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Toxicological assessment of potable reuse and conventional drinking waters

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Potable reuse, the process of treating wastewater to drinkable standards, offers a reliable and sustainable solution to cities and regions facing shortages of clean water. However, implementation is hindered by perceptions of poor water quality and potential health threats. Herein, we compare water samples from potable reuse systems with conventional drinking waters based on the analysis of Chinese hamster ovary cell cytotoxicity contributed by disinfection by-products (DBPs) and sewage-derived anthropogenic contaminants. In all cases, the cytotoxicity of potable reuse waters is lower than that of drinking waters derived from surface waters. The median contribution to total cytotoxicity was 0.2% for regulated DBPs and 16% for the unregulated DBPs of current research interest. Nonvolatile, uncharacterized DBPs and anthropogenic contaminants accounted for 83% of total cytotoxicity. Potable reuse waters treated by reverse osmosis are not more cytotoxic than groundwaters. Even in the absence of reverse osmosis, reuse waters are less cytotoxic than surface drinking waters. Our results suggest that potable reuse can provide a safe, energy-efficient and cost-effective alternative water supply.

Prolonged droughts induced by climate change and rising water demands in urban areas due to population growth are making the current reliance on freshwater sources for drinking water unsustainable. Many utilities are considering potable reuse of municipal wastewater, which can be a local, reliable and sustainable option to augment drinking water supplies. In coastal areas, potable reuse systems often employ microfiltration (MF), reverse osmosis (RO), and the ultraviolet/hydrogen peroxide advanced oxidation process (UV/H_2O_2 AOP) to remove sewage-derived microbial and chemical contaminants[1–3]. RO-based potable reuse is less energy intensive (1.0–2.5 kWh m^{-3}) (refs. [4,5]) than seawater desalination (3–6 kWh m^{-3}) (refs. [6,7]) and ~40% less costly[4]. For inland utilities, RO-free potable reuse trains based on ozonation and biologically active filtration (O_3/BAF) are attractive alternatives that avoid the challenge of discharging RO concentrate[3] and consume less energy (<0.5 kWh m^{-3}) (refs. [4,5]). Yet the association of potable reuse water with sewage has promoted adverse perceptions of water quality[8,9] that hinder the adoption of potable reuse as a sustainable and cost-effective alternative to seawater desalination, particularly for RO-free systems where contaminant removal is expected to be less efficient than in RO-based trains. These perceptions can drive the incorporation of additional treatment processes that increase the energy intensity and cost of potable reuse for only marginal water-quality improvements. Thus, quantitative evaluations of potable reuse water quality are critical.

Previous studies[10–13] have characterized potable reuse water quality by combining targeted analyses of specific contaminants with bioassays, which can capture the biological effects of uncharacterized contaminants. These studies indicate that reuse water, whether produced using RO-based[10–13] or RO-free[11] systems, is not more toxic than conventional drinking water. However, the chemical analyses and extraction procedures employed to prepare samples for bioassays in these and other studies on reuse water quality focused on sewage-derived anthropogenic contaminants, primarily pharmaceuticals and personal care products (PPCPs)[10,11,13–15]. The occurrence

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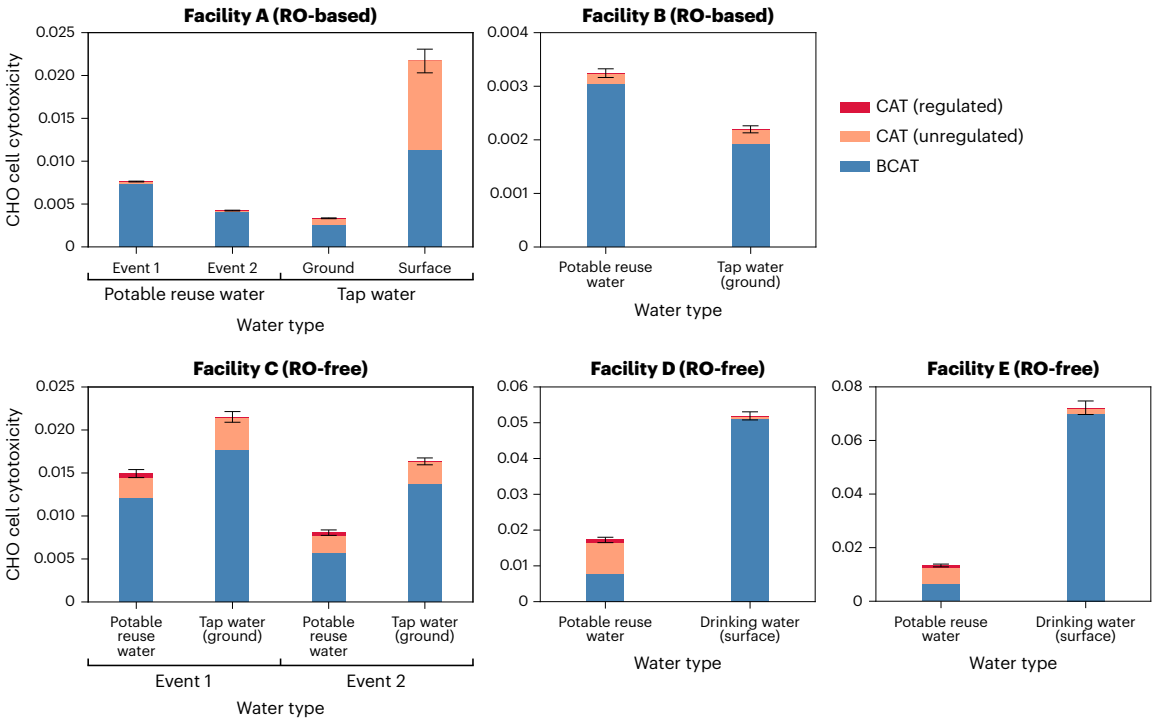

Fig. 1 | Comparison of potable reuse and conventional drinking-water quality. Total CHO cell cytotoxicity, represented by the sum of CAT (contributed by regulated and unregulated DBPs) and BCAT for the final reuse water, for each potable reuse facility and the local conventional drinking water. Error bars denote standard errors of the total cytotoxicity (uncertainty calculations are described in Supplementary Note 3).

and toxicity of other contaminant classes in reuse water are relatively unexplored.

One often-overlooked contaminant class in potable reuse water is disinfection by-products (DBPs), which occur at concentrations far closer to levels of potential human health concern than do PPCPs[14]. DBPs form when disinfectants are applied (1) during reuse treatment (for example, chloramination to control biofouling of MF and RO membranes[1–3]), (2) after reuse treatment and before transport to environmental or engineered buffers and (3) during subsequent drinking-water treatment[16]. While >700 DBPs have been identified, research has focused on a smaller pool that includes the trihalomethanes (THMs) and haloacetic acids (HAAs) regulated in many countries as well as unregulated classes such as haloacetonitriles (HANs) and haloacetaldehydes (HALs)[17]. Unlike PPCPs, many of these DBPs are low-molecular-weight neutral compounds with electron-withdrawing substituents that are poorly removed by RO and AOPs[18–22]. Although previous studies have suggested that disinfectants applied during[11] or after[10–13] treatment increased the toxicity of reuse waters, DBPs were not measured except in one study[13] that reported THM and HAA concentrations.

Unfortunately, bioassays do not capture the effects of many DBPs of current research focus because these DBPs are (semi-)volatile and are lost during water sample extraction[23]. The innovative purge and cold-trap approach developed by Stalter et al.[24] could potentially capture a wide range of (semi-)volatile DBPs for bioassays, but <32% of HANs in conventional drinking waters was retained[24]. Accordingly, bioassays conducted on whole-water extracts are measuring primarily the toxicity of nonvolatile, largely uncharacterized DBPs (representing >50% of total organic halogen)[25] and sewage-derived anthropogenic chemicals.

Of the (semi-)volatile DBPs, ~100 have been analysed for Chinese hamster ovary (CHO) cell cytotoxicity[26], resulting in a large database of lethal concentration 50 (LC_{50}) values (the concentration of a DBP associated with 50% reduction in CHO cell density). For defined mixtures of (semi-)volatile DBPs, the bioassay response is within 12% of the cytotoxicity predicted by weighting individual DBP concentrations by CHO cell LC_{50} values and then summing the toxic potency-weighted concentrations (calculated additive toxicity (CAT); equation (1))[27]. CAT calculations indicate that potable reuse waters, even those from RO-free treatment trains, have comparable or lower cytotoxicity associated with (semi-)volatile DBPs relative to conventional drinking waters[28]. While previous studies had combined the toxicity of (semi-)volatile DBPs with that of nonvolatile DBPs and other contaminant classes to assess conventional drinking-water quality[24,29], this combination has not been applied to reuse waters.

Using CHO cell cytotoxicity as the metric, this study combines the CAT of known (semi-)volatile DBPs with the bioassay response of nonvolatile, largely uncharacterized DBPs and sewage-derived anthropogenic contaminants to assess potable reuse water quality. This study relies on CHO cell chronic cytotoxicity for fundamental and practical reasons. Fundamentally, cytotoxicity is a broad metric of toxicity because many modes of toxic action can reduce cell growth. Practically, CHO cell cytotoxicity is the only toxicity endpoint for which LC_{50} values for many (semi-)volatile DBPs are available and the predictive nature of CAT calculations has been demonstrated. Potable reuse waters, whether produced by RO-based or RO-free treatment trains, are of comparable or higher quality than local conventional drinking waters from the same catchments. It remains unclear whether DBPs are more cytotoxic than sewage-derived anthropogenic chemical; however, the regulated THMs and HAAs, in addition to the unregulated (semi-)volatile DBPs of current research interest, account for <50% of the total cytotoxicity in most of the potable reuse and conventional drinking waters. Our results demonstrate that potable reuse can provide a sustainable source of clean water.

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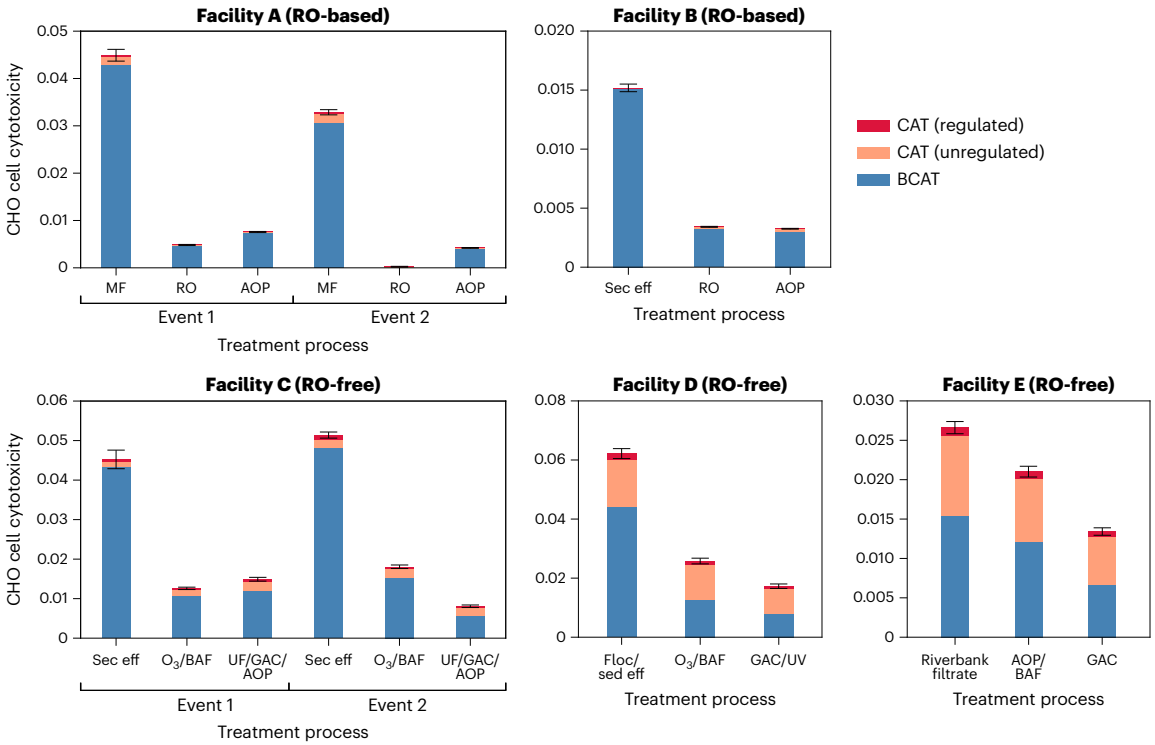

Fig. 2 | Effect of advanced treatment processes on cytotoxicity. Total CHO cell cytotoxicity, represented by the sum of CAT (contributed by regulated and unregulated DBPs) and BCAT for samples collected along RO-based and RO-free potable reuse treatment trains. Error bars represent standard errors of the total cytotoxicity (uncertainty calculations are described in Supplementary Note 3). sec eff, secondary biological wastewater treatment; floc/sed eff, flocculation and sedimentation; riverbank filtrate, riverbank filtration.

Results

Extraction-method validation

To assess the cytotoxicity of nonvolatile, uncharacterized DBPs and anthropogenic contaminants, we concentrated 10 l water samples 50,000 times using solid-phase extraction (SPE) cartridges packed with Sepra ZTL sorbent. Analysis of CHO cell cytotoxicity for various dilutions of each extract revealed the concentration factor (CF) relative to the 10-l sample that exerted 50% reduction in cell density (LC_{50}). The inverse of this CF provides the bioassay-based CAT (BCAT; equation (2)), which can be directly compared with the CAT of the (semi-)volatile DBPs that are typically lost during extraction (equation (1)).

$$\mathrm{CAT} = \sum_{i=1}^{n}\left(\frac{[\mathrm{DBP}]_i}{\mathrm{LC}_{50_i}}\right) \quad (1)$$

$$\mathrm{BCAT} = \frac{1}{(\mathrm{CF})_{\mathrm{LC}_{50}}} \quad (2)$$

Extraction of 10 l deionized (DI) water using Sepra ZTL sorbent resulted in negligible cytotoxicity (BCAT = 0; Supplementary Fig. 1a). Adding an anion exchange sorbent to SPE cartridges, or using Dupont Amberlite™ XAD resins for SPE as in many previous DBP studies[12,13,30–36], resulted in substantially higher BCAT values, suggesting the leaching of cytotoxic materials from these sorbents. SPE with only Sepra ZTL sorbent also maximized the recovery of cytotoxins from a chlorinated surface water (Supplementary Fig. 1b). The potable reuse facilities using the UV/H_2O_2 AOP applied 5–6.5 mg l^{-1} H_2O_2, but the residual H_2O_2 is difficult to quench or measure when chloramines, or the thiosulfate used to quench chloramines, are present (Supplementary Note 1). We conducted a control experiment to examine whether unreacted H_2O_2 could cause artefacts in bioassays. DI water containing 3.5 mg l^{-1} H_2O_2 and chloramines exhibited a detectable but low cytotoxic response (BCAT = 0.011; Supplementary Fig. 1a) after extraction and analysis; these results indicate that caution is needed when examining the cytotoxicity of UV/H_2O_2 AOP-treated waters since a portion of cytotoxicity could be attributable to residual H_2O_2.

Potable reuse and conventional drinking water cytotoxicity

We collected 10 l water samples along five potable reuse facilities in the United States, chloraminated the samples using protocols similar to those used by the facilities, extracted the samples using SPE and analysed the extracts for cytotoxicity. We also collected conventional drinking waters from the same locations and processed them similarly. For each extract, we calculated BCAT from the LC_{50} value using equation (2). Separately, we measured concentrations of (semi-)volatile DBPs (10 THMs, 10 HAAs, 4 HANs, 4 HALs, 4 haloacetamides (HAMs) and chloropicrin; Supplementary Tables 1–8 (the supplementary tables are also provided in Excel format in Supplementary Data 1) and Supplementary Note 2) in each water and calculated CAT using equation (1). The concentrations of these (semi-)volatile DBPs retained in the extracts were measured, and the CAT associated with these DBPs was subtracted from BCAT to avoid double counting their contribution to total cytotoxicity.

Figure 1 shows the total cytotoxicity, calculated as the sum of CAT and BCAT, for individual reuse waters compared with conventional drinking waters from the same catchment. Very low levels of cytotoxicity were observed in groundwater-derived drinking waters and RO-based reuse waters from Facilities A and B. At Facility A, AOP treatment increased the BCAT relative to the chloraminated RO permeate (Fig. 2), due partly to H_2O_2 addition. Although AOP treatment probably modifies cytotoxicity, the cytotoxicity of the chloraminated RO permeate samples provides a rough estimate of the cytotoxicity of the final reuse waters since H_2O_2 had not yet been added. All RO-free reuse

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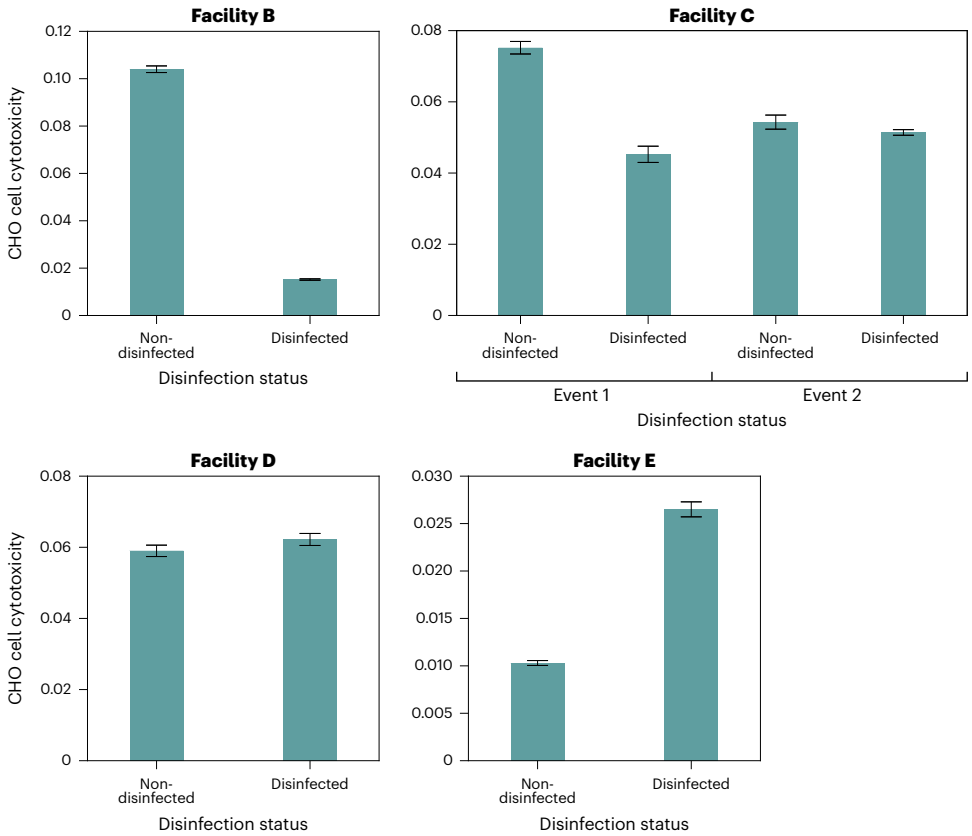

Fig. 3 | Contributions of DBPs and anthropogenic chemicals towards cytotoxicity. Total CHO cell cytotoxicity, represented by the sum of BCAT and CAT, for advanced treatment train influents collected from Facilities B, C, D and E with and without chloramine disinfection. Error bars represent standard errors of the total cytotoxicity (uncertainty calculations are described in Supplementary Note 3).

waters exhibited lower cytotoxicity than their associated drinking waters. A portion of the cytotoxicity of the reuse waters from Facility C, an RO-free train that uses UV/H_2O_2 AOP as the final treatment unit, was probably attributable to residual H_2O_2. The conventional drinking water related to Facility C is a groundwater with higher total organic carbon (0.6–0.7 mg l^{-1}) than those related to Facilities A and B (<0.5 mg l^{-1}; Supplementary Table 9).

