response and genotoxicity in water samples collected at this specific water treatment plant [5]. In that study, water samples were collected in November 2019 and May 2020 and the raw water from November had both a relatively high oxidative stress activity (69.8 tBHQeq/L) and was genotoxic. The raw water collected in May 2020 had a clearly lower oxidative stress activity (8.3 tBHQeq/L), but was still genotoxic. Furthermore, we

have previously reported a case from a different water utility, where the drinking water had markedly higher oxidative stress activity after artificial infiltration, as compared to the raw water being infiltrated [2].

Shi et al. [19] evaluated source and drinking water from Chinese rivers and lakes for genotoxicity with both micronucleus assay and Ames test (assessing mutagenicity). Raw water from one of the three water sources was found to be genotoxic in the micronucleus assay, but the genotoxicity was removed by conventional water treatment methods. Feretti et al. [20] studied genotoxicity in raw and finished drinking water in four distribution systems in Sardinia, Italy, using a battery of in vitro tests. Genotoxicity was observed in raw water, which was not reduced by the pre-oxidant/disinfection treatment. In another system, genotoxicity was introduced by the pre-oxidant/disinfection process from non-genotoxic raw water.

In this study, the oxidative stress activity is expressed as bioanalytical equivalent concentrations, namely tBHQ equivalents. In the literature, there are a number of studies reporting oxidative stress activity in surface and drinking water, but only reporting the effect concentration induction ratio 1.5 ( $EC_{IR1.5}$ ), expressed as relative enrichment factor (REF). For the following, it is worth mentioning that a lower effect concentration of a sample expressed as REF is indicating a higher oxidative stress activity. The highest oxidative stress responses observed in raw and drinking water in this study are in a similar range as what has been reported by others in similar water types. For example, Escher *et al* [21] reported  $EC_{IR1.5}$  values of 7.5–12, Farre et al [22] values of 7.8–9.9 and Hashmi et al [23] a value of 12, all in surface waters. Our highest observed oxidative stress response (November 2021) had an  $EC_{IR1.5}$  value of 7.1. In finished drinking water,  $EC_{IR1.5}$  values have been reported in the range of 2.7 to >150 [21, 24, 25]. In our study, the finished drinking water sample having the highest potency for oxidative stress (November 2021) had an  $EC_{IR1.5}$  value of 12.8. It should, however, be noted that many of the studies in the literature include final chlorination of the water with considerably higher doses than what is the case in Swedish drinking water production, which might cause formation of oxidative stress-inducing disinfection byproducts.

#### AhR activity observed in most samples

AhR activity was observed in all analyzed samples from the full-scale process, including raw water, finished drinking water and samples from the distribution network (Additional file 2: Table S3). Observed activities varied over the year of sampling, with the lowest activities ( $\leq 63$  pg TCDDeq/L) being observed in January and May while the highest activities were observed in April

(in the range of 640–1100 pg TCDDeq/L). In general, the removal efficiency for the AhR activity observed in raw water was limited in the full-scale process, in some cases even negative (i.e., higher activity in the finished drinking water than in the raw water).

We have previously [5] reported AhR activity in the raw water to this drinking water facility in the range of 19–35 pg TCDDeq/L and in drinking water ranging from below the limit of detection to 52 pg TCDDeq/L. The activities observed in this study are in most cases, and especially in April 2021, higher than those reported in the previous study. In another study in seven drinking water treatment plants in Sweden using river water as raw water, AhR activities ranged from below the detection limit to 2 157 pg TCDDeq/L in raw water and were below the detection limit in all drinking water samples [2]. Furthermore, we have reported AhR activities in drinking water from five Swedish drinking water facilities ranging from below the limit of detection to 290 pg TCDDeq/L [13]. In the same study, we analyzed drinking water from the same facilities that had been stored in glass bottles for around 25 years and found AhR activities in those stored waters in the range of 160 to 1000 pg TCDDeq/L. Escher et al. [12] have reported an AhR activity of 24 pg TCDDeq/L in drinking water (calculated from the EC<sub>10</sub> value [26]), although with a different cell model than used in this study.

#### **Observed estrogenic effects were far below the proposed health-based trigger value**

Estrogenic effects were observed in both the raw lake water and treated drinking water at six out of nine sampling occasions (Additional file 2: Table S4). The estrogenicity ranged from 7 to 317 pg E2eq/L and 11–79 pg E2eq/L for raw water and drinking water, respectively. The highest estrogenic activity in raw water was observed in September and in drinking water in June. Estrogenic activities were also observed in samples collected on the distribution network, on multiple occasions. The removal efficiency for the estrogenic effects varied between the sampling occasions, but was high (93%) in the case, where the estrogenic activity in the raw water was the highest (September 2021).

The European Union has established a guidance value of 1 ng E2/L in the watch list of substances of concern for water intended for human consumption [18, 27]. Furthermore, California State Water Resources Control Board has established a monitoring trigger level of 3.5 ng E2eq/L for estrogenic effects in treated wastewater intended for potable reuse [28]. The estrogenic effects

observed in these samples, both the raw water and the drinking water, as well as the samples from the distribution network, are well below these suggested trigger values and does not pose a risk to human health.

