nature sustainability

Article

https://doi.org/10.1038/s41893-022-00985-7

Toxicological assessment of potable reuse and conventional drinking waters

Received: 3 May 2022

Accepted: 19 September 2022

Published online: 27 October 2022

Check for updates

Stephanie S. Lau[1], Katherine Bokenkamp[2,3], Aleksander Tecza[2,3], Elizabeth D. Wagner[2,3], Michael J. Plewa[2,3] and William A. Mitch[1]

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

[1]Department of Civil and Environmental Engineering, Stanford University, Stanford, CA, USA. [2]Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA. [3]Safe Global Water Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
e-mail: wamitch@stanford.edu

https://doi.org/10.1038/s41893-022-00985-7

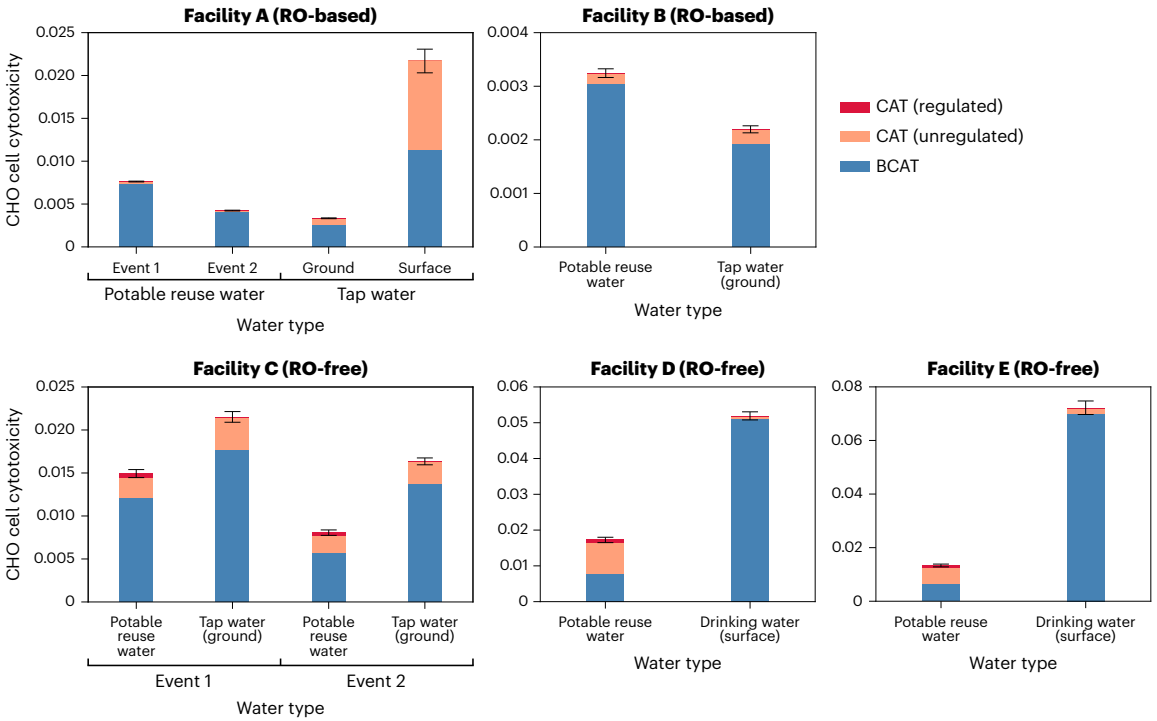

Fig. 1 | Comparison of potable reuse and conventional drinking-water quality. Total CHO cell cytotoxicity, represented by the sum of CAT (contributed by regulated and unregulated DBPs) and BCAT for the final reuse water, for each potable reuse facility and the local conventional drinking water. Error 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.

