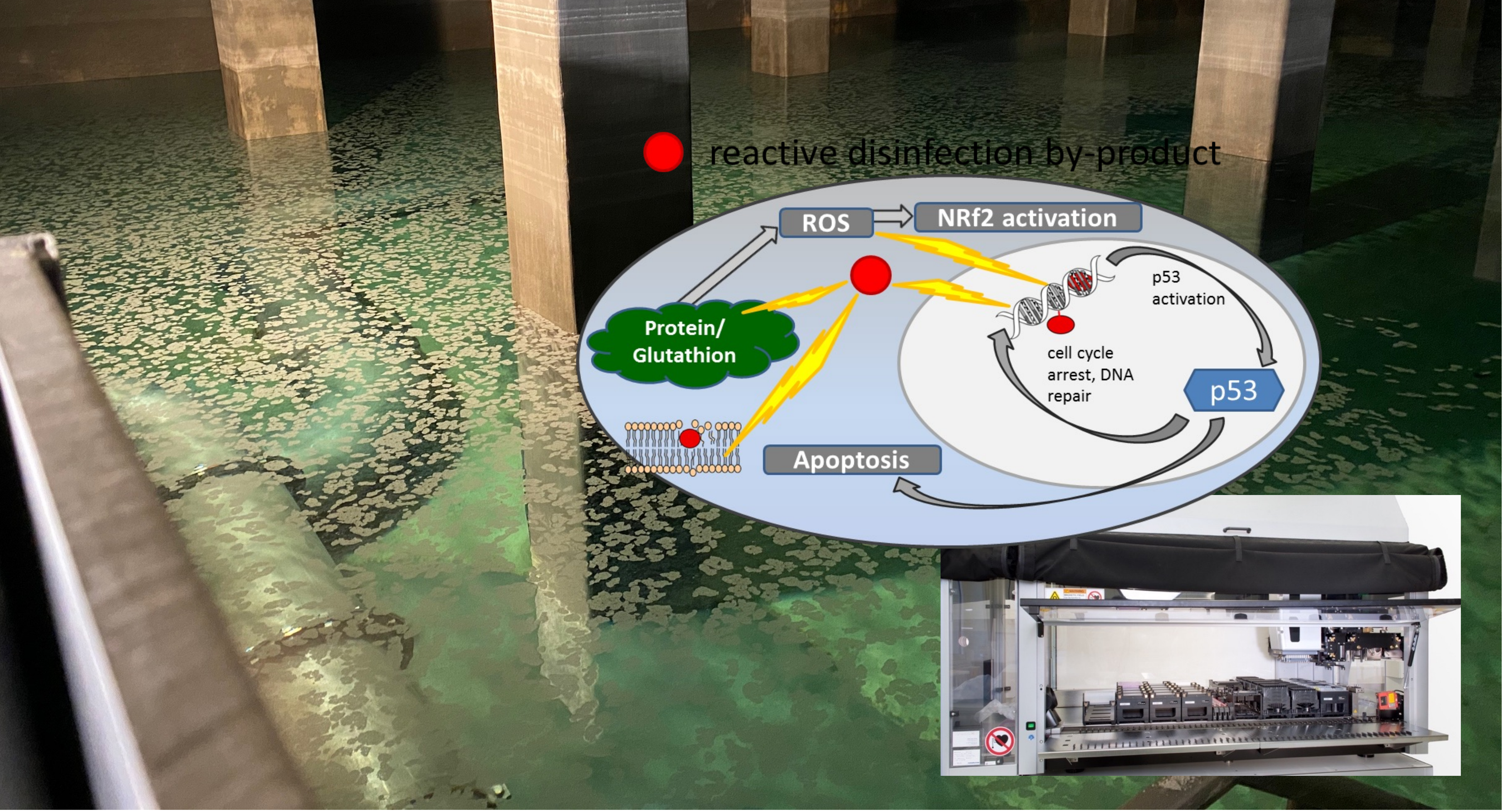


*In vitro B*ioassays for the Evaluation of Drinking Water

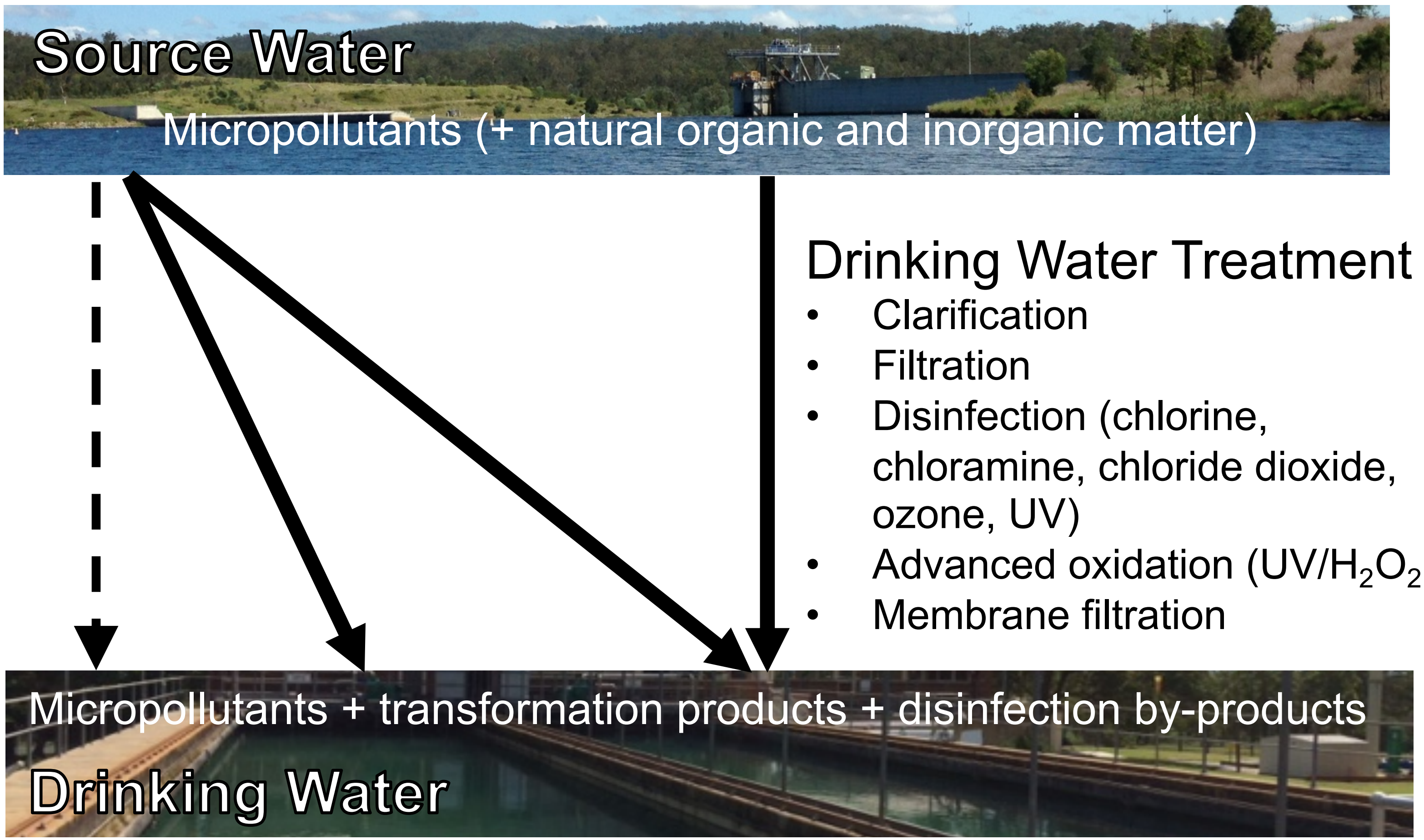
Beate Escher, Daniel Stalter, Peta Neale and many more…..
Eberhard Karls University Tübingen, Germany
Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany
The University of Queensland, QAEHS, and Griffith University, Brisbane, Australia

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