Contributors to cytotoxicity in municipal wastewaters

Figure 3 compares the total CHO cell cytotoxicity (including CAT and BCAT) of the municipal wastewater effluents entering Facilities B–E before and after application of chloramines; a sample from Facility A before disinfection was not available. The cytotoxicity of effluents before disinfection reflects the maximum contribution of sewage-derived anthropogenic contaminants as subsequent potable reuse treatment processes partially remove these contaminants. The cytotoxicity of the effluents before disinfection ranged from ~0.01 at Facility E to ~0.1 at Facility B (Fig. 3), indicating substantial variation in the levels of anthropogenic contaminants in the sewage. Facility E indicated that the source water is a river dominated by wastewater effluents; the low cytotoxicity of the Facility E influent may be due to removal of anthropogenic contaminants by riverbank filtration upstream of reuse treatment. Facility B indicated that agriculture-related wastewaters contribute to the reuse train influent, suggesting that pesticides may have contributed to the high cytotoxicity.

Reactions with disinfectants can degrade some sewage-derived anthropogenic chemicals, forming transformation products that would be considered DBPs. Disinfection of Facility B influent reduced total cytotoxicity more than fivefold, suggesting the degradation of anthropogenic contaminants. Disinfection of Facility C and D influents produced negligible net change or a decrease in total cytotoxicity. At Facility E, disinfection increased the cytotoxicity of the riverbank filtrate 2.5-fold, suggesting that DBPs were more important contributors to cytotoxicity than were anthropogenic contaminants. It is not possible to differentiate between the contributions of anthropogenic contaminants and uncharacterized DBPs towards the bioassay response (BCAT) of the wastewater after disinfection. Nonetheless, the total cytotoxicity after disinfection partially reflects the cytotoxicity of DBPs, as evidenced by CAT accounting for 29% and 42% of the total cytotoxicity of the disinfected Facility D and Facility E influent waters, respectively (Fig. 2).

Uncharacterized nonvolatile contaminants

Across all waters, the CAT associated with regulated DBPs (four THMs and five HAAs) contributed only 0.2% to total cytotoxicity on a median basis (Supplementary Table 10). The median contribution of regulated DBPs was higher in the waters of RO-free reuse trains (5%) than for other water categories. The median contribution of (semi-)volatile unregulated DBPs to total cytotoxicity across all waters was 16%. Again, their median contribution to total cytotoxicity was higher in RO-free reuse waters (35%) than for other water categories. HANs, HALs and HAMs accounted for the majority of CAT in waters from RO-based reuse systems (Supplementary Fig. 2). In waters from RO-free reuse systems, HANs and HAMs were the dominant contributors to CAT (Supplementary Fig. 3). HALs dominated CAT in conventional drinking waters derived from Facility A and Facility B groundwater sources (Supplementary Fig. 4a), whereas HANs, HAMs and HALs were important contributors to CAT in the groundwater-derived drinking waters from

Facility C (Supplementary Fig. 4b) and the surface-water-derived drinking waters from Facilities D and E (Supplementary Fig. 4c).

The dominant component of total cytotoxicity across all waters was BCAT (83% on a median basis; Supplementary Table 10). Although unregulated (semi-)volatile DBPs were important contributors to the total cytotoxicity of RO-free reuse waters, BCAT still constituted 62% (on a median basis) of the total cytotoxicity of these waters. BCAT encompasses nonvolatile, largely uncharacterized DBPs and sewage-derived anthropogenic chemicals; the relative importance of these two contaminant classes to BCAT is unclear.

Measuring known DBPs in water samples and calculating CAT require less time than extracting 10 l water samples and conducting the bioassay. Unfortunately, CAT does not correlate with BCAT (Supplementary Fig. 5), as expected if (semi-)volatile DBPs are minor contributors towards total cytotoxicity. This lack of correlation between CAT and BCAT is also apparent in Fig. 1. For example, the surface-water-derived drinking water from Facility A and the potable reuse waters from Facilities D and E featured high CAT but moderate BCAT levels (Fig. 1). By contrast, the surface-water-derived drinking waters from Facilities D and E exhibited high BCAT but low CAT.

Cytotoxicity reduction by RO and O_3/BAF

Figure 2 shows CAT and BCAT for samples collected along the five potable reuse systems. For the RO-based reuse trains, Facility A applies chloramines upstream of MF to control biofouling, while Facility B applies chloramines and ozone. RO reduced the cytotoxicity of the wastewaters entering Facilities A and B by 77–99%, predominantly by reducing BCAT, the fraction containing higher-molecular-weight compounds. Previous research has demonstrated that the low-molecular-weight neutral DBPs that dominate CAT are poorly rejected by RO[18–20,22]. BCAT was not detectable in RO permeate during the second sampling event at Facility A. Although substantially reduced in the other two RO permeate samples, the dominance of BCAT over CAT suggests the occurrence of uncharacterized compounds that have sufficiently low molecular weight to pass through RO membranes but that can be retained by the SPE extraction protocol used to prepare samples for bioassays. Residual H_2O_2 in the final reuse waters prevented the evaluation of the effect of AOP treatment on cytotoxicity since a portion of the BCAT could be attributable to residual H_2O_2.

Process trains for RO-free reuse are more diverse (Supplementary Table 11 and Supplementary Note 4). Facility C treats wastewater effluent with O_3/BAF, ultrafiltration, granular activated carbon (GAC) and UV/H_2O_2 AOP. Facility D treats wastewater effluent by flocculation/sedimentation, O_3/BAF, GAC and UV disinfection. Facility E treats the water from an effluent-dominated river by riverbank filtration, softening, UV/H_2O_2 AOP, BAF and GAC. O_3/BAF treatment in Facilities C and D reduced cytotoxicity to levels comparable to conventional disinfected surface waters (Figs. 1 and 2). Although the effect of ultrafiltration/GAC/AOP treatment at Facility C is difficult to assess because residual H_2O_2 contributes to the measured BCAT, the results suggest the importance of GAC for reducing cytotoxicity. For sampling event 1 at Facility C, the observed cytotoxicity of the reuse water increased slightly after GAC treatment when the GAC was nearly exhausted (81% dissolved organic carbon (DOC) breakthrough). For sampling event 2, after the GAC had been replaced (36% DOC breakthrough), the cytotoxicity of the reuse water declined by 55% after GAC treatment (Fig. 2). Indeed, the cytotoxicity of GAC-treated waters increased with DOC across Facilities C–E (Supplementary Fig. 6).

Discussion

Potable reuse provides drought-prone regions with a secure water supply that is less energy intensive and costly than seawater desalination. Unfortunately, unfavourable perceptions of its quality, driven by its sewage origin, have hindered implementation. To address pathogen risk, California's potable reuse regulations[37] require log-removals for viruses and protozoa. Addressing chemical contaminants has been more difficult, partly because a lack of holistic evaluations of chemical exposure in reuse waters has inhibited the identification of toxicity drivers. Previous studies that indicated that reuse water was of comparable quality to conventional drinking waters focused either on analysis of specific DBPs (CAT) or bioassay analysis of nonvolatile components (BCAT), sometimes coupled with chemical analysis of anthropogenic contaminants. While not all DBPs and anthropogenic contaminants were captured by our CAT and BCAT analyses, and the assumption that cytotoxicity is additive has not been validated for all DBPs and anthropogenic contaminants, combining CAT and BCAT provides the broadest comparison of potable reuse and conventional drinking-water quality to date. Our results indicate that potable reuse treatment trains, whether RO-based or RO-free ($n = 7$), produce waters of lower cytotoxicity than surface-water-derived conventional drinking waters ($n = 3$; one-sided t test $P = 0.0016$; power = 0.98). The cytotoxicity of RO-treated reuse waters was comparable to that of conventional groundwaters. Since many modes of toxic action can reduce cell growth, chronic cytotoxicity provides a broad metric for chemical exposure.

Regulated THMs and HAAs contributed little to cytotoxicity in all waters. To reduce THM and HAA formation, many utilities have altered disinfectants (for example, from free chlorine to chloramines[31]). As each disinfection scheme forms different DBPs[38], identifying the toxicity drivers in disinfected waters is important for protecting human health[39]. This study concurs with previous research indicating that unregulated (semi-)volatile DBPs contribute more to cytotoxicity than regulated DBPs across many water types[40–42]. More importantly, our results demonstrate that nonvolatile DBPs and anthropogenic contaminants (represented by BCAT) always contributed more to cytotoxicity than (semi-)volatile DBPs, although the contribution of (semi-)volatile DBPs (represented by CAT) approached that of BCAT in 5 out of 28 samples. Our findings regarding the importance of the nonvolatile fraction concur with two recent studies. A study that separated the volatile and nonvolatile fractions in disinfected municipal wastewater effluent found that combining the volatile and nonvolatile fractions in bioassays led to only a 20–30% increase in CHO cell cytotoxicity and induction of oxidative stress compared with the nonvolatile fraction alone[43]. Another study found that removing volatile DBPs in a chlorinated model surface water by nitrogen sparging did not reduce the developmental toxicity of the water[44]. These results suggest that the current focus on (semi-)volatile DBPs may be unwarranted and indicate the need to redirect efforts towards identifying toxicity drivers within the nonvolatile fraction.

Comparing the cytotoxicity of the influents to potable reuse trains before and after disinfection to elucidate the importance of sewage-derived anthropogenic contaminants relative to DBPs provided mixed results. Chloramination of the influent at Facility B, which receives agricultural wastewaters, reduced cytotoxicity, possibly by degrading toxic pesticides; this finding concurs with the high cytotoxicity in agricultural wastewaters observed in previous research that evaluated only nonvolatile contaminants[32,45]. However, the notable contribution of (semi-)volatile DBPs (CAT) to the cytotoxicity in the chloraminated influents at Facilities D and E suggests the importance of DBPs. While further characterization of nonvolatile contaminants is needed, the importance of DBPs relative to sewage-derived anthropogenic contaminants is expected to increase as anthropogenic contaminants are removed through the treatment trains. Moreover, many anthropogenic contaminants, including pharmaceuticals[46] and pesticides[47], react rapidly with chlorine; when disinfectants are applied within reuse trains, the associated transformation products would be considered as DBPs.

Fears about potable reuse water quality could prompt additional treatment requirements, increasing the energy consumption and costs of potable reuse. California's draft requirements for direct potable

reuse mandate O_3/BAF treatment upstream of MF/RO/AOP[48,49]. While the combination of O_3/BAF and MF/RO/AOP increases pathogen removal, we found that MF/RO/AOP already delivers water with low cytotoxicity, comparable to the cytotoxicity of conventional groundwaters. Even for RO-free trains, O_3/BAF/GAC treatment reduced cytotoxicity to levels below those in conventional surface waters. Although the correlation between CHO cell cytotoxicity and human toxicity has not been established, this work evaluated cytotoxicity because cytotoxicity is a broad metric that captures the effects of many different toxicity pathways, and the information needed for calculating CAT on the basis of cytotoxicity is available[26]. Our approach of combining CAT and BCAT provides an overall estimate of cytotoxicity contributed by mixtures of (semi-)volatile DBPs as well as nonvolatile, largely unknown DBPs and anthropogenic chemicals. Future research should examine a greater number of potable reuse systems to delineate the effect of different treatment processes on cytotoxicity. Beyond characterizing drivers of cytotoxicity within the nonvolatile fraction, research is needed to expand this type of holistic evaluation to other toxicity endpoints (for example, genotoxicity). Nonetheless, the current results are encouraging for the development of potable reuse as a safe, energy-efficient and cost-effective alternative water supply.

Methods

Reagents

Ammonium chloride (NH_4Cl, ACS grade), dimethyl sulfoxide (DMSO, ≥99.7%), hydrogen peroxide (H_2O_2, 30%), methanol (Optima grade, ≥99.9%), sodium hypochlorite (NaOCl) solution (5.65–6.00%), sodium sulfate ($NaSO_4$, ACS grade), sodium thiosulfate ($Na_2S_2O_3$, ACS grade) and sulfuric acid (H_2SO_4, ACS Plus grade) were from Fisher Scientific. Methyl *tert*-butyl ether (MtBE, ≥99.5%) was from Sigma–Aldrich. Ascorbic acid was from Alfa Aesar.

Stock solutions of the four regulated THMs (THM4), a mix of eight DBPs included in US Environmental Protection Agency (EPA) Method 551.1 (bromochloroacetonitrile, trichloroacetaldehyde, trichloronitromethane, dibromoacetonitrile, dichloroacetonitrile, 1,1-dichloro-2-propanone, trichloroacetonitrile and 1,1,1-trichloro-2-propanone) and stock solutions of 1,2-dibromopropane (the internal standard used in analysis of halogenated (semi-)volatile DBPs) were purchased from AccuStandard. Dichloroacetamide (98+%) was from Alfa Aesar. Bromochloroiodomethane (95+%), bromodichloroacetaldehyde (90+%), bromochloroacetamide (99+%), bromodiiodomethane (90–95%), chlorodiiodomethane (90–95%), dibromoacetamide (99+%), dibromochloroacetaldehyde (90+%), dibromoiodomethane (90–95%) and dichloroiodomethane (95+%) were from CanSyn Chem. Corp. Stock solutions containing the five regulated HAAs (HAA5) plus bromochloroacetic acid, bromodichloroacetic acid and dibromochloroacetic acid were from Sigma–Aldrich. Iodoacetic acid (≥98%), iodoform (triiodomethane, 99%), tribromoacetaldehyde (97%) and trichloroacetamide (99%) were also from Sigma–Aldrich.

Sampling and disinfection protocols

Samples were collected along potable reuse treatment trains and from some conventional drinking-water facilities upstream of disinfection. Supplementary Note 4 describes the process units in the potable reuse treatment trains. Supplementary Table 9 provides basic water-quality parameters. These samples were chloraminated in the lab for three days using similar procedures as those used by the facilities (described for each sample in Supplementary Note 5) in two aliquots: (1) duplicate 60 ml glass vials with minimal headspace for the analysis of (semi-) volatile DBPs and (2) 10 l amber glass bottles for preparing extracts for bioassays. After three days of chloramination, the 60 ml samples were quenched using ascorbic acid (33 mg l^{-1}) and extracted into MtBE using modified EPA Methods 551.1[50] (for (semi-)volatile halogenated DBPs) and 552.3[51] (for HAAs). The MtBE extracts were analysed by gas chromatography mass spectrometry. Full descriptions of DBP analysis procedures and analytical methods are in Supplementary Notes 6 and 7, respectively. The 10 l samples were quenched with $Na_2S_2O_3$, acidified to ~pH 3.7 using H_2SO_4 and extracted by SPE. Tap-water samples, which already contained disinfectants, were quenched, acidified and extracted without further chloramination.

Extraction protocol

The SPE method developed by Stalter et al.[24], which can capture ~50% of total organic halogen (TOX) in 2 l disinfected water with Strata-X SPE cartridges (Phenomenex), was scaled up for 10 l extractions. SPE cartridges were packed with 2.5 g Sepra ZTL (Phenomenex), a sorbent similar to the Strata-X (Phenomenex) sorbent that Stalter et al.[24] found to optimize DBP recovery (which was not available in bulk packaging). Extractions were conducted at ~pH 3.7 to maximize DBP stability[52]. Supplementary Note 8 describes the SPE procedure. Additional extractions were conducted to evaluate whether adding an anion exchange sorbent (1 g Phenomenex Sepra ZT-SAX) as the bottom layer in SPE cartridges could capture HAAs and other anionic DBPs. We also compared the two SPE methods with the more established XAD resin extraction[53]. Details of these alternative extraction procedures are also in Supplementary Note 8.

As Strata-X cartridges can leach toxic materials at extraction pH ≤ 1.5 (ref. [24]), the three extraction methods were tested for background toxicity by extracting 10 l DI water dosed with free chlorine and quenched with $Na_2S_2O_3$. Recovery of CHO cell cytotoxicity was assessed by extracting a surface water (10 l) that was chlorinated for 24 hours and quenched with $Na_2S_2O_3$ in duplicate; the results of the BCAT evaluations for the duplicate chlorinated aliquots (0.093 average; 0.083–0.105 range) provide an indication of the error in BCAT determinations. Another control experiment was conducted to examine whether unreacted H_2O_2 in AOP-treated reuse waters causes artefacts in the cytotoxicity assay. A single extraction (Sepra ZTL) was performed of DI water (10 l) dosed with H_2O_2 and monochloramine (NH_2Cl) and then quenched with $Na_2S_2O_3$. Supplementary Note 1 details the experimental conditions.

CHO cell chronic cytotoxicity assay

Water extracts were analysed for chronic cytotoxicity using CHO K1 cells[26,54], and the resulting concentration–response curves are presented in Supplementary Figs. 7–11. The regression analyses of the concentration–response curves and the generation of the mean LC_{50} ± standard error values and the statistical analyses of the data are presented in Supplementary Table 12. Details of the bioassay were published[26] and are described in Supplementary Note 9 and Supplementary Fig. 12.

Data availability

The datasets generated and/or analysed during the current study are available in Supplementary Tables 1–8 for (semi-)volatile DBPs and in Supplementary Data 2 for the cytotoxicity bioassay results. Data associated with Supplementary Tables 1–12 are provided in Supplementary Data 1. Source data are provided with this paper.

References

1. Gerrity, D., Pecson, B., Trussell, R. S. & Trussell, R. R. Potable reuse treatment trains throughout the world. *Aqua* **62**, 321–338 (2013).
2. Pecson, B. M., Trussell, R. S., Pisarenko, A. N. & Trussell, R. R. Achieving reliability in potable reuse: the four Rs. *J. Am. Water Works Assoc.* **107**, 48–58 (2015).
3. Marron, E. L., Mitch, W. A., von Gunten, U. & Sedlak, D. L. A tale of two treatments: the multiple barrier approach to removing chemical contaminants during potable water reuse. *Acc. Chem. Res.* **52**, 615–622 (2019).

