Effect-based *in vitro* methods to monitor hazardous chemicals in the environment.

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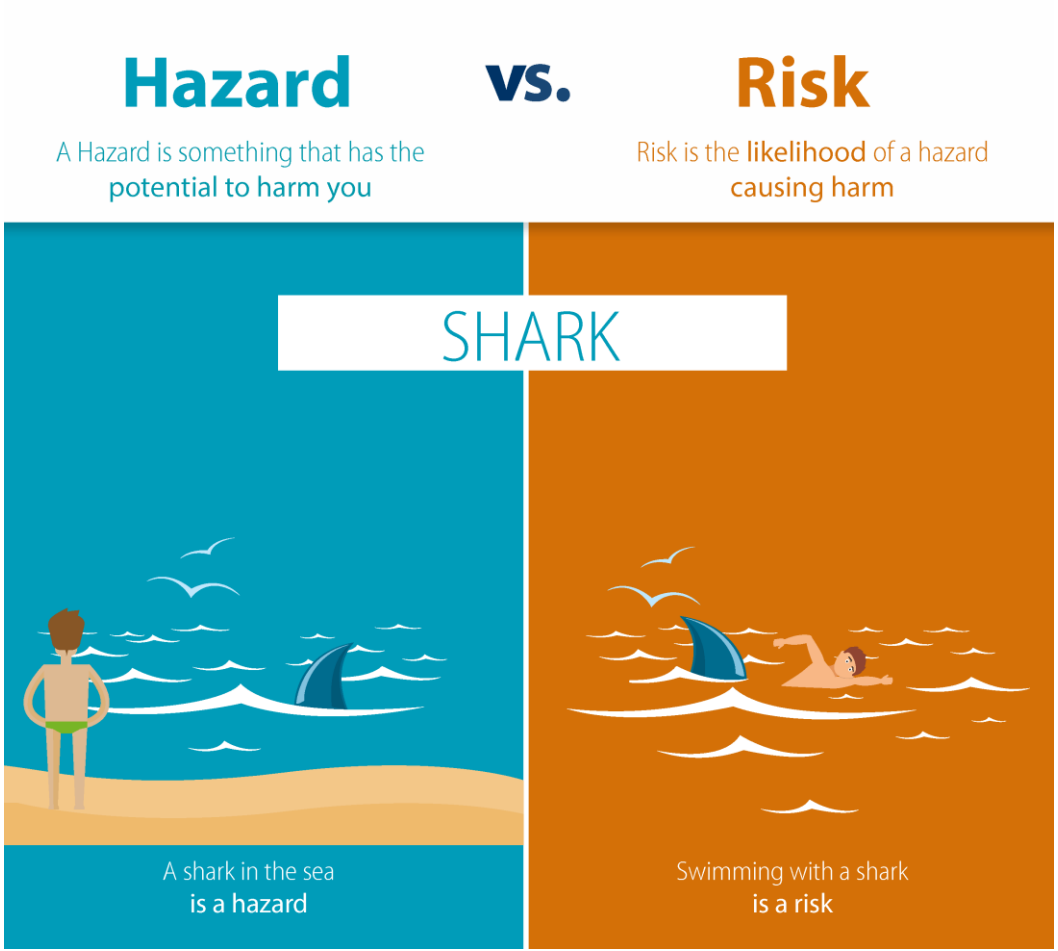
Disclosure statement

- Co-founder of a startup company providing effect-based testing services to the water sector