We have previously reported low or undetectable levels of estrogenicity in raw and drinking water from Sweden. In samples from seven facilities along river Göta Älv, Sweden, no estrogenicity was observed neither in the raw water nor in the finished drinking water [2]. In an earlier study conducted at Görväln drinking water facility, we observed estrogenic effects in the range of 20 pg E2eq/L in raw and treated water [5]. In another study, we compared the estrogenic activity in drinking water from five Swedish drinking water treatment plants (DWTPs) and found that three out of five samples were inactive and the remaining two had an estrogenicity in the range of 3–16 pg E2eq/L [13]. Neale et al. [25] have reported multiple occasions of estrogenicity above 1 ng E2eq/L in the raw water, in a study of the drinking water quality in the greater Paris area. The applied drinking water treatment technologies could, however, efficiently reduce the estrogenicity in the water. Shi et al. [19] have also reported high removal efficiency of estrogenic effects during drinking water treatment in Chinese facilities producing drinking water from surface water. However, in one of the plants, the finished drinking water had estrogenic activities in the range of 4–5 ng E2eq/L, which is above the guidance level and monitoring trigger level mentioned above. Enault et al. [29] summarized estrogenicity in water from both conventional DWTPs and advanced DWTPs (including oxidation, adsorption and/or membrane filtration) in a recent review on effect-based water quality monitoring. In conventional DWTPs, the removal efficiency for estrogenicity was >80% for approximately 80% of the studied sites and for the plants with advanced processes, around 95% of the studied sites removed any estrogenicity to below the limit of detection.

#### **Only few observations with androgenic and antiandrogenic effects**

Androgenic effects in the raw and drinking water were only observed at three sampling occasions (Additional file 2: Table S5). All samples showing androgenic effects had a BEQ value clearly below the proposed effect-based trigger values for human consumption ranging from 4.5 to 11 ng DHTeq/L [30, 31]. No antiandrogenic effects were observed in the samples except for in one raw water sample, one after sand filtration and one sample from the distribution network, but very close to the limit of detection (Additional file 2: Table S6).

### **Pilot GAC filtration was efficiently removing oxidative stress, AhR activity and genotoxic effects**

Different adsorption filters and biofilters were investigated to evaluate the removal of biological activities observed in the feed water. Details on the materials used in each column, as well as the contact time, are presented in the results tables. All filters were fed with sand filtrate water, so the measured activity for a specific filter should be compared to the sand filtrate sample for that sampling occasion, to evaluate the removal efficiency. The results from full-scale BAC reference (Norit 830, 4–6 min) is given for comparison with the pilot results and especially pilot column BAC-6 (6 min EBCT) having similar operating conditions to the full-scale filter.

#### **Biofiltration (BAC filters)**

The pilot filters BAC-6 (6 min EBCT) and BAC-20 (20 min EBCT) were filled with saturated GAC, that had been used for 10–15 years in the full-scale process and can, therefore, be considered as biofilters. Filtralite was used in pilot column BF-20 (20 min EBCT) which was filled with new filter material upon the start of the pilot trials in March 2021 and was expected to develop into a biofilter over time, but to have no or very limited removal initially. Filtralite, being an expanded clay material, is not supposed to have any adsorptive properties and removal is only expected through biodegradation.

Regarding genotoxicity, the full-scale BAC reference filter that was sampled monthly from September to November 2021 was not able to remove the genotoxicity measured in the feed (sand filtrate) water, nor was the pilot filter BAC-6 (samples in November 2021), both with short EBCT:s of approximately 6 min (Table 4). Meanwhile, the Filtralite filter (BF-20) and BAC filter BAC-20 having EBCT:s of 20 min produced water without genotoxic activity in September and October 2021, indicating that a longer residence time in the filters could increase the removal of the compound(s) causing genotoxic effects. However, as genotoxic activity was detected in effluent water from these filters in November 2021 it seems that the removal efficiency can vary over time which raises questions regarding the suitability of biofiltration for stable removal of genotoxic activity.

Oxidative stress was detected in the feed (sand filtrate) water at all three sampling events, September to November 2021, when this parameter was analyzed in the biofilter effluents, and the removal was practically non-existent in most biofilters, with a few exceptions (Table 3).

Removal of AhR activity was limited in the full-scale BAC and the Filtralite filter (BF-20), even if they were able to reduce or even eliminate the activity in the feed water at times (Additional file 2: Table S3).

Estrogen receptor activity was detected in the feed water at two out of three sampling events, September to November 2021, and in October 2021 also in two of the biofilters (full-scale BAC reference and BF-20) while no ER activity was detected in any of the other biofilter effluent samples (Additional file 2 Table S4). This indicates that ER activating compounds are more biodegradable compared to those inducing oxidative stress and AhR activity.

#### **Adsorption filters (GAC filters)**

Most pilot GAC filters were filled with new GAC (type specified in results tables) prior to the start of the pilot trials in March 2021 while filters GAC5-10 (10 min EBCT) and GAC5-20 (20 min EBCT) were filled with new GAC and put in operation in November 2022. All GAC filters, except GAC5-10, had an EBCT of 20 min.

