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**Point-of-use water filters can effectively remove disinfection by-products and toxicity from chlorinated and chloraminated tap water**

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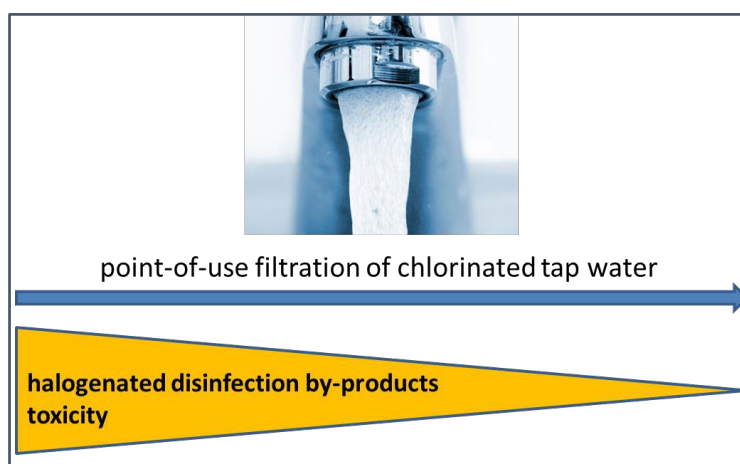
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Tap water filters were evaluated for their efficacy to abate fluoride, bacteria, adsorbable organic halogens (sum parameter of halogenated DBPs), and mixtures of bioactive DBPs quantified by cell-based bioassays.



## Water impact statement

The life-long consumption of chlorinated drinking water may pose a health risk due to the toxic properties of some disinfection by-products. Point-of-use water filters can effectively remove disinfection by-products as evidenced by abatement of organic halogenated compounds as well as reduction of biological activity of the water constituents determined with bioassays for cytotoxicity, oxidative stress response, and genotoxicity.

## Abstract

Epidemiological risk estimates point toward potential health risks posed by disinfection by-products (DBPs) in chlorinated drinking water. Point-of-use filters can effectively remove regulated DBPs from tap water but the removal of unknown DBPs and toxicity has not yet been assessed.

We evaluated 11 tap water filters for their efficacy to abate fluoride, bacteria, and adsorbable organic halogens (AOX) as sum parameter of known and unknown halogenated DBPs. Biological effects were quantified in water samples enriched with solid phase extraction by use of the Microtox assay for bacterial cytotoxicity, the AREc32 assay for oxidative stress response, and the umuC assay for genotoxicity.

Six out of 11 filters effectively removed chlorinated and brominated organic halogens by >60%. Reverse osmosis and one activated carbon based gravity filter were most effective (>94% AOX removal). Four out of five non-membrane pressure filters were less effective for AOX abatement than four out of five activated carbon based gravity filters. Renewal of the filter cartridges significantly improved AOX removal efficacies. Fluoride was removed by >83% only by reverse osmosis and two filters specifically designed for fluoride removal by use of activated alumina. Bacterial counts increased after filtration with most filters, indicating biofilm growth on the filter matrix. Cartridge renewal decreased bacterial counts. Seven out of 11 filters reduced cytotoxicity, oxidative stress response, and genotoxicity by >60%.

Activated carbon based tap water filters could provide an important short-term public health benefit through removal of halogenated DBPs, but regular filter cartridge exchange is critical to maintain a good filter efficacy.

## 1. Introduction

Implementation of drinking water disinfection and filtration effectively reduced pathogen-related waterborne diseases, such as typhoid fever and cholera, and therefore can be regarded as one of the most important public health advances of the last century.<sup>1-3</sup> However, the discovery of disinfection by-products (DBPs)<sup>4</sup>—formed by the reaction between chemical disinfectants and natural organic matter (NOM) as well as inorganic precursors (e.g., bromide)—raised concerns due to their suspected adverse health effects.<sup>2, 5-8</sup> Therefore, application of chlorinous disinfectants is a balancing act between disinfection and the formation of DBPs.<sup>8</sup> Epidemiological studies suggested an increased risk of bladder cancer after life-long ingestion of chlorinated drinking water.<sup>9-12</sup> Findings of other diseases being associated with DBP exposure were less consistent.<sup>11</sup> Based on epidemiological studies, the US-EPA calculated that 2 – 17% of the bladder cancer cases could be avoided if the exposure to DBPs was absent.<sup>13</sup> It has to be emphasized, however, that the causation of urinary bladder cancer by DBP exposure has not been proven but remains a “working hypothesis”.<sup>11</sup> The proof of causality between exposure to complex mixtures and adverse health effects is very challenging.<sup>14</sup>

More than 40 years of DBP research has led to an improved understanding of drinking water treatment processes as well as improved drinking water quality.<sup>9</sup> Nevertheless, compounds that could explain the epidemiological outcomes have not been found.<sup>9</sup> Given that >50% of the total organic halogens are unknown,<sup>15</sup> unknown DBPs could be responsible for increased cancer risks. Unknown DBPs occur—most likely—at low concentrations and thus individual DBPs would need to have an extreme potency if they are to close the gap between toxic effects of known DBPs and potential adverse health outcomes caused by DBPs. Thus, it is

highly likely that effects are triggered by additive mixture effects of unknown and known DBPs with similar mechanisms of action.

Point-of-use (PoU) filtration devices may reduce potential risks posed by DBP exposure. PoU filters should be considered as an end-of-pipe solution for distributions systems with chlorinous disinfectants. Preferably, in optimized drinking water systems, the use of chlorine can be minimized or avoided.<sup>3</sup>

It is estimated that in China (Beijing, Guangzhou, and Shanghai) and in the US around 11 – 30% of the population use household water purifiers<sup>16</sup> and in Korea 90% of the population avoids to drink water directly from the tap.<sup>17</sup> Even simple PoU filters, such as pitcher filters or other low-cost activated carbon filters, effectively reduced concentrations of trihalomethanes (THMs) or haloacetic acids (HAAs)<sup>16, 18, 19</sup> as well as MX (3-chloro-4-(dichloromethyl)-5-hydroxy-5H-furan-2-one).<sup>20</sup>

In this study we assessed the DBP removal efficiency of 11 different PoU tap water filters by analyzing the total organic halogen (AOX) concentration as a sum parameter for halogenated DBPs. We further assessed the toxicity removal by analyzing the cytotoxicity, oxidative stress response induction, and genotoxicity of tap waters before and after filtration. Fluoride was quantified as its removal could be a desired or non-desired side effect of PoU water filtration. Finally, we quantified the bacterial count to test if water filters reduce or increase the number of bacteria in tap water. These results could aid DBP exposure studies and epidemiological studies, in particular when bioanalytical results are included in studies assessing exposure and health consequences of DBPs as recommended by Villanueva et al.<sup>14</sup>

## 2. Material & Methods

### 2.1. Materials

Organic solvents (methanol, MeOH; methyl tert-butyl ether, MTBE) as well as concentrated sulfuric acid were purchased at a purity of  $\geq 99.9\%$  from Sigma Aldrich (Australia). Ultrapure water was prepared with a Milli-Q water purifier (Merck-Millipore, USA) from reverse osmosis treated tap water.