Challenge for (drinking) water quality assessment

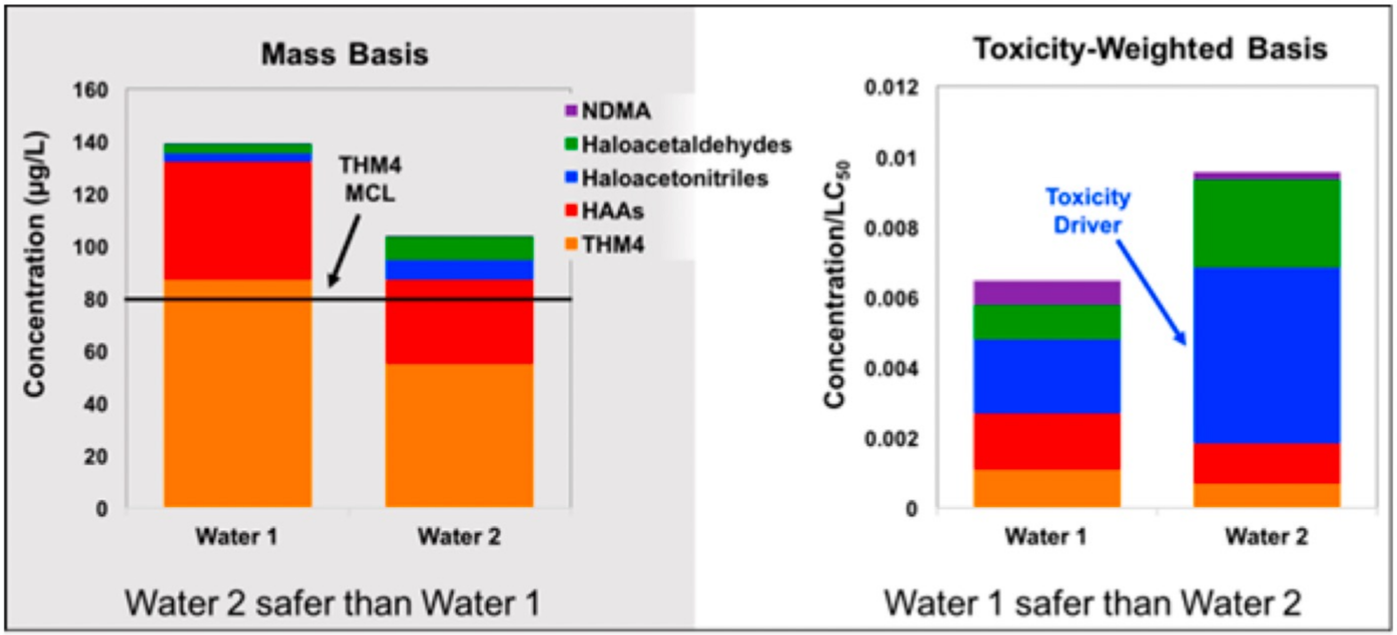
100'000s of micropollutants and 1000s of disinfection by-products (DBP), individually often below limit of detection but ALL potentially acting together in mixtures



Neale PA, Escher BI. 2019. *In vitro* bioassays to assess drinking water quality. *Current Opinion in Environmental Science & Health* 7:1-7.