4. Giammar, D. E. et al. Cost and energy metrics for municipal water reuse. *ACS EST Engg.* **2**, 489–507 (2022).
5. Sim, A. & Mauter, M. S. Cost and energy intensity of US potable water reuse systems. *Environ. Sci. Water Res. Technol.* **7**, 748–761 (2021).
6. Kim, J., Park, K., Yang, D. R. & Hong, S. A comprehensive review of energy consumption of seawater reverse osmosis desalination plants. *Appl. Energy* **254**, 113652 (2019).
7. Quon, H. et al. Pipe parity analysis of seawater desalination in the United States: exploring costs, energy, and reliability via case studies and scenarios of emerging technology. *ACS EST Engg.* **2**, 434–445 (2022).
8. Fielding, K. S., Gardner, J., Leviston, Z. & Price, J. Comparing public perceptions of alternative water sources for potable use: the case of rainwater, stormwater, desalinated water, and recycled water. *Water Resour. Manage.* **29**, 4501–4518 (2015).
9. Barnes, J. L., Krishen, A. S. & Hu, H. Untapped knowledge about water reuse: the roles of direct and indirect educational messaging. *Water Resour. Manage.* **35**, 2601–2615 (2021).
10. Escher, B. I. et al. Evaluation of contaminant removal of reverse osmosis and advanced oxidation in full-scale operation by combining passive sampling with chemical analysis and bioanalytical tools. *Environ. Sci. Technol.* **45**, 5387–5394 (2011).
11. Macova, M. et al. Bioanalytical tools for the evaluation of organic micropollutants during sewage treatment, water recycling and drinking water generation. *Water Res.* **45**, 4238–4247 (2011).
12. Dong, S. et al. Toxicological comparison of water, wastewaters, and processed wastewaters. *Environ. Sci. Technol.* **53**, 9139–9147 (2019).
13. Page, M. A. et al. Composite toxicity assays for enhanced assessment of decentralized potable reuse systems. *Environ. Sci. Water Res. Technol.* **6**, 3306–3315 (2020).
14. *Water Reuse: Potential for Expanding the Nation's Water Supply Through Reuse of Municipal Wastewater* (National Research Council, 2012); https://doi.org/10.17226/13303
15. Leusch, F. D. L. & Snyder, S. A. Bioanalytical tools: half a century of application for potable reuse. *Environ. Sci. Water Res. Technol.* **1**, 606–621 (2015).
16. *Potable Reuse: Guidance for Producing Safe Drinking-Water* (WHO, 2017).
17. Li, X.-F. & Mitch, W. A. Drinking water disinfection byproducts (DBPs) and human health effects: multidisciplinary challenges and opportunities. *Environ. Sci. Technol.* **52**, 1681–1689 (2018).
18. Agus, E. & Sedlak, D. L. Formation and fate of chlorination by-products in reverse osmosis desalination systems. *Water Res.* **44**, 1616–1626 (2010).
19. Linge, K. L. et al. Formation of halogenated disinfection by-products during microfiltration and reverse osmosis treatment: implications for water recycling. *Sep. Purif. Technol.* **104**, 221–228 (2013).
20. Doederer, K., Farré, M. J., Pidou, M., Weinberg, H. S. & Gernjak, W. Rejection of disinfection by-products by RO and NF membranes: influence of solute properties and operational parameters. *J. Membr. Sci.* **467**, 195–205 (2014).
21. Chuang, Y.-H., Parker, K. M. & Mitch, W. A. Development of predictive models for the degradation of halogenated disinfection byproducts during the UV/H_2O_2 advanced oxidation process. *Environ. Sci. Technol.* **50**, 11209–11217 (2016).
22. Zeng, T., Plewa, M. J. & Mitch, W. A. *N*-Nitrosamines and halogenated disinfection byproducts in US. Full advanced treatment trains for potable reuse. *Water Res.* **101**, 176–186 (2016).
23. Lau, S. S., Forster, A. L., Richardson, S. D. & Mitch, W. A. Disinfection byproduct recovery during extraction and concentration in preparation for chemical analyses or toxicity assays. *Environ. Sci. Technol.* **55**, 14136–14145 (2021).
24. Stalter, D. et al. Sample enrichment for bioanalytical assessment of disinfected drinking water: concentrating the polar, the volatiles, and the unknowns. *Environ. Sci. Technol.* **50**, 6495–6505 (2016).
25. Krasner, S. W. et al. Occurrence of a new generation of disinfection byproducts. *Environ. Sci. Technol.* **40**, 7175–7185 (2006).
26. Wagner, E. D. & Plewa, M. J. CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: an updated review. *J. Environ. Sci.* **58**, 64–76 (2017).
27. Lau, S. S. et al. Assessing additivity of cytotoxicity associated with disinfection byproducts in potable reuse and conventional drinking waters. *Environ. Sci. Technol.* **54**, 5729–5736 (2020).
28. Chuang, Y.-H., Szczuka, A. & Mitch, W. A. Comparison of toxicity-weighted disinfection byproduct concentrations in potable reuse waters and conventional drinking waters as a new approach to assessing the quality of advanced treatment train waters. *Environ. Sci. Technol.* **53**, 3729–3738 (2019).
29. Hebert, A. et al. Bioanalytical assessment of adaptive stress responses in drinking water: a predictive tool to differentiate between micropollutants and disinfection by-products. *Water Res.* **132**, 340–349 (2018).
30. Le Roux, J. et al. Chloramination of wastewater effluent: toxicity and formation of disinfection byproducts. *J. Environ. Sci.* **58**, 135–145 (2017).
31. Yang, Y. et al. Toxic impact of bromide and iodide on drinking water disinfected with chlorine or chloramines. *Environ. Sci. Technol.* **48**, 12362–12369 (2014).
32. Dong, S., Lu, J., Plewa, M. J. & Nguyen, T. H. Comparative mammalian cell cytotoxicity of wastewaters for agricultural reuse after ozonation. *Environ. Sci. Technol.* **50**, 11752–11759 (2016).
33. Dong, S., Masalha, N., Plewa, M. J. & Nguyen, T. H. Toxicity of wastewater with elevated bromide and iodide after chlorination, chloramination, or ozonation disinfection. *Environ. Sci. Technol.* **51**, 9297–9304 (2017).
34. Liu, C. et al. Toxicity of chlorinated algal-impacted waters: formation of disinfection byproducts vs. reduction of cyanotoxins. *Water Res.* **184**, 116145 (2020).
35. Allen, J. M. et al. Making swimming pools safer: does copper–silver ionization with chlorine lower the toxicity and disinfection byproduct formation? *Environ. Sci. Technol.* **55**, 2908–2918 (2021).
36. Allen, J. M. et al. Drivers of disinfection byproduct cytotoxicity in US drinking water: should other DBPs be considered for regulation? *Environ. Sci. Technol.* **56**, 392–402 (2022).
37. Olivieri, A. W., Pecson, B., Crook, J. & Hultquist, R. California water reuse—past, present and future perspectives. *Adv. Chem. Pollut. Environ. Manage. Prot.* **5**, 65–111 (2020).
38. Shah, A. D. & Mitch, W. A. Halonitroalkanes, halonitriles, haloamides, and *N*-nitrosamines: a critical review of nitrogenous disinfection byproduct formation pathways. *Environ. Sci. Technol.* **46**, 119–131 (2012).
39. Richardson, S. D. & Plewa, M. J. To regulate or not to regulate? What to do with more toxic disinfection by-products? *J. Environ. Chem. Eng.* **8**, 103939 (2020).
40. Szczuka, A. et al. Regulated and unregulated halogenated disinfection byproduct formation from chlorination of saline groundwater. *Water Res.* **122**, 633–644 (2017).
41. Furst, K. E., Pecson, B. M., Webber, B. D. & Mitch, W. A. Tradeoffs between pathogen inactivation and disinfection byproduct formation during sequential chlorine and chloramine disinfection for wastewater reuse. *Water Res.* **143**, 579–588 (2018).

42. Furst, K. E., Coyte, R. M., Wood, M., Vengosh, A. & Mitch, W. A. Disinfection byproducts in Rajasthan, India: are trihalomethanes a sufficient indicator of disinfection byproduct exposure in low-income countries? *Environ. Sci. Technol.* **53**, 12007–12017 (2019).
43. Wu, Q.-Y. et al. Non-volatile disinfection byproducts are far more toxic to mammalian cells than volatile byproducts. *Water Res.* **183**, 116080 (2020).
44. Li, Y. et al. Volatile DBPs contributed marginally to the developmental toxicity of drinking water DBP mixtures against *Platynereis dumerilii*. *Chemosphere* **252**, 126611 (2020).
45. Dong, S., Massalha, N., Plewa, M. J. & Nguyen, T. H. The impact of disinfection *Ct* values on cytotoxicity of agricultural wastewaters: ozonation vs. chlorination. *Water Res.* **144**, 482–490 (2018).
46. Postigo, C. & Richardson, S. D. Transformation of pharmaceuticals during oxidation/disinfection processes in drinking water treatment. *J. Hazard. Mater.* **279**, 461–475 (2014).
47. Magara, Y., Aizawa, T., Matumoto, N. & Souna, F. Degradation of pesticides by chlorination during water purification. *Water Sci. Technol.* **30**, 119–128 (1994).
48. *A Proposed Framework for Regulating Direct Potable Reuse in California* 2nd edn (California State Water Resources Control Board, 2019).
49. *A Proposed Framework for Regulating Direct Potable Reuse in California, Addendum: Early Draft of Anticipated Criteria for Direct Potable Reuse* (California State Water Resources Control Board, 2021).
50. *Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid–Liquid Extraction and Gas Chromatography with Electron-Capture Detection* Revision 1.0 (US EPA, 1995).
51. *Method 552.3 Determination of Haloacetic Acids and Dalapon in Drinking Water by Liquid–Liquid Microextraction, Derivatization, and Gas Chromatography with Electron Capture Detection* Revision 1.0 (US EPA, 2003).
52. Chinn, R. et al. Solid-phase extraction of 35 DBPs with analysis by GC/ECD and GC/MS. In *Proc. 2007 American Water Works Association (AWWA) Water Quality Technology Conference* 1–20 (2007).
53. Richardson, S. D. *XAD Resin Extraction of Disinfectant By-Products Drinking Water* SOP-RSB-003.1, Revision No. 1 (US EPA, 2011).
54. Wagner, E. D., Rayburn, A. L., Anderson, D. & Plewa, M. J. Analysis of mutagens with single cell gel electrophoresis, flow cytometry, and forward mutation assays in an isolated clone of Chinese hamster ovary cells. *Environ. Mol. Mutagen.* **32**, 360–368 (1998).

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Author contributions

W.A.M. and M.J.P. designed the project. S.S.L. and W.A.M. collected water samples, measured DBP concentrations and compiled all the data. S.S.L. prepared samples for bioassays. K.B., A.T., E.D.W. and M.J.P. performed the bioassays and data analysis. S.S.L., W.A.M. and M.J.P. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

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Toxicological assessment of potable reuse and conventional drinking waters

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Supplementary Information

Toxicological assessment of potable reuse and conventional drinking waters

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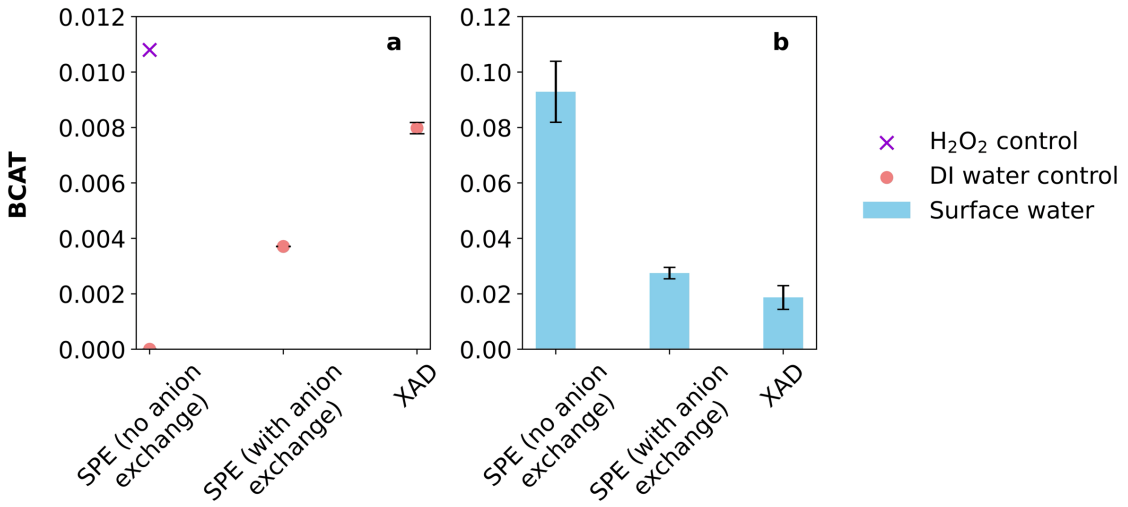
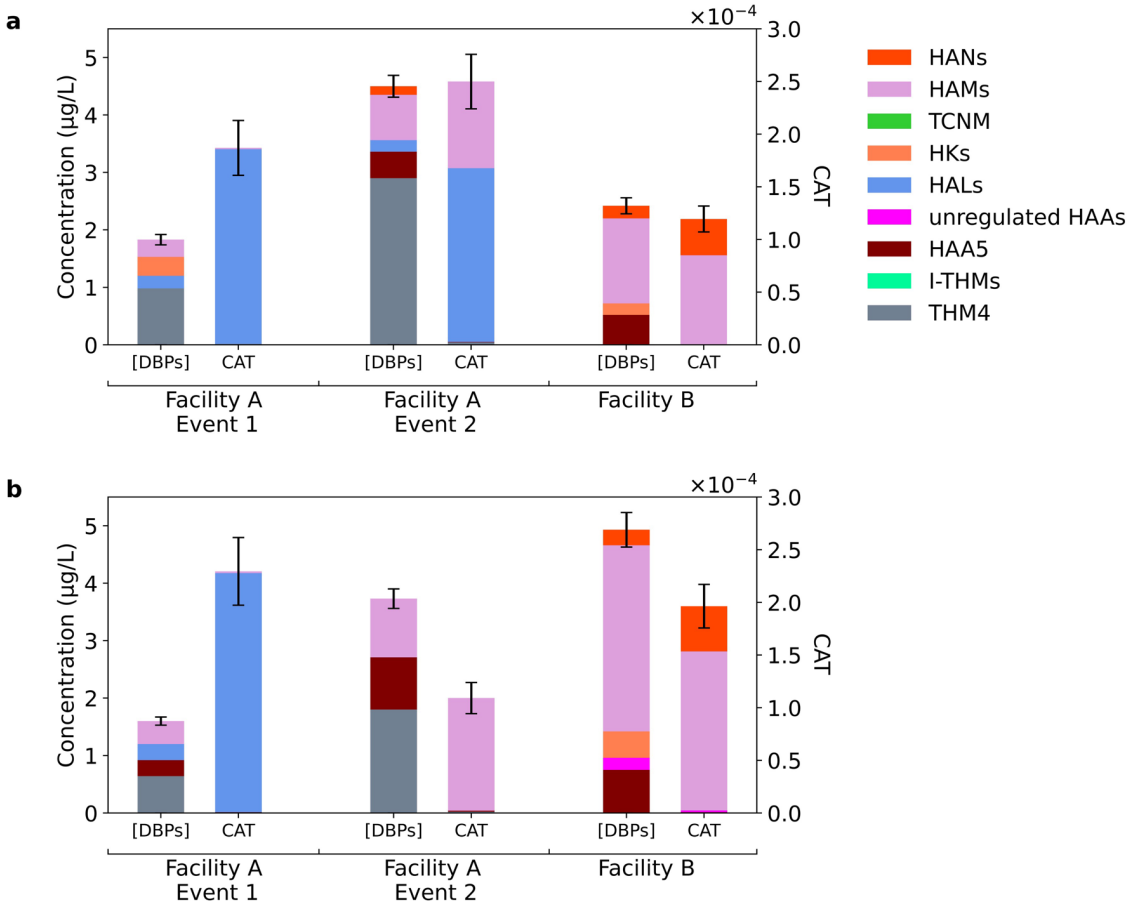


Supplementary Fig. 1: Results from control experiments. Bioassay-based calculated additive toxicity (BCAT) values obtained from extractions of 10 L of **(a)** deionized (DI) water[a] and **(b)** a chlorinated surface water[b] by three methods. BCAT for DI water containing hydrogen peroxide (H_2O_2)[c] is also shown in panel A. Uncertainties in the DI water control and H_2O_2 control data were determined from the standard error of the CHO cell LC_{50} value of each water obtained from a single extraction. Error bars in the surface water data represent ranges of duplicate extractions.

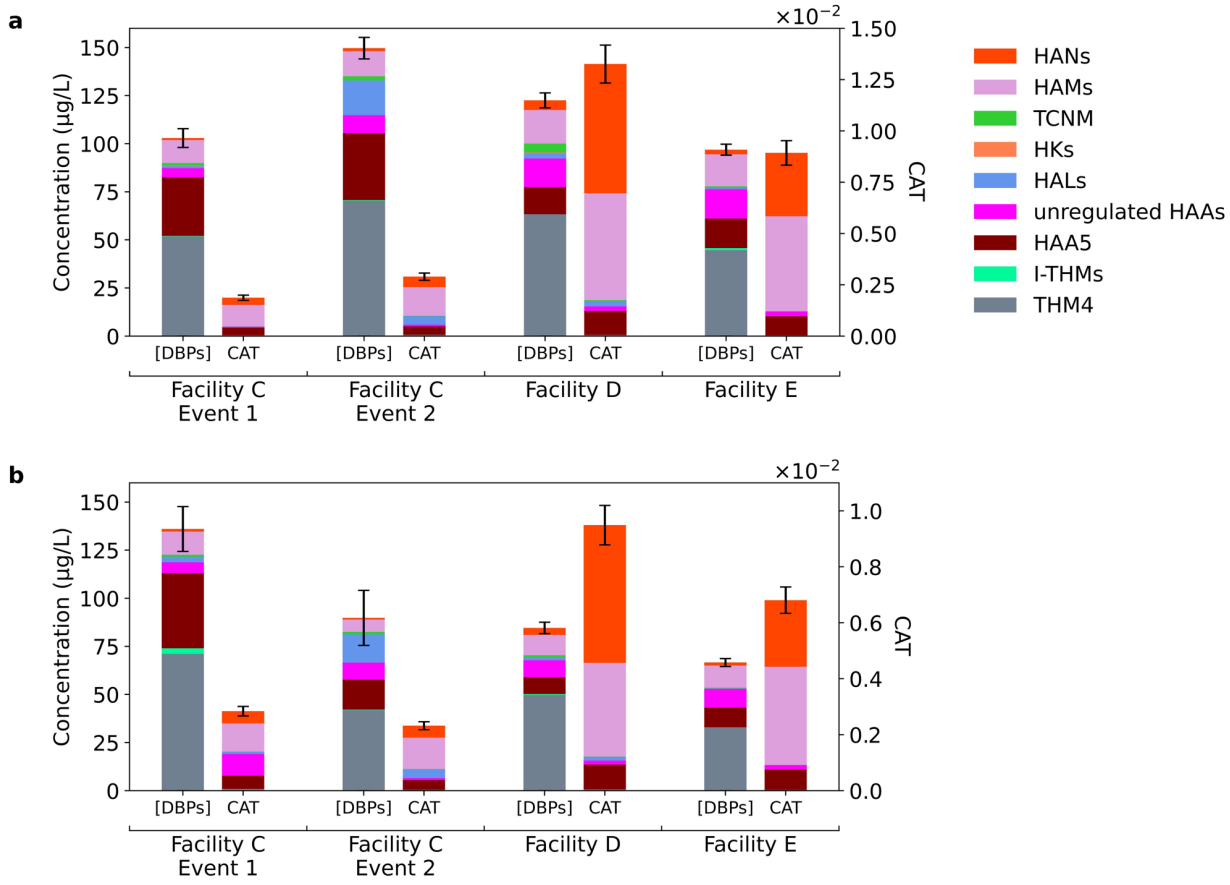
[a] DI water control: 10 L of DI water buffered at pH 8 with 4 mM of sodium borate; 1.5 mg/L of free chlorine was added and then immediately quenched with 1.1-fold molar excess of sodium thiosulfate ($Na_2S_2O_3$)

