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Water Impact Statement

Cell-based composite toxicity assays are a beneficial tool for validating direct potable reuse systems. They can augment conventional chemical species monitoring approaches by quantifying toxicological signatures of trace mixtures of known and unknown oxidation byproducts and other contaminants.

ARTICLE

Composite Toxicity Assays for Enhanced Assessment of Decentralized Potable Reuse Systems

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Martin A. Page,*[a] Shengkun Dong,[bd] Nedal Massalha,[be] Bruce MacAllister,[a] Andy Y. Hur,[a,b] Paul Bandstra,[c] Elizabeth D. Wagner,[b] and Michael J. Plewa [b]

Decentralized direct potable reuse systems present new opportunities and potential risks for resilient and sustainable facilities of the future. This study explored the use of advanced cell-based composite toxicity methods to augment the assessment of known and unknown chemicals in waters from decentralized direct potable reuse systems. The decentralized reuse systems were equipped with newly-developed low energy membranes and advanced oxidation technologies for the control of the full spectrum of contaminants found in wastewater effluent. The feed and product waters from these systems were tested for conventional chemical contaminants, personal care and pharmaceutical products, composite cytotoxicity and composite genotoxicity. The composite toxicity assays confirmed the high degree of purification in the decentralized potable reuse systems and responded accordingly when known contaminants were detected at levels approaching or exceeding regulatory limits. The composite toxicity assays identified potential risks for water samples that would not be considered contaminated or a risk to health based on conventional contaminant monitoring. Unknown disinfection byproducts appeared to be the causal factors due to increases in cytotoxicity that occurred during the disinfection step. It is recommended that composite toxicity assays be considered to augment validation and optimization of direct potable reuse systems to minimize potential health risks associated with the additive effects of known and unknown contaminants.

Introduction

Resilient and sustainable facilities of the future will be equipped with advanced wastewater treatment capabilities that automatically recover purified water for decentralized direct potable reuse. Decentralized potable reuse technologies are currently being developed, tested, and in some cases implemented for situations where environmental, economic or security factors necessitate their due consideration. Exemplary decentralized potable reuse scenarios include progressive sustainable buildings, resilient facilities in which critical operations must be maintained at all times, space missions supported by astronauts, and expeditionary military operations.[1] While the scale of decentralized reuse systems may be small relative to municipal systems, the operations being supported can have global impacts.

The capability to safely treat and reuse wastewater for potable applications has already been adopted at municipal scale by many water-stressed communities around the world.[2] In decentralized applications, however, direct potable reuse systems are still an emerging capability and may present different levels of risk due to decreased dilution of contaminants inherent to their smaller scale, potentially requiring higher levels of treatment.[3] This challenge is augmented by the lack of a skilled operators, practical limits on product water sampling and analysis, and automation gaps for small scale systems. While the latter challenges have been largely overcome for residential water purification systems that treat shallow groundwater, the use of wastewater as a source presents greater risk for system failure through fouling or other mechanisms. Collectively, these challenges require additional due diligence in the validation of decentralized direct potable reuse systems.

As with centralized systems, the primary health risk for decentralized direct potable reuse systems is associated with pathogens.[4] Indeed, modelling of pathogen loading in small scale systems clearly showed how these systems can present high peaks in influent pathogen concentrations when contamination events occur.[3] In addition to microbial risks, it is also critical for decentralized DPR systems to manage peaks in chemical contaminant loadings, including trace pollutants, as well as chemicals that might affect the treatment process performance. Therefore, engineering performance in terms of general water quality parameters, regulated contaminants,

[a] US Army ERDC. Champaign, IL, USA *Martin.A.Page@usace.army.mil
[b] University of Illinois at Urbana-Champaign, Urbana IL, USA
[c] Suez Water Technologies and Solutions
[d] Sun Yat-sen University, Guangdong, China
[e] The Galilee Society, Shefa-Amr, and University of Haifa, Israel

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and those of emerging concern should be carefully considered. Further, the potential generation of trace advanced oxidation or disinfection byproducts (DBPs), both known and unknown, is a particular challenge for assessing both decentralized and centralized systems.[5]

The use of composite cell-based toxicity assays that measure cytotoxicity, genotoxicity, and other molecular signatures of potential biological consequence has been explored for assessing chemical quality of water, including known and unknown DBPs.[6] Compared to identification and quantification of specific species, these methods provide quantitative comparisons that identify potentially harmful biological activity of the mixture of contaminants present, known and unknown.[7] The ability to assess additive effects was demonstrated in a recent controlled study with individual contaminants and mixtures.[8] Other recent studies showed significant differences in cytotoxicity for various water types, with toxicity response signals decreasing with increasing water quality, and providing resolution over a range of water qualities, including wastewater, wastewater treated for environmental discharge, natural potable water sources, potable water from distribution systems, and a limited set of samples from water reuse systems.[9] The composite cell-based toxicity methods used indicated that the approach may have promise for assessing technology performance in advanced treatment systems with increased sensitivity and precision.[10] In the state of California in the United States, bioassays for estrogenic activity are being incorporated into regulatory guidance for direct potable reuse systems.[11] Improved understanding of bioassay methods and their application could augment potable water quality monitoring approaches that have historically focused heavily on monitoring for known, individual contaminants.

The objective of this study was to use of composite cell toxicity methods to augment the assessment of decentralized direct potable reuse systems. Composite mammalian cell cytotoxicity and genotoxicity signatures were compared to measurements of conventional chemical contaminants as well as trace personal care and pharmaceutical products (PCPPs). Two existing decentralized reuse systems were studied, and an additional mobile advanced treatment trailer was designed and deployed to generate water quality data for this study. Water samples included synthetic and actual wastewater effluents, as well as advanced-treatment water samples collected during various phases of development and testing of decentralized potable water reuse systems. Of particular relevance were samples from advanced oxidation and residual disinfection under different conditions. The study results can inform the future use of composite toxicity assays in the optimization of advanced treatment process design and operation.