Challenge for (drinking) water quality assessment

- Chemicals with dominant concentrations in drinking water are not necessarily drivers of mixture effects and risk



Environmental Science & Technology

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Feature

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Drinking Water Disinfection Byproducts (DBPs) and Human Health Effects: Multidisciplinary Challenges and Opportunities

Xing-Fang Li*,† and William A. Mitch*,‡

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Bioassays for (drinking) water quality assessment

- Goal: Protection of all aquatic life against chronic effects and human health with respect to long-term intake of drinking water
  - Micropollutants from source water
  - Disinfection by-products formed during drinking water treatment
- Measure: *in vitro* and low-complexity *in vivo* bioassays (animal protection, low sample volume, low cost, large sample numbers)

Protection target

Ecosystem health

Human health (drinking water)

Acute effects

Chronic effects

bioassays

Bacteria
Algae
Daphnia
Fish Embryo Toxicity

Proxies for chronic effects (environment and human health): MOA-specific indicators

Endocrine effects
1. ER
2. Anti AR
3. GR

Metabolism
1. AhR
2. PPARy
3. PXR

Reactive Toxicity:
1. Oxidative stress
2. Genotox

Neurotoxicity

Fish Embryo Toxicity (as proxy of develop-mental effects)

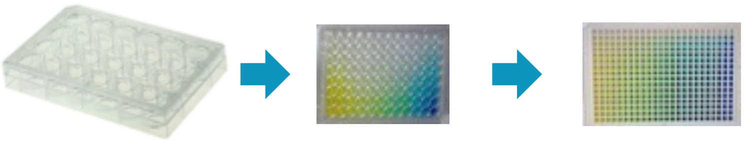
Selection of test batteries of bioassays

Test batteries can be purpose-built for specific applications (modular set up)

- Profiling of single chemicals for chemical risk assessment (ToxCast, Tox21)
- Assessment of treatment efficacy of natural and engineered treatment systems
- Monitoring of drinking water quality: source water versus drinking water disinfection by-products
- Surveillance and compliance monitoring of water quality (effect-based trigger values (EBT))
- Benchmarking chemicals in diverse environmental samples (sediment, biota, human biomonitoring)

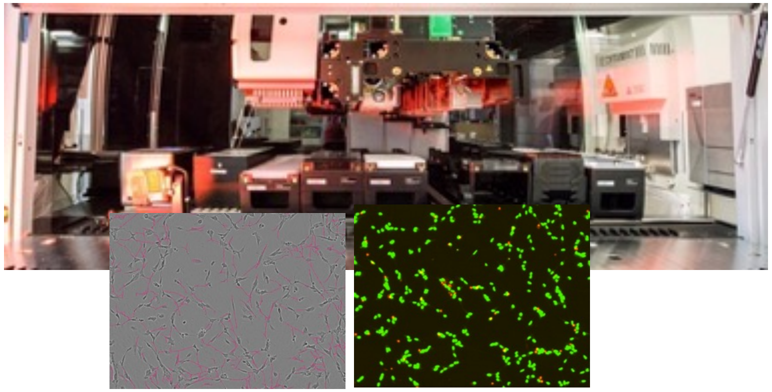
High throughput screening (HTS)

- Pipetting robots: large numbers of bioassays
- Well-plates: low volume requirement
  - Bacteria (30 min, 40-200 µL, 96/384)
  - Cell-based bioassays (24h, 40-100 µL, 96/384)
  - Algae (24-74h, 300 µL, 24/96)
  - Daphnia (48h, 1 mL, 12/24)
  - Fish Embryo Toxicity (FET) (24-120h, 2 mL, 12/24/96)

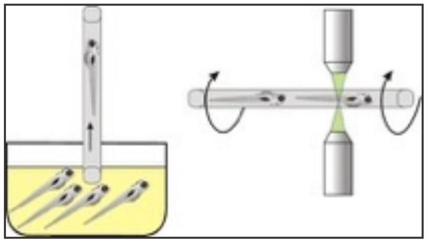


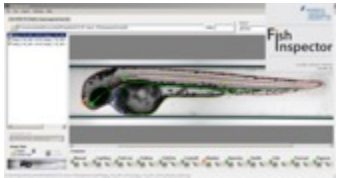
12/24 well plates   96 well plates   384 well-plates

HTS robotic system for cellular assays



HTS system for fish embryo toxicity test (VAST imager)





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Effects in drinking water are low- therefore we need to enrich water, e.g., by SPE

step 1
solid phase extraction

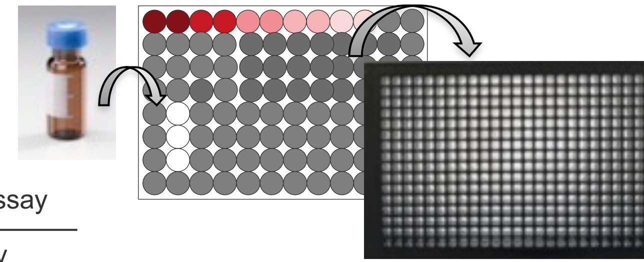
enrichment

$$\frac{V_{water}}{V_{extract}}$$

steps 2-5

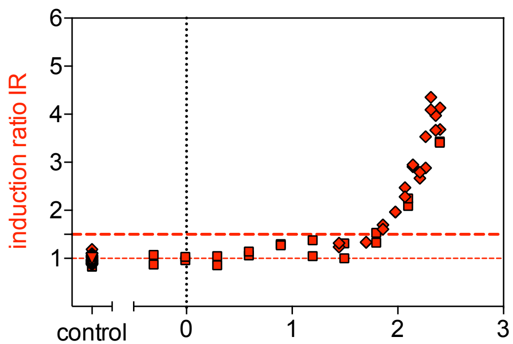
dilution

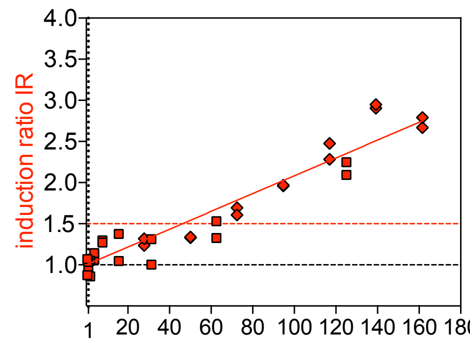
$$\frac{\text{volume of extract added to assay}}{\text{total volume of assay}}$$