GAC filtration was very efficient in reducing the genotoxic activity in the feed water and no genotoxic effects could be detected in any of the GAC filtrates for the entire duration of the study, with the exception of one sample: GAC1-20-a in November 2022 (Table 4). This filter had treated 39 898 bed volumes at that time.

Oxidative stress was analyzed for the pilot filters in the fall of 2021 (Table 3). For the first two sampling occasions in September and October all GAC filters had reduced the oxidative stress response to <LOD. In November, however, activities were detected in GAC1-20-a and GAC2-20 indicating that compounds causing oxidative stress may exhibit earlier breakthrough compared to the genotoxic activity. As GAC3-20 and GAC4-20 still reduced the oxidative stress to <LOD further studies are needed to conclude the behavior of oxidative stress inducing compounds during GAC filtration and how the GAC type may influence the removal efficiency and duration.

The GAC filters were very efficiently removing AhR-active compounds with the exception of GAC1-20-a, where the filtrate had AhR activity close to the LOD during two of the three sampling events. This difference in performance is not expected to be an effect from different GAC types used as the duplicate filter GAC1-20-b was performing similar to the other filters. The effective removal could be an effect of hydrophobic properties of AhR-active compounds.

No estrogenic activity could be detected in any of the GAC filtrates for the two sampling events, where ER-active compounds were measured in the feed water indicating that these compounds are not only biodegradable but also readily adsorbable. Sampling over a longer period of GAC operation is needed to further study when these compounds would exhibit breakthrough.

In summary, adsorption function in the GAC filters was essential to ensure a stable removal of compounds causing genotoxicity. In this study there were no observed differences in performance from different GACs used nor between GAC filters with 10 and 20 min EBCT but an earlier breakthrough would be expected with 10 min EBCT as a double volume of water is filtered compared to 20 min EBCT. AhR-active compounds was only effectively removed in the GAC filters, while the biological filters had limited effect. This shows that adsorption was needed to reduce the presence of the AhR-active compounds present in the untreated water. ER-activating compounds were effectively removed in both GAC and BAC filters indicating that removal can occur both through adsorption and biodegradation. Compounds causing oxidative stress was less effectively removed overall and already after a few months operation activities could be detected in the filtrate samples of most GAC filters indicating an earlier breakthrough of these compounds compared to those causing ER-AhR or genotoxic activity.

### The need for a new approach for drinking water safety

Numerous studies [2, 3, 7] have shown that the well-known and often analyzed single chemicals in most cases only explain a small fraction of the toxicity in environmental water samples, measured with effect-based methods *in vitro*. The majority of the effects are instead attributed to unknown chemicals or mixture effects, also when hundreds of single chemicals are included in the analysis—or the fact that compounds can cause biological effects also below their chemical detection limit. In this study, we report that both the raw water and the treated water being distributed to consumers were contaminated with unknown compound(s) causing oxidative stress and genotoxicity, at multiple occasions. In some cases, the genotoxic effects were also observed in samples collected from consumers tap. All this while all regulated chemical parameters were below legal thresholds. This clearly shows that the current drinking water regulation is not comprehensive enough, as chemical hazards of such severity as linked to genotoxicity, can be present in the water without being discovered by the mandated testing.

The efficient removal of the genotoxic activity during pilot scale GAC filtrations shows that with the right

treatment, public health can be protected. In order to optimize current treatment or to choose the right new treatments when complementing existing treatment plants or building new ones, effect-based methods need to be used so that health hazards from unknown compounds and mixtures can be discovered and handled accordingly.

Effect-based methods are ideal tools to complement current efforts for chemical safety in drinking water, as they can detect effects of both known and unknown chemicals as well as mixture effects. They can thereby serve as an early screening tool for chemical hazards and play an important role in the risk-based approach mandated in the recently revised European Drinking Water Directive. A recent proposal from the Commission of the European Union is mandating the use of effect-based methods in the environmental monitoring of hazardous chemicals in groundwater and surface waters, which highlights that the effect-based methods are gaining regulatory acceptance [32].

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12302-024-00875-z>.

**Additional file 1.** Details regarding sampling procedure, sample preparation, effect-based methods and cell culturing conditions.

**Additional file 2.** Limits of detection, correlation analyzes, calculated BEQs for AhR, ER and AR assays.

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### Author contributions

JL, EL, HE and AO planned the study. GM and ES performed all laboratory analyses. JL handled the data and performed all data analysis and calculations of BEQ values. JL, EL and HE and AO participated in the interpretation of the results. JL, EL and AO wrote the main manuscript text. All authors critically reviewed the manuscript.

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### Availability of data and materials

Data are provided within the manuscript or Additional files.

### Declarations

#### Competing interests

JL and A.O. are the founders and owners of BioCell Analytica Uppsala AB, a company providing effect-based testing services to the water sector. E.L., G.M., E.S. are or have been employees of the same company. All other 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.

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