### 2.2. Water filter and sampling

We tested 11 different PoU tap water filters (Table S1) located within the greater Brisbane area as well as Coffs Harbour (Australia) for their capacity to remove AOX and fluoride and ten of them for their performance to reduce biological effects. All drinking water treatment plants in the investigated areas use chlorination or chloramination for disinfection of reservoir water after coagulation and clarification/sand filtration. The distribution systems are connected with all treatment plants in the investigated areas and thus the tap waters could be a mixture originating from different drinking water treatment plants with differing disinfection methods. The AOX composition of the water samples is stated in Table S2. We assessed one reverse osmosis filter, five non-membrane pressure filters, which are directly attached to a tap, and five gravity filters. Most filters tested in this study were designed for removal of chlorine, chloramine, microorganisms, and some for the additional removal of organic contaminants (e.g., reverse osmosis or filter with activated carbon).

To assess the filter efficiency, tap water was sampled before and after filtration headspace-free in amber glass bottles with PTFE-lined screw caps. Prior to sampling we let the tap

water run >3 minutes and sampling was performed causing minimized turbulence (sampling with low water flow and the bottle held at an angle) to reduce the loss of volatile DBPs. The samples were extracted and analyzed on the same day. For each filter, water was sampled two to ten times at different time points. All filters were tested within the recommended service intervals and are described in more detail in Table S1 including the filtered water volume before sampling and the recommended lifetime of the filter cartridge. Filters D, E, G, and H were assessed before (old cartridge) and after exchange of the filter cartridge (new cartridge).

### 2.3. AOX analysis

Analysis of AOX was performed as described previously<sup>21, 22</sup> and quantified as adsorbable organic chlorine (AOCl), bromine (AOBr), and iodine (AOI). Before AOX analysis, samples were acidified to approximately pH 2 using 70% HNO<sub>3</sub> (10 µL per 10 mL sample) and quenched with a sodium sulfite solution (100 mM, 10µL per 10 mL sample). Next, 10 mL was loaded on two consecutive activated carbon cartridges (40 mg activated carbon per glass column with 3 mm inner diameter; CPI International, California, USA). To minimize the loss of volatile analytes during AOX analyses, we used a 10 mL gas-tight glass syringe to sample the water without headspace and to load the sample onto the activated carbon cartridges without contact to ambient air. After enrichment, the cartridges were washed with 10 mL of 5 g/L nitrate as HNO<sub>3</sub> to remove inorganic halides. We used a Mitsubishi AQF-2100 automated furnace unit to incinerate the activated carbon in the presence of oxygen for 260 seconds at 1000°C. The gases from the pyrolysis process, containing the halide ions from the organic contaminants, were collected in a Mitsubishi GA-210 absorption unit containing 10



mL of absorption solution (ultrapure water with 0.003% hydrogen peroxide) resulting in a one-fold relative enrichment. The absorption solution also contained phosphate (1 mg/L) as internal standard to take into account volume variations of absorption solution injected into the IC by the absorption module. Next, 1000 µL of the absorption solution was injected into a Dionex ICS-2100 Ion Chromatograph (Thermo Fisher Scientific, Australia). A Dionex IonPac AS11-HC column (with IonPac AG11-HC guard column) was used with eluent generated using a Dionex Eluent Generator Cartridge III with 30 – 75 mM KOH, a flow rate of 1 mL/min, and conductivity detection. The limits of detection were 1.9 µg/L (Cl<sup>-</sup>), 0.3 µg/L (Br<sup>-</sup>), and 0.7 µg/L (I<sup>-</sup>) and limits of quantification were 19 µg/L (Cl<sup>-</sup>), 3 µg/L (Br<sup>-</sup>) and 7 µg/L (I<sup>-</sup>). The coefficient of variation was below 10% between measurements. Recovery of five haloacetic acids was between 87 and 116%.<sup>23</sup> AOX removal efficiency by the tested filters were calculated according to equation 1.

$$\Delta\text{tap}_{\text{AOX}} (\%) = \left( 1 - \frac{\text{AOX}_{\text{filtered}}}{\text{AOX}_{\text{tap}}} \right) \times 100 \quad \text{equation 1}$$

## 2.4. Fluoride analysis

We quantified fluoride after direct injection of water samples (1000 µL) into the Dionex ICS-2100 ion chromatograph with the same columns and sequence as mentioned in the previous section. The limit of quantification for fluoride analysis was 100 µg/L and thus well below the measured concentrations. The coefficient of variation between measurements was below 20%. Fluoride removal was calculated according to equation 2.

$$\Delta\text{tap}_{\text{fluoride}} (\%) = \left( 1 - \frac{\text{fluoride}_{\text{filtered}}}{\text{fluoride}_{\text{tap}}} \right) \times 100 \quad \text{equation 2}$$

169

170 **2.5. Bacteria count**

171 We used the flow cytometer Accuri C6 (BD Biosciences) to count bacteria in the water  
 172 samples.<sup>24</sup> For the bacteria counts we sampled water in sterilized glass vials with PTFE-lined  
 173 screw caps. 500 µL from each sample was added to a 2 mL microtube (three replicates per  
 174 sample). 5 µL of SYBR Green I nucleic acid stain (100x diluted from stock) was added,  
 175 vortexed, and incubated in the dark for 10 minutes at approximately 40°C. 50 µL of sample  
 176 was measured at a flow rate of 30 µL/min using fluorescent detectors 1 (FL1: 530 ± 15 nm)  
 177 and 3 (FL3: > 670 nm). Data were collected as events per µL and gating was used to isolate  
 178 the bacteria cell counts from the sample matrix. The system was back-flushed between  
 179 samples to reduce contamination. Stained Milli-Q water was used with each sample run as a  
 180 negative control. We calculated the bacteria removal by the tested filters as absolute values  
 181 (counts per µL) according to equation 3. Positive values indicate an increase in bacteria  
 182 counts per µL.

$$183 \quad \Delta_{\text{tap}_{\text{bacteria}}} (\text{count}/\mu\text{L}) = \text{bacteria count}_{\text{filtered}} - \text{bacteria count}_{\text{tap}} \quad \text{equation 3}$$

184

185 **2.6. Bioassays**

186 For the bioanalytical assessment, water samples were acidified to pH 1.5 using sulfuric acid  
 187 and solid phase extracted (SPE) with TELOS ENV cartridges (Kinesis, Australia; 1 g sorbent  
 188 per 2 L of sample) as described previously.<sup>22</sup> The 10,000-fold enriched SPE extracts were  
 189 spiked to the bioassay medium at a maximum relative enrichment factor (REF) of 100  
 190 corresponding to a 1% solvent concentration (methanol) in the bioassays.