[b] Chlorinated surface water: 10 L of raw surface water buffered at pH 8 with 4 mM of sodium borate; free chlorine was added to achieve a residual of ~1 mg/L as Cl_2 after 24 hours at room temperature (22 ± 1 °C) and then quenched with 1.1-fold molar excess of $Na_2S_2O_3$

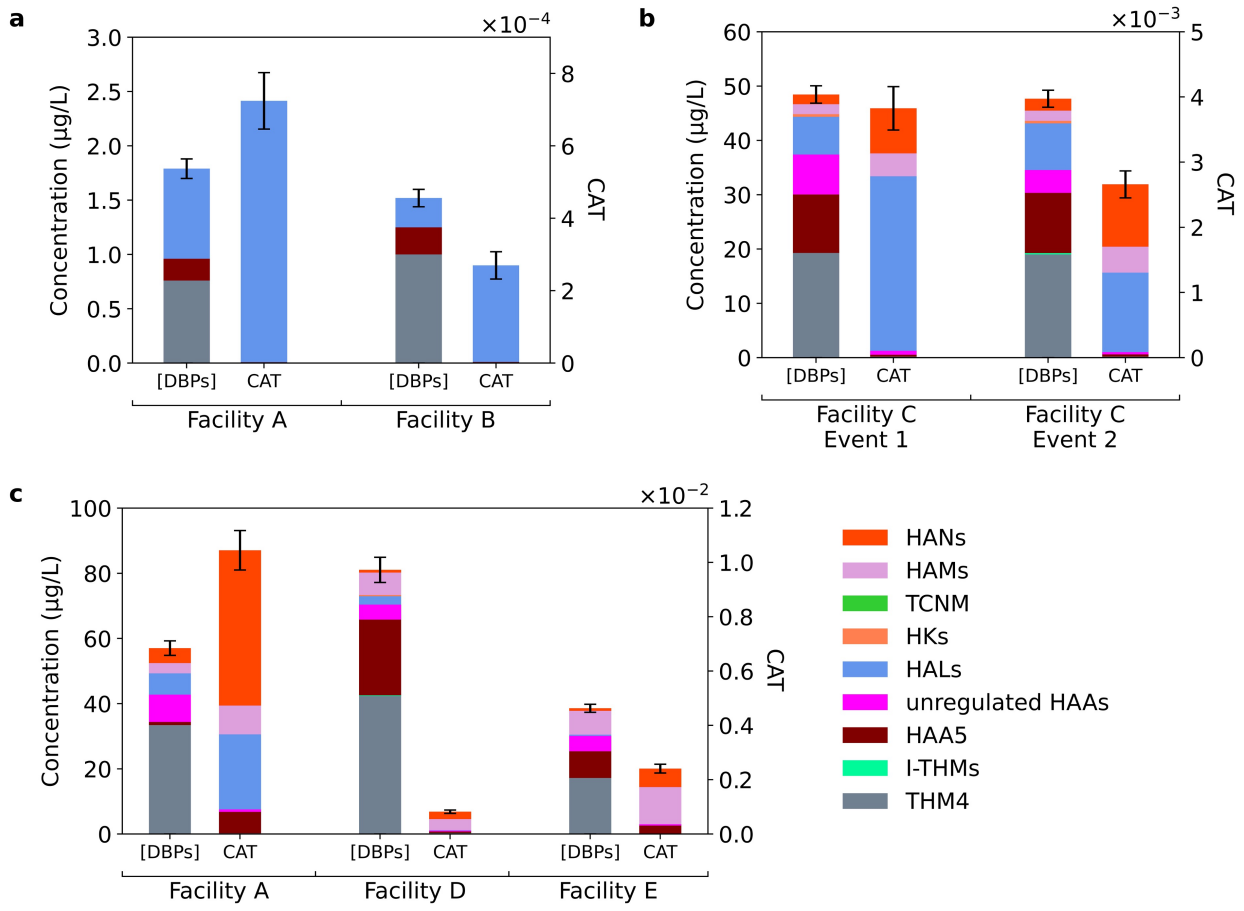
[c] H_2O_2 control: 10 L of DI water buffered at pH 8 with 4 mM of sodium borate; H_2O_2 (3.5 mg/L) and monochloramine (NH_2Cl; 3.5 mg/L as Cl_2) were added; after ~16 hours at room temperature (22 ± 1 °C), the total chlorine residual was quenched with 1.1-fold molar excess of $Na_2S_2O_3$



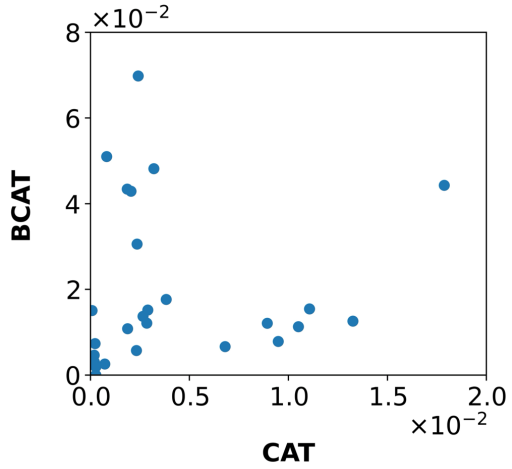
Supplementary Fig. 2: (Semi-)volatile DBPs in RO-based reuse waters. Total concentrations and calculated additive toxicity (CAT) of known, (semi-)volatile DBPs in the potable reuse waters from Facilities A and B after **(a)** RO and **(b)** UV/H_2O_2 AOP treatment. HANs = haloacetonitriles; HAMs = haloacetamides; TCNM = trichloronitromethane (chloropicrin); HKs = haloketones, HALs = haloacetaldehydes; other HAAs = unregulated haloacetic acid; HAA5 = five regulated haloacetic acids; I-THMs = iodinated trihalomethanes; THM4 = four regulated trihalomethanes. Error bars denote standard errors of the total DBP concentrations or total cytotoxicity indices (uncertainty calculations are described in Supplementary Note 3).



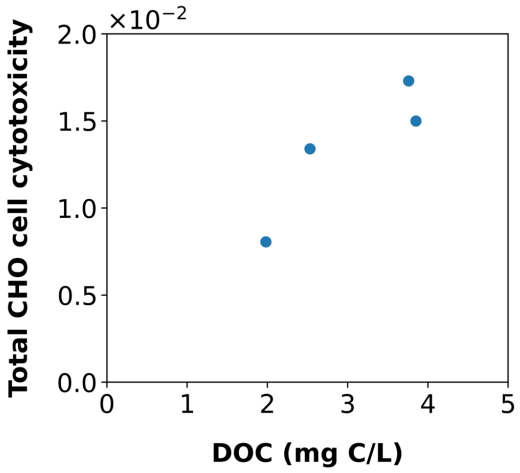
Supplementary Fig. 3: (Semi-)volatile DBPs in RO-free reuse waters. Total concentrations and calculated additive toxicity (CAT) of known, (semi-)volatile DBPs in the **(a)** BAF samples and **(b)** final potable reuse waters from Facilities C, D, and E. HANs = haloacetonitriles; HAMs = haloacetamides; TCNM = trichloronitromethane (chloropicrin); HKs = haloketones, HALs = haloacetaldehydes; other HAAs = unregulated haloacetic acid; HAA5 = five regulated haloacetic acids; I-THMs = iodinated trihalomethanes; THM4 = four regulated trihalomethanes. Error bars denote standard errors of the total DBP concentrations or total cytotoxicity indices (uncertainty calculations are described in Supplementary Note 3).



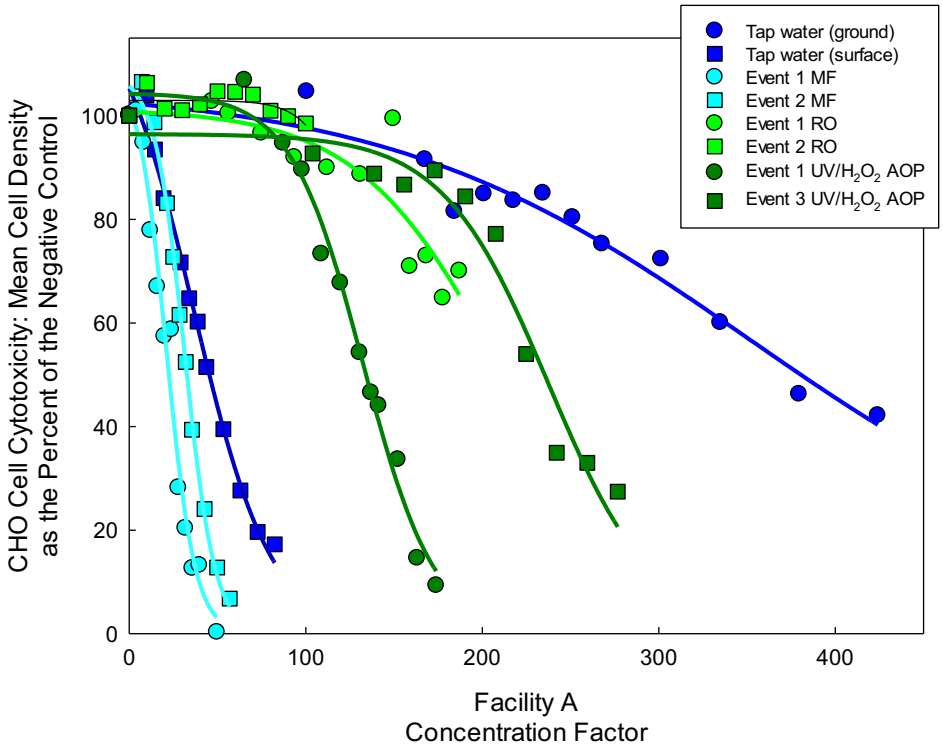
Supplementary Fig. 4: (Semi-)volatile DBPs in conventional drinking waters. Total concentrations and calculated additive toxicity (CAT) of known, (semi-)volatile DBPs in conventional drinking waters derived from **(a)** groundwaters near Facilities A and B, **(b)** groundwaters near Facility C, and **(c)** surface waters near Facilities A, D, and E. HANs = haloacetonitriles; HAMs = haloacetamides; TCNM = trichloronitromethane (chloropicrin); HKs = haloketones, HALs = haloacetaldehydes; other HAAs = unregulated haloacetic acid; HAA5 = five regulated haloacetic acids; I-THMs = iodinated trihalomethanes; THM4 = four regulated trihalomethanes. Error bars denote standard errors of the total DBP concentrations or total cytotoxicity indices (uncertainty calculations are described in Supplementary Note 3).



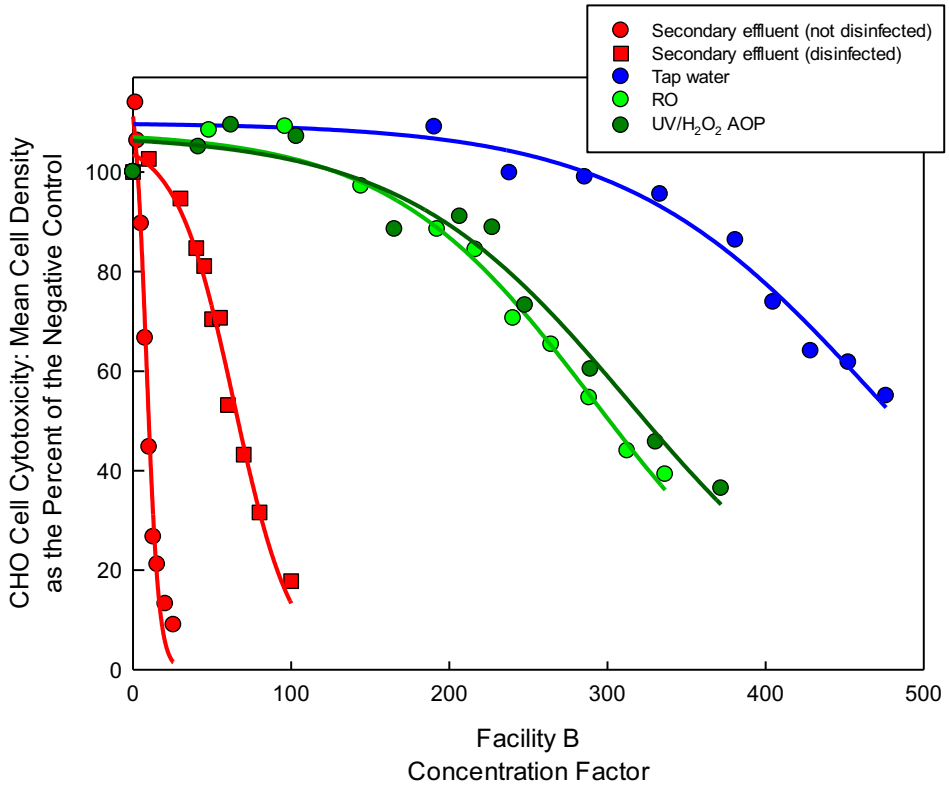
Supplementary Fig. 5: Relationship between BCAT and CAT. Bioassay-based calculated additive toxicity (BCAT) indices versus calculated additive toxicity (CAT) indices for disinfected water samples collected along RO-based and RO-free potable reuse treatment trains as well as disinfected conventional drinking waters. The data expressed no significant correlation between these two variables; $r = 0.129$; $P = 0.51$.



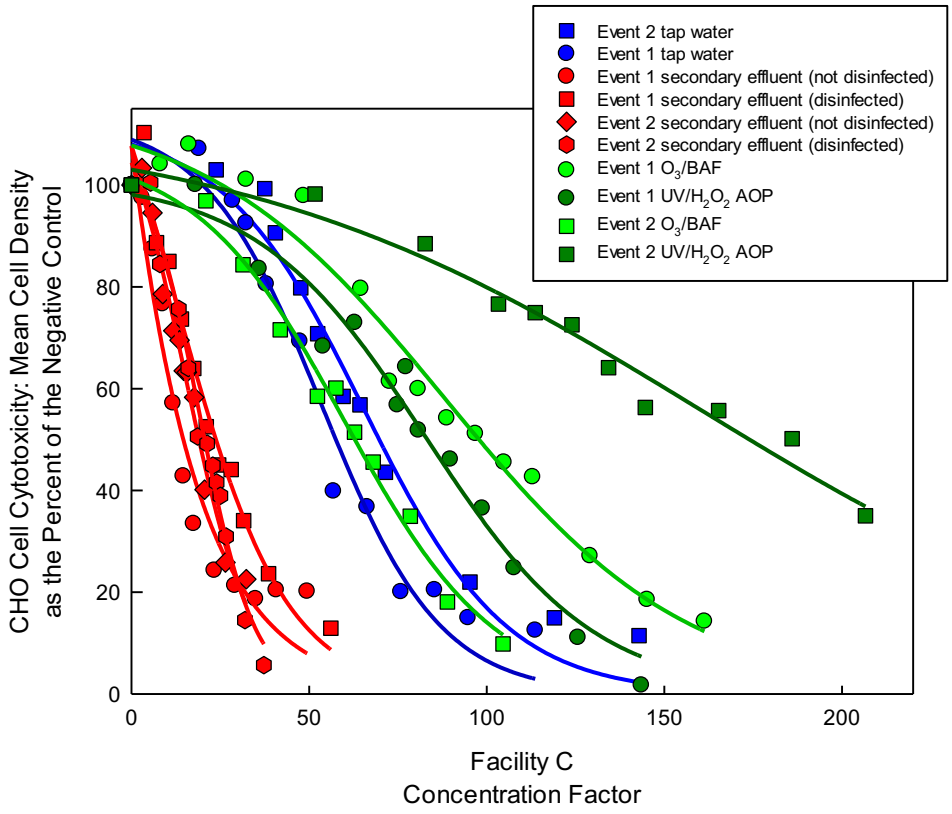
Supplementary Fig. 6: Correlation ($r = 0.89$) between cytotoxicity and DOC. Total Chinese hamster ovary (CHO) cell cytotoxicity, represented by the sum of bioassay-based calculated additive toxicity (BCAT) and calculated additive toxicity (CAT) indices, as a function of dissolved organic carbon (DOC) concentration for the final waters from RO-free potable reuse treatment trains incorporating GAC treatment (Facilities C, D, and E).



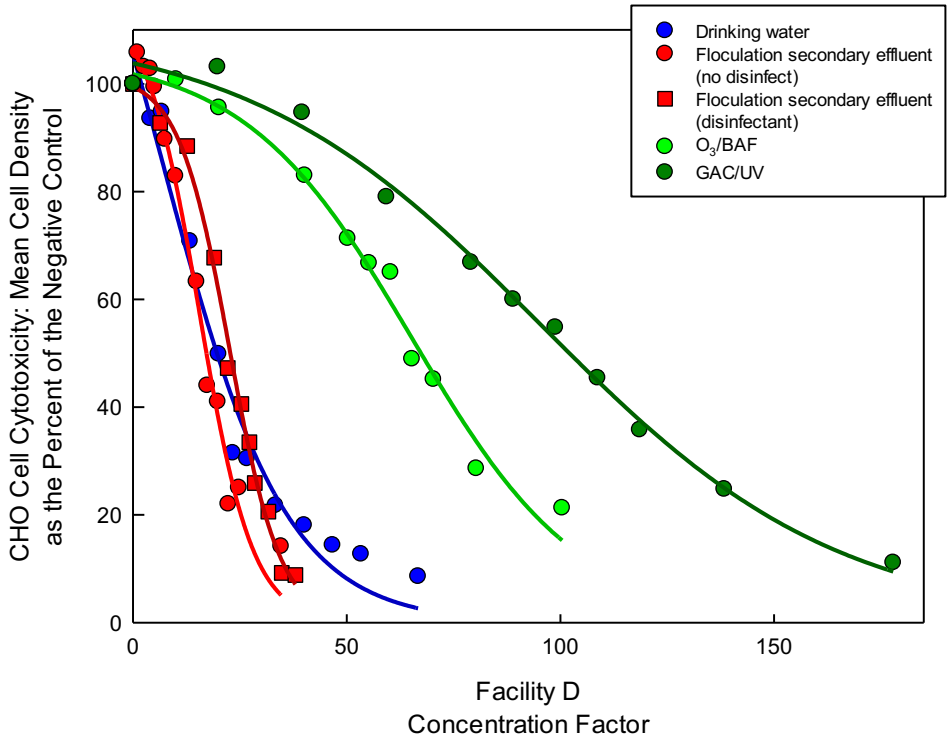
Supplementary Fig. 7: CHO cell cytotoxicity for water samples from Facility A. Cytotoxicity concentration-response curves are presented for each concentrated water sample derived from Facility A. MF = microfiltration. RO = reverse osmosis. UV/H_2O_2 AOP = UV/hydrogen peroxide advanced oxidation process. The statistical analyses of the data derived from these concentration-response curves is presented in Supplementary Table 12. The treatment processes in the potable reuse treatment trains for Facility A is presented in Supplementary Note 4.