Experimental

Water Treatment Systems

Three decentralized water reuse systems were assessed as a part of this study (Table 1). These included the Gray Water Treatment and Reuse System (G-WTRS), which is designed for reuse of gray water in deployed military settings; the WaterCycle building-scale direct potable reuse system that is operational in the state of Ohio, and a mobile Direct Potable Reuse Trailer (DPRT) that was built to support this study. The G-WTRS is housed in a shipping container and can treat up to 30,000 gpd (114,000 Lpd) of gray water from showers, sinks, and laundry systems in military contingency bases. The system is designed to recover 80% of the influent as purified water that presents no health risk relative to potable water supply when reused in showering, laundry, and hand washing.[12] The product water is not used or approved for consumptive reuse activities and does not include an AOP process, providing an interesting case alongside the DPR systems with AOP units. The G-WTRS was designed and built by US Army ERDC with support from Highland Engineering (Howell, MI, USA). The treatment train includes a self-cleaning mechanical screen filter with 15 µm cut-off (Eaton), a custom-built, patented intermittently-operated BAC filtration system (US Army ERDC), hollow fiber ultrafiltration membranes (Dow Integra), low-pressure UV disinfection (20 mJ/cm^2), reverse osmosis membranes (Dow HRLE 990), and residual chlorination with on-site chlorine generation (Miox). The WaterCycle system (Tangent LLC) is a building-scale direct potable reuse at the Western Reserve Land Conservatory in Moreland Hills, Ohio, USA and has been operational since May of 2016. The on-site wastewater treatment system uses aerobic bioreactor technology with an HRT of 6 h followed by rapid sand filtration. The water is then processed through an advanced treatment system that includes ultrafiltration, reverse osmosis, and advanced oxidation, followed by conditioning and chlorination. The system can treat up to 500 gpd (1,900 Lpd) and operates at a recovery of 75%. A mobile DPR trailer (DPRT) was designed and assembled to support this project. The treatment flow rate was 2.5 gpm (9.5 Lpm) and the product water recovery ratio was 50%. The mobile DPR trailer has multiple treatment configurations, but the treatment sequence used in this study included biogically-active activated carbon (BAC) filtration, ultrafiltration, reverse osmosis,

Table 1. Experimental matrix for decentralized advanced water treatment and reuse studies. G-WTRS and DPRT tests were for demonstations purposes only (without actual water reuse).

Reuse System	Treatment Train	Challenge Water Type	COD (mg/L)	TOC (mg/L)	Turb-idity (NTU)	Challenge Test Site
G-WTRS	MSF, IOBAC, UF, UV, RO, Cl2	Gray Water	284 ± 59	40 ± 11	31 ± 19	Fort Leonard Wood, Missouri, USA
WaterCycle Building DPR	UF, IX, RO, AOP, Cl2	On-Site WWT Effluent	56 ± 6	14	6.2	Moreland Hills, Ohio, USA
Mobile DPR Treatment Trailer	BAC, UF, RO, AOP, Cl2	Synthetic WWT Effluent	45 ± 8	8.4 ± 1.9	17 ± 6	ERDC-Champaign, Illinois, USA
Mobile DPR Treatment Trailer	BAC, UF, RO, AOP, Cl2	Centralized WWT Effluent	12 ± 7	2.2	4.8	Tobyhanna Army Depot Pennsylvania, USA

advanced oxidation with UV light and hydrogen peroxide (UV-H_2O_2), and chlorination with sodium hypochlorite. The BAC filter had an Empty-Bed-Contact-Time (EBCT) of 20 minutes and was pre-treated with 1 L of bioaugmentation seed

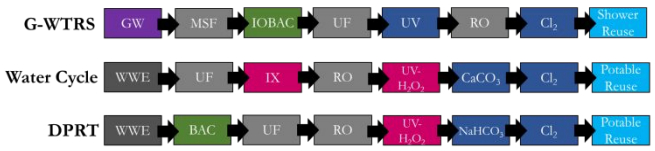

Figure 1. Advanced water treatment trains included the Gray Water Treatment and Reuse System (G-WTRS), the WaterCycle building scale direct potable reuse system, and the Direct Potable Reuse Trailer (DPRT). GW- Gray Water. MSF- Mechanical Screen Filter. IOBAC- Intermittently-Operated Biologically-active Activated Carbon filter. UF- Ultrafilter. RO- Reverse Osmosis. WWE- Wastewater Effluent; IX- Ion exchange; UV-H_2O_2- Ultraviolet Hydrogen Peroxide advanced oxidation; $CaCO_3$- Calcite; Cl2- chlorination with hypochlorite.

(Microbe Lift) 24 h prior to each test, followed by pre-equilibration of the filter with source water for 8 h at the design flow rate. The GAC mesh size was 8 *x* 16, and the bed height was 30 inches (76 cm). The PVDF hollow fiber ultrafiltration membrane had a cut-off of 0.02 microns and was operated at a feed pressure of 35 psi with 2-min air scour and backwash cycles every 30 min. The reverse osmosis membrane was an ultra-low-energy element developed by GE Global Research[13] and manufactured by Suez Water Technologies & Solutions. The RO system was operated at a feed pressure of 85 psi and a permeate recovery of 60%. The UV-H_2O_2 system was set to dose at 10 mg/L of hydrogen peroxide followed by a UV does of 900 mJ/cm^2. Hydrogen peroxide was quenched with either sodium bisulfite or sodium hypochlorite prior to residual disinfection with sodium hypochlorite at 2-4 mg/L as Cl_2.

Challenge Water Sources

The water sources used to challenge the advanced treatment systems during experimentation included gray water from shower and laundry systems in a training area at Fort Leonard Wood (Missouri, USA), synthetic wastewater effluent, building scale wastewater effluent (Moreland Hills, OH, USA), and wastewater effluent from a centralized wastewater treatment plant (Tobyhanna Army Depot, Pennsylvania, USA). General properties of the wastewaters are provided in Table 1. The synthetic wastewater effluent was prepared by subjecting 20 L of synthetic concentrated wastewater (COD 1400 mg/L) to batch aerobic treatment for 6 hours followed by sedimentation for 2 hours, and subsequent dilution of decanted water into de-chlorinated tap water in a 500 gallon tank. The wastewater was augmented with trace levels of common over-the-counter medicines and personal care products, BPA, insect repellent, and artificial sweeteners, as well as pre-formed DBPs generated in the laboratory by reacting 0.5% sodium hypochlorite with 0.2% beef extract broth. A summary of the target final concentrations of the synthetic wastewater ingredients is provided in Supplemental Information Table S.4.