The bacterial cytotoxicity assay using *Aliivibrio fischeri* (formerly termed *Vibrio fischeri*) bioluminescence inhibition (Microtox) was selected because of its high responsiveness to DBPs.<sup>7</sup> This Microtox assay was performed as described previously.<sup>25, 26</sup> The luminescence output of the bacteria was measured prior to addition of sample and after 30 min incubation. The luminescence inhibition was normalized to the solvent control and phenol served as positive control.

The Nrf2-ARE oxidative stress response pathway has been demonstrated in previous studies to be an important step in the toxicity pathway of mono-HAAs<sup>27</sup> and appears to play a central role for the toxicity of many more DBPs.<sup>7</sup> The AREc32 assay was performed as described previously.<sup>28</sup> The used cell line is the human breast cancer cell line MCF7 stably transfected with an ARE reporter plasmid coupled to a luciferase reporter gene.<sup>29</sup> The amount of produced luciferase is directly proportional to the ARE activated and thus also to the causative chemical stressor. We used t-butylhydroquinone (tBHQ) as positive control.<sup>30</sup>

The umuC assay with *Salmonella typhimurium* (TA1535/pSK1002) was selected as a bacterial screening tool for genotoxicity (Reifferscheid *et al.* 1991). This assay detects the activation of the cellular SOS-response, which is a global response to DNA damage to induce DNA repair mechanisms, and hence detects a response to genotoxic effects. The assay was performed without metabolic activation with S9 because in the umuC assay S9 addition leads to reduced genotoxicity for most DBPs<sup>7</sup> and reduced effects in whole drinking water samples.<sup>31, 32</sup> 4-Nitroquinoline-1-oxide (4NQO) served as positive control. For all assays we ran a negative control and a positive control on each multi-well plate for every experiment.

The assessment endpoint for the Microtox was the 50% effect concentration (EC<sub>50</sub>) derived from a log-logistic concentration-effect curve.<sup>33</sup> For oxidative stress response (AREc32) and

214 genotoxicity (umuC) we used the induction ratio (IR). IR is defined as the ratio of effect of  
 215 the sample divided by the average effect observed in the controls. The effect concentration  
 216 that elicits an IR of 1.5 ( $EC_{IR1.5}$ ; i.e., 1.5-fold or 50% effect increase compared to the negative  
 217 control), is the assessment endpoint for these assays.<sup>33</sup> We selected this benchmark value  
 218 because for reporter gene assays often no maximum response can be obtained due to  
 219 cytotoxic interferences.<sup>33, 34</sup> Therefore, we used the linear part of the concentration-effect  
 220 curves up to an induction ratio (IR) of 5. IR is the ratio of measured signal to the signal of the  
 221 negative control. We derived the  $EC_{IR1.5}$  by use of linear regression and selected the  
 222 threshold of 1.5 because (i) it is employed in several guideline documents, e.g., OECD  
 223 guideline for the umuC genotoxicity assay, (ii) it differs significantly from the negative  
 224 control, and (iii) it can be derived when the maximum of the dose-response curve is not  
 225 known.<sup>33</sup> It has been demonstrated before that the  $EC_{IR1.5}$  is a statistically sound and robust  
 226 benchmark value for the umuC,<sup>35</sup> for the AREc32,<sup>30</sup> and for the Geneblazer reporter gene  
 227 assays.<sup>33</sup> Removal of effect ( $\Delta tap_{bioassay}$ ) was calculated according to equation 4.

$$\Delta tap_{bioassay} (\%) = \left( 1 - \frac{EC_{tap}}{EC_{filtered}} \right) \times 100 \quad \text{equation 4}$$

### 3. Results & Discussion

#### 3.1. Removal of adsorbable organic halogens (AOX)

We assessed the removal of AOX as sum parameter of halogenated organic compounds by the selected filtration devices (Figure 1). At the time of sampling the filters were in use for differing durations (Table S1). AOX was chosen because its concentration is often positively correlated with biological effects<sup>36</sup> indicating that it is a useful parameter to assess the removal of toxicologically relevant contaminants.

As expected, reverse osmosis (Filter A) was most effective for AOX removal together with the gravity filter B (contains activated carbon). Both filters removed >94% of AOCl, AOBr, and AOI (Table S2). The portable filter C was slightly less effective with removal rates >74%. Filter D was the most effective pressure filter. Its removal efficiency increased significantly after cartridge exchange ( $p < 0.05$ , one-way ANOVA with Tuckey's post-test), in particular for the more polar chlorinated organic compounds (AOCl, from 40 to 70%, filter D). The same is true for filter E where AOCl removal increased from 50 to 80% after cartridge exchange. Filter G was the least effective gravity filter in our study for the removal of AOX but again removal efficiency increased after cartridge exchange (for AOCl from 20 to 50%). AOX removal was least effective with the pressure filters H, I, J, and K. However, for filter H AOX removal increased after cartridge exchange (e.g., from 26 to 43% for AOCl).

For most filters, removal efficacy of AOBr was generally higher than for AOCl, which indicates that the sum of brominated organic compounds are likely less polar than chlorinated organic compounds, which facilitates sorption of AOBr to the carbon filter.<sup>37</sup>

This is an important aspect because brominated organic compounds are usually more

potent in bioassays<sup>2, 6</sup> and hence their removal can be considered as more important. For AOI, the removal pattern was less consistent and the removal efficiencies were often lower than for AOB<sub>r</sub>. Iodinated organic compounds are commonly more toxic than their brominated analogues but the AOI concentration was approximately 10-times lower than AOB<sub>r</sub> in most cases (Table S2).

<<Figure 1>>

**3.2. Removal of fluoride**

Fluoridation of drinking water is mandatory in our sampling areas and the detected fluoride concentrations matched the target concentration of the water providers (0.7 – 1 mg/L).<sup>38, 39</sup> Only filters specifically designed for fluoride removal through addition of activated alumina to the filter matrix (filters B and I) as well as reverse osmosis (filter A) removed fluoride effectively by >83% (Figure 2).

