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Integration of target analyses, non-target screening and effect-based monitoring to assess OMP related water quality changes in drinking water treatment

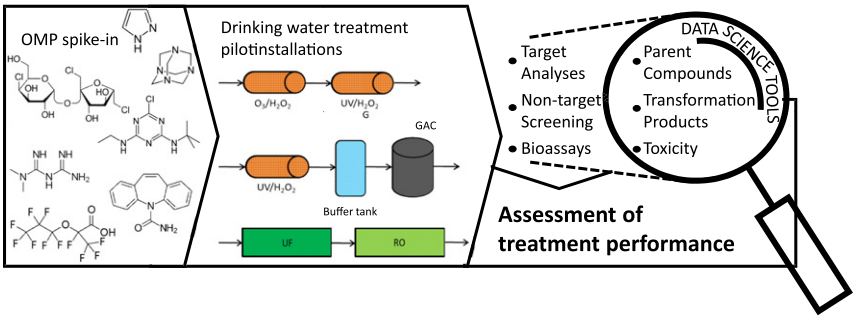
Andrea M. Brunner [a,*,1], Cheryl Bertelkamp [a,1], Milou M.L. Dingemans [a], Annemieke Kolkman [a], Bas Wols [a], Danny Harmsen [a], Wolter Siegers [a], Bram J. Martijn [b], Wim A. Oorthuizen [c], Thomas L. ter Laak [a,d]

[a] KWR Water Research Institute, Nieuwegein, the Netherlands
[b] PWN Technologies, Andijk, the Netherlands
[c] Dunea, Zoetermeer, the Netherlands
[d] Univerisity of Amsterdam, Amsterdam, the Netherlands

HIGHLIGHTS

- Combination target analyses, NTS and bioassays essential to determine water quality
- Performance assessment of OMP removal with different drinking water treatments
- Monitoring of parent compounds, transformation products and toxicity
- Focus on features that may pose a risk to human and environmental health
- Removal for some OMPs (e.g. methenamine, melamine, TFA) measured for the first time

GRAPHICAL ABSTRACT



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ABSTRACT

The ever-increasing production and use of chemicals lead to the occurrence of organic micro-pollutants (OMPs) in drinking water sources, and consequently the need for their removal during drinking water treatment. Due to the sheer number of OMPs, monitoring using targeted chemical analyses alone is not sufficient to assess drinking water quality as well as changes thereof during treatment. High-resolution mass spectrometry (HRMS) based non-target screening (NTS) as well as effect-based monitoring using bioassays are promising monitoring tools for a more complete assessment of water quality and treatment performance. Here, we developed a strategy that integrates data from chemical target analyses, NTS and bioassays. We applied it to the assessment of OMP related water quality changes at three drinking water treatment pilot installations. These installations included advanced oxidation processes, ultrafiltration in combination with reverse osmosis, and granular activated carbon filtration. OMPs relevant for the drinking water sector were spiked into the water treated in these installations. Target analyses, NTS and bioassays were performed on samples from all three installations. The NTS data was screened for predicted and known transformation products of the spike-in compounds. In parallel, trend profiles of NTS features were evaluated using multivariate analysis methods. Through integration of the chemical data

Abbreviations: GAC, granular activated carbon; methoxymethyl, hexa; HMMM, melamine; HC, hierarchical clustering; HRMS, high-resolution mass spectrometry; NTS, non-target screening; OMP, organic micro-pollutant; RO, reverse osmosis; TFA, trifluoroacetic acid; TPPO, triphenylphosphine oxide; UF, ultra-filtration.

* Corresponding author.
E-mail address: andrea.brunner@kwrwater.nl (A.M. Brunner).

[1] These authors contributed equally to this work.

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with the biological effect-based results potential toxicity was accounted for during prioritization. Together, the synergy of the three analytical methods allowed the monitoring of OMPs and transformation products, as well as the integrative biological effects of the mixture of chemicals. Through efficient analysis, visualization and interpretation of complex data, the developed strategy enabled to assess water quality and the impact of water treatment from multiple perspectives. Such information could not be obtained by any of the three methods alone. The developed strategy thereby provides drinking water companies with an integrative tool for comprehensive water quality assessment.

1. Introduction

1.1. Organic micro-pollutants (OMPs) and drinking water

With the production and use of chemicals exponentially on the rise, the occurrence of organic micro-pollutants (OMPs) in the environment, and consequently in drinking water sources, is increasing. Facilitated by improved analytical techniques, OMPs have been detected in low concentrations (ng/L - μg/L) in a number of drinking water sources, both in The Netherlands and worldwide (Kolpin et al., 2002; Verliefde et al., 2007; Mompelat et al., 2009; Houtman et al., 2014). Drinking water companies are faced with the challenge to adequately remove these compounds and prevent their presence in drinking water. To gain more insight into the OMP removal capacity of different drinking water treatment processes, the Dutch surface water companies assess OMP removal capacity of processes that are currently operated or aimed to be implemented in the near future every 5 years with spike-in pilot-scale experiments. The OMPs spiked into the treatment feeds are selected based on presence in Dutch surface water, toxicity, available knowledge on potential effective treatment options, chemical properties, detectability and availability.

In past pilot-scale experiments as well as in a large number of studies investigating OMP removal in different treatment processes (Verliefde et al., 2009; Wols et al., 2013), OMP removal was typically assessed through target analyses. However, water treatment processes can potentially result in the formation of transformation products (TPs) some of which have been shown to pose environmental and human health risks similar to or greater than the parent compounds they originate from (Belfroid et al., 1998; Sinclair and Boxall, 2003; de Jongh et al., 2012). Target analyses of a defined number of regulated priority substances alone are therefore not sufficient to assess drinking water quality. In particular, as target analyses do not allow distinction between removal and transformation of a compound. Instead, comprehensive non-target screening (NTS) and effect-based bioassays can be applied to detect a multitude of chemicals and their effect simultaneously. Such methods can support the water sector in realistically assessing the potential human and environmental health risks of (emerging) OMPs.

1.2. Non-target screening for comprehensive monitoring

NTS analyses based on high-resolution mass spectrometry combined with liquid chromatography enable the monitoring of OMPs in water in the ng / L range. However, the structural identification of unknown compounds from NTS data remains challenging. The presence of a compound in a database is often the decisive factor in the identification of a detected feature (accurate mass – retention time pair), as a database entry turns an "unknown unknown" into a "known unknown" (McEachran et al., 2017; Schymanski and Williams, 2017). Based on the database information, an accurate mass (MS1) based suspect screening can be performed, and consecutively a fragmentation (MS2) based similarity search against a spectral library or in silico predicted spectra of the compound. However, TPs are only beginning to be listed in chemical databases (Bayerisches Landesamt für Umwelt, 2018). If transformation products are lacking in the available databases they can be predicted on the basis of "metabolic logic" (Schollee et al., 2015), i.e. the mass shifts indicative of transformation processes are used to link parent compounds and TPs, and of well-known (bio) transformation rules (Gao et al., 2010; Lee et al., 2017)..

Alternatively, data science methods can be used to interpret NTS data. Thereby, water quality changes across water treatment steps can be assessed without identification of the detected features (Schollée et al., 2016; Schollee et al., 2018). For instance, these strategies can reveal shifts in polarity and mass of compounds, as well as newly formed compounds, i.e. transformation products as a result of a specific process. Thereby, they expose differences between the treatment steps of the drinking water treatment, including aspects that remain elusive when only target compounds are monitored. Furthermore, the NTS in combination with hazard based prioritization can facilitate defining risk based monitoring strategies as demanded by the European Drinking Water Directive (Commision, 2015), and ultimately safeguarding of water resources (Brack et al., 2019b) and drinking water quality (van Wezel et al., under review).

1.3. Bioassays to monitor the biological effects of mixtures of chemicals

Complementary to chemical analyses, bioassays are increasingly applied for water quality monitoring to measure the combined effects of low-level mixtures of chemicals (Brack et al., 2019a). Many biological test systems, such as isolated receptors, cell models, tissues or small organisms, have been developed to measure effects of chemicals on generic to specific biological processes. Several of these are already applied as bioanalytical tools for water quality (van der Oost et al., 2017; De Baat et al., 2019), and many more are considered as candidate tests. Although bioassay data cannot be used for comprehensive risk assessments, observed effects can reveal the presence of one or more chemicals causing effects in biological test systems relevant to human health and/or the environment. As different chemicals cause different effects, water quality monitoring requires a relevant and efficient set of bioassays, based on health effects of water relevant chemicals (Escher et al., 2014), environmental pressures (Boelee et al., 2019) or expected emissions (Leclerc et al., 2019). Due to a lack of regulation, different sets can be selected on a case-by-case basis. The most appropriate approach to interpret bioassay data also differs per application. The removal of positive responses, or trend analysis of repeated measurements may be sufficient to evaluate the impact of a (additional) water treatment step. However, not every positive response in a bioassay is associated with a potential risk to human and/or environmental health. Therefore, individual effect levels at which potential risks cannot be excluded, so called effect-based trigger values (EBT), need to be derived for each bioassay and for potential risks on human and environmental health (Brand et al., 2013; Escher et al., 2015; van der Oost et al., 2017).