Water Sample Collection

Composite water samples were prepared by collecting 4 L increments throughout the duration of the tests for a total of 28 L. All samples were stored in Amber Type III soda-lime glass jugs that meet EPA performance-based specifications for semi-volatile organics (Thermo Scientific 2452360). Samples were stored on wet ice until processing in accordance with hold time and preservation requirements for each water quality analysis method. Due to the sensitivity of some of the methods to particulates, unfiltered water samples were clarified on-site using a clean microfiltration membrane cartridge filter. In the clarification step, the first 4 L of filtered water were discarded for each sample.

Water Quality Analyses

A 4 L portion of each sample was used for conventional water quality analyses, including measurement of TOC using a QbD1200 automated TOC Analyzer (Hach, Loveland, CO) according to Standard Method 5310C. General water quality parameters (TOC, COD, turbidity, chlorine, pH) were measured in the field or at the ERDC laboratory per standard methods recommended by EPA or APHA Chemical contaminant measurements were performed by contract labs, including National Testing Laboratories (Ypsilanti, MI, USA) and Eurofins Eaton Analytical (Monrovia, CA, USA). National Test Labs analyzed pollutants listed on the EPA primary and secondary contaminant lists using standard methods. Eaton Analytical used LC-MS-MS to analyze 100 personal care and pharmaceutical products commonly found in wastewater. Individual parameter measurements for each sample are provided in the Supplemental Information.

Water Sample Processing for Toxicity Analyses

20 L of each sample was delivered on ice within 24 h of collection to the University of Illinois at Urbana-Champaign for toxicity analyses. Within 24 h of sample receipt, the organics from each water sample were extracted over XAD resins and concentrated in spectroscopy grade ethyl acetate. Organic agents were extracted from the water samples using XAD 2/8 columns. A combination of XAD resins was employed for extraction of organic micropollutants from water samples as recommended by the U.S. EPA.[14] XAD-2 resin (Amberlite XAD-2, Millipore Sigma) isolated polyfunctional organic acids, aliphatic acids with 5 or fewer carbons, and low molecular weight solutes while XAD-8 resin (DAX-8, Millipore Sigma) isolated hydrophobic acid fractions, aliphatic carboxylic acids, aromatic carboxylic acids, phenols, and humic substances.[15] We previously employed XAD resins to isolate organics from water samples for toxicological and chemical analyses, and the recovery of organics from different water types was between 64.6% and 69.5%.[16] Virtually all of the cytotoxic- and genotoxic-responsive agents were recovered from water samples by XAD resins.[17] Before extraction, 110 mL (wet

volume) of the XAD-2 and XAD-8 resins were consecutively washed using Soxhlet extractions with spectroscopy grade solvents: methanol (400 mL), followed by ethyl acetate (400 mL), and finally methanol (400 mL), each for 24 h, respectively. A chromatography column (i.d. × length: 35 mm × 700 mm with a 1 L reservoir) was plugged with glass wool; this was followed by a 1:1 v/v mixture of XAD-2 and XAD-8 resins. The amount of resin was based on the volumetric ratio of water extract to resin and did not exceed 770:1. The packed column was rinsed with three resin volumes of deionized–distilled water, two resin volumes of 0.1 N HCl, one resin volume of 0.1 N NaOH, one resin volume of 0.1 N HCl, and two resin volumes of deionized–distilled water. Each acidified water sample was transferred onto the resin packed resin bed. The XAD resins were eluted with 400 mL of spectroscopy grade ethyl acetate, and the ethyl acetate extract was separated from the residual water. After being dried over anhydrous sodium sulfate, the extracts were concentrated to a volume of 2–3 mL using a vacuum rotary evaporator, and these were concentrated further under a gentle stream of nitrogen. The extracts were solvent exchanged into dimethyl sulfoxide (DMSO) and stored in amber vials with Teflon septa at −20 °C until use for toxicological analyses. The final concentration factor was 1×10^5.

CHO Cells for Toxicity Studies

Chinese hamster ovary (CHO) cell line K1, AS52 (clone 11–4–8) was employed for the mammalian cell-based cytotoxicity and genotoxicity analyses.[18] CHO cells were provided (in 1986) by the Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN. The CHO cells were maintained in Hams F12 medium containing 5% fetal bovine serum (FBS), 1% L-glutamine, and 1% antibiotics (0.25 µg/mL amphotericin B, 100 µg/mL streptomycin sulfate, and 100 units/mL sodium penicillin G in 0.85% saline) at 37 °C in a mammalian cell incubator with a humidified atmosphere of 5% CO_2.

Cytotoxicity Analyses

By measuring the reduction in cell viability in comparison to that in untreated controls, cytotoxicity captures a wide array of toxic insults and adverse biological impacts. This assay measures cytotoxicity as the reduction in cell density after exposure of CHO cells to extracted water sample for 72 h (a chronic exposure encompassing 3–4 cell divisions) compared to that in untreated concurrent controls.[19] For each experiment, a dilution series (generally 10 concentrations) was prepared by diluting the extracted water sample into cell culture medium just prior to cell treatment. CHO cells (3×10^3 cells per well) were exposed to these treatment dilutions in 96-well microplates covered with AluminaSeal that prevented volatilization during the 72 h exposure period. After incubation in a mammalian cell incubator, the cell density per microplate well was determined by histological staining using crystal violet and absorbency at 595 nm using a microplate reader. The resulting data were saved as an Excel file. The dilution series generated from the extracted water sample represented a range of concentration factors for the organics in the original water sample. The range in concentration factors was selected to span concentrations that induced no significant reduction in growth to concentrations that reduced cell density per microplate well. A cytotoxicity concentration–response curve for each extracted water sample was generated from the summary data of the combined replicate experiments. The concentration factor associated with a 50% reduction in cell density compared to the negative controls (LC_{50}) was calculated using regression analyses of the concentration–response curve. Detailed procedures for this assay and its use with water samples and individual chemical contaminants were published.[20] The use of the CHO assay were used to develop QSAR assessment models for DBPs.[21]