<<Figure 2>>

In some countries, fluoride is added to the drinking water supply to improve dental health and in some regions, fluoride occurs naturally in water sources.<sup>40, 41</sup> Fluoride has been demonstrated to effectively reduce dental decay while it is often emphasized that fluoride acts topically (at the surface of the teeth) without a clear benefit in actually ingesting it.<sup>41</sup>

Also detrimental effects of high fluoride concentrations in drinking water (e.g., fluorosis) are documented and the range between beneficial and detrimental concentrations is very narrow.<sup>41-43</sup> In geographical areas with high fluoride concentrations in drinking water, fluoride removal with PoU filters can reduce the prevalence of fluorosis syndrome<sup>43</sup> but fluoride filters might also counteract tooth decay prevention measures in other regions.

### 3.3. Effect on bacteria count

Bacterial counts in most tap water samples were within the range observed in previous studies (<200/ $\mu$ L).<sup>44-46</sup> Bacteria counts were only effectively reduced with reverse osmosis (filter A) and filter D after cartridge exchange (>89% removal compared to tap water, Figure S1). For most filters, bacteria counts increased considerably after filtration, indicating biofilm growth in the filter medium. This is supported by the decreased bacteria counts after filter exchange (filter D, E, G, H). In our study we did not discriminate between pathogenic and non-pathogenic bacteria and hence increased bacteria counts are not necessarily of concern provided that no pathogens enter the filter medium. Biofilm on the sorbent material might even support removal of toxic organic contaminants from drinking water.<sup>47</sup>

### 3.4. Effect on cytotoxicity, oxidative stress, and genotoxicity

All tap water samples had to be enriched to exert biological effects in the bioassays. Relative enrichment factors of >3 were necessary to exceed the effect threshold for the most sensitive bioassay (AREc32, Table S4). Most filters removed cytotoxic effects effectively by >60% compared to tap water except for filters G (pitcher filter) and I (pressure filter; Figure 3). Reverse osmosis was most effective, followed by filter D (after cartridge exchange;

pressure filter) and filter B (gravity filter). Filter cartridge exchange after the recommended exchange interval increased removal of cytotoxicity significantly for filters D, G, and H ( $p < 0.05$ , one-way ANOVA with Tukey's post-test).

<<Figure 3>>

The oxidative stress response activation was reduced by >70% after filtration except for filters F, G, and I (Figure 4). Again, reverse osmosis (A), filter B, and D (after cartridge exchange) were most effective at reducing biological effects by >97%. The pitcher filter G was least effective with 37% effect removal after cartridge exchange. Cartridge exchange increased the oxidative stress removal significantly for filters D, E, and H ( $p > 0.01$ , one-way ANOVA with Tukey's post-test).

<<Figure 4>>

All filters, except filter I, removed genotoxic effects by >60% (for filter D, G, and H only after cartridge exchange; Figure S2). Filters A, B, and E were most effective with removal rates >85%. Cartridge exchange increased the genotoxicity removal significantly for filters E, G, and H ( $p > 0.01$ , one-way ANOVA with Tukey's post-test).

All of these assays detect toxicity caused by reactive compounds.<sup>7</sup> The observed effect decrease after filtration demonstrates that water filters can effectively remove such



toxicologically relevant compounds from tap water. However, the removal efficacy can vary considerably between 25 and 100%. Reverse osmosis (filter A) was most effective in most cases but also low cost activated carbon based gravity filters can effectively remove toxicologically relevant compounds. However, it is important to exchange the filter cartridge regularly according to recommendations of the manufacturer to maintain a good filter capacity. The only filter without a carbon-based sorbent was least effective for toxicity removal (filter I).

### 3.5. Reverse osmosis as best performing filter?

Reverse osmosis (RO) was one of the most effective systems to remove contaminants from tap water. This can be expected because RO is known to be one of the most efficient methods for trace organic contaminant removal from water (e.g. wastewater).<sup>33</sup> However, RO also removes minerals and according to the World Health Organization, very large numbers of people consume levels of calcium and magnesium that are insufficient to support their physiological needs.<sup>48</sup> Depending on the mineral content of drinking water, which varies greatly based on the geographical area, drinking water can contribute considerably to the calcium and magnesium intake and hence to overall nutrition.<sup>49</sup> In case of mineral-rich drinking water, mineral removal by RO filtration could counteract potential health benefits by DBP removal.<sup>50</sup> Simple carbon-based gravity filters or pressure filters do not remove geogenic minerals because those are in ionic form<sup>49</sup> and hence too polar to sorb to the filter material.

### 3.6. Alternative DBP mitigation strategies

Centralized DBP mitigation strategies would be most desirable to provide safe drinking water to the whole population and also to minimize dermal and inhalation exposure to DBPs during showering and bathing.<sup>2</sup> The reduction of the DBP formation potential at drinking water treatment plants, *e.g.*, via more effective removal of precursor compounds through physical-chemical treatment processes such as reverse osmosis or activated carbon filtration,<sup>51, 52</sup> would be very costly<sup>8</sup> and hence is unrealistic for most countries. In countries with newer and well-maintained drinking-water distribution infrastructure, such as Switzerland, the Netherlands, Austria, and Germany, drinking water distribution is possible without residual disinfectants<sup>3, 8, 44</sup> with similar or lower waterborne disease outbreaks compared to systems with residual disinfectant.<sup>3</sup> This can be achieved if three factors are considered: (1) Effective measures for water resources protection and water pollution control, (2) adapted multibarrier treatment including biological filtration to abate natural organic matter<sup>44, 45</sup> and (3) maintenance of the distribution system to avoid leakage.<sup>3</sup> However, the required infrastructure investments are in the range of tens of millions USD for a 100,000 inhabitant city<sup>3</sup> and hence, such a strategy is often out of reach for lower income countries. In contrast, it can be expected that the use of PoU filters is restricted to the more health-conscious and more prosperous part of the population. Considering approximate annual costs in the range of 50 USD for a well-working activated carbon-based PoU filter, centralized solutions might be more cost-efficient in the long run to provide safe drinking water to the whole population.

#### 4. Conclusions

The water filters evaluated in our study effectively removed adsorbable organic halogenated compounds, a sum parameter for DBPs, by 28 – 94%. Bioassays indicative of cytotoxicity and reactive toxicity endpoints, such as oxidative stress and genotoxicity, revealed an effective removal of toxicologically relevant contaminants from tap water (i.e., reduction of effect concentration by 25 – 100%). Taking into account the potential increase in bladder cancer incidents through DBP exposure, the removal of DBPs from chlorinated tap water using point-of-use filters could be an important public health benefit.<sup>13</sup>

In our study, besides reverse osmosis, only filters with activated carbon removed organic contaminants and toxicity effectively, in particular low-cost gravity filters with a cartridge consisting of an outer ceramic shell with a granular activated carbon core. Filter cartridges need to be replaced on a regular basis to maintain good filter efficacy and to minimize bacterial growth in the sorbent material.