relative enrichment factor REF = enrichment factor$_{SPE}$ x dilution factor$_{assay}$

logREF = 0, REF = 1 -> same concentration as original sample





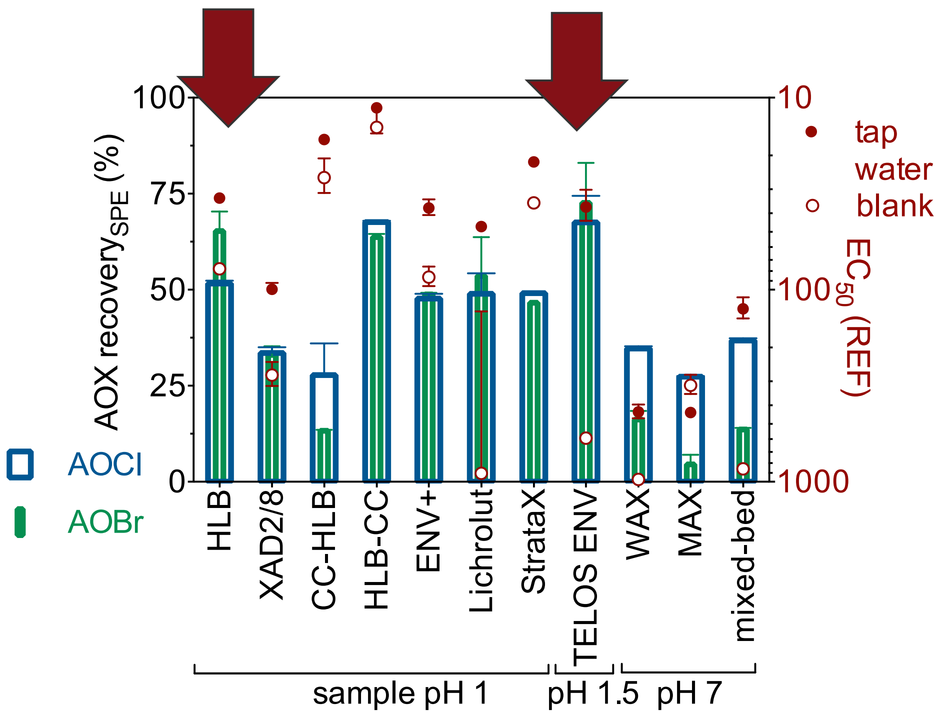
dilution ← → **enrichment**

enrichment →

How can we capture all chemicals in DW?

- Enrichment of volatile DBPs with purge and (cold) trap method

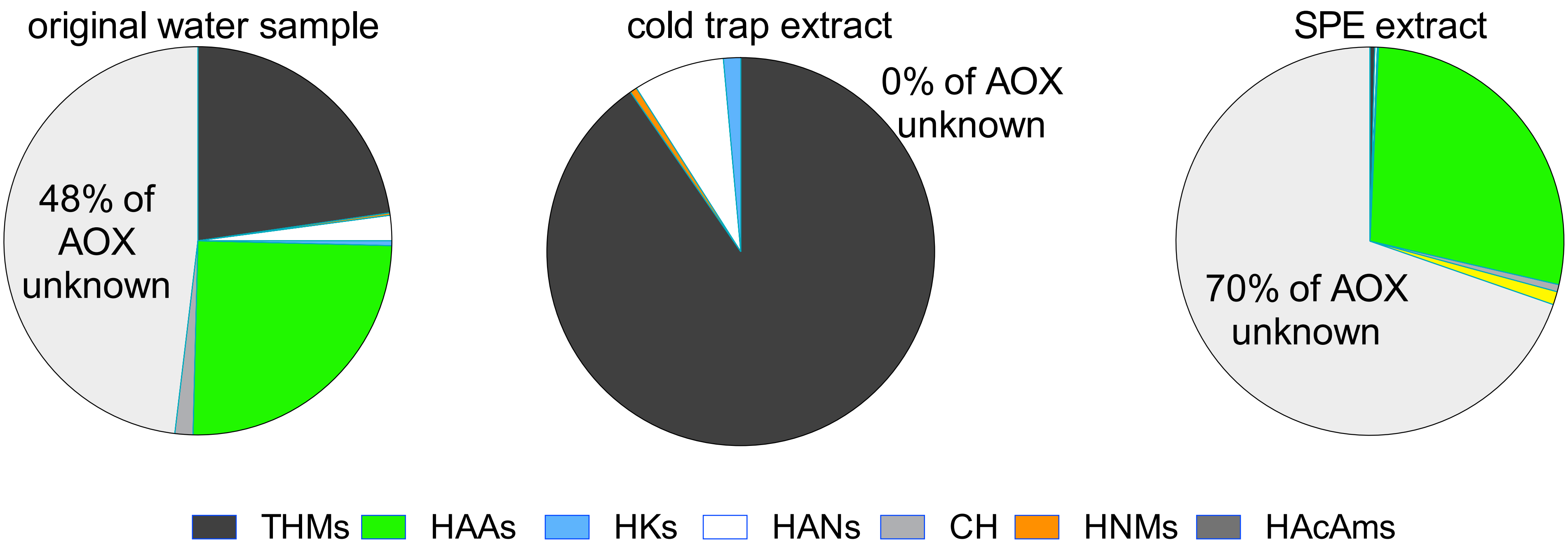
- Improved SPE method for very polar and charged DBPs: TELOS ENV at pH 1.5 or HLB



Stalter, …Escher, B.I. (2016). Sample Enrichment for Bioanalytical Assessment of Disinfected Drinking Water: Concentrating the Polar, the Volatiles, and the Unknowns. ES&T, 50: 6495-6505.