1.4. Comprehensive monitoring of OMPs in drinking water treatment trains

Together, the chemical and effect based methods can potentially reveal the cause of an effect and the effect itself. Consequently, combining

these methods has the potential to improve water quality monitoring (Altenburger et al., 2019). Here, we describe such a combined application of target analyses, non-target and effect-based screening to comprehensively assess OMP related water quality changes in drinking water treatment trains at three pilot installations that apply advanced treatment technologies (Company A advanced oxidation (O_3/H_2O_2-UV/H_2O_2); Company B ultrafiltration (UF) followed by reverse osmosis (RO) and Company B advanced oxidation (UV/H_2O_2) followed by granular activated carbon filtration (GAC)). OMP concentrations in the influent water of these pilot installations are close to their detection limit (ng/L – μg/L) rendering assessment of removal percentages difficult. Therefore, OMPs were spiked into the source water at higher concentrations. The removal of these compounds had not previously been assessed in the running full-scale treatment plant for the separate processes. For the NTS data analysis, we built on recent NTS work describing water quality changes in drinking and waste water treatment (Schollee et al., 2018; Brunner et al., 2019b), and further developed the data science tools applied there to also include effect-based bioassay data.

The combination of target, non-target and effect-based screening data allowed the monitoring of OMPs and transformation products, and the comparison of changes in water quality in the different water treatment trains. Overall, the developed strategy provides the drinking water companies and other water quality managers with a valuable new tool to assess novel (drinking) water treatment steps.

2. Material and methods

2.1. Selection of organic micro-pollutants for spike-in

Priority compounds were selected based on their presence in Association of River Water Works (RIWA) databases, research reports, the Water Framework Directive (WFD) guideline, and substances proposed by the water utilities. In addition, selection was based on exceedance of the drinking water standard or target value (0.01 μg / L for genotoxic compounds, 0.10 μg / L for other biologically active compounds and 1.00 μg / L for other anthropogenic compounds without known specific biological activity (Mons et al., 2013)) in several years between 2011 and 2015 more than twice a year, or frequent detection in concentrations above 50% of the standard or target value. Selected compounds were further filtered for toxicity, available knowledge on potential effective drinking water treatment processes, chemical properties, reference compounds and practical issues such as detectability and availability. The following OMPs were selected for the spike-in: acesulfame-K, aniline, barbital, 1H-benzotriazole, carbamazepine, carbendazim, diatrozoic acid, diclofenac, demethenamid, dimethomorph, furosemide, phenobarbital, gabapentin, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (HFPO-DA = GenX), glyphosate, hexa(methoxymethyl)melamine (HMMM), hydrochlorothiazide, melamine, metformin, methenamine, 4-methyl-1H-benzotriazole, 5-methyl-1H-benzotriazole, propranolol, pyrazole, sucralose, terbuthylazine, tetraglyme, trifluoroacetic acid (TFA), tiamulin, triphenylphosphine oxide (TPPO), and tramadol. These are mostly industrial chemicals and pharmaceuticals but also fungicides, herbicides, sweeteners and a contrast medium are comprised. Safe levels for chronic oral exposure to these compounds and the resulting health-based (provisional) guideline values for drinking water were obtained from Baken et al. (2018) (Baken et al., 2018), and from the open database of the Dutch National Institute for Public Health and the Environment (RIVM). They are included in the Supplementary Material file SI_targets.xlsx.

Spike-in concentrations of the selected compounds were based on the detection limit of the target method, the flow rate of the installation, dosing time and the maximum removal efficiency. For most compounds, concentrations exceeded 100× the limit of quantification. Exceptions were barbital (factor 10), phenobarbital (factor 20), HFPO-DA (factor 50). Detailed information (e.g. target concentration, feed concentration in the different pilots, LOQ, InChIKey, SMILES, CAS #, DTXSID) on the spike-solution is provided in SI_Targets.xlsx.

2.2. Set-up pilot installations

Drinking water company A produces drinking water from surface water. Water is taken from the "Afgedamde Maas" which is a tributary of the river Meuse. This tributary functions as a sedimentation and storage basin. To reduce the phosphate concentration of the river water, $FeSO_4$ is dosed into the river. In Brakel the water is treated with micro sieves after which it is transported to Bergambacht. In Bergambacht the water is further treated with rapid sand filters from where it is transported to the dune area. In the dune area the water infiltrates and is abstracted after approximately 2 months. After abstraction from the dunes, the water is softened and treated with powdered activated carbon, aeration, and rapid sand filtration. As a final step the water is treated with slow sand filters.

Drinking water company A considers to expand their treatment plant with an advanced oxidation process, particularly for the removal of OMPs. The company intends to implement this process after the rapid sand filtration process in Bergambacht. Their pilot installation consisted of O_3/H_2O_2 followed by UV/H_2O_2. The pilot installation was fed with rapid sand filtrate from Bergambacht (flow = 5 m^3/h) to which the OMP mixture was continuously added (50 L/h) to obtain the target concentration (SI_Targets.xlsx). The pilot was operated for 1 h for each experiment. Two experiments were performed, one on October 5, 2017 and a second one on October 7, 2017 (replicates). Grab samples from the pilot (feed tank, after O_3/H_2O_2 and after UV/H_2O_2) were collected after the total volume of the pilot was flushed at least three times with the feed water. A schematic representation of the pilot installation and the process conditions under which the experiment was performed are shown in Fig. 1A and SI Table 1, respectively.

Drinking water company B also produces drinking water from surface water, their source is IJsselmeer water. The water is abstracted from the lake, treated with drum sieves, coagulation/sedimentation, rapid filters, and activated carbon filters. After this pre-treatment the water is treated in two lines (1) and (2). Line 1 consists of ultrafiltration (UF) followed by reverse osmosis (RO). Line 2 consists of UV/H_2O_2

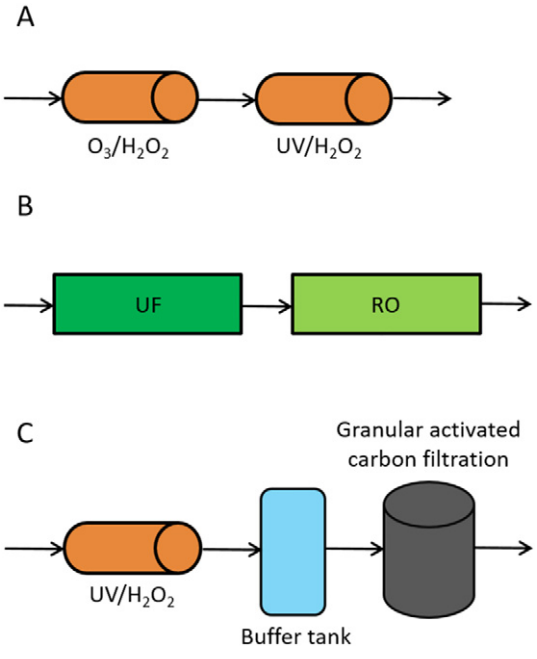

Fig. 1. Schematics of pilot installations.

followed by granular activated carbon (GAC) filtration from where the water is transported and infiltrated in the dune area. After abstraction from the dunes the water is treated with aeration, rapid filters, and UV post-disinfection or by softening, aeration, rapid filters, and chlorine dioxide dosing. The water is then mixed with the UF-RO treated water and distributed to the customer. To assess the robustness of different treatment processes for OMP removal, two pilot installations UF-RO Fig. 1 B and UV/H_2O_2 with subsequent activated carbon filtration Fig. 1C were selected.

The UF-RO pilot was fed with pre-treated IJsselmeer water (drum sieves, coagulation/sedimentation and rapid sand filters). The RO pilot was fed with UF effluent in which the OMPs were dissolved to the final target concentration. The flow of the pilot containing RO membranes (Hydranautics ESPA 3, 4040, 7.9 m^2) was equal to 9.7 m^3/h. The UF-RO pilot (B) was operated from September 18 to 22, 2017. Grab samples from the pilot (influent RO, effluent RO) were collected at $T = 72$ h and $T = 96$ h (replicates).

Pilot installation C (UV/H_2O_2 followed by GAC) was fed from a tank with pre-treated IJsselmeer water (drum sieves, coagulation/sedimentation and rapid sand filters) in which OMPs (target concentration) as well as H_2O_2 was dosed (15 mg/L). The flow of the pilot was 18 m^3/h. After mixing the feed tank for 0.5 h, the pilot was operated for 15 min in total for each experiment. Grab samples were collected after the volume of the UV reactor was flushed at least 5 times with the feed water. After passing the UV/H_2O_2 process, the water was treated with GAC filtration (EBCT = 25–30 min). GAC was obtained from a full-scale filter that was already operated for 2 years (± 30,000–40,000 BV). Grab samples of the influent and effluent of the GAC filter were collected after approximately 9–10 BV. Experiments with pilot-installation C (UV/H_2O_2 followed by GAC) were performed on 4 and 5 October 2017 (replicates). The average DOC concentration of the feed water of pilot A in 2017 was 3.92 ± 0.27 mg/L ($n = 54$). For pilot B and C the average DOC concentration in 2017 in the feed water was 3.48 ± 0.29 mg/L ($n = 20$, see SI Table 2). As a result of pre-treatment, variations in DOC concentration for the feed waters were relatively small. Although variations in DOC can affect OMP removal, these effects were expected to be comparatively modest and investigating this effect was beyond the scope of this study.