Single Cell Gel Electrophoresis for Genotoxicity

The single cell gel electrophoresis (SCGE or comet) assay quantitatively measures genomic DNA damage such as DNA strand breaks, alkali-labile sites, incomplete excision repair sites, and interstrand cross-links in the nuclei of cells.[22] CHO cells (4×10^4) were treated in individual wells of a 96-well microplate with a series of concentrations of each extracted water samples for 4 h at 37 °C. For each experiment, a concurrent negative control, a concurrent positive control of 3.8 mM ethylmethanesulfonate, and nine concentrations of a specific CWS were conducted. After treatment, the cells were removed from the microplate wells using a trypsin-EDTA solution. An aliquot of the cell suspension was used to determine the acute cytotoxicity by employing trypan blue vital dye.[23] SCGE data were not used if the acute cytotoxicity exceeded 30%. The remainder of the cell suspension was incorporated into agarose microgels; the cell membranes were lysed in situ. The microgels were electrophoresed and stained with a fluorescent DNA binding dye to resolve the migration of damaged DNA streaming from the nucleus. The microgels were analyzed with a Zeiss fluorescence microscope with an excitation filter of 546/10 nm and a barrier filter of 590 nm. A computerized image analysis system (Comet Assay IV; Instem PLC, Staffordshire, U.K.) was applied to measure a number of specific SCGE parameters of the nuclei per microgel. The fluorescent intensity of the DNA that migrated away from the nucleus (%Tail DNA) was the primary metric of DNA damage that was used for the concentration–response curves. A regression analysis of the SCGE concentration–response curve was conducted to obtain the concentration factor that induced a 50% Tail DNA value. The details of SCGE analyses for individual DBPs or extracted water samples have been published.[19]

Statistical analyses were conducted on the data for each toxicological end point assay (Tables S1–S3). After a concentration–response curve from combined replicate experiments was generated, a test for significance using a one-way analysis of variance (ANOVA) test was conducted. If a significant F value of $P \leq 0.05$ was obtained, a Holm–Sidak multiple comparison versus the control group analysis was

conducted with the power (1−β) ≥ 0.8 at α = 0.05 to identify the lowest concentration factor that was significantly different from the negative control.[24] After nonlinear regression analyses of the three data sets, LC_{50} values were determined for CHO cell cytotoxicity and 50% Tail DNA values for CHO cell genotoxicity. A bootstrap statistic was conducted for each assay data set, and mean toxicity index values (±SE) were calculated.[25] The cytotoxicity index (CTI) value is $LC_{50}^{-1} \times 10^3$; and the genotoxicity index (GTI) value is the 50%Tail $DNA^{-1} \times 10^3$. Pearson coefficient values and significance levels (*r*, *P*) were calculated to compare CTI and GTI values to water quality measurements including TDS, TOC, and PPCPs.

Results and discussion

Gray Water Treatment and Reuse System

A comparison of the influent gray water (GW) and G-WTRS product water (G-WTRS) showed a high degree of water quality improvement after treatment in terms of toxicity, general water quality parameters, and micropollutant levels (Figure 2). Individual parameter values are provided in Supplemental Information Tables S1.a-S1.f. The level of PPCPs detected in the gray water was 67,108 ng/L, which is relatively low compared to values reported for municipal wastewater influent and more consistent with levels found in wastewater effluents in this study and those studied previously.[26] PPCPs detected included ibuprofen, DEET, acesulfame-K, naproxen, propylparaben, sucralose, triclosan, theophylline, metformin, lidocaine, cotinine, caffeine, and acetaminophen. The gray water had a very high degree of cytotoxicity with a CTI > 100. Due to the high cytotoxicity, the gray water GTI was not measureable due to rapid cell death during the genotoxicity assay. After G-WTRS treatment, the CTI and GTI values were 5.4 and 0.15, respectively. These CTI and GTI values compare favourably with those reported in a study of municipal potable water supplies in which CTI values were 7.3 ± 3.1, and GTI values were 0.67 ± 0.21.[27] In the treated water, no primary or secondary drinking water contaminants were detected at levels of concern. The only water quality concerns observed during testing were associated with the chlorination process. The presence of TOC in the product water was significant at 2.45 mg/L, resulting in some haloacetic acid formation. Periodic ammonia spikes also created chlorine level control challenges. Aside from the ammonia issue, which was later addressed through modification of the treatment train, the water quality and cytotoxicity analyses were indicative of a high level of quality and low level of reuse risk with this system.

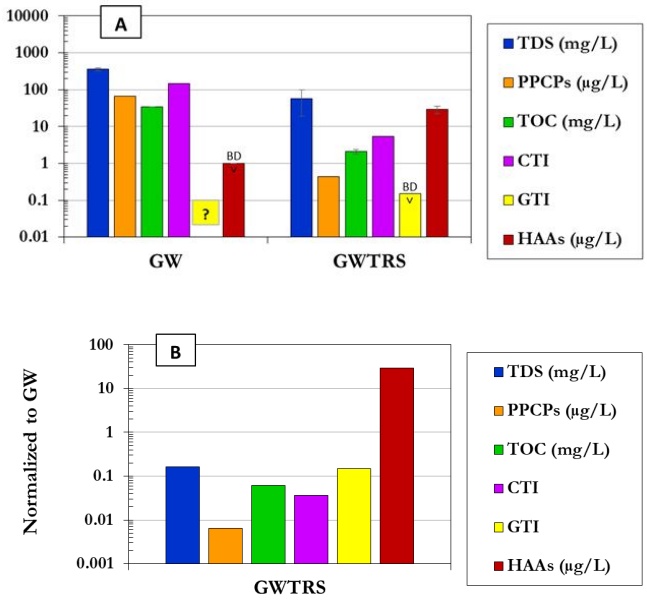

Figure 2. (A) Comparison of water quality and composite toxicity parameters before and after the Gray Water Treatment and Reuse System. GW- Gray Water. G-WTRS- Product Water from the Gray Water Treatment and Reuse System. **(B)** G-WTRS product water values normalized to the influent gray water values. *BD*- Below Detection. *?*- Unknown due to measurement method impairment by sample. *TDS*- Total Dissolved Solids. *PPCPs*- Pharmaceuticals and Personal Care Products. *TOC*- Total Organic Carbon. *CTI*- Cyto-Toxicity Index. *GTI*- Geno-Toxicity Index. *HAAs*- Halo-Acetic Acids.