The majority of the volatile DBPs is known! The unknowns remain in the SPE extract

- AOX in volatiles‘ extracts mainly from THMs
- AOX in SPE extracts mainly from HAAs but 70% of non-volatile AOX remains unknown



AOX = Absorbable organic carbon

Stalter, …Escher, B.I. (2016). Sample Enrichment for Bioanalytical Assessment of Disinfected Drinking Water: Concentrating the Polar, the Volatiles, and the Unknowns. Environmental Science & Technology, 50: 6495-6505.

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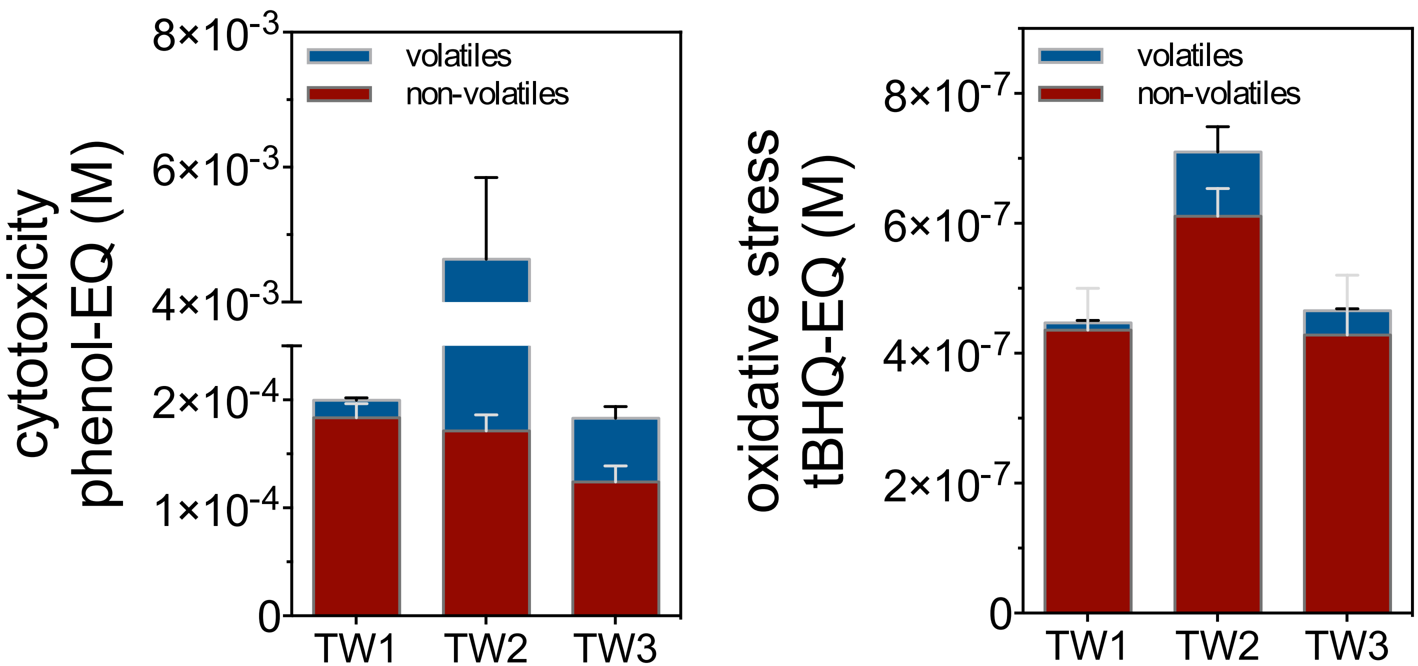
The majority of effect comes from non-volatiles

- The majority of cytotoxicity and oxidative stress response is in the non-volatile fraction of the DBPs
- Proportionally more effects than AOX ➡ non-volatiles more “toxic”?



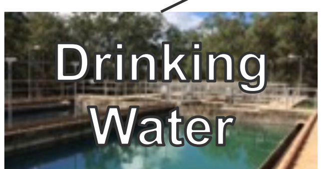
TW1 to 3: three different tap water samples from Brisbane, Australia

Stalter, …Escher, B.I. (2016). Sample Enrichment for Bioanalytical Assessment of Disinfected Drinking Water: Concentrating the Polar, the Volatiles, and the Unknowns. Environmental Science & Technology, 50: 6495-6505.

Mixture effects

Which chemicals contribute to the known effects? (tip of the iceberg)

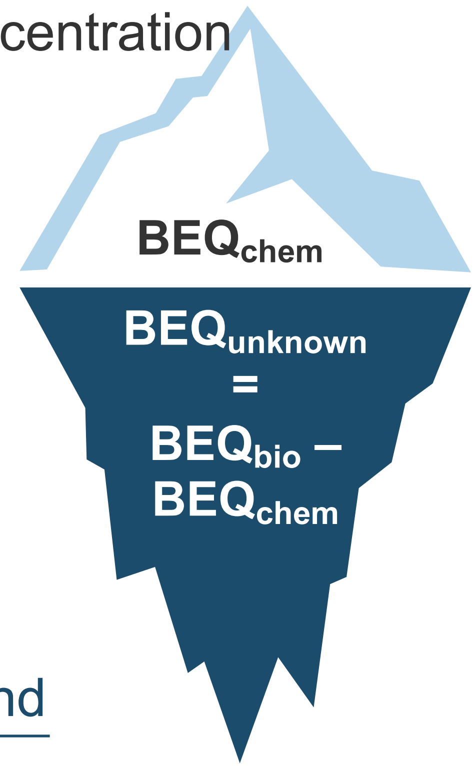
$$BEQ_{chem} = \sum_{i=1}^{n} \text{relative effect potency} \cdot \text{detected concentration}$$