Nevertheless, centralized solutions would be most desirable for providing safe drinking water free of DBPs to the whole population without residual disinfectants in the distribution system. Investments in water resource protection, infrastructure renewal and maintenance, as well as well-controlled water treatment including disinfection and removal of natural organic matter, might be more cost-effective in the long run than point-of-use filtration to protect the whole population from detrimental health effects.

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386

387 **Supplementary Information**

388 Additional figures and tables are available in the supplemental information file.

## References

1. Akin, E. W.; Hoff, J. C.; Lippy, E. C., Waterborne outbreak control - which disinfectant. *Environ. Health Perspect.* **1982**, *46*, (DEC), 7-12.
2. Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; DeMarini, D. M., Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat. Res.-Rev. Mutat.* **2007**, *636*, (1-3), 178-242.
3. Rosario-Ortiz, F. L.; Rose, J.; Speight, V.; Von Gunten, U.; Schnoor, J., How do you like your tap water? Safe drinking water may not need to contain a residual disinfectant. *Science* **2016**, *351*, (6276).
4. Rook, J. J., Formation of haloforms during chlorination of natural waters. *Water Treatment Examination* **1974**, *23*, (2), 234-243.
5. Plewa, M. J.; Wagner, E. D.; Muellner, M. G.; Hsu, K. M.; Richardson, S. D., Comparative mammalian cell toxicity of N-DBPs and C-DBPs. In *Disinfection by-products in drinking water: occurrence, formation, health effects, and control*, Karanfil, T.; Krasner, S. W.; Xie, Y., Eds. American Chemical Society: Washington, 2008; Vol. 995, pp 36-50.
6. Plewa, M. J.; Simmons, J. E.; Richardson, S. D.; Wagner, E. D., Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products. *Environ. Mol. Mutagen.* **2010**, *51*, (8-9), 871-878.
7. Stalter, D.; O'Malley, E.; Von Gunten, U.; Escher, B. I., Fingerprinting the reactive toxicity pathways of 50 drinking water disinfection by-products. *Water Res.* **2016**, *91*, 19-30.
8. Sedlak, D. L.; von Gunten, U., The Chlorine Dilemma. *Science* **2011**, *331*, (6013), 42-43.
9. Hrudey, S. E.; Fawell, J., 40 years on: what do we know about drinking water disinfection by-products (DBPs) and human health? *Water Science & Technology: Water Supply.* **2015**, *15*, (4), 667-674.
10. Cantor, K. P.; Villanueva, C. M.; Silverman, D. T.; Figueroa, J. D.; Real, F. X.; Garcia-Closas, M.; Malats, N.; Chanock, S.; Yeager, M.; Tardon, A.; Garcia-Closas, R.; Serra, C.; Carrato, A.; Castano-Vinyals, G.; Samanic, C.; Rothman, N.; Kogevinas, M., Polymorphisms in GSTT1, GSTZ1, and CYP2E1, disinfection by-products, and risk of bladder cancer in Spain. *Environ. Health Perspect.* **2010**, *118*, (11), 1545-1550.
11. Hrudey, S. E., Chlorination disinfection by-products, public health risk tradeoffs and me. *Water Res.* **2009**, *43*, (8), 2057-2092.
12. Villanueva, C. M.; Cantor, K. P.; Cordier, S.; Jaakkola, J. J. K.; King, W. D.; Lynch, C. F.; Porru, S.; Kogevinas, M., Disinfection byproducts and bladder cancer - A pooled analysis. *Epidemiology* **2004**, *15*, (3), 357-367.
13. Odom, R.; Regli, S.; Messner, M.; Cromwell, J.; Javdan, M., Benefit-cost analysis of the stage 1D/DBP rule. *Journal American Water Works Association* **1999**, *91*, (4), 137-147.
14. Villanueva, C. M.; Kogevinas, M.; Cordier, S.; Templeton, M. R.; Vermeulen, R.; Nuckols, J. R.; Nieuwenhuijsen, M. J.; Levallois, P., Assessing Exposure and Health Consequences of Chemicals in Drinking Water: Current State of Knowledge and Research Needs. *Environ. Health Perspect.* **2014**, *122*, (3), 213-221.
15. Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.; Scilimenti, M. J.; Onstad, G. D.; Thruston, A. D., Occurrence of a new generation of disinfection byproducts. *Environ. Sci. Technol.* **2006**, *40*, (23), 7175-7185.
16. Wright, J. M.; Murphy, P. A.; Nieuwenhuijsen, M. J.; Savitz, D. A., The impact of water consumption, point-of-use filtration and exposure categorization on exposure