OMP removal efficiency was calculated according to Eq. (1).

$$R\ (\%) = \frac{C_{in} - C_{out}}{C_{in}} \cdot 100\% \quad (1)$$

In which: R (%) = OMP removal percentage (%), C_{in} = OMP influent concentration (μg/L), C_{out} = OMP effluent concentration (μg/L)

If the OMP concentration was below the detection limit, the removal percentage was calculated with 0.5 •detection limit (Haas and Scheff, 1990). If the "half detection limit" data were used, this was indicated in the results (result in italics).

To determine the average OMP removal of the measured replicates, the single OMP removal percentages need to be transformed to a logit-scale since it then can be assumed the result follows a normal distribution.

The transformation to a logit-scale was determined according to Eq. (2).

$$R^* = \ln\left(\frac{R}{1-R}\right) \quad (2)$$

In which: R* = logit transformed value of the OMP removal R ((C_{in}-C_{out})/C_{in}).

After determination of the average of the logit transformed removal, this value is transformed back to a percentage removal.

2.3. Target analyses

A detailed description of the five methods that were applied to monitor target chemical concentrations can be found in SI 1.3 Target analyses. Performance characteristics are listed in Supplemental information (SI_targetMethods.xlsx). Target chemical concentrations were determined in both duplicates from the experimental sampling.

2.4. Non-target screening

2.4.1. Sample preparation

50 mL measuring flasks were pre-rinsed with acetone, petroleum ether and the sample, prior to addition of internal standards (IS) to a final concentration of 0.98 μg / L atrazine-d5, 0.85 μg / L bentazone-d6, and 1 μg / L fenuron, chloroxuron and diuron for quality control. Next, samples were filtered with a 0.2 μm regenerated cellulose filter. Blank samples were prepared correspondingly, through spike-in of IS to ultra-pure water followed by filtering. NTS analyses were performed on one duplicate of the experimental sampling. Through filtering, each sample was split into three separate vials which constituted the technical triplicates in the LC-HRMS analyses. 100 μL of sample were injected into the LC-HRMS.

2.4.2. Non-target screening based on LC - HRMS

A Tribrid Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) with a heated electrospray ionization source was connected to a Vanquish HPLC system (Thermo Fisher Scientific). An XBridge BEH C18 XP column (150 mm × 2.1 mm I.D., particle size 2.5 μm, Waters, Etten-Leur, The Netherlands) was used in combination with a 2.0 mm × 2.1 mm I.D. Phenomenex SecurityGuard Ultra column (Phenomenex, Torrance, USA), at a temperature of 25 °C. The LC gradient started with 5% acetonitrile, 95% water and 0.05% formic acid (v / v / v), increased to 100% acetonitrile, 0.05% formic acid in 25 min, and then remained constant for 4 min. The flow rate was 0.25 mL / min. Mass calibration was performed with ESI positive and negative ion calibration solution (Pierce) to ensure a mass error smaller than 2 ppm. The evaporator and capillary temperature was set at 300 °C. Sheath, auxiliary and sweep gas were set to arbitrary units of 40, 10 and 5. The source voltage was 3.0 kV in positive mode, and − 2.5 kV in negative mode. The RF lens was set to 50%. Full scan high resolution mass spectra were recorded from m / z 80–1300 with a resolution of 120,000 FWHM. Quadrupole isolation was used for acquisition with a 5 ppm mass window. Data dependent acquisition was performed with High Collision Dissociation (HCD) energy of 35% and FT resolution of 15,000 FWHM. Samples were measured in triplicate. Blank samples were run every 5–10 samples to check for carry-over and contamination, as well as signal stability of the IS compounds. Signal intensity of the IS atrazine-d5 was used to assess potential matrix effects.

2.4.3. Data processing, analysis and interpretation

The non-target data were processed with Compound Discoverer 3.0 (Beta version, Thermo Fisher) for peak picking, componentization, and suspect screening. An overview of the Compound Discoverer workflow and parameters can be found in SI 1.4. The output of this is a feature list, i.e. a table with accurate mass / retention time pairs (features) and their intensity. The feature intensity is reported as peak area. Depending on the statistical analysis, the "Area" (response of each technical triplicate is reported individually) or the "Group Area" (median response of the triplicate) output was used. Only features that were 5 times the intensity of the blank were clustered in the treatment train specific heat maps.

For the spike-in compounds included in the chemical database of the US EPA, Chemistry Dashboard (McEachran et al., 2017), a suspect screening was carried out with an in-house curated suspect list that also included potential transformation products (TP) of the spike-in compounds with a 5 ppm error tolerance, and in the case of the spike-

in parent compounds an RT tolerance of 0.5 min. The suspect list consisted of both known and predicted TPs, a total of 9560 entries with 1629 unique masses, 1609 of which were within the recorded mass range (m / z 80–1300). 120 known TPs were retrieved from the water-relevant database STOFF-IDENT (Bayerisches Landesamt für Umwelt, 2018) (https://www.lfu.bayern.de/stoffident/#!home) and from data from the Bayerisches Landesamt für Umwelt, kindly supplied by Dr. Manfred Sengl. 450 predicted TPs were based on "metabolic logic" (Schollee, 2015), 184 on biotransformation rules from the EAWAG BDD database (Fenner et al., 2008) and 8806 TPs were predicted with the ozonation prediction tool from Lee et al. (Lee et al., 2017). The suspect list was generated in R based on the packages *RMassScreening* (https://github.com/meowcat/RMassScreening/) and *rcdk* (https://cran.r-project.org/package=rcdk), and is available in the SI_suspectlist_TP_allSources_revised.csv. The transformation reactions used in RMAssScreening are listed in the Supplementary Information in SI 1.5.

The Compound Discoverer output was imported into R Studio for further data analysis and visualization. R version 3.4.4 and R-Studio version 1.1.463 were used for the data analysis (RStudio Team, 2015; R Core Team, 2017). Data preprocessing in R included the application of a retention time cut-off of 2 min, and for the separate analyses of the three different treatment trains the removal of background features and a RT cut-off of 2.2 min. Principal Component Analysis (PCA) was performed on the data scaled to unit variance using the package *factoMineR* (https://cran.r-project.org/package=FactoMineR) in combination with the package *factoextra* (https://cran.r-project.org/package=factoextra) for visualization, and the feature "Areas". The PCA thus provided an overview of the differences between the samples and treatment groups (Masia et al., 2014). After normalization of the data through division of feature "Group Areas" across samples by the maximum "Group Area" of the respective feature, features were clustered together in a hierarchical clustering (HC) based on Pearson correlations as distance matrices and the Ward's minimum variance method (Ward, 1963; Schollée et al., 2016). Thereby, treatment induced trends in the features were revealed, i.e. clusters of features that decrease in intensity, increase or remain the same. The first two could represent parent substances and transformation products. To investigate this further, a theoretical number of clusters X per treatment train was calculated where

$$X = 3^{(n-1)}$$

n … number of samples (group) (Schollee et al., 2018).

Based on the hierarchical clustering with Pearson correlations as distance matrices, X can be used to cut the dendrogram generated by the clustering, resulting in a table of features per cluster. Clustering results were visualized in heat maps using the *pheatmap* package in R (Kolde, 2019).

2.5. Bioassays

To cover various health effects of water relevant chemicals a representative set of bioassays was selected (Escher et al., 2014); Effect-based measurements were performed with the Ames fluctuation test for mutagenicity (see SI 1.6) as described previously (Heringa et al., 2011) with minor modifications with regard to used Salmonella strains, and culture media, and CALUX tests for anti-androgenic activity (anti-AR), estrogenic activity (ER), polycyclic aromatic hydrocarbons (PAHs) and oxidative stress response (Nrf2) according to the supplier's protocols (BioDetection Systems b.v., Amsterdam, The Netherlands) (Murk et al., 1996; Sonneveld et al., 2005; van der Linden et al., 2008; Pieterse et al., 2013). In the Ames test, Nrf2-CALUX and PAH-CALUX, reactive toxicity-related effects can be measured. These are processes on which many different compounds, including the spike-in target compounds, can have an effect. Consequently, the respective tests can detect a wide range of compounds. The anti-AR CALUX and the ER CALUX tests, which both measure a form of hormone disruption, were selected as in earlier studies on the pilot-installations, effects had been found using these tests.

Duplicates were water samples of two independent spike–in experiments. Duplicates were used for all bioassays. Bioassay responses were categorized as active and inactive for the Ames tests, CALUX responses were benchmarked against EBTs for environmental toxicity (van der Oost et al., 2017) due to this EBT's availability for all selected CALUX bioassays. Details on the selection of bioassays, experimental conditions and data interpretation are presented elsewhere (Dingemans et al. in preparation). Bioassay responses were determined in both duplicates. The bioassay output was integrated with the non-target screening data through visualization in the HC heat maps.