Chemical Analysis → **BEQ_{chem}**

% contribution to effect

Bioanalysis → **BEQ_{bio}**



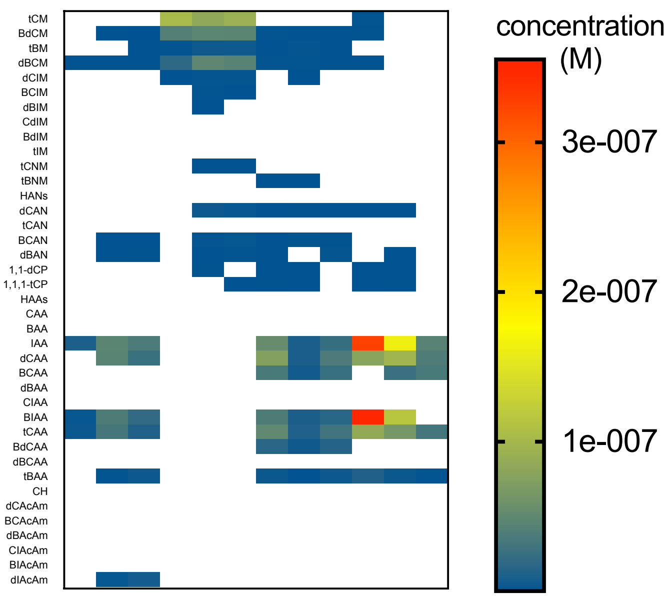
$$BEQ_{bio} = \frac{EC_{reference\ compound}}{EC_{sample}}$$

How much of the effect is unknown ? (submerged iceberg)

Mixture effects

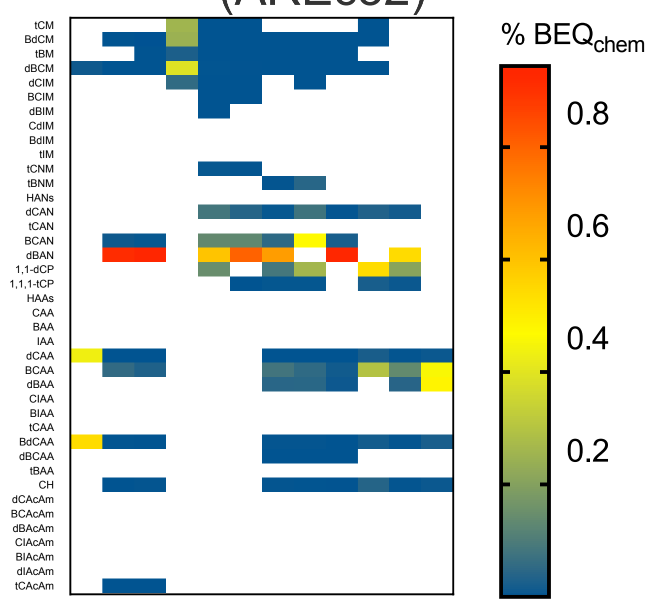
Which chemicals contribute to the known effects? (tip of the iceberg)

Concentrations



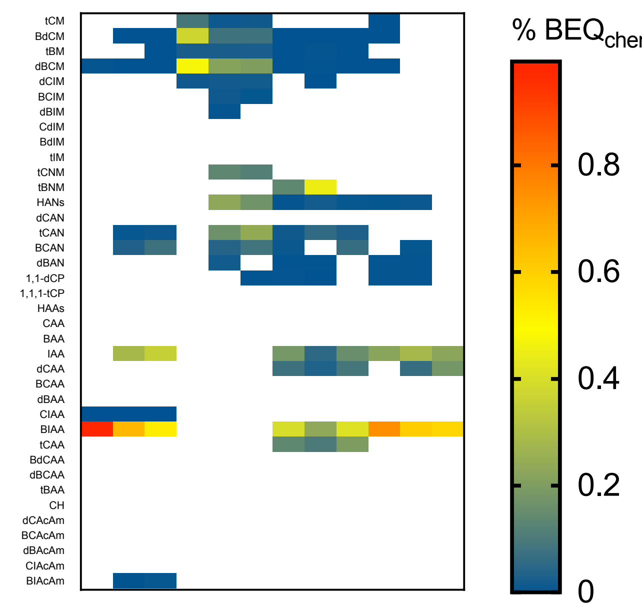
Trihalomethanes (TCM) & halocaetic acids (dCAA, tCAA) dominate

Oxidative stress response (AREc32)



Haloacetonitriles (dBAN) dominate

Genotoxicity (umuC)