- misclassification of ingested drinking water contaminants. *Sci. Total Environ.* **2006**, *366*, (1), 65-73.
17. Korea-Herald, Drink tap water? 'No way,' say Koreans - Health concerns linger despite state assurance. *The Korea Herald* **2015**, 26.05.2015 (<http://www.koreaherald.com/view.php?ud=20150526000906>).
18. Rahman, M. B.; Driscoll, T.; Clements, M.; Armstrong, B. K.; Cowie, C. T., Effects of tap water processing on the concentration of disinfection by-products. *J. Water Health* **2011**, *9*, (3), 507-514.
19. Leuesque, S.; Rodriguez, M. J.; Serodes, J.; Beaulieu, C.; Proulx, F., Effects of indoor drinking water handling on trihalomethanes and haloacetic acids. *Water Res.* **2006**, *40*, (15), 2921-2930.
20. Egorov, A. I.; Tereschenko, A. A.; Altshul, L. M.; Vurtiainen, T.; Samsonov, D.; LaBrecque, B.; Maki-Paakkanen, J.; Drizhd, N. L.; Fordb, T. E., Exposures to drinking water chlorination by-products in a Russian city. *Int. J. Hyg. Environ. Health* **2003**, *206*, (6), 539-551.
21. Farre, M. J.; Lyon, B. A.; de Vera, G. A.; Stalter, D.; Gernjak, W., Assessing adsorbable organic halogen formation and precursor removal during drinking water production. *Journal of Environmental Engineering* **2016**, *142*, (3), 04015087.
22. Stalter, D.; Peters, L. D.; O'Malley, E.; Tang, J. Y. M.; Revalor, M.; Farre, M. J.; Watson, K.; Von Gunten, U.; Escher, B. I., Sample enrichment for bioanalytical quality assessment of disinfected drinking water: concentrating the polar, the volatiles, the unknowns. *Environ. Sci. Technol.* **2016**, DOI: 10.1021/acs.est.6b00712.
23. Yeh, R. Y. L.; Farré, M. J.; Stalter, D.; Tang, J. Y. M.; Molendijk, J.; Escher, B. I., Bioanalytical and chemical evaluation of disinfection by-products in swimming pool water. *Water Res.* **2014**, *59*, (0), 172-184.
24. Neale, P. A.; Escher, B. I., Does co-extracted dissolved organic carbon cause artefacts in cell-based bioassays? *Chemosphere* **2014**, *108*, 281-288.
25. Stalter, D.; Dutt, M.; Escher, B., Headspace-free setup of in vitro bioassays for the evaluation of volatile disinfection by-products. *Chem. Res. Toxicol.* **2013**, *26*, 1605-1614.
26. Tang, J. Y. M.; McCarty, S.; Glenn, E.; Neale, P. A.; Warne, M. S. J.; Escher, B. I., Mixture effects of organic micropollutants present in water: Towards the development of effect-based water quality trigger values for baseline toxicity. *Water Res.* **2013**, *47*, (10), 3300-3314.
27. Pals, J.; Attene-Ramos, M. S.; Xia, M. H.; Wagner, E. D.; Plewa, M. J., Human cell toxicogenomic analysis linking reactive oxygen species to the toxicity of monohaloacetic acid drinking water disinfection byproducts. *Environ. Sci. Technol.* **2013**, *47*, (21), 12514-12523.
28. Escher, B. I.; van Daele, C.; Dutt, M.; Tang, J. Y. M.; Altenburger, R., Most oxidative stress response in water samples comes from unknown chemicals: the need for effect-based water quality trigger values. *Environ. Sci. Technol.* **2013**, *47*, (13), 7002-7011.
29. Wang, X. J.; Hayes, J. D.; Wolf, C. R., Generation of a stable antioxidant response element-driven reporter gene cell line and its use to show redox-dependent activation of Nrf2 by cancer chemotherapeutic agents. *Cancer Res.* **2006**, *66*, (22), 10983-10994.
30. Escher, B. I.; Dutt, M.; Maylin, E.; Tang, J. Y. M.; Toze, S.; Wolf, C. R.; Lang, M., Water quality assessment using the AREc32 reporter gene assay indicative of the oxidative stress response pathway. *J. Environ. Monit.* **2012**, *14*, (11), 2877-85.

31. Farré, M. J.; Day, S.; Neale, P. A.; Stalter, D.; Tang, J. Y. M.; Escher, B. I., Bioanalytical and chemical assessment of the disinfection by-product formation potential: Role of organic matter. *Water Res.* **2013**, *47*, (14), 5409-5421.
32. Neale, P. A.; Antony, A.; Bartkow, M. E.; Farre, M. J.; Heitz, A.; Kristiana, I.; Tang, J. Y. M.; Escher, B. I., Bioanalytical assessment of the formation of disinfection byproducts in a drinking water treatment plant. *Environ. Sci. Technol.* **2012**, *46*, (18), 10317-10325.
33. Escher, B. I.; Allinson, M.; Altenburger, R.; Bain, P. A.; Balaguer, P.; Busch, W.; Crago, J.; Denslow, N. D.; Dopp, E.; Hilscherova, K.; Humpage, A. R.; Kumar, A.; Grimaldi, M.; Jayasinghe, B. S.; Jarosova, B.; Jia, A.; Makarov, S.; Maruya, K. A.; Medvedev, A.; Mehinto, A. C.; Mendez, J. E.; Poulsen, A.; Prochazka, E.; Richard, J.; Schifferli, A.; Schlenk, D.; Scholz, S.; Shiraish, F.; Snyder, S.; Su, G. Y.; Tang, J. Y. M.; van der Burg, B.; van der Linden, S. C.; Werner, I.; Westerheide, S. D.; Wong, C. K. C.; Yang, M.; Yeung, B. H. Y.; Zhang, X. W.; Leusch, F. D. L., Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environ. Sci. Technol.* **2014**, *48*, (3), 1940-1956.
34. Escher, B. I.; Leusch, F. D. L., Bioanalytical tools in water quality assessment. In IWA Publishing: London, 2012.
35. Macova, M.; Toze, S.; Hodggers, L.; Mueller, J. F.; Bartkow, M.; Escher, B. I., Bioanalytical tools for the evaluation of organic micropollutants during sewage treatment, water recycling and drinking water generation. *Water Res.* **2011**, *45*, (14), 4238-4247.
36. Lyon, B. A.; Milsik, R. Y.; DeAngelo, A. B.; Simmons, J. E.; Moyer, M. P.; Weinberg, H. S., Integrated Chemical and Toxicological Investigation of UV-Chlorine/Chloramine Drinking Water Treatment. *Environ. Sci. Technol.* **2014**, *48*, (12), 6743-6753.
37. Kennedy, A. M.; Reinert, A. M.; Knappe, D. R. U.; Ferrer, I.; Summers, R. S., Full- and pilot-scale GAC adsorption of organic micropollutants. *Water Res.* **2015**, *68*, 238-248.
38. Seqwater, Fluoridation of the water supply. Fluoride Information Sheet Seqwater. <http://www.seqwater.com.au/sites/default/files/PDF%20Documents/Publications/20130107-Fluoride-information-sheet.pdf> **2011**.
39. MNCAHS, Mid North Coast Area Health Service Fluoride brochure. <http://www.coffsharbour.nsw.gov.au/places-for-living/drinking-water-supply/water-treatment/Pages/fluoridation.aspx> **2004**.
40. Johnston, R. B.; Berg, M.; Johnson, C. A.; Tilley, E.; Hering, J. G., Water and Sanitation in Developing Countries: Geochemical Aspects of Quality and Treatment. *Elements* **2011**, *7*, (3), 163-168.
41. Ayoob, S.; Gupta, A. K., Fluoride in drinking water: A review on the status and stress effects. *Crit. Rev. Environ. Sci. Technol.* **2006**, *36*, (6), 433-487.
42. Bretzler, A.; Johnson, C. A., The Geogenic Contamination Handbook: Addressing arsenic and fluoride in drinking water. *Applied Geochemistry* **2015**, *63*, 642-646.
43. Johnson, C. A.; Berg, M.; Sabatini, D., Towards sustainable safe drinking water supply in low-and middle-income countries: The challenges of geogenic contaminants and mitigation measures Foreword. *Sci. Total Environ.* **2014**, *488*, 479-480.
44. Hammes, F.; Berger, C.; Koster, O.; Egli, T., Assessing biological stability of drinking water without disinfectant residuals in a full-scale water supply system. *Journal of Water Supply Research and Technology-Aqua* **2010**, *59*, (1), 31-40.
45. El-Chakhtoura, J.; Prest, E.; Saikaly, P.; van Loosdrecht, M.; Hammes, F.; Vrouwenvelder, H., Dynamics of bacterial communities before and after distribution in a full-scale drinking water network. *Water Res.* **2015**, *74*, 180-190.