Risk = hazard x exposure

Adopted from EFSA

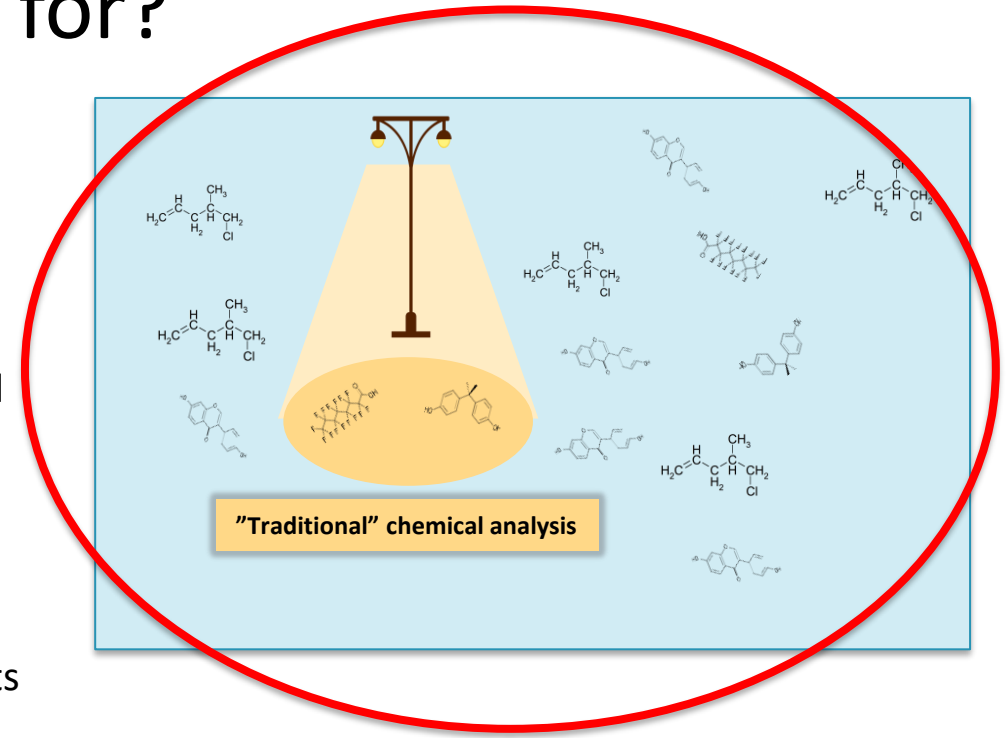
Pollutants in water

– a problem for both the environment and human health

- Tens of thousands of chemicals are spread into the environment
- Societal challenge of great concern, addressed in three UN Global Goals

What are we looking for?

- Chemicals in the environment – why worry? Potential biological effects!
- Limitations of environmental monitoring based on target screening
- Tens of thousands of chemicals are spread into the environment with very limited information on potential toxicity
- Need for a shift from analysis of a limited number of known pollutants to measurement of the total biological effects of pollutants



Looking under the streetlight – is it really a problem?

- Yes, it is!
- Numerous publications show that known/analyzed chemicals can only explain a small fraction of the observed biological effects in environmental water samples

Example:

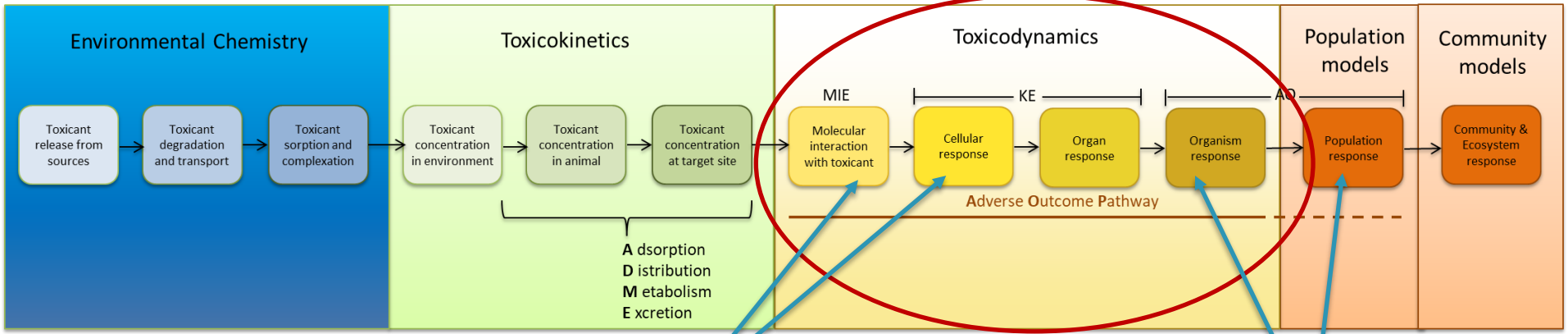
- Water samples from streams impacted by wastewater effluents
- Chemically characterized for 400 pollutants
- Effect-based assessment of bioactivities
- The 400 pollutants could only explain 0.2-1.6% of the observed effects on ER, AR and oxidative stress response
- 99% of the observed biological effects was cused by unknown chemicals