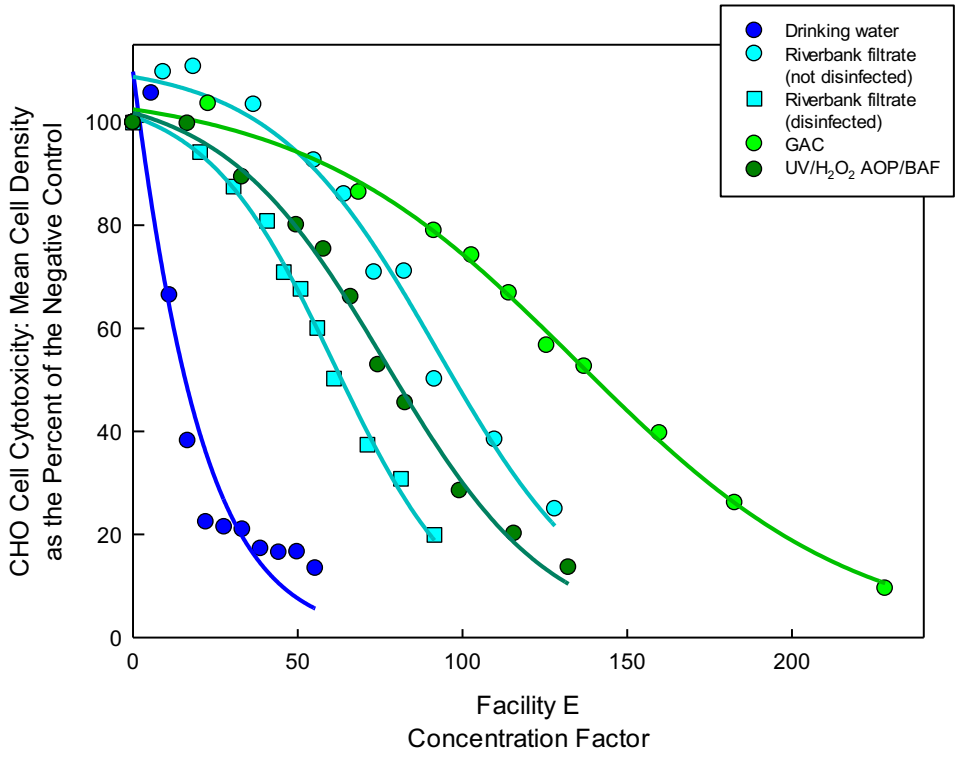
Supplementary Fig. 8: CHO cell cytotoxicity for water samples from Facility B. Cytotoxicity concentration-response curves are presented for each concentrated water sample derived from Facility B. RO = reverse osmosis. UV/H_2O_2 AOP = UV/hydrogen peroxide advanced oxidation process. The statistical analyses of the data derived from these concentration-response curves is presented in Supplementary Table 12. The treatment processes in the potable reuse treatment trains for Facility B is presented in Supplementary Note 4.



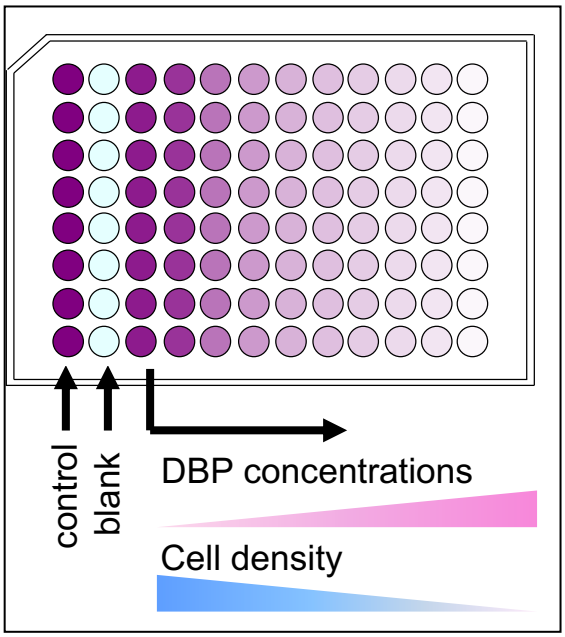
Supplementary Fig. 9: CHO cell cytotoxicity for water samples from Facility C. Cytotoxicity concentration-response curves are presented for each concentrated water sample derived from Facility C. MF = microfiltration. RO = reverse osmosis. BAF = biologically active filtration. UV/H_2O_2 AOP = UV/hydrogen peroxide advanced oxidation process. The statistical analyses of the data derived from these concentration-response curves is presented in Supplementary Table 12. The treatment processes in the potable reuse treatment trains for Facility C is presented in Supplementary Note 4.



Supplementary Fig. 10: CHO cell cytotoxicity for water samples from Facility D. Cytotoxicity concentration-response curves are presented for each concentrated water sample derived from Facility D. BAF = biologically active filtration. GAC = granular activated carbon filtration. The statistical analyses of the data derived from these concentration-response curves is presented in Supplementary Table 12. The treatment processes in the potable reuse treatment trains for Facility D is presented in Supplementary Note 4.



Supplementary Fig. 11: CHO cell cytotoxicity for water samples from Facility E. Cytotoxicity concentration-response curves are presented for each concentrated water sample derived from Facility E. GAC = granular activated carbon filtration. UV/H_2O_2 AOP = UV/hydrogen peroxide advanced oxidation process. The statistical analyses of the data derived from these concentration-response curves is presented in Supplementary Table 12. The treatment processes in the potable reuse treatment trains for Facility E is presented in Supplementary Note 4.



Supplementary Fig. 12: Illustration of the CHO cell cytotoxicity assay microplate.

Supplementary Table 1: DBP Concentrations[a] at Facility A, Event 1

	MF		RO		AOP	
	Average	Range	Average	Range	Average	Range
THMs						
TCM	4.03	0.27	0.61	0.11	0.45	0.03
BDCM	1.23	0.03	0.37	0.01	0.20	0.00
DBCM	0.27	0.00	< 0.20		< 0.20	
TBM	< 0.20		< 0.20		< 0.20	
DCIM	< 0.20		< 0.20		< 0.20	
BCIM	< 0.20		< 0.20		< 0.20	
DBIM	< 0.20		< 0.20		< 0.20	
CDIM	< 0.20		< 0.20		< 0.20	
BDIM	< 0.20		< 0.20		< 0.20	
TIM	< 0.20		< 0.20		< 0.20	
HANs						
DCAN	0.27	0.09	< 0.20		< 0.20	
TCAN	< 0.20		< 0.20		< 0.20	
BCAN	< 0.20		< 0.20		< 0.20	
DBAN	< 0.20		< 0.20		< 0.20	
HALs						
TCAL	0.66	0.01	< 0.20		< 0.20	
DBCAL	0.30	0.01	0.22	0.09	0.28	0.08
BDCAL	< 0.20		< 0.20		< 0.20	
TBAL	< 0.20		< 0.20		< 0.20	
HKs						
1,1-DCP	0.80	0.04	0.33	0.02	< 0.20	
1,1,1-TCP	< 0.20		< 0.20		< 0.20	
HNM						
TCNM	< 0.20		< 0.20		< 0.20	
HAMs						
DCAM	5.95	0.19	0.30	0.00	0.40	0.04
BCAM	2.33	0.16	< 0.20		< 0.20	
DBAM	0.59	0.08	< 0.20		< 0.20	
TCAM	< 0.20		< 0.20		< 0.20	
HAAs						
CAA	2.04	0.02	< 0.20		< 0.20	
DCAA	10.14	0.79	< 0.20		0.28	0.18
TCAA	2.85	0.60	< 0.20		< 0.20	
BAA	0.30	0.02	< 0.20		< 0.20	
BCAA	2.99	0.13	< 0.20		< 0.20	
DBAA	0.84	0.04	< 0.20		< 0.20	
BDCAA	< 0.20		< 0.20		< 0.20	
CDBAA	< 0.20		< 0.20		< 0.20	
TBAA	< 0.20		< 0.20		< 0.20	
IAA	0.23	0.07	< 0.20		< 0.20	

[a] Concentrations are averages of duplicate samples; units = µg/L

Supplementary Table 2: DBP Concentrations[a] at Facility A, Event 2

	MF		RO		AOP	
	Average	Range	Average	Range	Average	Range
THMs						
TCM	6.28	0.26	1.27	0.11	0.94	0.01
BDCM	2.56	0.03	1.11	0.07	0.70	0.00
DBCM	0.94	0.00	0.51	0.01	< 0.20	
TBM	< 0.20		< 0.20		< 0.20	
DCIM	< 0.20		< 0.20		< 0.20	
BCIM	< 0.20		< 0.20		< 0.20	
DBIM	< 0.20		< 0.20		< 0.20	
CDIM	< 0.20		< 0.20		< 0.20	
BDIM	< 0.20		< 0.20		< 0.20	
TIM	< 0.20		< 0.20		< 0.20	
HANs						
DCAN	0.76	0.02	< 0.20		< 0.20	
TCAN	< 0.20		< 0.20		< 0.20	
BCAN	< 0.20		< 0.20		< 0.20	
DBAN	< 0.20		< 0.20		< 0.20	
HALs						
TCAL	0.57	0.19	< 0.20		< 0.20	
DBCAL	0.23	0.02	0.20	0.10	< 0.20	
BDCAL	< 0.20		< 0.20		< 0.20	
TBAL	< 0.20		< 0.20		< 0.20	
HKs						
1,1-DCP	1.08	0.11	< 0.20		< 0.20	
1,1,1-TCP	< 0.20		< 0.20		< 0.20	
HNM						
TCNM	< 0.20		< 0.20		< 0.20	
HAMs						
DCAM	9.25	0.63	0.56	0.08	0.71	0.04
BCAM	2.74	0.30	0.24	0.11	0.31	0.04
DBAM	0.44	0.05	< 0.20		< 0.20	
TCAM	< 0.20		< 0.20		< 0.20	
HAAs						
CAA	2.06	0.34	< 0.20		< 0.20	
DCAA	13.29	1.15	0.46	0.12	0.91	0.31
TCAA	4.48	0.55	< 0.20		< 0.20	
BAA	0.36	0.01	< 0.20		< 0.20	
BCAA	3.27	0.48	< 0.20		< 0.20	
DBAA	0.86	0.19	< 0.20		< 0.20	
BDCAA	0.82	0.16	< 0.20		< 0.20	
CDBAA	0.36	0.12	< 0.20		< 0.20	
TBAA	< 0.20		< 0.20		< 0.20	
IAA	0.24	0.09	< 0.20		< 0.20	

[a] Concentrations are averages of duplicate samples; units = µg/L

Supplementary Table 3: DBP Concentrations[a] in Drinking Waters Near Facility A

	Tap water (ground)		Tap water (surface)	
	Average	Range	Average	Range
THMs				
TCM	< 0.20		1.77	0.01
BDCM	< 0.20		8.29	0.21
DBCM	0.41	0.01	15.81	0.20
TBM	0.35	0.01	7.54	0.10
DCIM	< 0.20		< 0.20	
BCIM	< 0.20		< 0.20	
DBIM	< 0.20		< 0.20	
CDIM	< 0.20		< 0.20	
BDIM	< 0.20		< 0.20	
TIM	< 0.20		< 0.20	
HANs				
DCAN	< 0.20		0.52	0.01
TCAN	< 0.20		< 0.20	
BCAN	< 0.20		1.58	0.05
DBAN	< 0.20		2.51	0.05
HALs				
TCAL	< 0.20		0.83	0.06
DBCAL	0.62	0.03		
BDCAL	< 0.20		3.99	0.02
TBAL	0.22	0.11	1.75	0.11
HKs				
1,1-DCP	< 0.20		< 0.20	
1,1,1-TCP	< 0.20		< 0.20	
HNM				
TCNM	< 0.20		< 0.20	
HAMs				
DCAM	< 0.20		0.23	0.00
BCAM	< 0.20		1.00	0.04
DBAM	< 0.20		1.90	0.09
TCAM	< 0.20		< 0.20	
HAAs				
CAA	< 0.20		< 0.20	
DCAA	< 0.20		1.20	0.90
TCAA	< 0.20		0.98	0.11
BAA	< 0.20		0.97	0.39
BCAA	< 0.20		4.23	0.42
DBAA	0.20	0.12	6.50	0.15
BDCAA	< 0.20		3.08	0.85
CDBAA	< 0.20		< 0.20	
TBAA	< 0.20		1.06	0.47
IAA	< 0.20		< 0.20	

[a] Concentrations are averages of duplicate samples; units = µg/L

Supplementary Table 4: DBP Concentrations[a] at Facility B

	Sec eff		RO		AOP		Tap water (ground)	
	Average	Range	Average	Range	Average	Range	Average	Range
THMs								
TCM	0.25	0.02	< 0.20		< 0.20		< 0.20	
BDCM	< 0.20		< 0.20		< 0.20		< 0.20	
DBCM	< 0.20		< 0.20		< 0.20		0.43	0.06
TBM	< 0.20		< 0.20		< 0.20		0.57	0.02
DCIM	< 0.20		< 0.20		< 0.20		< 0.20	
BCIM	< 0.20		< 0.20		< 0.20		< 0.20	
DBIM	< 0.20		< 0.20		< 0.20		< 0.20	
CDIM	< 0.20		< 0.20		< 0.20		< 0.20	
BDIM	< 0.20		< 0.20		< 0.20		< 0.20	
TIM	< 0.20		< 0.20		< 0.20		< 0.20	
HANs								
DCAN	0.24	0.00	0.22	0.01	0.27	0.02	< 0.20	
TCAN	< 0.20		< 0.20		< 0.20		< 0.20	
BCAN	< 0.20		< 0.20		< 0.20		< 0.20	
DBAN	< 0.20		< 0.20		< 0.20		< 0.20	
HALs								
TCAL	< 0.20		< 0.20		< 0.20		< 0.20	
DBCAL	< 0.20		< 0.20		< 0.20		< 0.20	
BDCAL	< 0.20		< 0.20		< 0.20		< 0.20	
TBAL	< 0.20		< 0.20		< 0.20		0.27	0.02
HKs								
1,1-DCP	0.54	0.03	0.20	0.02	0.46	0.04	< 0.20	
1,1,1-TCP	< 0.20		< 0.20		< 0.20		< 0.20	
HNM								
TCNM	< 0.20		< 0.20		< 0.20		< 0.20	
HAMs								
DCAM	3.94	0.71	1.25	0.02	2.83	0.40	< 0.20	
BCAM	< 0.20		0.23	0.01	0.41	0.12	< 0.20	
DBAM	< 0.20		< 0.20		< 0.20		< 0.20	
TCAM	< 0.20		< 0.20		< 0.20		< 0.20	
HAAs								
CAA	1.07	0.09	< 0.20		< 0.20		< 0.20	
DCAA	6.59	1.32	0.52	0.08	0.75	0.14	< 0.20	
TCAA	2.68	0.95	< 0.20		< 0.20		< 0.20	
BAA	< 0.20		< 0.20		< 0.20		< 0.20	
BCAA	< 0.20		< 0.20		0.21	0.04	< 0.20	
DBAA	< 0.20		< 0.20		< 0.20		0.25	0.04
BDCAA	< 0.20		< 0.20		< 0.20		< 0.20	
CDBAA	< 0.20		< 0.20		< 0.20		< 0.20	
TBAA	< 0.20		< 0.20		< 0.20		< 0.20	
IAA	< 0.20		< 0.20		< 0.20		< 0.20	

[a] Concentrations are averages of duplicate samples; units = µg/L

Supplementary Table 5: DBP Concentrations[a] at Facility C, Event 1

	Sec eff		O_3/BAF		UF/GAC/AOP		Tap water (ground)	
	Average	Range	Average	Range	Average	Range	Average	Range
THMs								
TCM	94.79	0.32	33.77	2.16	51.14	0.26	10.08	0.62
BDCM	15.02	0.26	13.50	0.86	15.87	0.02	6.87	0.09
DBCM	1.17	0.02	4.45	0.26	4.07	0.02	2.31	0.00
TBM	< 0.20		< 0.20		< 0.20		< 0.20	
DCIM	0.32	0.02	0.28	0.01	2.45	0.02	< 0.20	
BCIM	< 0.20		< 0.20		0.46	0.03	< 0.20	
DBIM	< 0.20		< 0.20		< 0.20		< 0.20	
CDIM	< 0.20		< 0.20		< 0.20		< 0.20	
BDIM	< 0.20		< 0.20		< 0.20		< 0.20	
TIM	< 0.20		< 0.20		< 0.20		< 0.20	
HANs								
DCAN	1.66	0.08	0.76	0.02	0.98	0.01	1.13	0.03
TCAN	< 0.20		< 0.20		< 0.20		< 0.20	
BCAN	0.27	0.00	0.30	0.00	0.37	0.03	0.67	0.01
DBAN	< 0.20		< 0.20		< 0.20		< 0.20	
HALs								
TCAL	< 0.20		1.07	0.00	2.65	0.85	2.56	0.22
DBCAL	< 0.20		< 0.20		< 0.20		2.72	0.30
BDCAL	< 0.20		< 0.20		0.21	0.09	1.68	0.02
TBAL	< 0.20		< 0.20		< 0.20		< 0.20	
HKs								
1,1-DCP	0.47	0.22	0.27	0.03	< 0.20		< 0.20	
1,1,1-TCP	< 0.20		< 0.20		< 0.20		0.50	0.00
HNM								
TCNM	0.40	0.05	1.33	0.10	1.07	0.07	< 0.20	
HAMs								
DCAM	18.17	1.44	9.01	1.02	9.26	0.69	0.83	0.04
BCAM	1.89	0.01	2.23	0.05	2.33	0.09	0.66	0.16
DBAM	< 0.20		0.69	0.02	0.49	0.02	0.32	0.01
TCAM	0.68	0.07	< 0.20		< 0.20		< 0.20	
HAAs								
CAA	4.60	0.02	1.59	0.26	2.63	0.00	0.40	0.15
DCAA	38.65	3.47	17.38	1.56	20.28	1.33	3.54	0.47
TCAA	49.25	7.07	10.32	1.32	14.91	0.96	6.34	0.98
BAA	0.37	0.02	0.39	0.01	0.50	0.09	< 0.20	
BCAA	3.28	0.25	4.10	0.11	4.36	0.14	1.47	0.01
DBAA	< 0.20		0.80	0.29	0.70	0.12	0.48	0.00
BDCAA	5.44	3.15	< 0.20		< 0.20		5.23	0.91
CDBAA	0.27	0.11	0.69	0.41	0.90	0.18	0.65	0.02
TBAA	< 0.20		< 0.20		< 0.20		< 0.20	
IAA	< 0.20		< 0.20		0.40	0.09	< 0.20	