3. Results and discussion

3.1. OMP removal assessed with target analyses

Most OMPs are removed to a large extent (>80%) as illustrated in Table 1 that provides an overview of the effectiveness of different treatment processes (or combinations) for the dosed OMPs. OMP removal with the separate processes is provided in the SI, Section 2.1, Table 6. There seems to be at least one effective treatment process for almost every OMP, an exception is melamine which shows no removal with O_3/H_2O_2-UV/H_2O_2 and relatively low removal with both UF-RO (63%) and UV/H_2O_2-GAC (52%).

None of the investigated pilot installations is capable of removing all dosed OMPs to a large extent. This implies that a combination of processes is required for the removal of all OMPs. Drinking water treatment plants in The Netherlands are designed according to the multi barrier treatment concept, which means that there is always more than one barrier in place for a contaminant (e.g. OMPs) and the treatment trains comprise more technologies than those of the pilot installations. Accordingly, for OMPs that show no or moderate removal in the investigated processes in the different pilots of the current study, removal efficiency in the remaining processes of the full-scale drinking water treatment plant should be assessed to prevent their presence in drinking water. OMPs that are poorly removed (<60%) in one of the investigated treatment processes are discussed in the following.

In accordance with previous studies, the benzotriazoles showed poor removal with pilot-scale RO, but good removal with a pilot-scale ozonation installation (Weiss et al., 2006; Albergamo et al., 2019).

As reported previously by Wols et al. (2013), but in contradiction to Macerak et al., 2018, metformin showed lower removal with UV/H_2O_2 (51%). These discrepancies could be caused by the different water qualities used in these studies (e.g. differences in organic matter content which could result in more/less competition with metformin removal). Macerak et al., 2018 used deionized water and observed high metformin removal. Wols et al. (2013) used both MilliQ water and pre-treated surface water (Meuse water, DOC = ± 5 mg/L) in the experimental set-up and observed lower metformin degradation for the Meuse water compared to the MilliQ water. The current study also used pre-treated surface water (DOC = 3.92 ± 0.27 mg/L (n = 54)) and thus lower metformin removal could be expected. When UV/H_2O_2 was followed by GAC filtration, metformin could be removed up to 97%. This is contradiction to the study of Scheurer et al., 2012 who reported ineffective removal of metformin with GAC filtration. However, Scheurer et al., 2012 used sodium azide to suppress biological activity in the filter while in the current study biological activity was not inactivated. Biological degradation might thus have been responsible for metformin removal. This is corroborated by the fact that WWTP sludge can readily remove metformin (Oosterhuis et al., 2013; ter Laak et al., 2014). Besides, Scheurer et al., 2012 used virgin GAC in a small-scale filter test, while the current study used GAC obtained from a full-scale filter already treated with 30,000–40,000 BV in the pilot experiment. In addition, the current

Table 1
Average removal (%) with (minimal, maximal) of two replicates for the different pilots.

	Average removal (%) (min;max) ($n = 2$)		
	Company A (O3/H2O2 - UV/H2O2)	Company B (UF-RO)	Company B (UV/H2O2-GAC)
Tiamuline	*99.7 (99.7; 99.8)*	*99.6 (99.4; 99.7)*	*99.2 (99.2; 99.3)*
Diatrizoic acid	81.5 (81.0; 82.0)	*99.5 (99.4; 99.6)*	96.3 (95.9; 96.8)
Dimethenamid	*99.6 (99.6; 99.6)*	*99.4 (99.2; 99.5)*	*99.5 (99.5; 99.5)*
Dimethomorph	98.2 (98.2; 98.2)	*99.4 (99.2; 99.5)*	*99.4 (99.4; 99.4)*
TPPO	95.0 (94.6; 95.4)	*99.3 (99.2; 99.3)*	*99.5 (99.5; 99.5)*
HFPO-DA	1.3 (0.0; 7.7)	*98.9 (98.6; 99.2)*	41.9 (40.0; 43.8)
Furosemide	*99.1 (99.1; 99.1)*	*98.9 (98.5; 99.2)*	*98.4 (98.4; 98.5)*
Terbuthylazine	73.9 (73.7; 74.1)	*98.8 (98.5; 99.0)*	*98.9 (98.9; 98.9)*
Gabapentine	84.7 (84.4; 85.0)	98.5 (98.5; 98.5)	97.1 (96.7; 97.5)
Acesulfame-K	96.5 (96.5; 96.6)	98.3 (98.1; 98.5)	*99.5 (99.5; 99.5)*
Methenamine	80.2 (78.3; 82.0)	*98.2 (96.4; 99.1)*	52.7 (51.9; 53.6)
Trifluoracetic acid (TFA)	1.6 (1.5; 1.6)	97.8 (97.3; 98.2)	15.9 (15.3; 16.7)
Carbamazepine	*99.6 (99.6; 99.6)*	97.7 (97.1; 98.2)	*99.5 (99.5; 99.5)*
Tramadol	98.4 (98.3; 98.5)	97.7 (97.0; 98.2)	*99.5 (99.4; 99.5)*
Fenobarbital	90.5 (90.0; 91.0)	*96.7 (96.0; 97.3)*	*96.9 (96.7; 97.1)*
Hydrochloorthiazide	98.0 (97.6; 98.3)	95.0 (92.8; 96.5)	*99.4 (99.4; 99.4)*
Diclofenac	*95.6 (95.5; 95.8)*	*94.5 (92.9; 95.8)*	*94.4 (94.4; 94.4)*
HMMM	86.9 (85.2; 88.5)	*93.5 (93.2; 93.8)*	*93.3 (93.2; 93.5)*
Sucralose	53.8 (53.1; 54.6)	*93.4 (91.9; 94.6)*	*94.0 (93.9; 94.0)*
Barbital	73.9 (73.6; 74.2)	*93.1 (91.7; 94.3)*	*94.3 (94.0; 94.6)*
Propranolol	*99.8 (99.8; 99.8)*	90.3 (86.2; 93.3)	*99.7 (99.6; 99.7)*
Tetraglyme	91.0 (90.8; 91.3)	87.0 (85.2; 88.6)	*99.5 (99.5; 99.5)*
Metformine	40.3 (40.0; 40.7)	80.3 (74.5; 85.1)	97.5 (97.0; 97.9)
Carbendazim	95.3 (95.1; 95.6)	78.5 (75.0; 81.6)	*98.4 (98.3; 98.4)*
Melamine	0.0 (0.0; 0.0)	62.9 (55.3; 70.0)	51.9 (51.9; 51.9)
Aniline	*99.5 (99.5; 99.5)*	59.1 (47.3; 70.0)	*99.4 (99.4; 99.4)*
4-Methyl-1H-benzotriazole	96.0 (95.8; 96.2)	42.3 (31.3; 54.3)	*99.4 (99.4; 99.4)*
5-Methyl-1H-benzotriazole	97.6 (97.6; 97.6)	27.3 (14.3; 45.7)	*99.6 (99.6; 99.6)*
Pyrazole	92.5 (92.3; 92.7)	19.3 (8.8; 37.3)	*97.2 (97.2; 97.3)*
1H-Benzotriazool	96.6 (96.3; 96.8)	15.6 (8.2; 27.7)	*99.6 (99.6; 99.6)*

study showed low metformin removal with the O_3/H_2O_2 process (27%). This is in line with the results presented by Knol et al., 2015 (10–35% metformin removal), but deviates from the study of Scheurer et al., 2012 who demonstrated higher metformin removal (50–70%) with ozone alone (batch tests). Again, these discrepancies could be the result of different water qualities and/or experimental set-up, as the DOC might scavenge reactive species formed by the oxidation processes, resulting in a lower removal using surface water instead of MiliQ water..

Removal of pyrazole with the different treatment technologies was in line with previously published reports describing low removal with RO and efficient removal with advanced oxidation and biological degradation (Bertelkamp et al., 2016).

Ozonation poorly removed sucralose (±33% in the O_3/H_2O_2 process) in accordance with what Hollender et al. (Hollender et al., 2009) observed (±31% sucralose removal) in a full-scale ozonation process of a waste water treatment. However, this is in contrast to the study of Bourgin et al., 2017 where O_3/H_2O_2 pilot scale treatment resulted in 20–90% depending on the O_3 and H_2O_2 dose applied. Again, differences between studies can likely be explained by lower NOM or inorganic ion content resulting in less competition between sucralose and organic matter/inorganic ions for OH radicals Xu et al., 2016. Moreover, Lester et al., 2014 reported that sucralose is not susceptible to direct UV photolysis and that the reaction with OH radicals is relatively slow. This could explain the lower removal (±20%) of sucralose with the UV/H_2O_2 process (following O_3/H_2O_2) investigated in the current study.

methenamine shows low removal (40–60%) in the UV/H_2O_2-GAC process. This is the first study that reports on methenamine removal capacity for different drinking water treatment processes.