Building Scale DPR System

For assessing the building scale DPR system, water samples were collected downstream of the on-site wastewater treatment unit (On-site WWE) and immediately downstream of the advanced treatment unit, prior to blending with any makeup water. An additional sample was collected from the existing on-site potable water system (On-site PWS), which treated local groundwater with ion exchange, a cartridge filter, and UV disinfection. Key water quality and toxicity data are shown in Figure 3. Individual parameter values are provided in Supplemental Information Tables S1.a-S1.f. For the wastewater effluent (WWE), the TOC was 10.2 mg/L, and the detected PPCPs (61,559 ng/L) were largely comprised of sucralose and acesulfame-K. Trace levels (< 20 ng/L) of BPA, butalbital, theobromine, TCPP, Lopressor, DEET, and dehydro nifedipine were also detected. Haloacetic acids were present, indicating that they were passing through the on-site wastewater treatment system. After advanced treatment, the DPR product water appeared to be of high quality, with CTI and GTI values comparable to or better than those of

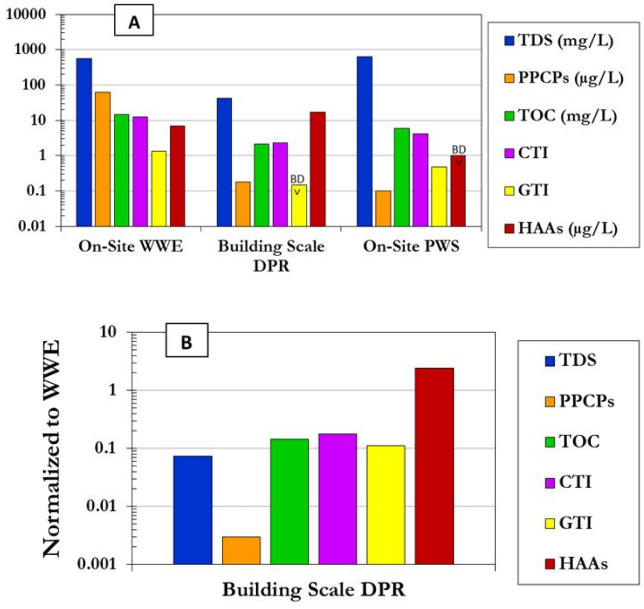

Figure 3. (a) Comparison of water quality and composite toxicity parameters before and after advanced treatment of effluent (WWE) from an on-site wastewater treatment system for direct potable reuse (DPR) at building scale. An existing on-site potable water system (PWS) was also studied for comparison. **(b)** DPR product water values normalized to the WWE source water values.

municipal potable water. Measured PCPPs totalled 162 ng/L. For this set of samples, the composite cytotoxicity and genotoxicity values from the DPR system and those from the on-site potable water treatment system fell within range with those from municipal potable water systems.[27] For this particular data set, the TOC and CTI values were similar in each sample.

Direct Potable Reuse Trailer Optimization Studies with Synthetic Wastewater Effluent

Before deploying the mobile DPR Trailer (DPRT) to a field test site, verification and optimization studies were performed using synthetic wastewater effluent at a controlled facility. These tests identified some important issues with the system. As shown in Figure 4, which represents data from three separate pilot tests, the cytotoxicity of the water actually increased as a result of treatment, despite drastic improvements in many of the key water quality parameters. Individual parameter values are provided in Supplemental Information Tables S2.a-S2.f. While one of the three chlorinated DPRT samples had HAAs totalling 187 µg/L, raising the average above the EPA maximum contaminant level, the other two samples had HAA values below 20 µg/L. However, the CTI values were consistently elevated in all three of these samples, indicating that the cytotoxicity was being driven by undetected contaminants. THMs were low or not detected. While HAAs were present at high levels in the influent, by design, the BAC, membranes, and UV-H_2O_2 removed these to below detection, as shown in the non-chlorinated sample (DPRT, no NaOCl). Clearly, DBP precursors were permeating the system and forming unknown DBPs with cytotoxic and genotoxic properties. An engineering analysis of the system noted that the use of bisulfite to quench H_2O_2 prior to chlorination resulted in very low pH levels (~4.9) when the chlorine was added to the system. Further, in two of the tests, solvents used in making repairs in the UV-H_2O_2 plumbing were also noted as potential DBP-precursor contributors, as trace levels of toluene (10-30 µg/L) were found in the treated water samples but not the SWWE. Prior to field testing, the system was modified to raise the pH with sodium carbonate prior to chlorination and thoroughly flushed to reduce potential leaching. The CTI and GTI values identified an issue that was not otherwise evident despite monitoring for over 300 individual chemical contaminants, with more than 95% of EPA primary contaminants compounds being below detection and none of those detected measuring within 50% of EPA maximum contaminant level (except for the single high HAA sample mentioned previously).

Direct Potable Reuse Trailer Field Demonstration Study

After making technical adjustments guided by the composite toxicity studies, the DPR trailer was field tested using wastewater effluent at a centralized wastewater plant at Tobyhanna Army Depot. Upon arrival at the test site, two on-site plumbing repairs were required due to PVC fittings cracking during transport of the system over 800 miles of highway. After the repair, the system was operated for 6 h prior to sampling, but trace amounts of BTEX compounds toluene and benzene (< 20 and 2 µg/L, respectively) were still present in the treated samples. These compounds were not present in the WWE challenge water going into the system, indicating they were leaching from the plumbing. Despite this issue, the results from the field test showed considerable

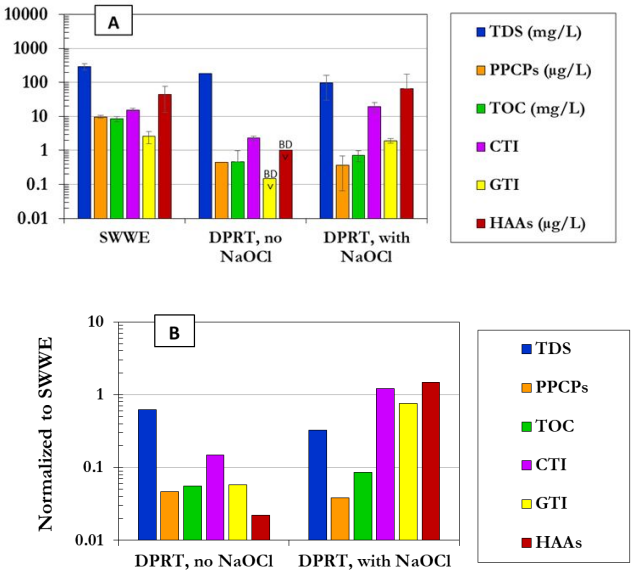

Figure 4. (A) Comparison of water quality and composite toxicity parameters before and after the advanced treatment of synthetic wastewater effluent (SWWE) by the mobile DPR trailer (DPRT, with NaOCl). A non-chlorinated sample (DPRT, no NaOCl) is provided for comparison. **(B)** DPRT product water values normalized to the SWWE