Neale et al, Science of the Total Environment, 2017, 576:785-795

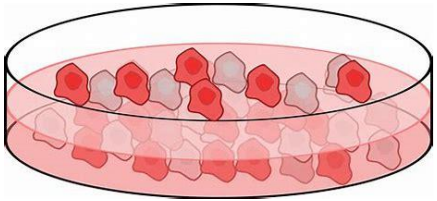
Target screening
- Concentrations of single compounds

Effect-based methods
- Integrating the effect of both known and unknown compounds and cocktail effects

Effect based methods - in vitro and *in vivo*



In vitro – cultured cells



In vivo – whole organisms

Effect-based *in vitro* methods

- Toxicity testing in the 21st century
- Cultured cells of, modified to respond to molecular events early in toxicity pathways
- Reporter gene assay (*e.g.* luciferase as reporter)
- Cost effective, high-throughput
    - 96 or 384 well plates

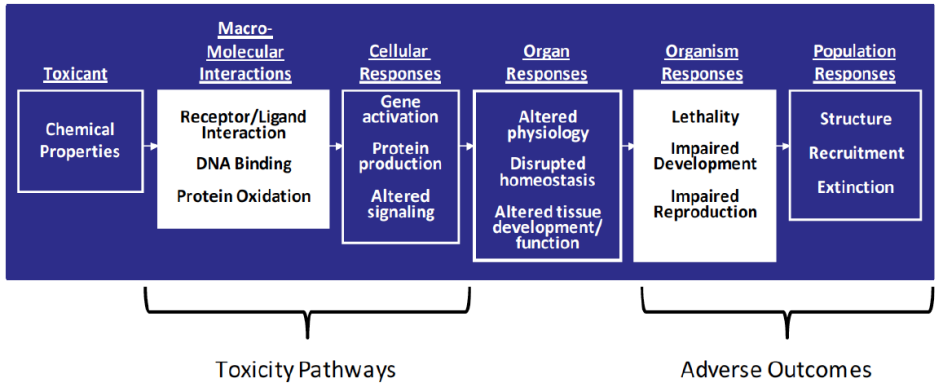
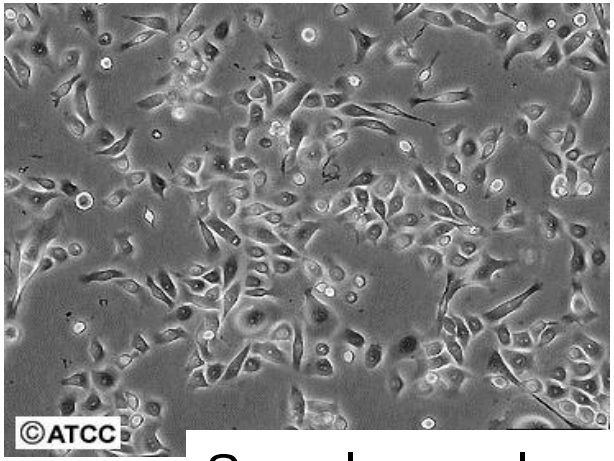
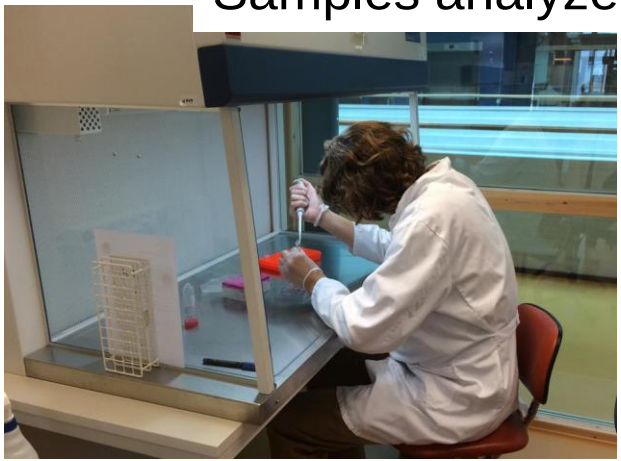