46. Lipphaus, P.; Hammes, F.; Kotzsch, S.; Green, J.; Gillespie, S.; Nocker, A., Microbiological tap water profile of a medium-sized building and effect of water stagnation. *Environ. Technol.* **2014**, *35*, (5), 620-628.
47. Reungoat, J.; Escher, B. I.; Macova, M.; Keller, J., Biofiltration of wastewater treatment plant effluent: Effective removal of pharmaceuticals and personal care products and reduction of toxicity. *Water Res.* **2011**, *45*, (9), 2751-2762.
48. Atkinson, S. A.; Costello, R.; Donohue, J. M., Overview of global dietary calcium and magnesium intakes and allowances. *Calcium and Magnesium in drinking-water: public health significance* **2009**, WHO Library Cataloguing-in-Publication Data, ISBN 978 92 4 156355 0 ([http://whqlibdoc.who.int/publications/2009/9789241563550\\_eng.pdf](http://whqlibdoc.who.int/publications/2009/9789241563550_eng.pdf)), 17-36.
49. Ong, C. N.; Grandjean, A. C.; Heaney, R. P., The mineral composition of water and its contribution to calcium and magnesium intake. *Calcium and Magnesium in drinking-water: public health significance* **2009**, WHO Library Cataloguing-in-Publication Data, ISBN 978 92 4 156355 0 ([http://whqlibdoc.who.int/publications/2009/9789241563550\\_eng.pdf](http://whqlibdoc.who.int/publications/2009/9789241563550_eng.pdf)), 37-58.
50. Zeng, H.; Shu, W. Q.; Chen, J. A.; Liu, L.; Wang, D. H.; Fu, W. J.; Wang, L. Q.; Luo, J. H.; Zhang, L.; Tan, Y.; Qiu, Z. Q.; Huang, Y. J., Experimental comparison of the reproductive outcomes and early development of the offspring of rats given five common types of drinking water. *Plos One* **2014**, *9*, (10), doi: 10.1371/journal.pone.0108955.
51. Watson, K.; Farre, M. J.; Knight, N., Comparing a silver-impregnated activated carbon with an unmodified activated carbon for disinfection by-product minimisation and precursor removal. *Sci. Total Environ.* **2016**, *542*, 672-684.
52. Watson, K.; Farré, M. J.; Knight, N., Enhanced coagulation with powdered activated carbon or MIEX® secondary treatment: A comparison of disinfection by-product formation and precursor removal. *Water Res.* **2015**, *68*, (0), 454-466.



## Figure legends

**Figure 1.** AOX removal by various water filters (Table S1; A: reverse osmosis; B: ceramic outer shell with granular activated carbon and activated alumina core; C, E, F: ceramic outer shell with granular activated carbon core; G: activated carbon and ion-exchange resin; D, H, J, K: carbon block; I: activated alumina without carbon); for filters D, E, G, H results are given before (old cart.) and after replacement of the filter cartridge (new cart.). Displayed is the arithmetic mean  $\pm$  standard deviation ( $n = 2 - 6$ ). Detailed results are given in Table S2.

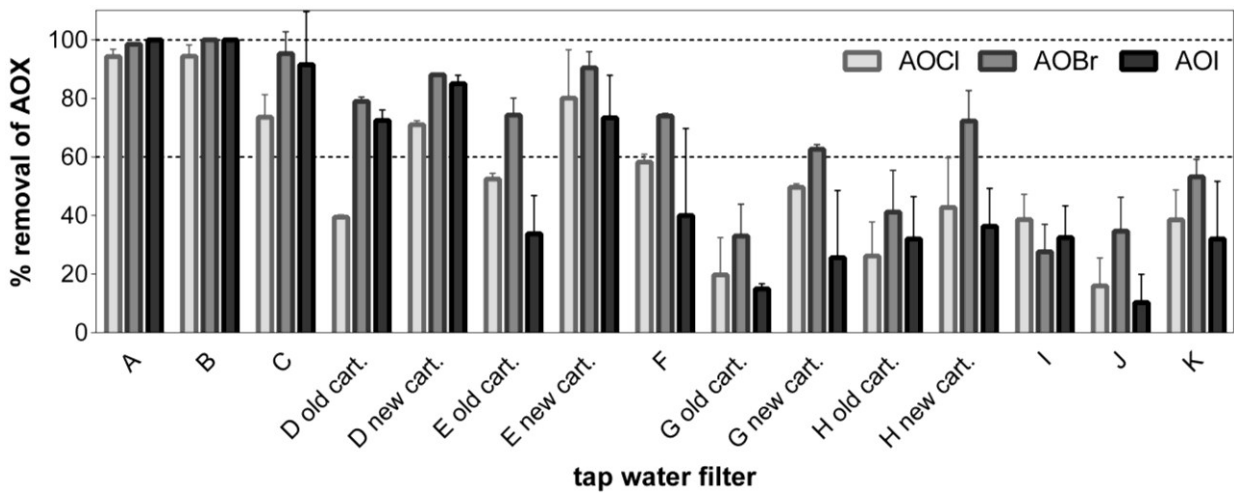
**Figure 2.** Fluoride concentrations before (tap) and after different water filters (Table S1; A: reverse osmosis; B: ceramic outer shell with granular activated carbon and activated alumina core; C, E, F: ceramic outer shell with granular activated carbon core; G: activated carbon and ion-exchange resin; D, H, J, K: carbon block; I: activated alumina without carbon); for filters D, E, G, H results are given before (old c.) and after replacement of the filter cartridge (new c.). Displayed is the median, box extends from the 25th to the 75th percentiles, and the whiskers from minimum to maximum ( $n = 1 - 6$ ). Detailed results are given in Table S2.