performance improvements relative to the synthetic wastewater challenge tests (Figure 5), likely because the pH was increased to 7.1 prior to chlorine dosing. Individual parameter values are provided in Supplemental Information Tables S3.a-S3.f. During the field test, samples were taken of the influent (WWE), after the BAC, UF, and RO filtration processes (RO), after advanced oxidation with UV-H_2O_2 (AOP), and then after chlorination (Cl_2). The challenge water (WWE) had high TDS but low TOC (2 mg/L). The concentration of monitored PPCPs was 110,563 ng/L, comprised primarily of sucralose (81%) and asulfame-K (7%). Additional PPCPs detected in the wastewater effluent at trace levels included albuterol, BPA, butalbital, carisoprodol, cotinine, DEET, diclofenac, dilantin, gemfibrozil, ibuprofen, iohexol, meprobamate, naproxen, salicylic acid, TDCPP, theobromine, warfarin, atenolol, caffeine, cimetidine, diuron, sulfamerazine, trimethoprim, amoxicillin, carbamazepine, diltiazem, lidocaine, lopressor, TCPP, TCEP, and fluoxetine. As treatment progressed, PPCPs were decreased to less than the detection limit for each of the PPCPs. The AOP process destroyed the small fraction of PPCPs remaining after RO filtration, while significant decreases in TOC were not observed between the BAC-UF-RO, +AOP, and +NaOCl samples. As with the SWWE

challenge studies (Figure 4), the CTI increased during the chlorination process (Figure 5), indicating DBP formation was still occurring. However, the CTI increased to a value of 7, which is comparable to the average cytotoxicity value reported for municipal potable water systems[26] and significantly lower than the value of 19 that was observed during the SWWE challenge studies (Figure 4). The pH adjustment prior to chlorination may have helped to reduce DBP formation, given that similar levels of residual solvent where observed in both tests. It is expected that addressing the aforementioned solvent leaching issue will result in lower formation of unknown chlorinate DBPs and further reduction of the composite toxicity index values.

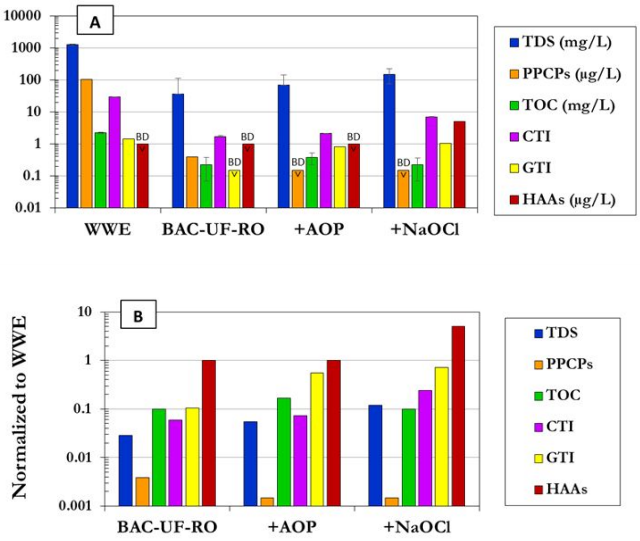

Figure 5. (A) Comparison of water quality and composite toxicity parameters before and after the advanced treatment of wastewater effluent (WWE) by the mobile DPR trailer during testing at the Tobyhanna Army Depot wastewater facility. Filtration-only (BAC-UF-RO) and filtration plus AOP (+AOP) samples are provided for comparison to the fully treated, chlorinated product water (+NaOCl). **(B)** DPRT product water values normalized to the WWE values.

Correlation Analysis

An important result from this study was that composite toxicity did not appear to correlate strongly with some of the key 'performance-indicator' parameters monitored during operation of DPR systems, such as TOC and TDS. Pearson correlation coefficients for a matrix of commonly measured water quality parameters plus the composite toxicity index values are presented in Table 2. Initially, all of the water samples were subjected to correlation analysis, with the exception of the gray water influent, which was deemed an outlier that might skew the data due to the high TOC and cytotoxicity. An additional analysis was performed using only the treated water samples, which increased the level of correlation of some parameters but did not change the overall outcome. The only significant correlations ($P < 0.05$) identified were COD:TOC (expected); CTI:GTI; and COD:TDS. When focusing solely on the treated water samples, the most notable change was an increased correlation ($P < 0.10$) between CTI and HAAs. While the strength of the correlation between composite toxicity index values and HAAs is limited, the trend is consistent with the notion that the chlorination step increases the toxicity of the water sample. The increase in toxicity of the chlorinated samples is not due to the presence of chlorine, or the lack of quenching in the present study. Previous studies determined that not quenching samples provided a better measure of toxicity that reflects the distribution network and that the toxicity was due to disinfection byproducts.[27] Thus, it is theorized that the increases in toxicity observed in the present study arise from unmeasured and possibly unknown DBPs. These data further demonstrate the absolute need to integrate both analytical biology and analytical chemistry when evaluating and optimizing new water reuse technologies.

Table 2. Correlation analysis of the total sample set as well as the subset of treated water samples. For each parmeter combination, correlation values are in the lower left half, and P-values are in the upper right half.