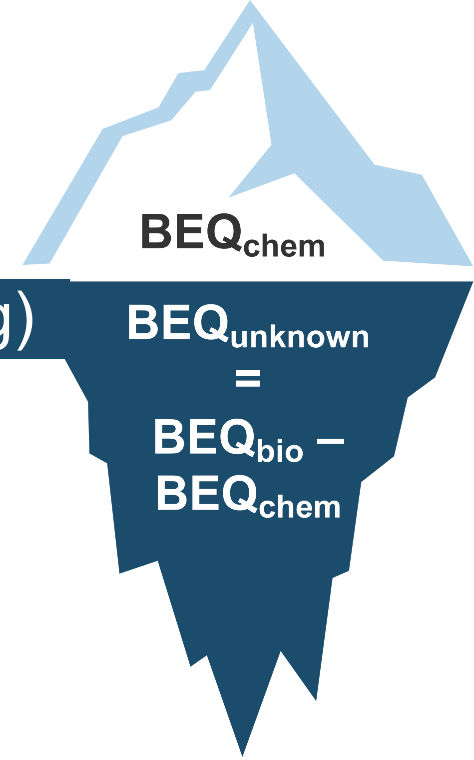
Haloacetic acids (BdCAA) dominate

Columns are different drinking water samples

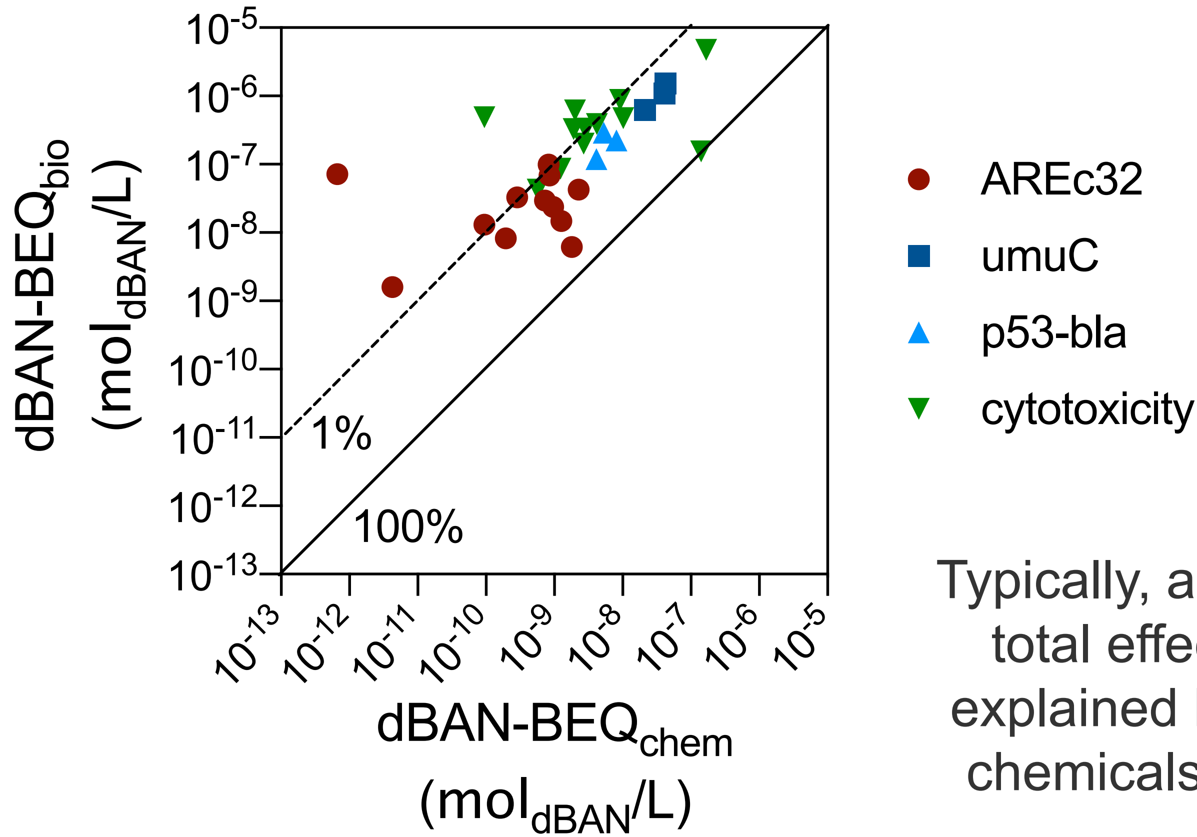
Stalter, D.; O'Malley, E.; von Gunten, U.; Escher, B. I. Mixture effects of drinking water disinfection by-products: implications for risk assessment. Environmental Science: Water Research & Technology 2020, 6, 2341-2351. DOI: 10.1039/c9ew00988d.

Mixture effects

How much of the effect is unknown ? (submerged iceberg)



Effects expressed as dibromoacetonitrile –equivalents (dBAN-BEQ)



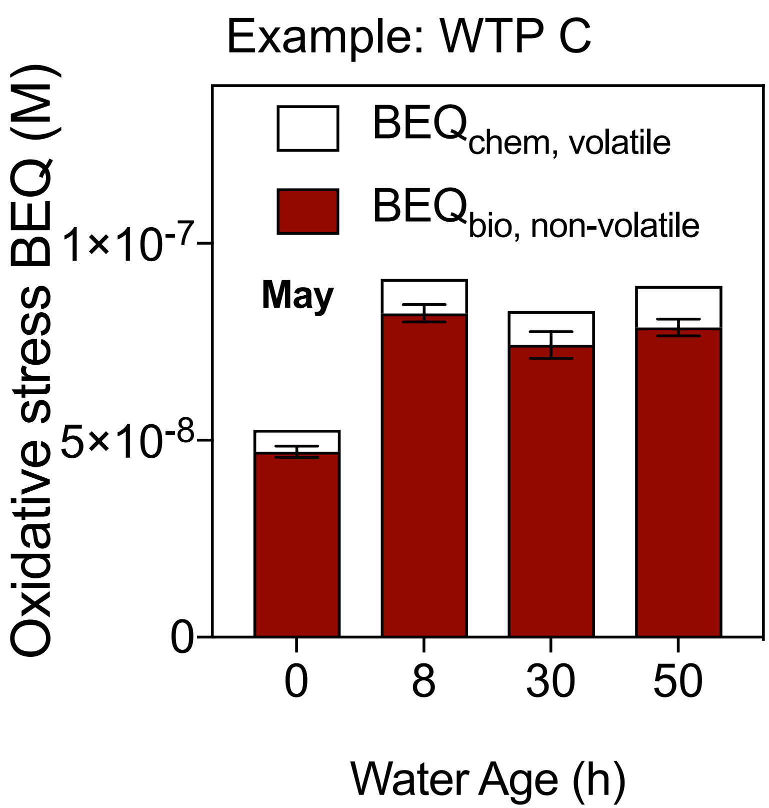
Typically, around 1% of total effect (BEQ_{bio} explained by detected chemicals (BEQ_{chem})

Stalter, D.; O'Malley, E.; von Gunten, U.; Escher, B. I. Mixture effects of drinking water disinfection by-products: implications for risk assessment. Environmental Science: Water Research & Technology 2020, 6, 2341-2351. DOI: 10.1039/c9ew00988d.