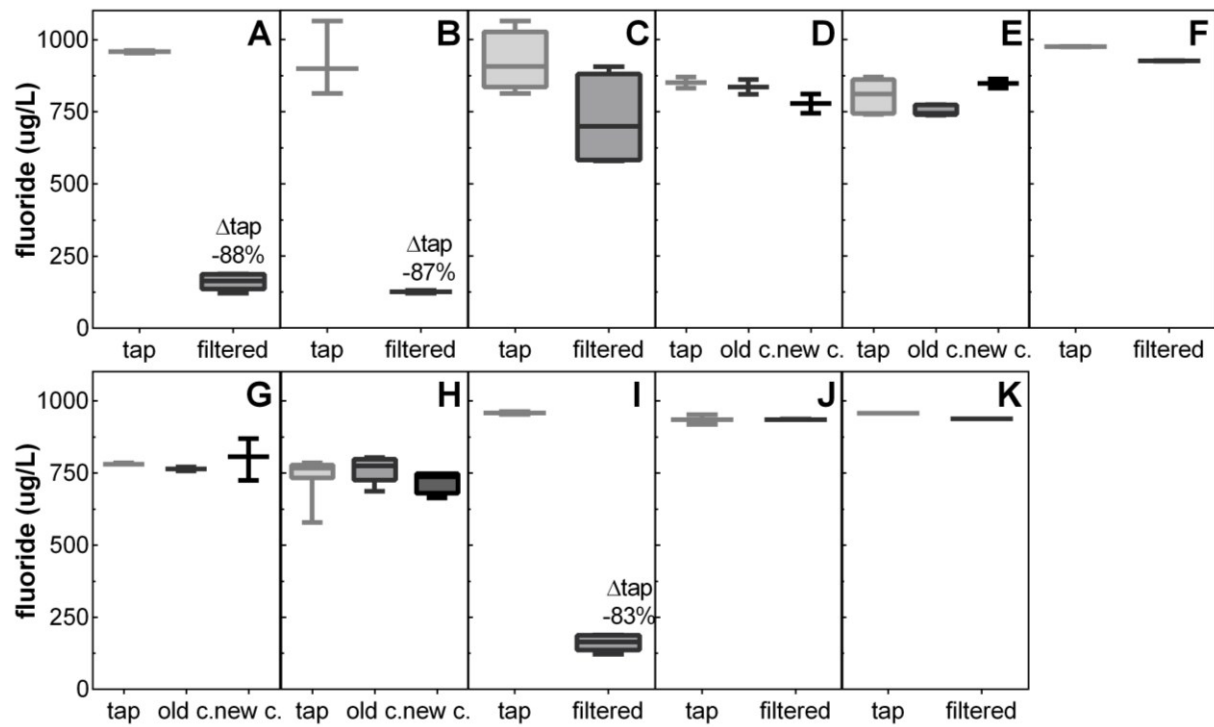
**Figure 3.** Cytotoxicity: 50% effect concentration ( $EC_{50}$ ) as relative enrichment factor (REF) before (tap) and after different water filters (Table S1; A: reverse osmosis; B: ceramic outer shell with granular activated carbon and activated alumina core; C, E, F: ceramic outer shell with granular activated carbon core; G: activated carbon and ion-exchange resin; D, H, J: carbon block; I: activated alumina without carbon) assessed with the *Aliivibrio fischeri* bioluminescence inhibition assay; for filters D, E, G, H results are given before (old c.) and after replacement of the filter cartridge (new c.). Displayed is the median, box extends from the 25<sup>th</sup> to the 75<sup>th</sup> percentiles, and the whiskers from minimum to maximum ( $n = 4 - 10$ ). Detailed results are given in Table S4.

**Figure 4.** Activation of Nrf2-mediated oxidative stress response assessed with the AREc32 assay: induction ratio of 1.5 ( $EC_{IR1.5}$ ) as relative enrichment factor (REF) before (tap) and after different water filters (Table S1; A: reverse osmosis; B: ceramic outer shell with granular activated carbon and activated alumina core; C, E, F: ceramic outer shell with granular activated carbon core; G: activated carbon and ion-exchange resin; D, H, J: carbon block; I: activated alumina without carbon); for filters D, E, G, H results are given before (old c.) and after replacement of the filter cartridge (new c.). Displayed is the median, box extends from the 25<sup>th</sup> to the 75<sup>th</sup> percentiles, and the whiskers from minimum to maximum ( $n = 4 - 10$ ). Detailed results are given in Table S4.

Figures

Figure 1:



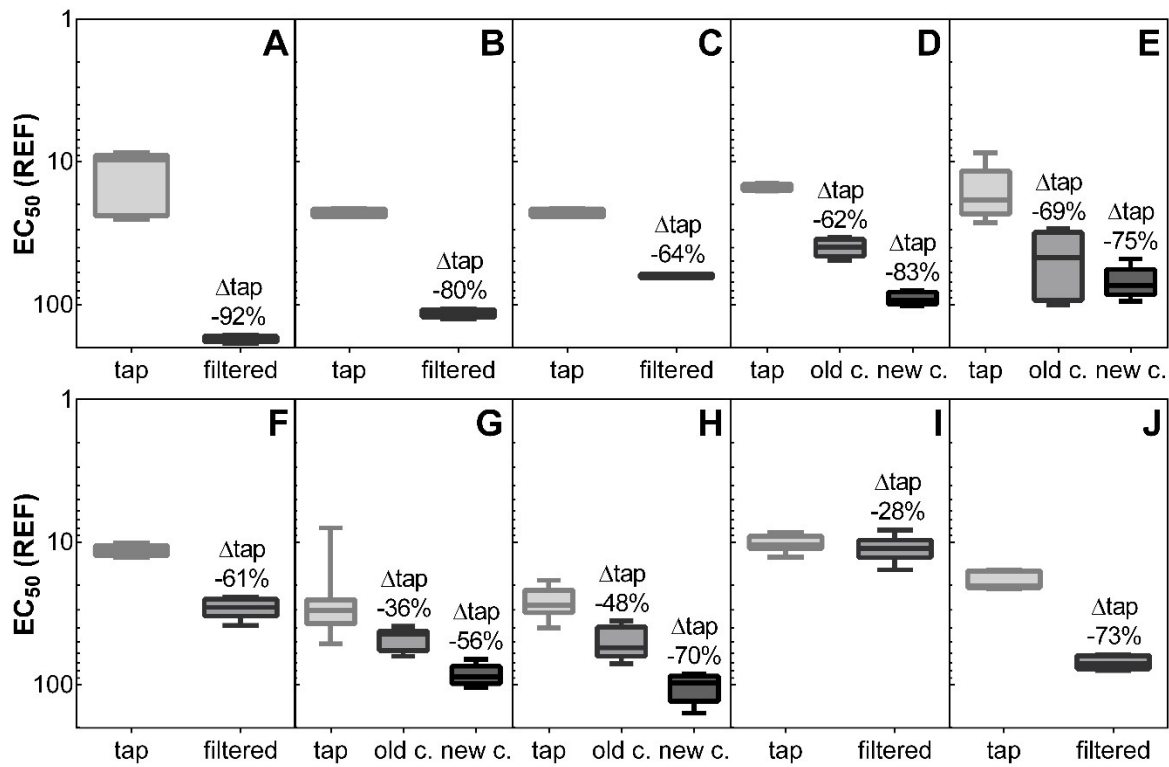
599 **Figure 2:**

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Figure 3:



**Figure 4:**