		P-Values (< 0.05, < 0.10)							
		CTI	**GTI**	**HAAs**	**THMs**	**TOC**	**COD**	**PPCPs**	**TDS**
Correlation Values Based on Total Sample Set	**CTI**		0.0129	0.1022	0.7023	0.6682	0.5698	0.0859	0.1771
	GTI	0.6057		0.5394	0.4161	0.2267	0.1177	0.6176	0.7343
	HAAs	0.4273	0.208		0.5698	0.9886	0.8083	0.6193	0.6901
	THMs	0.1037	0.2733	0.1929		0.7609	0.7522	0.7881	0.8577
	TOC	0.1162	0.397	0.0049	-0.104		0.00001	0.2983	0.7609
	COD	0.1537	0.4995	0.083	-0.1079	0.9492		0.5044	0.00013
	PPCPs	0.4427	0.0709	-0.1698	-0.0919	0.3453	0.2258		0.2851
	TDS	0.4387	0.1159	-0.136	-0.0674	-0.104	0.904	0.3453	
Correlation Values Based on Treated Water Samples Only	**CTI**		0.003	0.0721	0.6512	0.5292	0.1785	0.8151	0.0433
	GTI	0.8012		0.4393	0.1609	0.9163	0.6864	0.7861	0.2378
	HAAs	0.5618	0.2604		0.5692	0.7825	0.8083	0.2988	0.3617
	THMs	0.154	0.4538	0.1932		0.776	0.9235	0.9299	0.477
	TOC	0.2131	-0.0366	0.0944	0.0973		0.000218	0.6369	0.7475
	COD	0.4374	0.1377	0.083	0.0329	0.8926		0.504	0.4089
	PPCPs	-0.08	0.0928	-0.3549	0.0301	-0.1607	-0.2216		0.5891
	TDS	0.6165	0.3884	0.3053	0.2401	0.11	0.2774	-0.1835	

Conclusions

Small scale direct potable reuse systems featuring new low energy reverse osmosis membranes and advanced oxidation processes are capable of producing water of a quality similar to or better than current municipal potable water supplies when designed, assembled, and operated properly. By helping to account for unknown contaminants, composite toxicity assays represent an important tool for augmenting the evaluation of direct potable reuse and other advanced treatment systems. Based on the results of this study, disinfection byproducts appear to be important drivers of toxicity, but the specific drivers of toxicity in these samples may be unknown disinfection byproducts. Controlling DBP formation potential through pH adjustment prior to chlorination may help reduce DBP formation in some cases, particularly in the low pH environments downstream of RO systems and some AOP reactant quenching operations (i.e., H_2O_2 reduction). Several of the product waters from these systems had TOC above 0.5 mg/L but low levels of toxicity. Given the large array of organic and nitrogenous compounds

likely present in treated wastewater effluents, the complexity associated with byproduct formation from AOP and disinfection, and the potential for operational impacts at small scale, the incorporation of composite toxicity analyses into performance validation seems like a practical approach for addressing technical uncertainty with a single method. While not a replacement for chemical measurements, the incorporation of composite toxicity analysis can augment conventional water quality monitoring to potentially reduce risks associated with these exciting capabilities that will play an important role in a sustainable and resilient future.

Conflicts of interest

There are no conflicts of interest to declare.

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References

L. Mulford, E. Moore, D. MacNevin, and J. Ribotti, Potable reuse pilots and demonstrations; a review of flow, treatment, and cost, *Proceedings of the Water Environment Federation*, 2018, **15**, 1966; M. Page, A. Hur, B. MacAllister, E. Jenicek, and D. Cropek. Distributed water reuse systems for military applications. *WorldWater: Water Reuse and Desalination*, 2017, **8**, 13.

[2] D. Gerrity, B. Pecson, R.S. Trussell, and R.R. Trussell, Potable reuse treatment trains throughout the world, *AQUA,* 2013, **62**, 321.

[3] M.A. Jahne, M.E. Schoen, J.L. Garland, and N.J. Ashbolt. Simulation of enteric pathogen concentrations in locally-collected greywater and wastewater for microbial risk assessments *Microbial Risk Analysis,* 2017, **5**, 44; Sano, D., Haas, C.N. and Rose, J.B. *Global Water Pathogen Project.* 2019.

[4] WHO. Potable Reuse: Guidance for producing safe drinking-water. Geneva: World Health Organization; 2017.

[5] M. J. Plewa and E. D. Wagner, in *Occurrence, Formation, Health Effects, and Control of Disinfection By-Products*, 2015, **1190**, 3; S. D. Richardson and M. J. Plewa, To regulate or not to regulate? What to do with more toxic disinfection by-products? *Journal of Environ. Chem. Eng.*, 2020, **8**, 103939.

[6] S. D. Richardson, M. J. Plewa, E. D. Wagner, R. Schoeny and D. M. DeMarini, Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research, *Mutation Research*, 2007, **636**, 178; M. J. Plewa, E. D. Wagner, M. G. Muellner, K. M. Hsu and S. D. Richardson, in *Occurrence, Formation, Health Effects and Control of Disinfection By-Products in Drinking Water*, 2008, **995**, 36; B.I. Escher, M. Allinson, R. Altenburger, P.A. Bain, P. Balaguer, W. Busch, J. Crago, N.D. Denslow, E. Dopp, and K. Hilscherova. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environ. Sci. Technol.* 2014, 48, (3), 1940-1956.

[7] S.D. Melvin and F.D. Leusch, Removal of trace organic contaminants from domestic wastewater: A meta-analysis comparison of sewage treatment technologies. *Environment International,* 2016, 92, 183-188; K. Watson, G. Shaw, F.D. Leusch, and N.L. Knight, Chlorine disinfection by-products in wastewater effluent: bioassay-based assessment of toxicological impact. *Water Res.* 2012, 46, (18), 6069-6083; B.I Escher, C. van Daele, M. Dutt, J.Y. Tang, and R. Altenburger, Most oxidative stress response in water samples comes from unknown chemicals: the need for effect-based water quality trigger values, *Environ. Sci. Technol.,* 2013, **47**, 7002.

[8] S. S. Lau, X. Wei, K. Bokenkamp, E. D. Wagner, M. J. Plewa and W. A. Mitch, Assessing additivity of cytotoxicity associated with disinfection byproducts in potable reuse and conventional drinking waters, *Environ. Sci. Technol., 2020,* **54***,* 5729; D. Stalter, E. O'Malley, U. von Gunten and B. I. Escher, Mixture effects of drinking water disinfection by-products: implications for risk assessment, *Environ. Sci.: Water Res. Technol.* 2020, DOI: 10.1039/C9EW00988D; M. J. Plewa, E. D. Wagner and S. D. Richardson, TIC-Tox: A preliminary discussion on identifying the forcing agents of DBP-mediated toxicity of disinfected water, *Journal of Environmental Science*, 2017, **58**, 208.

[9] S. Dong, M. Page, N. Massalha, A. Hur, K. Hur, K. Bokenkamp, E.D. Wagner, and M.J. Plewa, Toxicological comparison of water, wastewaters, and processed wastewaters, *Environmental Science & Technology*, 2019 **53**, 9139.