[a] Concentrations are averages of duplicate samples; units = µg/L

Supplementary Table 6: DBP Concentrations[a] at Facility C, Event 2

	Sec eff		O_3/BAF		UF/GAC/AOP		Tap water (ground)	
	Average	Range	Average	Range	Average	Range	Average	Range
THMs								
TCM	111.62	1.09	50.76	4.76	23.62	5.95	10.07	0.66
BDCM	14.55	1.04	14.15	1.23	11.50	3.15	6.48	0.27
DBCM	1.42	0.05	5.08	0.15	6.28	1.30	2.45	0.02
TBM	< 0.20		< 0.20		0.20	0.01	0.43	0.08
DCIM	0.28	0.01	0.45	0.00	0.30	0.09	< 0.20	
BCIM	< 0.20		< 0.20		< 0.20		< 0.20	
DBIM	< 0.20		< 0.20		< 0.20		< 0.20	
CDIM	< 0.20		< 0.20		< 0.20		< 0.20	
BDIM	< 0.20		< 0.20		< 0.20		< 0.20	
TIM	< 0.20		< 0.20		< 0.20		< 0.20	
HANs								
DCAN	2.43	0.07	1.20	0.14	0.39	0.03	1.20	0.06
TCAN	< 0.20		< 0.20		< 0.20		< 0.20	
BCAN	0.42	0.08	0.41	0.05	0.48	0.05	1.00	0.07
DBAN	< 0.20		< 0.20		< 0.20		< 0.20	
HALs								
TCAL	33.80	0.24	16.80	6.01	13.80	9.22	3.93	0.07
DBCAL	< 0.20		< 0.20		< 0.20		< 0.20	
BDCAL	0.72	0.07	1.24	0.34	0.90	0.46	4.67	0.07
TBAL	< 0.20		< 0.20		< 0.20		< 0.20	
HKs								
1,1-DCP	< 0.20		< 0.20		< 0.20		< 0.20	
1,1,1-TCP	< 0.20		< 0.20		< 0.20		0.46	0.01
HNM								
TCNM	0.52	0.03	2.13	0.16	1.25	0.30	< 0.20	
HAMs								
DCAM	19.96	0.19	8.92	0.04	3.27	0.30	0.53	0.40
BCAM	2.82	0.30	3.12	0.23	2.29	0.09	0.80	0.57
DBAM	< 0.20		0.80	0.06	0.87	0.11	0.32	0.25
TCAM	1.02	0.01	0.24	0.01	< 0.20		0.21	0.00
HAAs								
CAA	7.31	0.26	2.41	0.04	1.46	0.21	0.91	0.50
DCAA	45.79	1.33	19.67	0.81	8.17	0.62	4.10	0.09
TCAA	63.75	2.84	11.30	0.25	4.49	0.29	5.61	0.41
BAA	0.83	0.04	0.43	0.01	0.39	0.00	< 0.20	
BCAA	3.84	0.04	4.38	0.06	3.06	0.19	1.67	0.07
DBAA	0.20	0.00	1.01	0.04	1.02	0.00	0.47	0.00
BDCAA	< 0.20		4.12	0.69	5.12	1.95	2.09	0.25
CDBAA	0.29	0.13	0.84	0.05	0.70	0.02	0.45	0.16
TBAA	< 0.20		< 0.20		< 0.20		< 0.20	
IAA	< 0.20		< 0.20		< 0.20		< 0.20	

[a] Concentrations are averages of duplicate samples; units = µg/L

Supplementary Table 7: DBP Concentrations[a] at Facility D

	Floc/sed eff		O_3/BAF		GAC/UV		Tap water (surface)	
	Average	Range	Average	Range	Average	Range	Average	Range
THMs								
TCM	44.51	3.48	6.61	0.23	3.85	0.01	33.56	1.53
BDCM	52.99	3.55	15.78	0.72	9.69	0.00	7.78	0.28
DBCM	36.20	1.59	27.49	1.14	20.58	0.08	0.96	0.02
TBM	5.62	0.01	13.43	0.61	15.55	0.24	< 0.20	
DCIM	0.52	0.01	< 0.20		< 0.20		0.24	0.01
BCIM	0.29	0.04	< 0.20		0.24	0.00	< 0.20	
DBIM	< 0.20		< 0.20		0.28	0.01	< 0.20	
CDIM	< 0.20		< 0.20		< 0.20		< 0.20	
BDIM	< 0.20		< 0.20		< 0.20		< 0.20	
TIM	< 0.20		< 0.20		< 0.20		< 0.20	
HANs								
DCAN	2.30	0.16	0.64	0.01	0.31	0.01	0.65	0.04
TCAN	< 0.20		< 0.20		< 0.20		< 0.20	
BCAN	3.51	0.14	1.53	0.02	0.94	0.05	0.22	0.00
DBAN	2.59	0.05	2.85	0.00	2.35	0.02	< 0.20	
HALs								
TCAL	11.39	0.04	1.88	0.06	0.98	0.15	2.58	0.09
DBCAL	< 0.20		< 0.20		< 0.20		< 0.20	
BDCAL	1.40	0.15	0.94	0.06	0.50	0.20	< 0.20	
TBAL	< 0.20		< 0.20		< 0.20		< 0.20	
HKs								
1,1-DCP	< 0.20		0.81	0.06	0.32	0.01	0.39	0.00
1,1,1-TCP	< 0.20		< 0.20		< 0.20		< 0.20	
HNM								
TCNM	1.04	0.03	4.61	0.22	1.21	0.03	< 0.20	
HAMs								
DCAM	12.99	1.69	3.19	0.02	1.40	0.09	5.72	0.07
BCAM	12.30	1.55	5.37	0.10	3.06	0.12	1.13	0.01
DBAM	7.85	0.78	8.93	0.04	6.11	0.21	< 0.20	
TCAM	0.38	0.01	< 0.20		< 0.20		< 0.20	
HAAs								
CAA	3.57	0.12	0.98	0.45	0.43	0.04	0.96	0.17
DCAA	17.52	3.98	4.00	1.71	1.56	0.11	10.43	4.26
TCAA	15.95	3.39	1.31	0.76	0.36	0.10	11.86	4.36
BAA	2.45	0.01	1.47	0.18	1.12	0.02	< 0.20	
BCAA	13.95	1.60	6.17	1.33	3.76	0.15	1.50	0.42
DBAA	7.73	0.26	6.31	0.96	5.32	0.59	< 0.20	
BDCAA	20.11	1.85	2.63	0.99	1.25	0.50	2.85	0.81
CDBAA	10.35	1.18	4.20	0.82	2.41	0.69	0.23	0.08
TBAA	1.76	0.12	1.90	0.42	1.33	0.79	< 0.20	
IAA	< 0.20		< 0.20		< 0.20		< 0.20	

[a] Concentrations are averages of duplicate samples; units = µg/L

Supplementary Table 8: DBP Concentrations[a] at Facility E

	Riverbank filtrate		AOP/BAF		GAC		Tap water (surface)	
	Average	Range	Average	Range	Average	Range	Average	Range
THMs								
TCM	10.80	0.30	7.41	0.35	4.42	0.06	6.58	0.38
BDCM	19.05	0.23	13.58	0.24	8.57	0.06	6.57	0.16
DBCM	20.58	0.34	16.84	0.16	12.74	0.20	3.65	0.04
TBM	6.72	0.14	6.89	0.07	7.24	0.31	0.39	0.00
DCIM	0.20	0.00	0.41	0.00	< 0.20		< 0.20	
BCIM	< 0.20		0.39	0.05	< 0.20		< 0.20	
DBIM	< 0.20		0.20	0.01	< 0.20		< 0.20	
CDIM	< 0.20		< 0.20		< 0.20		< 0.20	
BDIM	< 0.20		< 0.20		< 0.20		< 0.20	
TIM	< 0.20		< 0.20		< 0.20		< 0.20	
HANs								
DCAN	0.48	0.01	0.29	0.00	< 0.20		0.27	0.01
TCAN	< 0.20		< 0.20		< 0.20		< 0.20	
BCAN	1.04	0.04	0.74	0.03	0.46	0.02	0.32	0.00
DBAN	1.56	0.07	1.40	0.03	1.15	0.09	0.22	0.00
HALs								
TCAL	0.95	0.25	0.66	0.11	0.26	0.02	0.36	0.08
DBCAL	< 0.20		< 0.20		< 0.20		< 0.20	
BDCAL	< 0.20		< 0.20		< 0.20		< 0.20	
TBAL	< 0.20		< 0.20		< 0.20		< 0.20	
HKs								
1,1-DCP	< 0.20		< 0.20		< 0.20		< 0.20	
1,1,1-TCP	< 0.20		< 0.20		< 0.20		< 0.20	
HNM								
TCNM	0.55	0.01	0.83	0.00	0.36	0.01	< 0.20	
HAMs								
DCAM	6.10	1.41	3.80	0.52	1.85	0.12	3.43	0.04
BCAM	8.48	1.84	6.35	0.91	3.98	0.10	2.67	0.33
DBAM	8.23	2.60	6.52	0.54	5.69	1.02	1.20	0.23
TCAM	< 0.20		< 0.20		< 0.20		< 0.20	
HAAs								
CAA	0.98	0.05	0.89	0.23	0.45	0.03	0.66	0.12
DCAA	6.72	0.04	4.61	0.34	2.17	0.13	4.29	0.10
TCAA	2.58	0.06	1.44	0.06	0.53	0.09	1.72	0.06
BAA	1.30	0.05	1.13	0.12	0.89	0.03	0.36	0.06
BCAA	7.31	0.17	5.46	0.39	3.51	0.14	2.30	0.04
DBAA	8.18	0.10	7.29	0.32	6.15	0.33	1.16	0.02
BDCAA	5.64	0.47	3.32	0.20	1.47	0.28	1.48	0.12
CDBAA	6.38	0.68	4.47	0.40	3.08	0.91	0.94	0.03
TBAA	2.24	0.27	1.98	0.24	1.67	0.61	< 0.20	
IAA	< 0.20		< 0.20		< 0.20		< 0.20	

[a] Concentrations are averages of duplicate samples; units = µg/L

Supplementary Table 9: Basic Water Quality Parameters

Potable reuse facility	pH	UV_{254} (cm^{-1})	TOC (mg C/L)	SUVA (L/mg-M)	$[NH_4^+]$ (mg/L as N)	$[NO_2^-]$ (mg/L as N)	$[NO_3^-]$ (mg/L as N)	$[Br^-]$ (mg/L)
A								
Reuse Event 1								
MF	7.1	0.1707	7.66	2.23	1.63	0.27	14.11	0.13
RO	4.9	0.0135	<0.50	—	0.580	<0.02	1.01	<0.10
AOP	6.0	0.0062	<0.50	—	0.289	<0.02	1.21	<0.10
Reuse Event 2								
MF	7.1	0.1819	7.58	2.40	1.57	<0.02	17.36	0.12
RO	5.3	0.0176	<0.50	—	0.456	<0.02	1.27	<0.10
AOP	6.8	0.0082	<0.50	—	0.284	<0.02	1.51	<0.10
Conventional drinking waters								
Tap water (ground)	7.5	0.0061	<0.50	—	<0.015	<0.02	2.81	<0.10
Tap water (surface)	7.6	0.0444	2.01	2.21	0.446	<0.02	0.11	0.15
B								
Secondary effluent	7.3	0.2479	9.81	2.53	35.9	<0.02	2.74	0.41
RO	4.8	0.0036	<0.50	—	NM [a]	<0.02	0.74	<0.10
AOP	4.8	0.0049	<0.50	—	NM [a]	<0.02	0.89	<0.10
Tap water (ground)	6.9	0.0060	<0.50	—	<0.015	<0.02	3.56	<0.10
C								
Event 1								
Secondary effluent	7.7	0.1697	5.85	2.90	0.042	0.04	2.59	<0.10
O_3/BAF	7.6	0.0733	4.27	1.72	<0.015	0.04	1.52	<0.10
UF/GAC/AOP	7.4	0.0459	3.47	1.32	<0.015	<0.02	3.18	<0.10
Tap water (surface)	NA [b]	0.0252	0.60	4.20	<0.015	<0.02	<0.10	<0.10
Event 2								
Secondary effluent	7.5	0.1635	6.04	2.71	0.017	0.04	0.95	<0.10
O_3/BAF	7.3	0.0762	4.67	1.63	<0.015	0.04	1.37	<0.10
UF/GAC/AOP	7.4	0.0225	1.67	1.35	<0.015	<0.02	1.90	<0.10
Tap water (surface)	NA [b]	0.0327	0.73	4.48	<0.015	<0.02	<0.10	<0.10

Potable reuse facility	pH	UV_{254} (cm^{-1})	TOC (mg C/L)	SUVA (L/mg-M)	$[NH_4^+]$ (mg/L as N)	$[NO_2^-]$ (mg/L as N)	$[NO_3^-]$ (mg/L as N)	$[Br^-]$ (mg/L)
D								
Floc/sed effluent	7.8	0.1531	6.44	2.38	0.071	<0.02	0.25	0.32
O_3/BAF	7.5	0.0662	4.45	1.49	<0.015	<0.02	1.57	0.27
GAC/UV	7.4	0.0331	3.38	0.98	<0.015	<0.02	1.68	0.27
Drinking water (surface)	6.8	0.0477	1.98	2.41	0.048	<0.02	<0.10	<0.10
E								
Riverbank filtrate	7.7	0.0700	2.95	2.37	<0.015	<0.02	1.80	0.12
AOP/BAF	7.6	0.0489	2.80	1.75	<0.015	<0.02	1.64	0.12
GAC	7.4	0.0380	2.15	1.77	<0.015	<0.02	1.66	0.16
Drinking water	6.9	0.0323	1.69	1.91	<0.015	<0.02	0.23	<0.10

[a] NM = not measured; assumed complete rejection of NH_4^+ by RO

[b] NA = not analyzed; tap water samples from Facility C were acidified to ~pH 3.7 after collection and before transport to Stanford University

Supplementary Table 10: Contributions of Contaminant Classes to Total Cytotoxicity [a]

Water Category	CAT (regulated) [b]	CAT (unregulated) [c]	BCAT [d]
Potable reuse waters			
RO-based (n = 3)	0.02% (0.01–0.05%)	3.0% (2.6–6.0%)	97% (94–97%)
RO-free (n = 4)	5.0% (3.5–5.6%)	35% (15–49%)	60% (45–81%)
Conventional drinking waters			
Groundwater (n = 4)	0.17% (0.10–0.30%)	17% (12–22%)	83% (78–88%)
Surface water (n = 3)	0.17% (0–0.43%)	3.0% (1.4–48%)	97% (52–98%)
Median across all categories	0.2%	16%	83%

[a] Reported values = median (range)

[b] Calculated additive toxicity for regulated DBPs determined using equation 1 (main text)

[c] Calculated additive toxicity for unregulated, known DBPs determined using equation 1 (main text)

[d] Bioassay-based calculated additive toxicity for uncharacterized DBPs and anthropogenic chemicals determined using equation 2 (main text)

Supplementary Table 11: Potable Reuse Treatment Processes[a]

Facility	Treatment processes[b]
A	secondary effluent – chloramination – **MF** – **RO** – **UV/H_2O_2 AOP**
B	**secondary effluent** – chloramination – ozonation – **MF** – **RO** – **UV/H_2O_2 AOP**
C	**secondary effluent** – ozonation – **BAF** – ultrafiltration – GAC – **UV/H_2O_2 AOP**
D	secondary effluent – flocculation – **sedimentation** – ozonation/chloramination – **BAF** – GAC – **UV**
E	secondary effluent – **riverbank filtration** – chemical softening – UV/H_2O_2 AOP – **BAF** – **GAC**

[a] The treatment stages after which potable reuse waters were collected are shown in bold.

[b] MF = microfiltration
RO = reverse osmosis
UV/H_2O_2 AOP = UV/hydrogen peroxide advanced oxidation process
BAF = biologically active filtration
GAC = granular activated carbon filtration

Supplementary Table 12: Summary of CHO Cell Chronic Cytotoxicity Results

Sample № Figure №	Lowest Cytotoxic Conc. Factor [a]	Mean LC_{50} ± SE Conc. Factor [b]	r^2 [c]	ANOVA Statistic [d]
Facility A				
Reuse Event 1				
MF	11.90	23.27 ± 0.68	0.97	$F_{11,90}$ =170; $P \leq 0.001$
RO	130.84	215.64 ± 8.39	0.78	$F_{11,99}$ = 20.2; $P \leq 0.001$
AOP	97.83	135.23 ± 1.11	0.98	$F_{11,93}$ = 134; $P \leq 0.001$
Reuse Event 2				
MF	21.33	32.66 ± 0.54	0.99	$F_{10,92}$ = 231; $P \leq 0.001$
RO	NS	NA	NA	NS
AOP	138.71	239.44 ± 6.31	0.93	$F_{10,98}$ = 67.7; $P \leq 0.001$
Conventional drinking waters				
Tap water (ground)	184.15	387.10 ± 4.49	0.95	$F_{12,63}$ = 38.5; $P \leq 0.001$
Tap water (surface)	14.54	45.32 ± 0.97	0.98	$F_{11,103}$ = 184; $P \leq 0.001$
Facility B				
Secondary effluent (not disinfected)	5.09	9.62 ± 0.13	0.98	$F_{10,81}$ = 193; $P \leq 0.001$
Secondary effluent (disinfected)	40.0	66.36 ± 1.35	0.99	$F_{10,81}$ = 75.4; $P \leq 0.001$
RO	192.31	302.69 ± 3.15	0.97	$F_{10,81}$ = 75.5; $P \leq 0.001$
AOP	247.93	328.32 ± 8.19	0.94	$F_{10,81}$ = 52.1; $P \leq 0.001$
Tap water (ground)	380.95	518.38 ± 13.98	0.92	$F_{10,76}$ = 35.0; $P \leq 0.001$
Facility C				
Event 1				
Secondary effluent (not disinfected)	5.81	13.29 ± 0.31	0.99	$F_{11,103}$ = 234; $P \leq 0.001$
Secondary effluent (disinfected)	10.51	23.03 ± 1.21	0.97	$F_{11,104}$ = 86.2; $P \leq 0.001$
O_3/BAF	64.52	92.41 ± 2.20	0.97	$F_{14,101}$ = 84.5; $P \leq 0.001$
UF/GAC/AOP	35.89	81.96 ± 2.82	0.97	$F_{12,102}$ = 94.4; $P \leq 0.001$
Tap water (surface)	37.88	55.41 ± 1.55	0.98	$F_{11,84}$ = 123; $P \leq 0.001$
Event 2				
Secondary effluent (not disinfected)	8.80	18.40 ± 0.67	0.98	$F_{11,101}$ = 58.6; $P \leq 0.001$
Secondary effluent (disinfected)	7.98	20.72 ± 0.33	0.98	$F_{12,101}$ = 190; $P \leq 0.001$
O_3/BAF	21.38	65.22 ± 1.46	0.98	$F_{10,106}$ = 225; $P \leq 0.001$
UF/GAC/AOP	82.64	173.62 ± 7.85	0.95	$F_{10,106}$ = 49.5; $P \leq 0.001$
Tap water (surface)	40.48	67.85 ± 1.38	0.98	$F_{11,106}$ = 185; $P \leq 0.001$
Facility D				
Floc/sed effluent (not disinfected)	9.90	16.94 ± 0.46	0.98	$F_{12,99}$ = 96.3; $P \leq 0.001$
Floc/sed effluent (disinfected)	12.66	21.62 ± 0.60	0.99	$F_{10,103}$ = 123; $P \leq 0.001$
O_3/BAF	40.16	66.75 ± 0.79	0.98	$F_{10,105}$ = 101; $P \leq 0.001$
GAC/UV	59.29	102.01 ± 1.41	0.99	$F_{10,106}$ = 130; $P \leq 0.001$
Drinking water (surface)	13.33	19.60 ± 0.47	0.99	$F_{11,103}$ = 185; $P \leq 0.001$

Sample № Figure №	Lowest Cytotoxic Conc. Factor [a]	Mean LC_{50} ± SE Conc. Factor [b]	r^2 [c]	ANOVA Statistic [d]
Facility E				
Riverbank filtrate (not disinfected)	73.17	97.37 ± 2.45	0.97	$F_{10,83} = 62.7$; $P \leq 0.001$
Riverbank filtrate (disinfected)	30.49	61.58 ± 1.05	0.99	$F_{10,83} = 86.8$; $P \leq 0.001$
AOP/BAF	49.56	77.89 ± 1.99	0.99	$F_{10,83} = 97.7$; $P \leq 0.001$
GAC	68.48	140.34 ± 1.05	0.99	$F_{10,85} = 130$; $P \leq 0.001$
Drinking water	11.06	14.31 ± 0.52	0.97	$F_{10,83} = 142$; $P \leq 0.001$
Method blanks *(extractions of deionized water)*				
SPE (no anion exchange)	NS	NA	NA	NS
SPE (with anion exchange)	156.52	269.70 ± 0.31*	0.93	$F_{10,110} = 4.05$; $P \leq 0.001$
XAD	93.46	125.32 ± 3.18	0.98	$F_{11,94} = 156$; $P \leq 0.001$
H_2O_2 control	56.25	92.86 ± 2.54	0.98	$F_{10,92} = 165$; $P \leq 0.001$
Control experiments *(extractions of chlorinated surface water)*				
SPE (no anion exchange)				
Replicate 1	5.00	9.50 ± 0.60	0.98	$F_{11,84} = 68.8$; $P \leq 0.001$
Replicate 2	5.00	12.02 ± 0.13	0.98	$F_{12,91} = 459$; $P \leq 0.001$
SPE (with anion exchange)				
Replicate 1	18.07	33.60 ± 1.23	0.96	$F_{14,81} = 165$; $P \leq 0.001$
Replicate 2	19.61	39.04 ± 0.56	0.98	$F_{10,98} = 513$; $P \leq 0.001$
XAD				
Replicate 1	32.19	41.88 ± 0.33	0.99	$F_{14,77} = 172$; $P \leq 0.001$
Replicate 2	30.24	65.21 ± 0.75	0.94	$F_{13,94} = 78.3$; $P \leq 0.001$

[a] Lowest cytotoxic concentration was the lowest concentration factor of the sample that induced a statistically significant reduction in cell density as compared to the negative control.