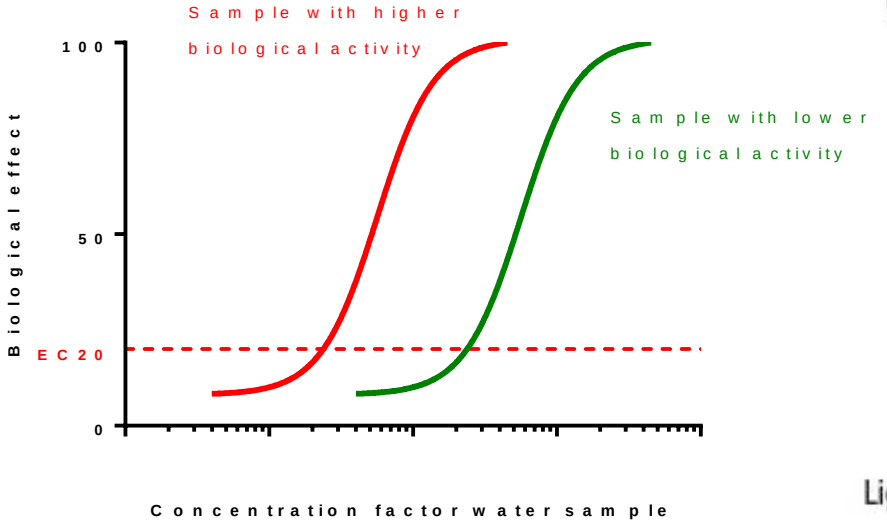
Figure: US Environmental Protection Agency