[10] S. D. Richardson and M. J. Plewa, To regulate or not to regulate? What to do with more toxic disinfection by-products? *Journal of Environmental & Chemical Engineering*, 2020, **8**, 103939.

[11] California Environmental Protection Agency. *Water Quality Control Policy for Recycled Water*. 2019.

[12] U.S. Army Public Health Center. Environmental Health Engineering Assessment No. S.0052748-19. 2019.

[3] Wang, Hua. Membrane Bioreactor/Ultra Low Energy Reverse Osmosis Membrane Process for Forward Operating Base. Final Report SERDP Project ER-2238. 2014.

[14] G. Crumley, XAD resin extraction disinfectant by-products in drinking water: EPA SOP RSB-003.0, 2003, 1.

[5] A. T. Chow, Comparison of DAX-8 and XAD-8 resins for isolating disinfection byproduct precursors, *Journal of Water Supply: Research & Technology, AQUA*, 2006, **55**, 45.

[6] H. Liberatore, M. J. Plewa, E. D. Wagner, J. Vanbriesen, D. Burnett, L. Cizmas and S. D. Richardson, Identification and comparative mammalian cell cytotoxicity of new iodo-phenolic disinfection byproducts in chloraminated oil and gas wastewaters, *Environmental Science and Technology Letters*, 2017, **4**, 475; M. J. Plewa, E. D. Wagner, D. H. Metz, R. Kashinkunti, K. J. Jamriska and M. Meyer, Differential toxicity of drinking water disinfected with combinations of ultraviolet radiation and chlorine, *Environmental Science & Technology*, 2012, **46**, 7811; C. H. Jeong, E. D. Wagner, V. R. Siebert, S. Anduri, S. D. Richardson, E. J. Daiber, A. B. McKague, M. Kogevinas, C. M. Villanueva, E. H. Goslan, W. Luo, L. M. Isabelle, J. F. Pankow, R. Grazuleviciene, S. Cordier, S. C. Edwards, E. Righi, M. J. Nieuwenhuijsen and M. J. Plewa, Occurrence and toxicity of disinfection byproducts in European drinking waters in relation with the HIWATE epidemiology study, *Environmental Science and Technology*, 2012, **46**, 12120.

[17] J. Le Roux, M. J. Plewa, E. D. Wagner, M. Nihemaiti, A. Dad and J. P. Croue. Chloramination of wastewater effluent: Toxicity and formation of disinfection byproducts, *Journal of Environmental Science*, 2017, **58**, 135.

[8] E. D. Wagner, A. L. Rayburn, D. Anderson and M. J. Plewa, Calibration of the single cell gel electrophoresis assay, flow cytometry analysis and forward mutation in Chinese hamster ovary cells, *Mutagenesis*, 1998, **13**, 81.

[19] M. J. Plewa, Y. Kargalioglu, D. Vankerk, R. A. Minear and E. D. Wagner, Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products, *Environmental Molecular Mutagenesis*, 2002, **40**, 134-142; M. J. Plewa and E. D. Wagner, *Mammalian Cell Cytotoxicity and Genotoxicity of Disinfection By-Products*, Water Research Foundation, Denver, CO, 2009.

[20] E. D. Wagner and M. J. Plewa, CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: an updated review, *Journal of Environmental Science*, 2017, **58**, 64.

[2] L.T. Qin, X. Zhang, Y.-H. Chen, L.-Y. Mo, H.-H. Zeng, Y.-P. Liang, H. Lin and D.-Q. Wang, Predicting the cytotoxicity of disinfection by-products to Chinese hamster ovary by using linear quantitative structure–activity relationship models, *Environmental Science & Pollution Research*, 2019, 1; Z. Zhang, Q. Zhu, C. Huang, M. Yang, J. Li, Y. Chen, B. Yang and X. Zhao, Comparative cytotoxicity of halogenated aromatic DBPs and implications of the corresponding developed QSAR model to toxicity mechanisms of those DBPs: Binding interactions between aromatic DBPs and catalase play an important role, *Water Research*, 2020, **170**, 115283.

[22] R. R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J. C. Ryu and Y. F. Sasaki, Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, *Environmental Molecular Mutagenesis*, 2000, **35**, 206; M. S. Rundell, E. D. Wagner and M. J. Plewa, The comet assay: genotoxic damage or nuclear fragmentation? *Environmental Molecular Mutagenesis*, 2003, **42**, 61.

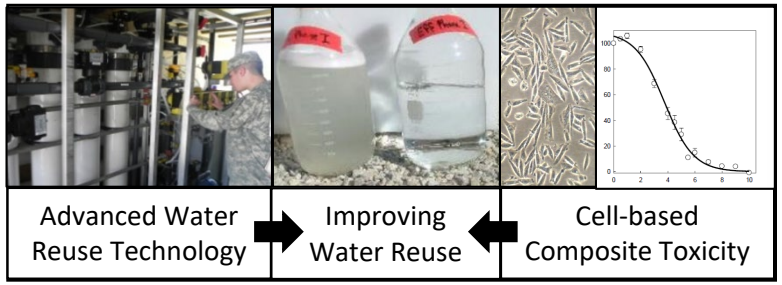
[23] H. J. Phillips, in *Tissue Culture: Methods and Applications*, eds. P. F. Kruse and M. J. Patterson, 1973, p. 406.

[24] G. E. P. Box, W. G. Hunter and J. S. Hunter, *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building*, 1978.

[25] A. J. Canty, A. C. Davison, D. V. Hinkley and V. Ventura, Bootstrap diagnostics and remedies, *Canadian Journal of Statistics*, 2006, **34**, 5-27.

[26] Yang, Yi, Yong Sik Ok, Ki-Hyun Kim, Eilhann E. Kwon, and Yiu Fai Tsang. Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review, *Science of the Total Environment*, 2017, **596**, 303.

[27] C. H. Jeong, E. D. Wagner, V. R. Siebert, S. Anduri, S. D. Richardson, E. J. Daiber, A. B. McKague, M. Kogevinas, C. M. Villanueva, E. H. Goslan, W. Luo, L. M. Isabelle, J. F. Pankow, R. Grazuleviciene, S. Cordier, S. C. Edwards, E. Righi, M. J. Nieuwenhuijsen and M. J. Plewa, Occurrence and toxicity of disinfection byproducts in European drinking waters in relation with the HIWATE epidemiology study, *Environmental Science & Technology*, 2012, **46**, 12120.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