[b] The LC_{50} value is the concentration factor of the water sample, determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative controls. The mean and the standard error (SE) of each LC_{50} value were derived from multiple regression analyses using bootstrap statistics.

[c] The r^2 is the coefficient of determination for the regression analysis of the concentration-response data upon which the LC_{50} value was calculated.

[d] The degrees of freedom for the between-groups and residual associated with the calculated *F*-test result and the resulting probability value.

NS = not statistically significant within the concentration range analyzed
NA = not applicable
* = value derived by extrapolation

Supplementary Note 1: Validation of extraction methods

Background toxicity of the three extraction methods (SPE without anion exchange sorbent, SPE with anion exchange sorbent, and XAD resin extraction) was assessed by extracting 10 L of DI water buffered at pH 8 with sodium borate (4 mM), dosed with 1.5 mg/L of sodium hypochlorite (NaOCl), and then quenched with 1.1-fold molar excess of sodium thiosulfate ($Na_2S_2O_3$). All quenched water samples were acidified to ~pH 3.7 (SPE) or pH 1–2 (XAD) using sulfuric acid (H_2SO_4) shortly before extraction. The DI water control experiments involved a single extraction per extraction method. Full details of the extraction methods are in Supplementary Note 8.

To determine the recovery of CHO cell cytotoxicity, 10 L of a surface water was collected, filtered using 0.7-µm glass fiber filters (Whatman), buffered at pH 8 with sodium borate (4 mM), dosed with NaOCl to achieve a residual of ~1 mg/L as Cl_2 after 24 h at room temperature (22 ± 1 °C), and then quenched with 1.1-fold molar excess of $Na_2S_2O_3$. These control experiments involving chlorinated surface water were conducted in duplicate, with each set of duplicate extractions occurring on the same day.

A single control experiment was conducted to assess the effect of unreacted H_2O_2 on bioassays. DI water (10 L) was buffered at pH 8 using sodium borate (4 mM) and then dosed with 3.5 mg/L of H_2O_2; the rationale for this H_2O_2 concentration is discussed below. A monochloramine (NH_2Cl) stock solution of ~40 mM was made by adding sodium hypochlorite (NaOCl; 80 mM) dropwise to a solution of ammonium chloride (NH_4Cl; 96 mM) while stirring; the NH_2Cl stock solution was standardized by UV absorbance at 245 and 295 nm (as described previously[1]) shortly before use. Next, NH_2Cl was added to the 10-L water sample at an initial concentration of 3.5 mg/L as Cl_2. The chloraminated water was left at room temperature (22 ± 1 °C) for ~16 h. Afterwards, the water was quenched with 1.1-fold molar excess of $Na_2S_2O_3$. The

quenched water was acidified to ~pH 3.7 with H_2SO_4 and extracted on the same day using SPE without anion exchange sorbent.

Potable reuse facilities applied 5–6.5 mg/L of H_2O_2 for UV/H_2O_2 AOP treatment (Supplementary Note 4); for Facilities A and B, ~3 mg/L chloramines were present in the RO permeate from chloramines applied upstream to control biofouling. Measuring residual H_2O_2 after AOP treatment is difficult because both chloramines and the thiosulfate used to quench chloramines interfere with the analysis of H_2O_2 by the horseradish peroxidase method.[2] However, the H_2O_2 concentration was expected to decrease somewhat from the levels applied at the facilities because H_2O_2 can react with the borate buffer[3] we used to maintain a pH of ~8 during the 3-day chloramination of water samples. Moreover, thiosulfate reacts slowly with H_2O_2, and some H_2O_2 might have been quenched over the several hours required for SPE by reactions with the thiosulfate used to quench chloramine residuals.[4] The 3.5 mg/L H_2O_2 concentration employed in the control analysis was employed to indicate whether H_2O_2 can interfere with the analysis rather than to quantitatively estimate the effect of residual H_2O_2 concentrations within the actual samples.

Supplementary Note 2: DBP acronyms

THMs = trihalomethanes

- TCM = chloroform
- BDCM = bromodichloromethane
- DBCM = dibromochloromethane
- TBM = bromoform
- DCIM = dichloroiodomethane
- BCIM = bromochloroiodomethane
- DBIM = dibromoiodomethane
- CDIM = chlorodiiodomethane
- BDIM = bromodiiodomethane
- TIM = iodoform

HANs = haloacetonitriles

- DCAN = dichloroacetonitrile
- TCAN = trichloroacetonitrile
- BCAN = bromochloroacetonitrile
- DBAN = dibromoacetonitrile

HALs = haloacetaldehydes

- TCAL = chloral hydrate
- BDCAL = bromodichloroacetaldehyde
- DBCAL = dibromochloroacetaldehyde
- TBAL = tribromoacetaldehyde

HKs = haloketones

- 1,1-DCP = 1,1-dichloropropanone
- 1,1,1-TCP = 1,1,1-trichloropropanone

HNM = halonitromethane

- TCNM = chloropicrin

HAMs = haloacetamides

- DCAM = dichloroacetamide
- BCAM = bromochloroacetamide
- TCAM = trichloroacetamide
- DBAM = dibromoacetamide

HAAs = haloacetic acids

- CAA = chloroacetic acid
- BAA = bromoacetic acid
- DCAA = dichloroacetic acid
- TCAA = trichloroacetic acid
- BCAA = bromochloroacetic acid
- DBAA = dibromoacetic acid
- BDCAA = bromodichloroacetic acid
- CDBAA = chlorodibromoacetic acid
- TBAA = tribromoacetic acid
- IAA = iodoacetic acid

Supplementary Note 3: Uncertainty calculations

The relative standard errors in DBP concentrations measured in duplicate samples are typically ≤ 10%. A recent study on the CHO cell cytotoxicity of haloacetonitriles (HANs) reported LC_{50} values with ≤ 6% relative standard errors, calculated using bootstrap statistics.[5] When calculating the uncertainties in CAT, we assumed a relative standard error of 10% for the concentrations and CHO cell LC_{50} values of individual (semi-)volatile DBPs. The standard errors of toxic potency-weighted DBP concentrations (i.e., [DBP]/LC_{50}) were obtained by propagating the standard errors of DBP concentrations and CHO LC_{50} values. The standard errors of the sums of toxic potency-weighted DBP concentrations (i.e., CAT) were determined by propagating the standard errors of individual toxic potency-weighted DBP concentrations.

For bioassays results, the standard errors of CHO cell LC_{50} values were determined by bootstrap statistics (Supplementary Note 9). The standard errors in BCAT were calculated from propagating the standard errors of the LC_{50} values determined for the SPE extracts. To avoid overestimating the BCAT of SPE extracts, we calculated the CAT contributed by (semi-)volatile DBPs that were retained in the extracts and subtracted it from the BCAT values obtained from bioassays. The standard errors of these "net" BCAT values were obtained by propagating the standard errors of the experimental BCAT and the CAT of (semi-)volatile DBPs in the extracts.

The standard errors of the total cytotoxicity indices (i.e., sums of CAT and "net" BCAT shown in Figs. 1–3 in the main text) were determined by propagating the standard errors of CAT and BCAT. The standard errors of total DBP concentrations (Supplementary Figs. 2–4) were calculated by assuming a relative standard error of 10% for the concentrations of individual (semi-)volatile DBPs. The average cytotoxicity of the 7 RO-based and RO-free reuse effluents was compared to that of the 3 surface water-derived conventional drinking waters using a one-sided Student's *t*-test.

Supplementary Note 4: Potable reuse treatment trains

The major treatment processes used in the potable reuse trains sampled in this study are listed in Supplementary Table 11. Details for individual process trains for potable reuse and conventional drinking waters for each Facility are provided below. Basic water quality parameters of the potable reuse waters, as well as those of conventional drinking waters from the same locations, are shown in Supplementary Table 9. Total organic carbon (TOC) was measured using a Shimadzu TOC-L analyzer and non-purgeable organic carbon (NPOC) method. Ammonia (NH_4^+) and nitrite (NO_2^-) concentrations were determined using colorimetric methods (Hach Method 10205 and 8507, respectively).

Nitrate (NO_3^-) and bromide (Br^-) concentrations were measured using a Dionex Integrion High Performance Ion Chromatography (HPIC) system with a 25-µL sample loop, an IonPac AS19 analytical column, an IonPac AG19 guard column, a Dionex CR-ATC 600 continuously regenerated trap column, a Dionex ADRS 600 CO_2 suppressor, and a Dionex EGC 500 potassium hydroxide (KOH) generator. The eluent gradient profile was 5 mM KOH from 0–5 min, linear increase to 10 mM KOH at 6–11 min, linear increase to 15 mM KOH at 12–17, linear increase to 20 mM KOH at 18–23 min, linear increase to 40 mM KOH at 25–27.5 min, linear decrease to 5 mM KOH at 30–37 min, and then holding at 5 mM KOH at 30–37 min. The total run time was 37 min, and the eluent flow rate was 1 mL/min.

Descriptions of conventional drinking water and potable reuse treatment systems

Facility A: The conventional drinking water system servicing the area near Facility A features sections of the distribution system serviced predominantly by groundwater and other sections that are serviced predominantly by surface water. A grab sample was collected in May 2021

from a consumer tap within the region serviced by groundwater. Although the aquifer is within an urban area, impacts resulting from leaching from urban runoff are not expected to be important because the aquifer is confined, with a depth > 150 feet below ground surface. The groundwater is treated using chloramine disinfectant prior to distribution. A separate grab sample was collected in May 2021 from a consumer tap within the region serviced by a surface water supply. The surface water supply derives from the California Delta. Within the conventional drinking water treatment plant, the water is treated by sedimentation, ozonation, granular activated carbon filtration and then chloramination for distribution.

The potable reuse treatment train consists of application of chloramines to the secondary municipal wastewater effluent, microfiltration (MF), reverse osmosis (RO), and then the UV/hydrogen peroxide advanced oxidation process (AOP) using 6 mg/L hydrogen peroxide. Grab samples of the effluent of the MF, RO and AOP were collected on two different occasions, both during May 2021. For samples featuring < 2.5 mg/L as Cl_2 total chlorine residuals, the samples were treated with chloramines at Stanford, as described in Supplementary Note 5.

Facility B: The conventional drinking water system servicing the area near Facility B is supplied by groundwater. Although the aquifer underlies an agricultural region, leaching of agricultural contaminants from the surface is unlikely because the aquifer is confined and is > 180 feet below the surface. The groundwater is chlorinated prior to distribution. One grab sample of tap water was collected during June 2021.

The potable reuse treatment train consists of application of chloramines and then ozone to the secondary municipal wastewater effluent, MF, RO, and then the UV/hydrogen peroxide advanced oxidation process (AOP) using 6.5 mg/L hydrogen peroxide. Grab samples of the secondary municipal wastewater effluent, RO effluent, and AOP effluent were collected during

one sample event in June 2021. These samples were treated with chloramines at Stanford, as described in Supplementary Note 5.

Facility C: The conventional drinking water system servicing the area near Facility C is supplied by groundwater. Contamination from surface runoff is unlikely because the aquifer is confined and is located > 100 feet below ground surface. The groundwater is chlorinated prior to distribution. Two grab samples were collected from the drinking water treatment plant effluent after chlorine disinfection, one during September 2021 and one during November 2021.

The potable reuse treatment train treats secondary municipal wastewater effluent by ozonation (5 mg/L), biofiltration, ultrafiltration, granular activated carbon filtration (GAC), and the UV/hydrogen peroxide advanced oxidation process (AOP). Two grab samples were collected from each of the secondary municipal wastewater effluent, the biofiltration effluent and the AOP effluent, once during September 2021 and once during November 2021. The samples were treated with chlorine and then chloramines at Stanford, as described in Supplementary Note 5.

Facility D: The conventional drinking water system servicing the area near Facility D treats a surface water by coagulation, sedimentation, filtration, chlorination for primary disinfection and chloramination to maintain a distribution system residual. One grab sample of filter effluent was collected prior to chlorine disinfection in July 2021. The sample was treated with chlorine and then chloramines at Stanford, as described in Supplementary Note 5.

The potable reuse treatment train consists of application of chloramines to secondary municipal wastewater effluent, flocculation, sedimentation, ozonation, biological activated carbon (BAC), granular activated carbon (GAC) and UV disinfection. Grab samples were collected from the effluents of the sedimentation, BAC and UV process units during one sample

event in July 2021. Samples were treated with chlorine and then chloramines at Stanford, as described in Supplementary Note 5.

Facility E: The conventional drinking water is a pristine surface water. After pre-treatment with chlorine and potassium permanganate, the water is treated by coagulation, flocculation, sedimentation, and biological activated carbon, blending with the potable reuse water (see below), chlorine for primary disinfection and then chloramination for distribution. A grab samples was collected from the biological activated carbon effluent during one sample event in June 2021. Samples were treated with chlorine and then chloramines at Stanford, as described in Supplementary Note 5.

The potable reuse treatment train treats an effluent-dominated river by riverbank filtration, chemical softening, the UV/hydrogen peroxide AOP, biofiltration, GAC, blending with the conventional source water (see above), chlorine for primary disinfection and then chloramination for distribution. Grab samples were collected from the effluents of the riverbank filtration, biofiltration and GAC process units during one sample event in June 2021. Samples were treated with chlorine and then chloramines at Stanford, as described in Supplementary Note 5.

Supplementary Note 5: Chloramination protocols

Grab samples were collected in 4-L amber glass bottles (if the samples contained disinfectants) or PTFE bottles (for all other samples) and transported to Stanford University within 24 hours. Secondary wastewater effluents and other waters collected from RO-free potable reuse treatment trains were filtered using 0.7-µm glass fiber filters (Whatman). All samples were transferred to amber glass bottles before chloramination.

Facility A: The local conventional drinking water was collected at the tap, quenched with 1.1-fold molar excess of sodium thiosulfate ($Na_2S_2O_3$), acidified to ~pH 3.7, and extracted by SPE without further addition of disinfectants. For samples collected along the potable reuse treatment train, sodium borate buffer (4 mM) was added to maintain the pH at ~8. Some of the potable reuse samples (MF effluent from event 1, MF effluent and RO permeate from event 2) had a total chlorine residual of ≥ 2.5 mg/L as Cl_2 at the time of collection; disinfectant was not added to these samples in the lab. For the samples with a total chlorine residual of < 2.5 mg/L, pre-formed NH_2Cl was added to achieve a dose of 2.5 mg/L as Cl_2. All 10-L water samples were kept in amber glass bottles at room temperature (22 ± 1°C) for 3 days. After 3 days of chloramination, the residual was measured and quenched using 1.1-fold molar excess of $Na_2S_2O_3$. The quenched water samples were acidified to ~pH 3.7 using H_2SO_4 and then extracted by SPE.

Facility B: The local conventional drinking water was collected at the tap, quenched with 1.1-fold molar excess of $Na_2S_2O_3$, acidified to ~pH 3.7, and extracted by SPE without further addition of disinfectants. For samples collected along the potable reuse treatment train, sodium borate buffer (4 mM) was added to maintain the pH at ~8. NH_2Cl was added to the secondary wastewater effluent and RO permeate to achieve an initial concentration of 2.5 mg/L as Cl_2; NH_2Cl was added to the AOP water at 3.5 mg/L to ensure a measurable residual in the presence

of H_2O_2 after 3 days. All the 10-L water samples were kept in amber glass bottles at room temperature (22 ± 1°C) for 3 days. After 3 days of chloramination, the water samples were quenched using 1.1-fold molar excess of $Na_2S_2O_3$, acidified to ~pH 3.7, and extracted by SPE. To assess the toxicity of wastewater contaminants other than DBPs, another 10-L sample of non-disinfected secondary wastewater effluent was adjusted to ~pH 3.7 and extracted by SPE.

Facility C: The local conventional drinking water was collected at the tap, quenched with 1.1-fold molar excess of $Na_2S_2O_3$, acidified to ~pH 3.7, and extracted by SPE. For samples collected along the potable reuse treatment train, sodium borate buffer (4 mM) was added to maintain the pH at ~8. Free chlorine was added to the water samples such that the total chlorine residual was ~2.25 mg/L as Cl_2 after 80 minutes at room temperature (22 ± 1°C). After 80 minutes of chlorine contact time, ammonium chloride (NH_4Cl) was added to the samples such that $[\text{free chlorine}]_o/[NH_4Cl]_o = 4.5$ on a weight basis to form NH_2Cl *in situ*. After 3 days of chloramination, all water samples were quenched using 1.1-fold molar excess of $Na_2S_2O_3$, acidified to ~pH 3.7, and extracted by SPE.