Aniline removal with the advanced oxidation pilots (O_3/H_2O_2-UV/H_2O_2 and UV/H_2O_2-GAC) was high in the current study (80–99%) consistent with studies of Mestankova et al. (2016) and Benito et al. (2017) who reported high reactivity of aniline towards UV, O_3 and H_2O_2 or combinations of the aforementioned found in lab-scale experiments (Mestankova et al., 2016; Benito et al., 2017). Aniline removal with GAC filtration could not be determined in the current study since almost complete removal was observed after UV/H_2O_2 treatment. However, Suresh et al. (2012, 2013) reported effective removal of aniline with GAC in batch sorption studies (Suresh et al., 2012; Suresh et al., 2013). UF-RO was found to be less effective (40–60%) for aniline removal in the current study, in line with the results of Guo et al., 2009 who reported <10% aniline removal with UF (lab-scale) which could be explained by the much smaller molecular size of aniline compared to the molecular weight cut off (MWCO) of the UF membrane. In addition, Gomez et al., 2009 demonstrated that RO (lab-scale set-up) could be more effective in aniline removal (79–92%), but this rejection will decrease in full-scale installations as shown by Verliefde et al. (2009).

Some OMPs show poor removal in several of the pilot installations: Melamine is not removed well with the advanced oxidation processes, but shows slightly better removal when GAC is added. With UF-RO melamine removal is higher (60–80%), but still a significant percentage remains in the water. To the best of the authors' knowledge this is the first study that reports on melamine removal capacity for different drinking water treatment processes.

HFPO-DA does not seem susceptible to advanced oxidation techniques; however, improvement in removal can be achieved when GAC is added (pilot B). Hopkins et al., 2018 also reported that GAC/PAC were moderately effective in HFPO-DA removal, but that desorption from the carbon could pose a risk. This same study concluded that coagulation, flocculation, sedimentation, filtration, disinfection with free chlorine, ozonation, biofiltration, and disinfection with medium-pressure ultraviolet (UV) lamps were ineffective in removing HFPO-DA from the water. In addition to GAC/PAC, anion exchange also showed moderate HFPO-DA removal. Hopkins et al., 2018 hypothesized that nanofiltration (NF)/reverse osmosis (RO) would be effective in HFPO-DA removal, as confirmed in the current study (HFPO-DA removal with RO >80%).

TFA does not seem susceptible to either advanced oxidation or GAC. However, good removal of TFA is observed with RO (>80%) consistent

with a study of Scheurer et al. (2017) (Scheurer et al., 2017). To the best of the authors' knowledge, this is the first study that investigated TFA removal with UV/H_2O_2 which was shown to be ineffective.

3.2. Non-target screening reveals trend profiles of OMPs and transformation products

To allow monitoring beyond the spike-in compounds, in particular of transformation products that are formed during water treatment, NTS and bioassays were performed in addition to the target analyses. In the NTS, analyses based on high-resolution mass spectrometry combined with liquid chromatography, a total of 2821 and 1180 features were detected, across all samples using positive and negative ionization, respectively (see SI_allFeatures_pos_neg.xlsx for the full list of features, including information of their monoisotopic mass, RT, signal intensities, molecular formula and when available score from MS2 mass spectral matching with the spectral library mzCloud). Application of a retention time cut-off of 2 min and removal of features that were also present in the background resulted in 927 and 310 features in positive and negative ionization mode, respectively (SI Table 7). By means of suspect screening based on accurate mass and retention time, the non-target data was searched for the spike-in compounds, i.e. parent compounds. 26 features could be matched to parent compounds, 20 in positive and 6 in negative ionization mode (SI Table 8). 21 of the parent compound matches could be substantiated by comparison of the experimental MS2 spectra with mzCloud library spectra (https://www.mzcloud.org/) with mzCloud identity scores ranging from 73.1 to 99.8. Barbital was detected both ionization modes. Melamine and dimethomorph had split peaks, i.e. two features with the same accurate mass but different RT, potentially due to tautomerism (Klotz and Askounis, 1947). The 26 matched features thus corresponded to 23 detected compounds. Dimethenamid which is not listed among these was also detected after manual inspection, however, not using Compound Discoverer. Supplementary Information SI_Targets.xlsx includes a column specifying the reasons for not detecting the respective spike-in compounds with the NTS. In SI Table 8 the OMP removal rates calculated from the NTS analyses are compared to those from the target analyses, showing correspondence in most cases. Next, the NTS data was screened against an in-house generated suspect list of 9560 suspects, including known and predicted TPs of the spike-in parent compounds based on accurate mass. Thereby, 91 features - 53 in positive and 38 in negative ionization mode, could tentatively be assigned to one or more TPs of the suspect list based on their accurate mass. In the positive ionization mode data, 12 features had a single TP suspect match, the other 41 features matched multiple TPs of the same mass from the suspect list. In the negative ionization mode data, 2 features matched exactly one suspect list entry while the remaining 36 could be matched to multiple TPs. Further confirmation of the suspect candidates was carried out after prioritization (see below). The list of TP matches can be found in SI suspectsMatched.xlsx.

Despite tailored suspect lists of experimentally detected and predicted OMP transformation products, over 1000 features remained unmatched in the non-target data. These features included compounds that had not been spiked-in, but were present in the source water, and TPs thereof, as well as TPs of the parent compounds that had not been included in the suspect list used for screening. The unknown features account thus for 90% of the total feature number. However, as SI Fig. 1 illustrates, the contribution of these features to the overall feature intensities across samples is only around 15%. A prioritization strategy based on tailored suspect lists can thus explain 85% of the total intensity measured in samples, demonstrating that the majority of relevant features can be explained. Nevertheless, as the feature intensity does not necessarily reflect the concentration of a compound in a sample (Sjerps et al., 2016) or risk potential (Brunner et al., 2019a), it can still be relevant to consider low intensity features when comprehensively assessing water quality.

To utilize all information from the NTS data for water quality assessment including these unknown features, data science methods were applied to the data set. Principal Component Analysis (PCA) allowed visualization of sample similarity (see Fig. 2 and SI_PCA_var_contrib.xlsx for the contributions per variable, i.e. feature, for the PCA dimensions. The spike-in compounds are highlighted in yellow.). The first dimension (Dim1) explained 31% (pos) and 25% (neg) of the variation in the data and seemed to represent the total feature intensity, with an increase in intensity going from left to right. Blank and UF-RO samples showing lowest intensities, clustered together on the left, Company B samples at the x-axis, and Company A samples that had the highest overall response on the right. The PCA thereby showed that UF-RO removed most compounds, hence the clustering with the ultrapure water blank. The second dimension (Dim2), which explained 12% (pos) and 14% (neg) of the variation clearly separated Company A samples (red tint) from Company B samples (blue-green tint), both in positive and in negative ionization mode. The source water affected the clustering more than the spike-in compounds and most treatment steps, which can be explained by the fact that the spike-in compounds only account for around 1% of the total feature intensity observed (see SI Fig. 1).

3.3. Treatment train specific analyses per pilot installation

To assess water quality changes due to a specific drinking water treatment train in more detail, the non-target data of the 3 different pilot installations were analyzed individually. In these analyses, the triplicate samples were grouped and a more stringent RT cut-off of 2.2 min was applied. Only features that exceeded 5 times the intensity of the blank in a given sample were considered to calculate feature numbers and summed intensities. The resulting numbers of features detected in the samples, summed feature intensities and parent compound and TP matches are listed in Fig. 3.

To reveal trend profiles of features which can expose transformation product formation related to treatment steps, hierarchical clustering (HC) based on Pearson correlations was performed separately on the features of the three datasets. To be used in the HC, a feature needed to exceed 5 times the blank intensity in at least one of the samples of the treatment train. The resulting clusters could represent parent compounds and their transformation products and facilitate prioritization for identification. The HC output is visualized in the heat maps shown in Fig. 4 for positive ionization mode data. The negative ionization data is discussed in SI 2.2.2. It showed similar clusters and trend profiles, and can be found in SI Figs. 4, 5, 7 and 9.

In the heat maps, samples are columns (listed horizontally), and features are rows (clustered vertically). Normalized feature areas are represented in color ranging from blue (the least intense feature) to red (the most intense feature). Additional feature information is displayed in the columns left of the heat map. From left to right the columns indicate: the cluster number of the feature, whether the feature matches a suspect compound, whether it matches a parent compound, its molecular weight, and retention time. The trend profiles of the feature intensities, as well as molecular weight and retention time distribution of the features per cluster are shown in SI Figs. 3, 6 and 8. To account for toxicity in the prioritization of features, bioassay readouts of the CALUX assays and AMES test were superimposed on the HC heat maps. The assay response of the replicate analyzed with NTS was used for the integration. In the case of differing replicate responses, this could result in overestimation of activity. This is of particular importance for the Ames data where three responses differed between replicates (see SI Fig. 10). Due to the known occurrence of false positive responses in this test system, a sample is considered positive only if mutagenicity is observed in two independent replicates.