https://doi.org/10.1038/s41893-022-00985-7

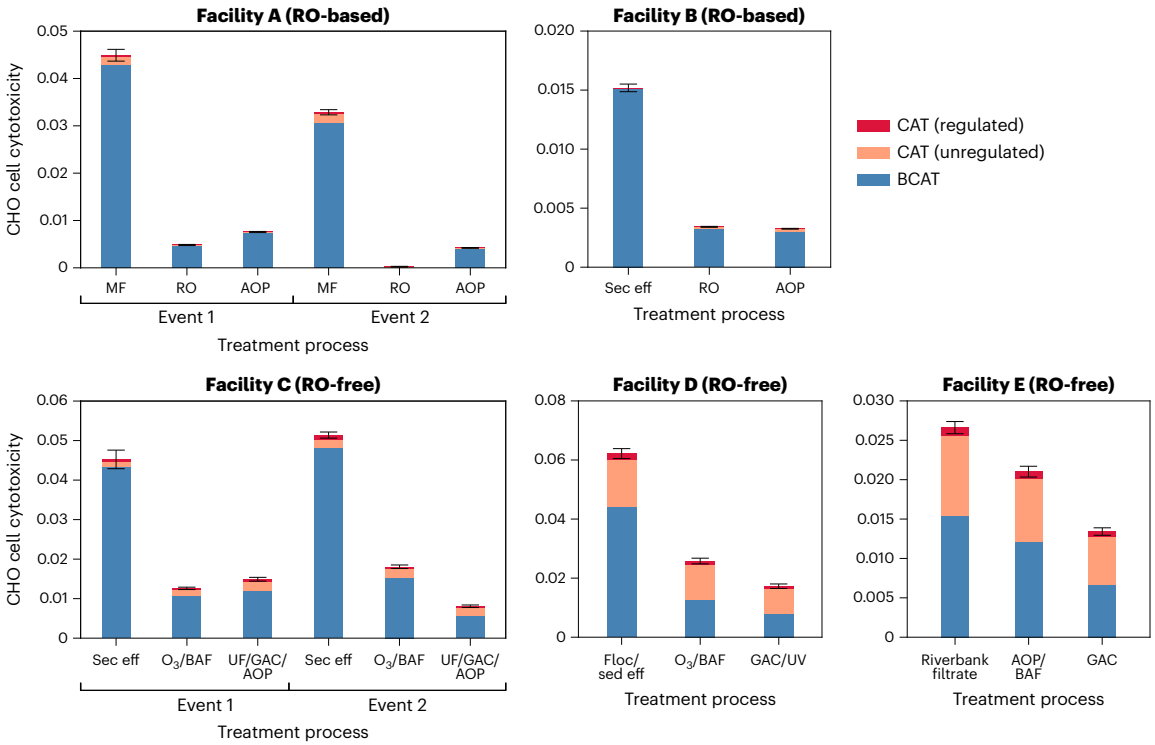

Fig. 2 | Effect of advanced treatment processes on cytotoxicity. Total CHO cell cytotoxicity, represented by the sum of CAT (contributed by regulated and unregulated DBPs) and BCAT for samples collected along RO-based and RO-free potable reuse treatment trains. Error bars represent standard errors of 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

https://doi.org/10.1038/s41893-022-00985-7

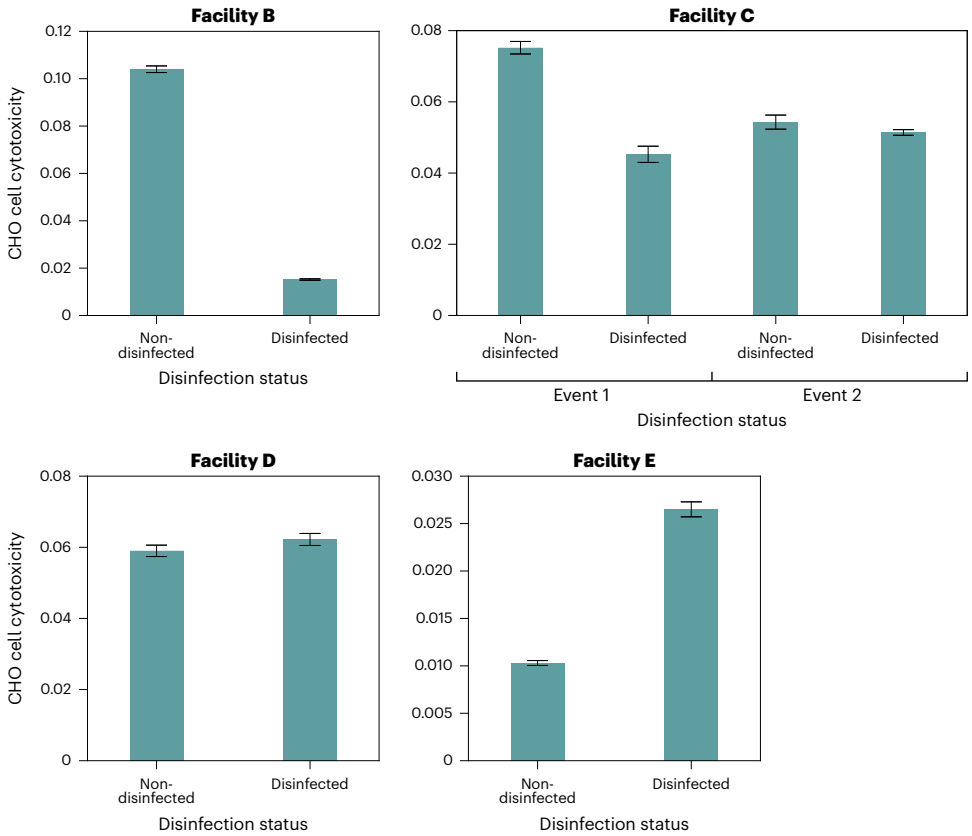

Fig. 3 | Contributions of DBPs and anthropogenic chemicals towards cytotoxicity. Total CHO cell cytotoxicity, represented by the sum of BCAT and CAT, for advanced treatment train influents collected from Facilities B, C, D and E with and without chloramine disinfection. Error bars represent standard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

Acknowledgements

This work was supported by funding from the National Science Foundation (CBET 1706154 to W.A.M. and CBET 1706575 to M.J.P.) and the Water Research Foundation (projects 4737 and 5140) to W.A.M. and M.J.P.

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

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41893-022-00985-7.

Correspondence and requests for materials should be addressed to William A. Mitch.

Peer review information *Nature Sustainability* thanks Martin A. Page and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.