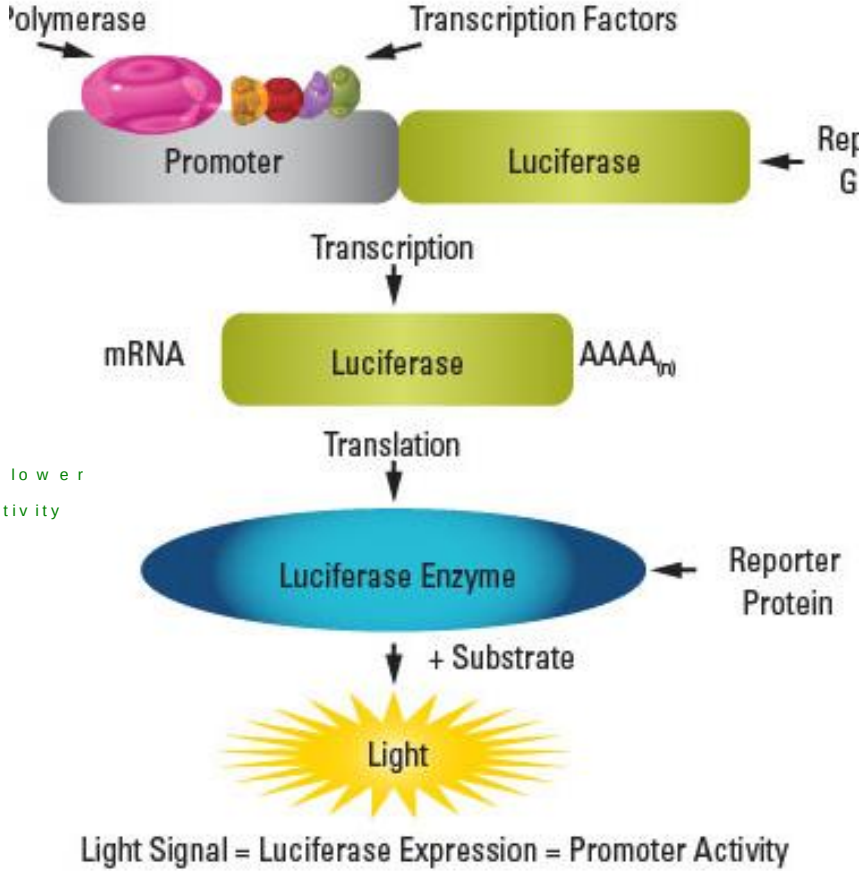


Samples analyzed in 384 well plates



In vitro assays





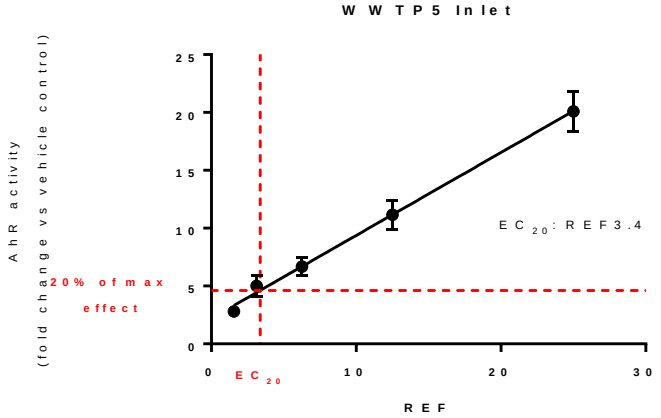
Possible metrics/outputs

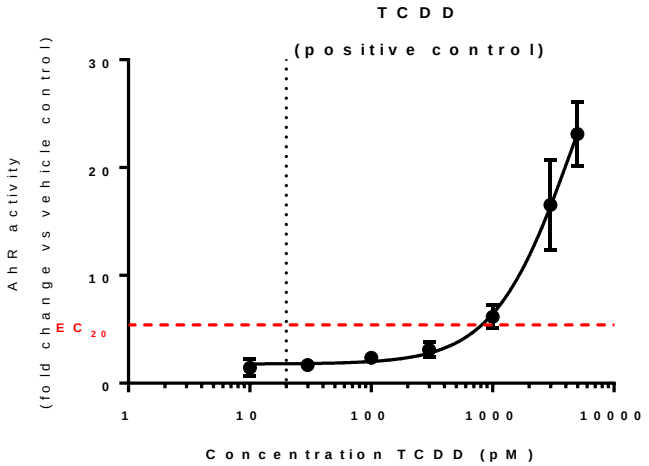
Preferred

- EC_{20}/EC_{50} expressed as REF
- Bioanalytical equivalent concentrations (BEQ) (”the observed activity corresponds to x ng/L of the known inducer y”)

Non-preferred

- LOEC/NOEC (depends on the REF values analyzed)





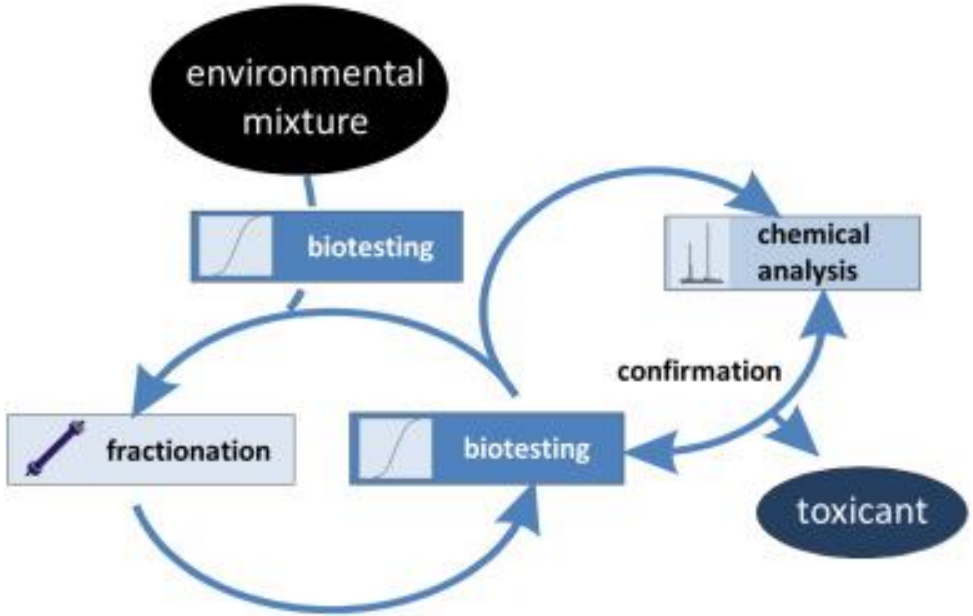
In vitro endpoints investigated

- **Oxidative stress (Nrf2 activity)**
  - Broad range of pollutants
- **Aryl hydrocarbon receptor (AhR) activation**
  - Broad range of pollutants, eg PAHs, dioxins etcetera
- **Estrogen receptor (ER) activation**
  - Sex hormones and a few pollutants
- **Androgen receptor (AR) activation and deactivation**
  - Sex hormones and a few pollutants
- **Genotoxicity**