The heat map visualization shows that in the data from all pilot installations features that represent parent compounds (indicated in turquoise in the third column from the left) cluster together in one cluster

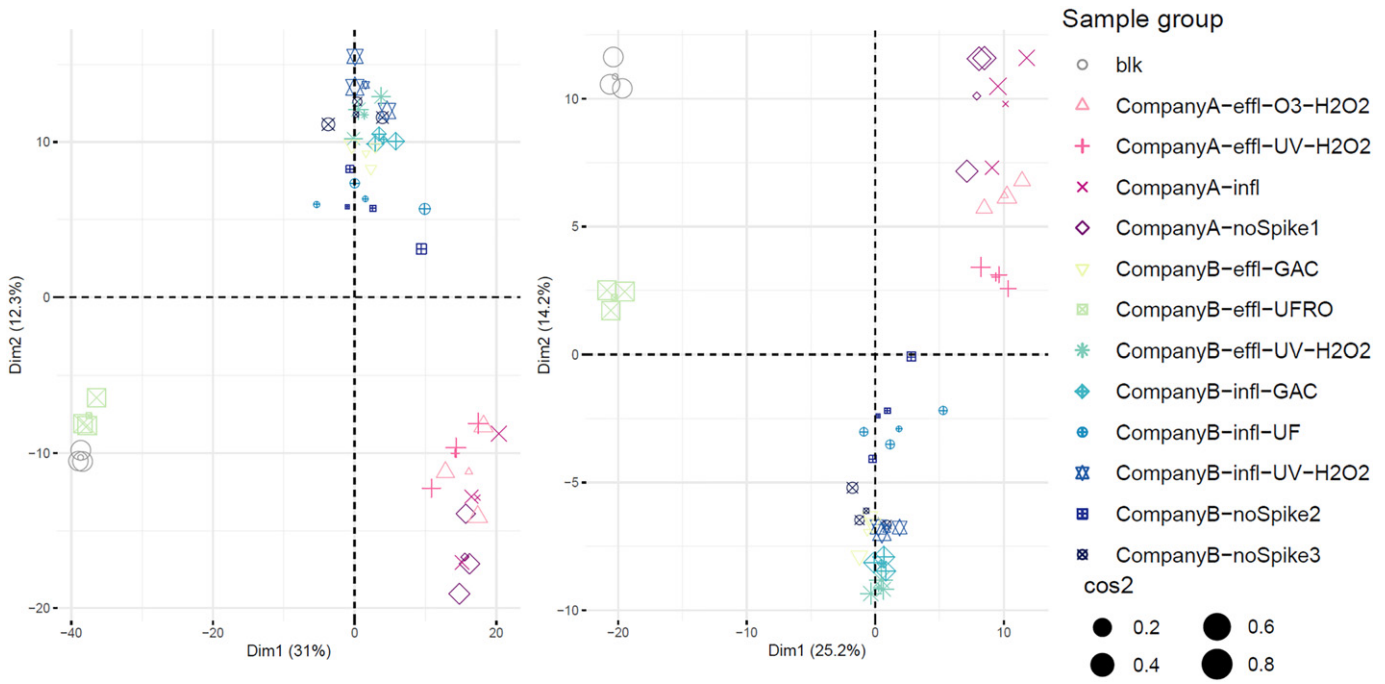

Fig. 2. PCA plot of features detected in (a) positive and (b) negative ionization mode. squared cosine of the observation (COS2) shows the importance of a component for a given observation.

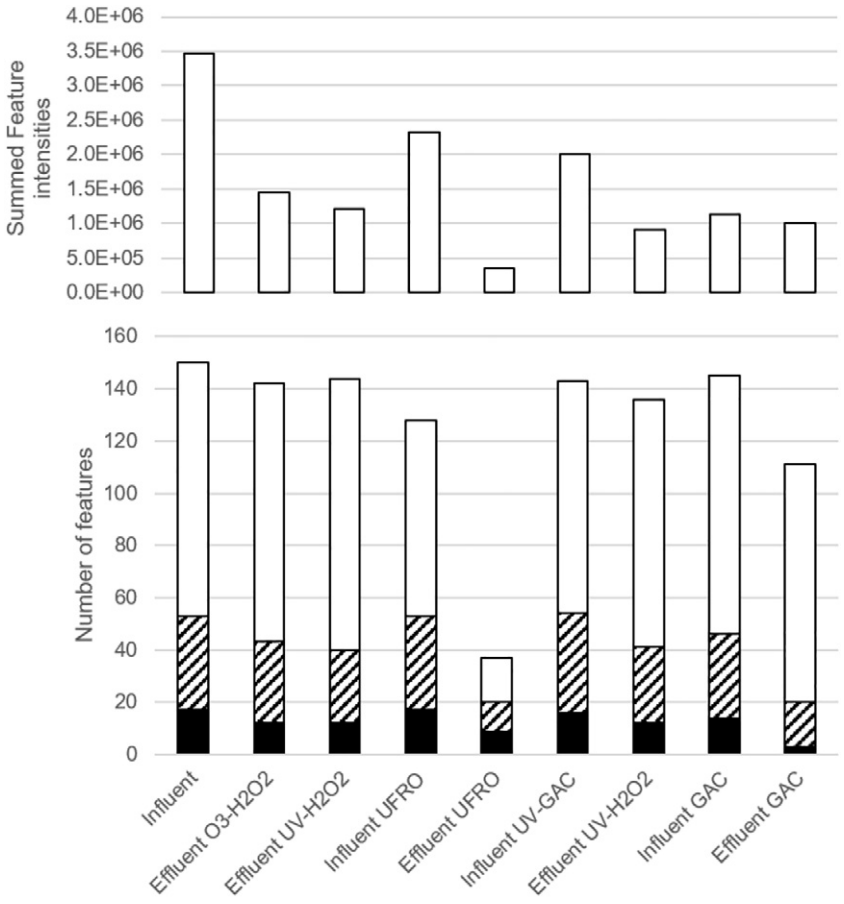

Fig. 3. Feature intensities (top) and numbers (bottom) after 2.2 min RT cut-off and 5× blank cut-off, with parent compound (black) and tp matches (striped) per treatment train. positive ionization mode data. summed feature intensities are added group areas.

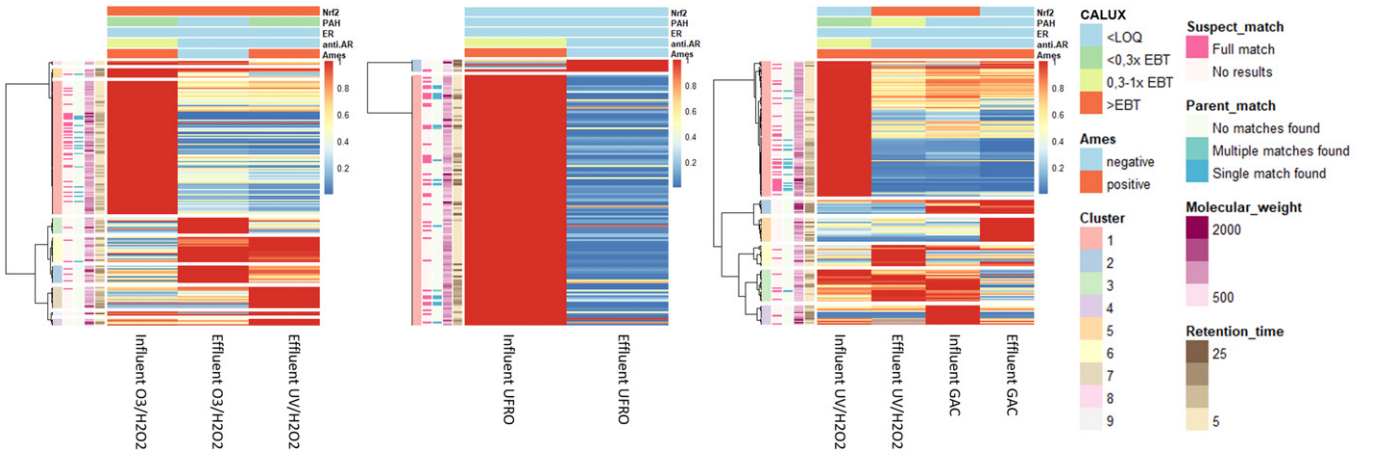

Fig. 4. Hierarchical clustering of features from pilot installation A. Company A, B. Company B ufro and C. Company B UV-GAC detected in positive ionization mode based on pearson correlation using the ward.d2 method and max normalized feature intensities.

(Cluster 1, indicated in the leftmost column in red. Note: cluster numbers are merely an aid for communication and do not have any significance.). This cluster exhibits a substantial decrease in feature intensity already in the first treatment step of each treatment train. The spike-in compounds are thus overall well removed during treatment. Because the parent compounds are also included in the TP suspect list, these are shown in both columns. Interestingly, this cluster also exhibited many TP suspect matches (indicated in pink in the second column from the left). These potential TPs could indicate that the parent compounds were already transformed in the influent or present in the source water.