Proposal: quantify volatile DBPs with chemical analysis and non-volatiles with bioassays

Volatiles:

- Calculate BEQ_{chem} from analysis of volatile chemicals
- All DBP with Henry Law Constant $>10^{-6}$ atm m^3/mol
- $BEQ_{chem} = \Sigma c_i \cdot REP_i$



Non-volatiles:

- Use SPE extracts to calculate BEQ_{bio}

$$BEQ_{bio,non\text{-}volatile} = \frac{EC(reference)}{EC(sample)}$$

Comparison $BEQ_{chem,volatiles}$ vs. $BEQ_{bio,SPE}$ indicates that volatiles' BEQ is less important than non-volatiles' BEQ

Hebert A, Felier C, Lecarpentier C, Neale P, Schlichting R, Thibert S, Escher B. 2018. Bioanalytical assessment of adaptive stress responses in drinking water as a tool to differentiate between micropollutants and disinfection by-products. Water Res 132:340-349.

Study of Drinking Water Quality in Drinking Water and its Distributions Networks

Chemical analysis of regulated DBPs
Trihalomethanes and -ethanes, nitrosamines, haloacetic acids, haloacetonitriles)

- Mainly volatile DBPs
- Their effect is well characterized in bioassays

- Their mixture effect can be calculated

Bioanalytical Assessment of adaptive stress responses
(oxidative stress, p53)

- Mainly triggered by DBPs but also micropollutants

- Differentiate between DBPs and MP by comparison before and after chlorination

Bioanalytical Assessment of hormone-like effects
(ER, AR, PR, GR, etc)

- Mainly triggered by micropollutants

- No effects detected on hormone receptors

Hebert A, Felier C, Lecarpentier C, Neale P, Schlichting R, Thibert S, Escher B. 2018. Bioanalytical assessment of adaptive stress responses in drinking water as a tool to differentiate between micropollutants and disinfection by-products. Water Res 132:340-349.
(1) Neale, P.; Feliers, C.; Glauch, L.; Lecarpentier, C.; Schlichting, R.; Thibert, S.; Escher, B. Application of in vitro bioassays for water quality monitoring in three drinking water treatment plants using different treatment processes including biological treatment, nanofiltration and ozonation coupled with disinfection. *Environ. Sci.: Water Res. Technol.* **2020**, *6* (9), 2444-2453. DOI: doi.org/10.1039/C9EW00987F.

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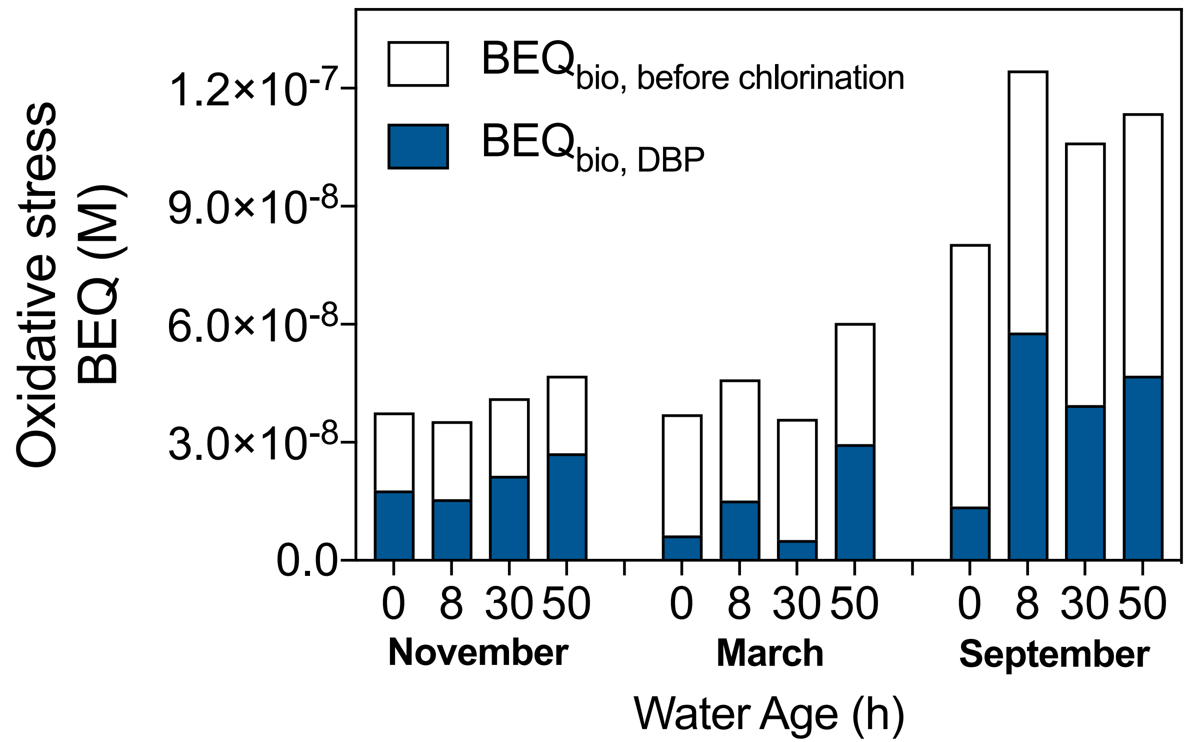
Can we differentiate between organic micropollutants and DBPs?

BEQ(after chlorination)=BEQ(before chlorination)+BEQ(DBPs formed)

Contribution of DBPs to effect was equal or smaller than the effect caused by micropollutants and organic matter in the treated water prior to chlorination



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Summary and Conclusions

- High-throughput screening bioassays for (drinking) water assessment
  - Many samples can be run, stringent quality control and data evaluation pipeline
- Versatile applications of bioanalytical tools
  - Removal efficacy of micropollutants in WTP
  - Assessment of treatment technologies
  - Formation of disinfection byproducts
  - Benchmarking of drinking water quality against other water types and across WTPs, countries and continents
- Mixture modelling
  - DBPs act concentration additive in mixtures
  - Differentiation between contribution of micropollutants and formation of DBPs
  - Effect of DBPs (often) lower than of micropollutants
  - Effects of volatile DBPs typically lower than of non-volatile DBPs