Quality controls:

- Cytotoxic concentrations excluded
- Positive control standard curve

Identify causative compounds



- EDA
- vEDA

Brack et al, Science of The Total Environment
Volume 544, 15 February 2016, Pages 1073-1118


Two examples
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Current and previous studies

- Removal-efficiency of chemical pollutants during waste water treatment
- Unidentified hazardous chemical in drinking water

SCIENTIFIC REPORTS

OPEN

In vitro bioanalytical evaluation of removal efficiency for bioactive chemicals in Swedish wastewater treatment plants

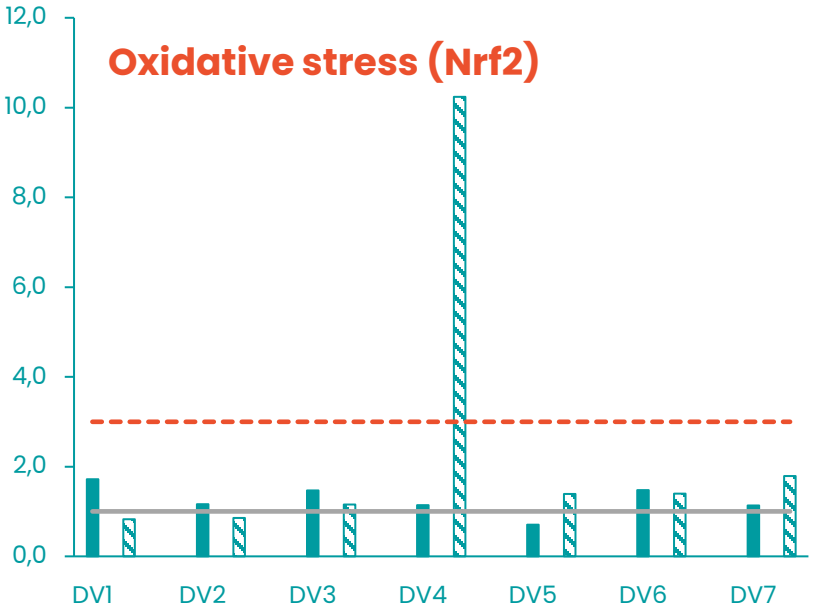
l: 16 January 2019
l: 29 April 2019
d online: 09 May 2019

Johan Lundqvist[1], Geeta Mandava[1], Sebastian Lungu-Mitea[1], Foon Yin Lai[2] & Lutz Ahrens[2]

Assay	WWTP1 Inlet	WWTP1 Outlet	WWTP2 Inlet	WWTP2 Outlet	WWTP3 Inlet	WWTP3 Outlet	WWTP4 Inlet	WWTP4 Outlet	WWTP5 Inlet	WWTP5 Outlet
AR agonism DHTEQ (nM)	60	0.2	0.6	0.3	0.7	0.3	40	0.25	38	n.d.
ER agonism E2EQ (pM)	930	30	130	30	100	10	930	4.0	130	4.0
Nrf2 activity tBHQEQ (µM)	2.5	1.1	n.d.	0.8	n.d.	n.d.	3.5	1.0	1.2	0.9
AhR activity TCDDEQ (pM)	580	490	730	350	610	300	1200	480	750	300
NFκB activity TNFαEQ (ng mL^{-1})	n.d.	n.d.	0.05	n.d.	n.d.	n.d.	0.2	n.d.	n.d.	n.d.

Lundqvist et al, 2019

Unknown pollutant in drinking water



- All chemical parameters were acceptable
- Unknown compound(s) with hazardous properties is contaminating the drinking water

Data from Oskarsson et al, 2021

Summary effect-based *in vitro* methods

- Integrates the effects of both known and unknown chemicals as well as mixture/cocktail effects

- High specificity and sensitivity – especially for the assays based on nuclear receptor activation (e.g. estrogenic activity)

- Possibility to combine with advanced chemical analysis to potentially identify currently unknown toxic environmental pollutants