3.4. Sequential advanced oxidation processes at pilot installation A

HC analyses of the samples from the sequential O_3/H_2O_2 - UV/H_2O_2 pilot installation at company A clustered 178 features and resulted in 9 defined clusters for data recorded in positive ionization mode (shown in Fig. 4). Parent compounds clustered together, predominantly in cluster 1 (indicated in the leftmost column left of the heat map in red) which was composed of compounds that were removed or transformed by the ozone treatment. In contrast, clusters 2 (in blue), 3 (in green), and 6 (yellow) showed an increasing feature intensity profile after ozonation and thusincluded substances that appeared to be generated by ozone treatment. In particular, cluster 2 consisted of 13 features, including the spike-in compound terbuthylazine despite its signal intensity decrease, as well as 3 TP suspect matches. The suspect matches were known and predicted TPs of the parent compounds tramadol and terbuthylazine, and TPs of hydrochlorothiazide or furesomide that were predicted with the O3 prediction tool (see Supplementary Material 2.2.3 Suspect matches per treatment process for full list of suspect candidates). The suspect matches with the known TPs Tramadol N-Oxide and Desethylterbuthylazine could be substantiated with MS2 spectral library matches in mzCloud with respective scores of 89.6 and 95.6, resulting in a confidence level 2/3 identification. The 16 features of cluster 6 included one O3 predicted TP of aniline with multiple possible structures, and the spike-in compound melamine. Melamine was not removed by ozone treatment, corroborating the target analyses. The seeming increase in signal intensity in the NTS data, however, is an artefact due to measurement inaccuracy. Cluster 3 consisted of 12 features of which one could be matched to an O_3 predicted TP of tiamulin. For all clusters that showed an increase in intensity there

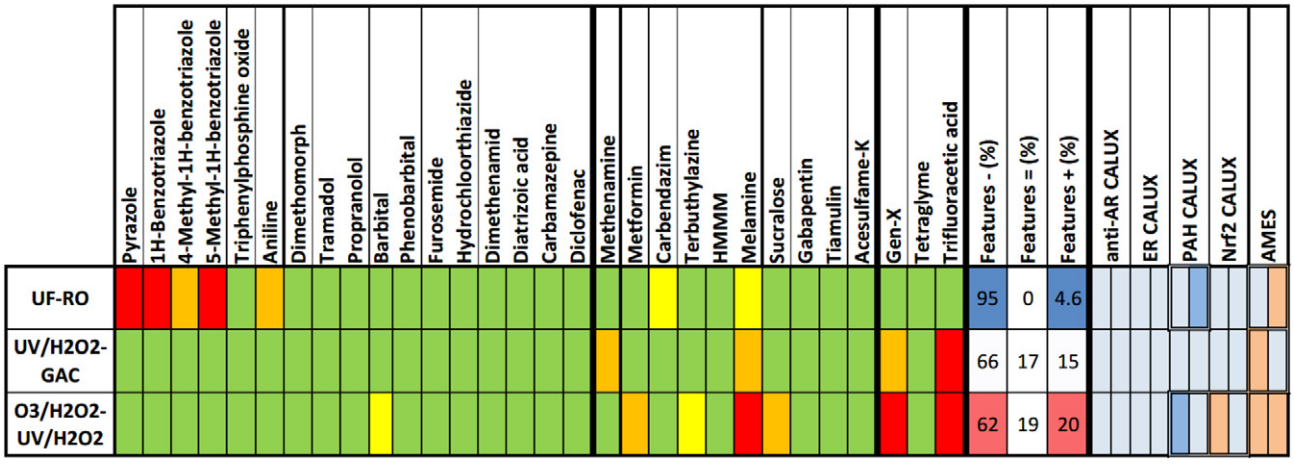

Fig. 5. Integrated results of target analyses, nts and bioassays per treatment train. omp removal rates from target analyses are indicated in green ≥80% removal, yellow = 60–80% removal, orange = 40–60% removal, and red ≤40% removal.percentage of nts features that decrease in intensity (features – (%)), stay the same (features = (%), and increase in intensity (features + (%). highest precentage (blue) and lowest percentage (red) of features that decrease, and lowest percentage (blue) and highest percentage (red) of features that increase. ames test light blue = negative result, orange = positive result; calux bioassays light blue = result under the detection limit, dark blue = result above the detection limit, orange = effect above ebt. double border emphasizes differing responses between replicates.

was thus at least one suspect match with a TP that was predicted to be formed by ozonation, emphasizing the usefulness of process-specific TP prediction tools. Furthermore, the matched suspects were all TPs of parent compounds that showed removal of >90%, with the exception of terbuthylazine which had an average removal of 45.2%. This indicates that indeed most TPs are formed from (at least some of) the target compounds that are best removed.

Compounds in clusters 3, and 8 were removed or transformed by UV/H_2O_2 in the second treatment step. Cluster 8 grouped compounds that were present in the influent already, and persistent against ozonation, but removed or transformed with UV treatment, while compounds in cluster 3 were generated by ozonation. Cluster 4 and 7 consisted of 5 and 16 features, respectively, representing compounds that were formed by UV/H_2O_2 treatment. One of these features could be matched to an O3 predicted TP of diclofenac. Cluster 2 and 6 contained compounds that were generated by ozone treatment and persistent against UV/H_2O_2 treatment. Clusters 5 and 9 included compounds that decreased by ozone treatment. Cluster 9 increased again after UV/H_2O_2 treatment. The 3 features of this cluster could not be matched to a TP suspect.

Furthermore, the addition of the bioassay responses to the non-target screening data showed that anti-AR CALUX response seemed to correlate with the feature intensity profiles of cluster 1. For none of the spiked-in compounds, effects in the anti-AR CALUX have been published. Carbendazim (0.43μg/L), dimethenamid (1.2 μg/L), dimethomorph (1.1μg/L), terbuthylazine (0.58μg/L), and tiamulin (2μg/L) give responses in analogous ToxCast in vitro test, however, not at the measured spike-in concentrations (see SI Table 9). Other cluster 1 features but the spike-in OMPs are thus more likely be responsible for the observed anti-AR response. Oxidative stress was observed across samples from all treatment steps. This could be related to persistent spiked or background OMPs, but also to residual oxidants from the UV/H_2O_2 and or O_3/H_2O_2 treatment. PAHs activity and mutagenicity were no longer observed after the O_3/H_2O_2 treatment step, but reappeared after UV/H_2O_2 treatment. The features that were formed during this treatment step could thus be responsible for the active bioassay responses. Based on their NTS trend profiles, i.e. present after the second treatment step of the pilot installation, and the positive Ames and PAH bioassay read-out, the features of clusters 4, 7 and 9 were therefore categorized as potentially harmful transformation products and therefore prioritized for identification ($n = 24$). This meant a 6× reduction in the number of features that needed to be identified. Only one of these features had a TP suspect match, namely an O3 predicted TP of diclofenac. Due to a lack of an MS2 spectrum, the feature could not be confirmed any further. The prioritized features are likely UV TPs of background OMPs and/or of ozonation TPs of the spike-in compounds, which we did not expect to find in chemical compound databases. As the spectral quality of the MS2 spectra of these features was low, identification of sub-structures based on spectral similarity with known compounds was not successful either. Prediction tools for UV induced processes could potentially improve the identification of these unknowns in the future.

3.5. Combination of UF and RO removes most compounds at plant B

For the UF-RO pilot installation at company B, visual inspection revealed that 2 clusters (rather than the theoretically expected 2) described the trend profiles of the 131 and 68 features detected in positive and negative ionization mode, respectively (see Fig. 3B and SI Figs. 4, 6 and 7). Again, parent compounds clustered together, in cluster 1, together with the majority of features which all were removed or transformed by the RO treatment. The second cluster contained merely 6 features that increased through RO treatment likely as a result of the increased salt concentration and consequently signal suppression (SI Fig. 6). One of these 6 features could be matched to three suspects of the same mass. These were generated by the O3 prediction software. Interestingly, features in this cluster had smaller molecular weight but higher retention times than those of cluster 1 (SI Fig. 6). None of the cluster 2 compounds resulted in an active response in any of the bioassays tested. A further identification of these compounds might thus not be critical, presuming that the selected bioassays cover the relevant toxicological endpoints.

3.6. Advanced oxidation followed by granular activated carbon treatment at plant B

The second pilot installation at company B combined UV/H_2O_2 and GAC and resulted in 176 features after RT cut-off and blank subtraction. The multiple treatment steps and resulting samples lead to a theoretical maximum of 27 clusters which upon visual inspection of the trend profiles of each cluster could be reduced to 6 bigger clusters for a clearer picture (Fig. 3C and SI Fig. 8). Cluster 1 contained parent compounds and other compounds that decreased due to the treatment. The 15 features of cluster 6 showed an increase in intensity after UV/H_2O_2 and are thus likely to be UV/H_2O_2 transformation products. Two of the features could be matched to O3 predicted TPs of Hydrochlorothiazide or Furosemide (see Supplementary Material 2.2.3.4), which are both target compounds that showed good removal (>95%) in the target analyses. Clusters 2 and 5 consisted of 10 and 17 features, respectively, which based on their intensity profiles are likely transformation products that originated in the buffer tank before the GAC and during the GAC. None of these features had a TP suspect match, emphasizing the need for better prediction of the biotic transformation processes that occur during drinking water treatment. Cluster 3 consisted of 23 features that were persistent or increased in the UV/H_2O_2 treatment, but removed by GAC. Among these were 3 TP suspect matches, namely O3 predicted TPs of aniline, diclofenac and terbuthylazine, which are spike-in target compounds that showed average removal rates of 99.4%, 94.4 and 87.8%, respectively. Based on a spectral match with mzCloud, the latter feature could be identified as desethylterbuthylazine. Cluster 4 contained substances that increased continuously during the treatment steps, but were removed by GAC. Similar clusters could be distinguished in the negative ionization data, with parent compounds detected in cluster 3. Interestingly, the features that were removed most, i.e. cluster 1 (pos) and cluster 3 (neg) exhibited later retention times than the other features, including those of the respective clusters, suggesting that the polarity of a compound affects the UV removal rates in line with Kusic et al. (SI Fig. 8, (Kušić et al., 2009)).