Facilities D and E: All samples were collected upstream of disinfection, and sodium borate buffer (4 mM) was added to all samples to maintain the pH at ~8. Free chlorine was added to the water samples such that the total chlorine residual was ~2.25 mg/L as Cl_2 after 80 minutes at room temperature (22 ± 1°C). After 80 minutes of chlorine contact time, ammonium chloride (NH_4Cl) was added to the samples such that $[\text{free chlorine}]_o/[NH_4Cl]_o = 4.5$ on a weight basis to form NH_2Cl *in situ*. After 3 days of chloramination, all water samples were quenched using 1.1-fold molar excess of $Na_2S_2O_3$, acidified to ~pH 3.7, and extracted by SPE.

Supplementary Note 6: DBP analysis

Formation of (semi-)volatile DBPs and HAAs was assessed by transferring each water sample into 60-mL glass vials (with minimal headspace) and chlor(am)inating the water under the same conditions as the corresponding 10-L sample for bioassays. After 3 days of chlor(am)ination, the 60-mL samples were quenched with ascorbic acid (33 mg/L). Halogenated volatile DBPs and HAAs were analyzed using modified EPA Methods 551.1[6] and 552.3,[7] respectively.

Halogenated volatile DBPs

After removing and discarding 10 mL of the quenched 60-mL water samples, 3 mL of methyl *tert*-butyl ether (MtBE; containing 300 μg/L of 1,2-dibromopropane as the internal standard) was added to each 50-mL sample. Sodium sulfate (~15 g; baked at 105 °C for ≥ 20 min before use) was added to the samples to increase the ionic strength, and the vials were shaken vigorously for 2 min. After waiting for ≥ 5 min for phase separation to occur, the upper MtBE layer was collected and dried by adding sodium sulfate. The dried MtBE extract was concentrated to 0.5 mL by N_2 blowdown prior to GC-MS analysis.

HAAs

After removing and discarding 10 mL of the quenched 60-mL water samples, concentrated sulfuric acid (~1.5 mL) was added to each 50-mL sample to acidify the water to pH 1–2. Next, sodium sulfate (~15 g; baked at 105 °C for ≥ 20 min before use) was added to the samples to increase the ionic strength. Then, 3 mL of MtBE (containing 300 μg/L of 1,2-dibromopropane as the internal standard) was added to the samples, and the vials were shaken vigorously for 3 min. After waiting for ≥ 5 min for phase separation to occur, the upper MtBE layer was transferred a 12-mL glass tube with a screw cap. Acidic methanol (3 mL; containing

10% sulfuric acid by volume) was added to the glass tube, which was then placed in a circulating water bath set at 50 °C for 2 hours. After 2 hours of incubation, the glass tube was removed from the water bath and allowed to cool to room temperature. Sodium sulfate solution (5 mL; 150 g/L) was added to the glass tube to enable separation between the MtBE and water/methanol phases. The bottom aqueous layer was removed from the glass tube. Next, saturated sodium bicarbonate solution (1 mL) was added to the MtBE extract to neutralize any remaining sulfuric acid. The MtBE layer was then collected and dried by adding sodium sulfate. The dried MtBE extract was concentrated to 0.5 mL by N_2 blowdown prior to GC-MS analysis.

Measuring (semi-)volatile DBPs and HAAs retained by SPE in 10-L extractions

Most (semi-)volatile DBPs and HAAs of current research interest are poorly retained by SPE.[8] Nonetheless, we analyzed the final extracts from 10-L SPE for any (semi-)volatile DBPs and HAAs that remained. The calculated additive toxicity (CAT) index contributed by these DBPs that were retained by SPE was subtracted from the bioassay-based calculated additive toxicity (BCAT) index of the SPE extract to avoid overestimating the total cytotoxicity of the waters (i.e., by including contributions of these retained DBPs in both CAT and BCAT). To measure the (semi-)volatile DBPs and HAAs retained by SPE in 10-L extractions, small volumes of the final DMSO extracts were diluted into 50 mL of DI water by a factor equal to the concentration factor achieved during extraction and concentration. The 50-mL samples were extracted into MtBE using the procedures described above and analyzed by GC-MS.

Supplementary Note 7: GC-MS methods

An Agilent 6890N gas chromatograph equipped with an Agilent HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness) and interfaced with an Agilent 5973N mass selective detector was used to analyze halogenated volatile DBPs (not including haloacetamides). Aliquots (2 μL) of MtBE samples were injected in splitless mode with the injection port temperature set at 170 °C. The GC oven was initially held at 35 °C for 20 min, ramping to 120 at 4 °C/min, then ramping to 280 at 59 °C/min and holding at 280 ºC for 2 min. The total run time was 45.96 min. The total column flow was constant at 1 mL/min. Halogenated volatile DBPs were ionized by electron ionization (EI) and detected in selected ion monitoring (SIM) mode.

Haloacetic acids (HAAs) were analyzed using the same GC-MS instrument described above. Aliquots (2 μL) of MtBE samples were injected in splitless mode with the injection port temperature set at 170 °C. The GC oven was initially held at 35 °C for 10 min, ramping to 120 at 5 °C/min, then ramping to 280 at 59 °C/min and holding at 280 ºC for 2 min. The total run time was 31.71 minutes. The total column flow was constant at 1 mL/min. HAAs were ionized by EI and detected in SIM mode.

An Agilent 7890A gas chromatograph equipped with an Agilent DB-1701 column (60 m × 0.25 mm, 1 μm film thickness) and interfaced with an Agilent 240 ion trap mass spectrometer was used to analyze haloacetamides (HAMs). Aliquots (5 μL) of MtBE samples were injected in programmed temperature vaporization (PTV) solvent vent mode. The temperature of the multimode inlet was held at 37 °C for 0.06 min, ramped to 230 ºC at 600 ºC/min and holding for 8 min, and then decreased to 37 °C at 5 °C/min. The GC oven was initially held at 37 °C for 1 min, ramped to 205 °C at 10 °C/min and held for 7 min, and then ramped to 280 at 40 °C/min and held for 2 min. The total analysis time was 28.675 min. The total column flow was constant

at 1 mL/min. Analytes were ionized by chemical ionization (CI) with methanol as the reagent gas and the resulting ions were prepared by selected ion storage (μSIS).

The quantitation ions and retention times of halogenated, (semi-)volatile DBPs analyzed by the GC-MS methods described above have been reported previously.[8] DBP concentrations below 0.2 μg/L (the method detection limits) were not used to determine CAT.

Supplementary Note 8: Extraction protocols

The SPE method using Strata-X cartridges (Phenomenex) described in Stalter et al.[9] was adapted to extract 10-L water samples in preparation for bioassays. SPE cartridges were packed in-house; each cartridge contained 2.5 g of Sepra ZTL (Phenomenex), which is a surface-modified styrene divinylbenzene sorbent that is similar to the sorbent pre-packed Strata-X cartridges. A strong anion exchange sorbent (1 g; Sepra ZT-SAX, Phenomenex) was added as the bottom layer of some SPE cartridges to assess its ability to enhance retention of HAAs and other DBPs that would be anionic at an extraction pH of 3.7.

For SPE without anion exchange sorbent, each 10-L water sample (quenched with sodium thiosulfate ($Na_2S_2O_3$) and acidified to ~pH 3.7 with sulfuric acid (H_2SO_4)) was divided into two 5-L aliquots and extracted using two SPE cartridges. Each cartridge containing 2.5 g of Sepra ZTL sorbent, was conditioned using 50 mL of methanol that was previously distilled by rotoevaporation and then equilibrated using 50 mL of DI water containing 0.01% (v/v) H_2SO_4. Afterwards, the water sample was loaded onto the SPE cartridges using a vacuum manifold. The flow rate was kept at ~11 mL/min. After the extractions were done, SPE cartridges were dried under vacuum overnight until no moisture remained. Each cartridge was eluted using 40 mL of distilled methanol, and the methanol eluted from the two cartridges used in each 10-L extraction was combined in a 125-mL round-bottom flask and concentrated to < 10 mL by rotoevaporation. The methanol was then transferred to a glass tube and further concentrated to < 5 mL using a gentle stream of nitrogen (N_2) gas. Next, solvent exchange was performed by adding 200 µL of dimethyl sulfoxide (DMSO) to the methanol and then concentrating the DMSO-methanol mixture by N_2 blowdown. As DMSO has a higher boiling point (189 °C) than methanol (64.7 °C), methanol would evaporate more readily than DMSO during N_2 blowdown. To calculate the

concentration factor, the final volume of the DMSO extract was measured using a 100-mL glass syringe.

For SPE with anion exchange sorbent, each SPE cartridge contained 1 g of Sepra ZT-SAX at the bottom and 2.5 g of Sepra ZTL on top. These cartridges were conditioned first using 50 mL of methanol and then 25 mL of 0.35% (v/v) formic acid in methanol. Next, the cartridge was equilibrated using 50 mL of DI water containing 0.01% (v/v) H_2SO_4 before extracting a 5-L aliquot of a 10-L quenched, acidified water sample. After extraction was finished, the SPE cartridge was eluted with 50 mL of MeOH followed by 25 mL of 0.35% (v/v) formic acid in methanol. The methanol extracts from the two cartridges used in each 10-L extraction were combined, concentrated by rotoevaporation and N_2 blowdown, and exchanged into DMSO.

XAD resin extractions were performed according to the U.S. EPA standard operating procedure.[10] A mix of Amberlite XAD-2 and Supelite DAX-8 resins (both from Supelco; mixed at 1:1 weight ratio) mix was washed consecutively with 0.1 N NaOH, DI water, and methanol before being Soxhlet-cleaned with methanol, ethyl acetate, and again methanol (detailed procedure has been described previously[8]). Soxhlet-cleaned XAD resins (28 mL) were added to a glass column. Each quenched 10-L water sample was acidified to pH 1–2 by adding 20 mL of H_2SO_4 for every liter of water before being manually poured over the XAD resins. The water drained from the glass column by gravity flow at ~50 mL/min. Afterwards, the XAD resins were eluted using 100 mL of ethyl acetate (EtOAc). The EtOAc extract was dried by adding sodium sulfate ($NaSO_4$) and concentrated to < 10 mL by rotoevaporation. The EtOAc was then transferred to a glass tube and further concentrated to < 5 mL by N_2 blowdown. Next, solvent exchange was performed by adding 200 µL of DMSO to the EtOAc (boiling point = 77.1 °C) and then concentrating the DMSO-EtOAc mixture by N_2 blowdown.

Supplementary Note 9: CHO cell chronic cytotoxicity assay

CHO cells and culture conditions

CHO cells are widely used for in vitro toxicology.[11,12] CHO cell line K1 AS52 Clone 11-4-8 expressed a stable chromosome complement and a consistent cell doubling time.[13,14] CHO cells were maintained in Ham's F12 medium containing 5% fetal bovine serum (FBS), 1% antibiotics (100 U/mL sodium penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B in 0.85% saline), and 1% glutamine at 37°C in a humidified atmosphere of 5% CO_2. The cells exhibit adherent, normal morphology, express cell contact inhibition and grow as a monolayer without expression of neoplastic foci. For maintenance when the culture became confluent cells were transferred to a new culture plate.

CHO cell chronic cytotoxicity assay

Chronic in vitro cytotoxicity captures a wide array of toxic insults and their adverse biological effects by measuring the reduction in cell viability over a 72-h exposure time of a test agent as compared to concurrent untreated negative controls. The experimental protocol for a concentrated water sample consisted of a range-finding experiment to define the concentration range that induced cell killing. After the range-finding experiment at least two repeated, focused experiments were conducted for each concentrated water sample. With each experiment, a dilution series (generally 10 concentrations) was prepared by diluting the concentrated water sample with F12 culture medium just prior to the experiment. A series of sample dilutions with 4–8 replicates per concentration was added to a sterile 96-well microplate and 3×10^3 CHO cells were added to each sample well as well as for the negative control. Cell culture medium blanks were included with each microplate. After the microplate was prepared it was covered with

Aluminaseal® to prevent volatilization. The microplate was incubated for 72 h in a humidified atmosphere of 5% CO_2 at 37 °C and the CHO cells proliferated. After exposure, the cell density per microplate was determined by histological staining using crystal violet and the absorbance at 595 nm was recorded using a microplate reader (Supplementary Fig. 12). All experiments were conducted under sterile conditions in a BSCII biological and chemical safety hood and the safety regulations of the University of Illinois were followed.

The details of the assay and improvements to the procedure were published.[12,15–17] Initially we experimentally determined that 3000 CHO cells seeded in a microplate well with a total volume of 200 µL F12 FBS medium in a 96-well microplate well allowed the cells to grow in a monolayer to near confluency after 72 h at 37 °C with or without AlumnaSeal™. Note that CHO K1 cells are immortalized but are not neoplastic, they express cell contact inhibition. This approach provided that the cells within each treatment group were exposed to the test agent over several cell generations. The summed cell volume of the initial cell seeding was 1.2×10^4 cubic microns. The volume of the F12 FBS medium in the microplate well (200 µL) was 2.0×10^{11} cubic microns. Thus, the initial ratio of the volume of the medium to the volume occupied by the cells was 1.7×10^7. This ratio of medium to cells preserves the pH of the media during the 72-h period. Approximately 12 h are required for the cells to attach to the bottom of the well and begin division. The cells then continue to grow throughout the remaining treatment time (60 h). Since the 72-h treatment time is precise as well as the number of cells that are seeded in each microplate well (except the blank wells), the assay is sensitive and reproducible. In the initial published calibration experiments throughout the 72-h period, the control wells demonstrated no difference in growth with or without AlumnaSeal™. For consistency and to reduce experimental variables AlumnaSeal™ was employed for all wells on the microplates used in this study.

After repeated experiments with replicate concentrations a cytotoxicity concentration-

response curve for each water sample was generated using the data from the combined replicate experiments. The concentration factor associated with a 50% reduction in cell density compared to negative controls (LC_{50}) was calculated from a non-linear regression of the concentration-response curve. Detailed descriptions of the CHO chronic cytotoxicity assay were published.[12,17,18] The CHO cell cytotoxicity concentration response curves for the water samples derived from Facility A–E are presented in Supplementary Figs. 7–11.

Statistical analyses of the CHO cell cytotoxicity data

For each Facility water sample a concentration-response curve was derived from the summed cytotoxicity data from repeated experiments (Supplementary Figs. 7–11). The lowest concentration factor that induced a significant level of cytotoxicity was determined using an analysis of variance (ANOVA) test statistic.[19] If a significant F value of $P \leq 0.05$ was obtained, a Holm-Sidak multiple comparison versus the control group analysis was conducted. The power of the test statistic ($1 - \beta$) was maintained as ≥ 0.8 at $\alpha = 0.05$. In this study nearly all of the ANOVA tests expressed a power of > 0.95. To determine significant differences among different water samples, a bootstrap statistic was used.[20,21] This approach generated a series of multiple regressions for each sample dataset and a series of LC_{50} values were derived for each water sample. Generating multiple LC_{50} values for each water sample allowed for the calculation of a mean LC_{50} value (± SE). Using these values ANOVA tests for significance of the cytotoxicity among individual water samples were conducted (Supplementary Table 12).

Supplementary References

1. Schreiber, I. M. & Mitch, W. A. Influence of the Order of Reagent Addition on NDMA Formation during Chloramination. *Environ. Sci. Technol.* **39**, 3811–3818 (2005).

2. Bader, H., Sturzenegger, V. & Hoigné, J. Photometric method for the determination of low concentrations of hydrogen peroxide by the peroxidase catalyzed oxidation of *N*,*N*-diethyl-*p*-phenylenediamine (DPD). *Water Res.* **22**, 1109–1115 (1988).

3. Kern, D. M. A polarographic study of the perborate complex. *J. Am. Chem. Soc.* **77**, 5458–5462 (1955).

4. Keen, O. S., Dotson, A. D. & Linden, K. G. Evaluation of hydrogen peroxide chemical quenching agents following an advanced oxidation process. *J. Environ. Eng.* **139**, 137–140 (2013).

5. Wei, X., Yang, M., Zhu, Q., Wagner, E. D. & Plewa, M. J. Comparative quantitative toxicology and QSAR modeling of the haloacetonitriles: Forcing agents of water disinfection byproduct toxicity. *Environ. Sci. Technol.* **54**, 8909–8918 (2020).

6. U.S. EPA. Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography With Electron-Capture Detection, Revision 1.0; Cincinnati, OH, 1995.

7. U.S. EPA. Method 552.3 Determination of Haloacetic Acids and Dalapon in Drinking Water by Liquid-Liquid Microextraction, Derivatization, and Gas Chromatography with Electron Capture Detection, Revision 1.0; Washington, D.C., 2003.

8. Lau, S. S., Forster, A. L., Richardson, S. D. & Mitch, W. A. Disinfection byproduct recovery during extraction and concentration in preparation for chemical analyses or toxicity assays. *Environ. Sci. Technol.* **55**, 14136–14145 (2021).

9. Stalter, D. *et al.* Sample enrichment for bioanalytical assessment of disinfected drinking water: Concentrating the polar, the volatiles, and the unknowns. *Environ. Sci. Technol.* **50**, 6495–6505 (2016).

10. Richardson, S. D. XAD resin extraction of disinfectant by-products drinking water (SOP-RSB-003.1, Revision No. 1); Environmental Protection Agency: Athens, GA, 2011.

11. Xu, X. *et al.* The genomic sequence of the Chinese hamster ovary (CHO)-K1 cell line. *Nat. Biotechnol.* **29**, 735–741 (2011).

12. Wagner, E. D. & Plewa, M. J. CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: An updated review. *J. Environ. Sci.* **58**, 64–76 (2017).

13. Wagner, E. D., Rayburn, A. L., Anderson, D. & Plewa, M. J. Analysis of mutagens with single cell gel electrophoresis, flow cytometry, and forward mutation assays in an isolated clone of Chinese hamster ovary cells. *Environ. Mol. Mutagen.* **32**, 360–368 (1998).

14. Wagner, E. D., Rayburn, A. L., Anderson, D. & Plewa, M. J. Calibration of the single cell gel electrophoresis assay, flow cytometry analysis and forward mutation in Chinese hamster ovary cells. *Mutagenesis* **13**, 81–84 (1998).

15. Plewa, M. J., Kargalioglu, Y., Vankerk, D., Minear, R. A. & Wagner, E. D. Development of quantitative comparative cytotoxicity and genotoxicity assays for environmental hazardous chemicals. *Water Sci. Technol.* **42**, 109–116 (2000).

16. Plewa, M. J., Kargalioglu, Y., Vankerk, D., Minear, R. A. & Wagner, E. D. Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products. *Environ. Mol. Mutagen.* **40**, 134–142 (2002).

17. Plewa, M. J. & Wagner, E. D. *Mammalian Cell Cytotoxicity and Genotoxicity of Disinfection By-Products*. (Water Research Foundation, 2009).

18. Plewa, M. J., Kargalioglu, Y., Vankerk, D., Minear, R. A. & Wagner, E. D. Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products. *Environ. Mol. Mutagen.* **40**, 134–142 (2002).

19. Box, G. E. P., Hunter, W. G. & Hunter, J. S. *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building*. (Wiley & Sons Inc., 1978).

20. Canty, A. J., Davison, A. C., Hinkley, D. V. & Ventura, V. Bootstrap diagnostics and remedies. *Can. J. Stat.* **34**, 5–27 (2006).

21. Singh, K. & Xie, M. *Bootstrap: A Statistical Method*. (Rutgers University, 2008).