Regarding potential toxicity indicated by the bioassays, positive responses in the Ames test were observed across all treatment steps albeit not in both replicates. As two positive responses are needed for a positive result, activated carbon treatment does seem to remove mutagenic compounds. PAH activity was persistent through UV treatment, but seemed to be removed by the storage step prior to GAC filtration. Nrf2 activity was induced by UV treatment and removed by GAC. The Nrf2 profile thus matched the features of cluster 3, consisting of UV-treatment induced TPs that are removed by GAC (see above).

Integration of the NTS feature intensity trend profiles and the bioassay data, i.e. the positive response in the AMES test, thus resulted in the prioritization of the features detected in clusters 2 and 5 ($n = 27$). Thereby, a 4× reduction of features that need to be identified could be achieved. Identified OMPs / TPs could then be assessed regarding their potential to account for the effect-based responses. The prioritized features are likely TPs formed through biotic processes and might be responsible for the mutagenicity observed in the AMES test. The lack of TP suspect matches in combination with the fact that these features might still be TPs and consequently missing from chemical compound databases, prevented the identification of these unknowns within the scope of the project.

3.7. Assessment of treatment performance through integration of target analyses, NTS and bioassays

To investigate whether integration of results from the three analysis techniques affected treatment performance assessment, the results of target analyses, NTS and bioassays were combined in Fig. 5. Despite few substances (the benzotriazoles, pyrazole and aniline) that exhibited removal <20% - 60%, UF-RO showed high average OMP removal. This corresponded to the NTS results, which showed that >95% of the detected features decreased in intensity following UF-RO. Accordingly, effect-based methods showed no measured effects above the EBT for all CALUX bioassay investigated and no mutagenic activity. However, roughly 5% of the features increased in intensity. This would possibly be caused by the salt concentration, which is higher in UF-RO influent than in effluent. As a result of the matrix effect a number of substances may not be detectable in the influent, but can be detected in the effluent due to the lower salt concentration. Though, based on the IS signal intensities, no matrix effects were observed.

The O_3/H_2O_2-UV/H_2O_2 process had an average OMP removal of 95%. This process combination resulted in a lower percentage of features that decreased in intensity (61.6%) than the UV/H_2O_2-GAC process. 18.5% of the feature intensities remained the same and 19.7% increased. For this process combination, an effect above the EBT could also be observed for Nrf2 activity and the AMES test was positive. As in practice the O_3/H_2O_2 - UV/H_2O_2 process is often followed by activated carbon filtration and dune infiltration, it is expected that these processes remove (part of) the OMPs and/or transformation products that cause the bioassay response, as seen with UV/H_2O_2 followed by GAC in pilot installation C.

UV/H_2O_2-GAC removed around 65% of features, 17% of the feature intensities remained the same and 15% increased. No effects above the EBT were observed for the samples collected after UV/H_2O_2-GAC.

4. Conclusions and outlook

4.1. Conclusions for the drinking water treatments studied

In the studied pilot installations at Company A and B, most OMPs are removed to a large extent (>80%). Moreover, there seems to be at least one effective treatment process for almost every OMP except melamine. None of the investigated pilot installations is capable of removing all dosed OMPs to a large extent which implies that always a combination of processes is required. To the best of the authors' knowledge, removal for some OMPs (e.g. methenamine, melamine, TFA) was described for the first time in this study.

In drinking water treatment, high OMP removal rates in combination with minimal transformation product formation and absence of positive bioassay responses are sought after. Yet, the formation of transformation products does not necessarily have to be problematic, as long as these are not harmful to human health. In theory, if the selected bioassays cover the complete range of human health risks and no positive response is measured above the EBT, the effect of detected transformation products can be presumed negligible. However, to date EBTs are not available for all bioassays. Furthermore, the selected bioassays do not cover all relevant biological effects in humans, nor correct for processes associated with the exposure in a human body (Dingemans et al., 2019). Prioritizing the treatment processes is therefore challenging, in particular as the conditions investigated in this study were limited to one type of water and one experiment per year for each process. In addition, more polar substances may not have been monitored in the NTS and bioassays (see below). Nevertheless, based on the integrated results from the target analyses, NTS and bioassays it could be concluded that all three pilot installations performed well. In the next 5-year cycle of the assessment of drinking water treatment processes by the Dutch surface water companies, it will be interesting to further investigate the effect of the source water quality on the OMP removal and TP formation capacity of the studied treatment processes, as well as the causes of certain bioassay responses, which might first require laboratory experiments under controlled conditions.

4.2. Synergy of the three methods

To investigate the complementarity of the three analysis techniques, the output was compared for the different treatment lines. The three techniques measure different parameters, so a strict (linear) correlation might not be expected. Nevertheless the following aspects were correlated.

First, the reduction of the summed feature intensities from the NTS data (Fig. 3) was compared to the average removal of the target compounds (Table 1 and Fig. 5). The comparison showed that the removal of the spike-in compounds (95–98.4% depending on the pilot installation) is much greater than the decrease in NTS feature intensities (2 to 5-fold). Despite the fact that the ion count MS response is not directly linked to the concentration, this suggested that the persistence of other OMPs from the source water and/or the formation of TPs were responsible for the higher response compared to the removal of spike-in parents quantified with the target approach. This is especially evident for the pilot installations using advanced oxidation processes. These are known to result in the formation of TPs. The comparison of target and NTS analyses thus indicates that with a better quantification and identification of the NTS results, the combination of techniques is better capable to define the chemical water composition as a whole and makes the differentiation of physical separation and chemical oxidation techniques more pronounced in the favor of physical techniques.

Second, a comparison of the target analysis and bioassay results (SI Fig. 10) showed a similar picture. The strong removal of the spike-in compounds was only reflected in the loss of the anti-AR CALUX test response, however, the other bioassay response patterns did not correlate to what was seen with the target analyses. Corresponding to what was seen with the NTS analyses, other persistent background OMPs and/or TPs that are formed by the different treatment steps seemed to be responsible for the bioassay results.

Third, a comparison of the bioassay and the NTS results showed that the correlation between these two techniques is stronger than for the spike-in target compounds, indicating that a NTS gives a better representation of the water quality than a series of spiked target compounds.

Furthermore, a prioritization approach was developed in which information from the feature intensity trend profiles across the treatment steps was combined with the bioassay readouts to select features that needed to be identified. Thereby, focus was on features that were relevant to human and environmental health, and the number of features that needed to be identified could be decreased significantly, saving time and labor.

Overall, we have tested three different analytical representations of water quality and measured it along 3 different treatment trains using quantitative target analysis, comprehensive NTS to cover a wider range of compounds and effect-based bioassays that can also detect mixture-toxicity of unknown compounds. The results show that there is correlation between the different approaches, but that there are also differences observed. Target screening is quantitative, but does not necessarily reflect the total chemical load nor the effects. NTS gives a better, but merely semi-quantitative representation of the water quality, while the bioassays reflect the combined biological activity of the mixtures of chemicals in water samples, which is lacking in the case of target analyses and NTS, but cannot be directly linked to individual or groups of chemicals. Bioassays can thus be used to evaluate the potential of water treatments to remove chemical hazards. The comparative assessment of treatments with bioassay readouts can indicate a risk to environmental or human health, and/or water treatment problems and support prioritization. In the absence of a positive bioassay readout, further identification of unknowns can be renounced, thereby circumventing the challenges in identifying unknown unknowns. Looking at water quality and changes due to treatment using these

Table 2
Complementation of performances of target analyses, nts and bioassays.

	Target analyses	Non-target screening	Bioassays
Quantitative analyses of chemicals	Yes	No	No
Quantitative analyses of effects	No	No	Yes
Very polar substances	Yes	Not possible with RP C18 (optimization needed)	Not possible with SPE extraction (optimization needed)
TP	No	Yes	Yes
Risk of unknowns	No	No	Yes
Identification of unknowns	No	Yes	No

methods, enables us to gain more insight in what is actually happening to water quality during the different treatment steps.

Despite the additional information that NTS and bioassays provide, target analysis is still required for quantitative monitoring of regulated compounds (European Commission, 1998), as well as for the analysis of specific compounds such as persistent and mobile organic chemicals (PMOCs). NTS is typically based on RP chromatography and thus not suitable for detecting very polar substances (see Table 2). Hydrophilic interaction liquid chromatography (HILIC) can alleviate the problem. However, analysis time and costs double when RP and HILIC are both performed on the same samples. Similarly, sample pre-treatment used for effect-based monitoring that consists of a solid phase extraction (SPE) can result in loss of highly polar compounds. Vacuum assisted evaporative concentration seems a promising alternative concentration method (Schollee et al., 2018; Mechelke et al., 2019).

Overall, none of the methods alone can provide a complete picture. Their synergistic integration supports the choice of using them in combination. Eventually, the more comprehensive the analytics the more comprehensive will be our view of the chemical water quality and the better the assessment of drinking water treatment performance.

Declaration of competing interest

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.

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Appendix A. Supplementary data

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