

Comparison of different genotoxicity tests *in vitro* for their sensitivity to detect toxic effects of micropollutants in water samples

H. Bielak | J. Richard | A. Simon | E. Dopp

AIMS OF THE STUDY

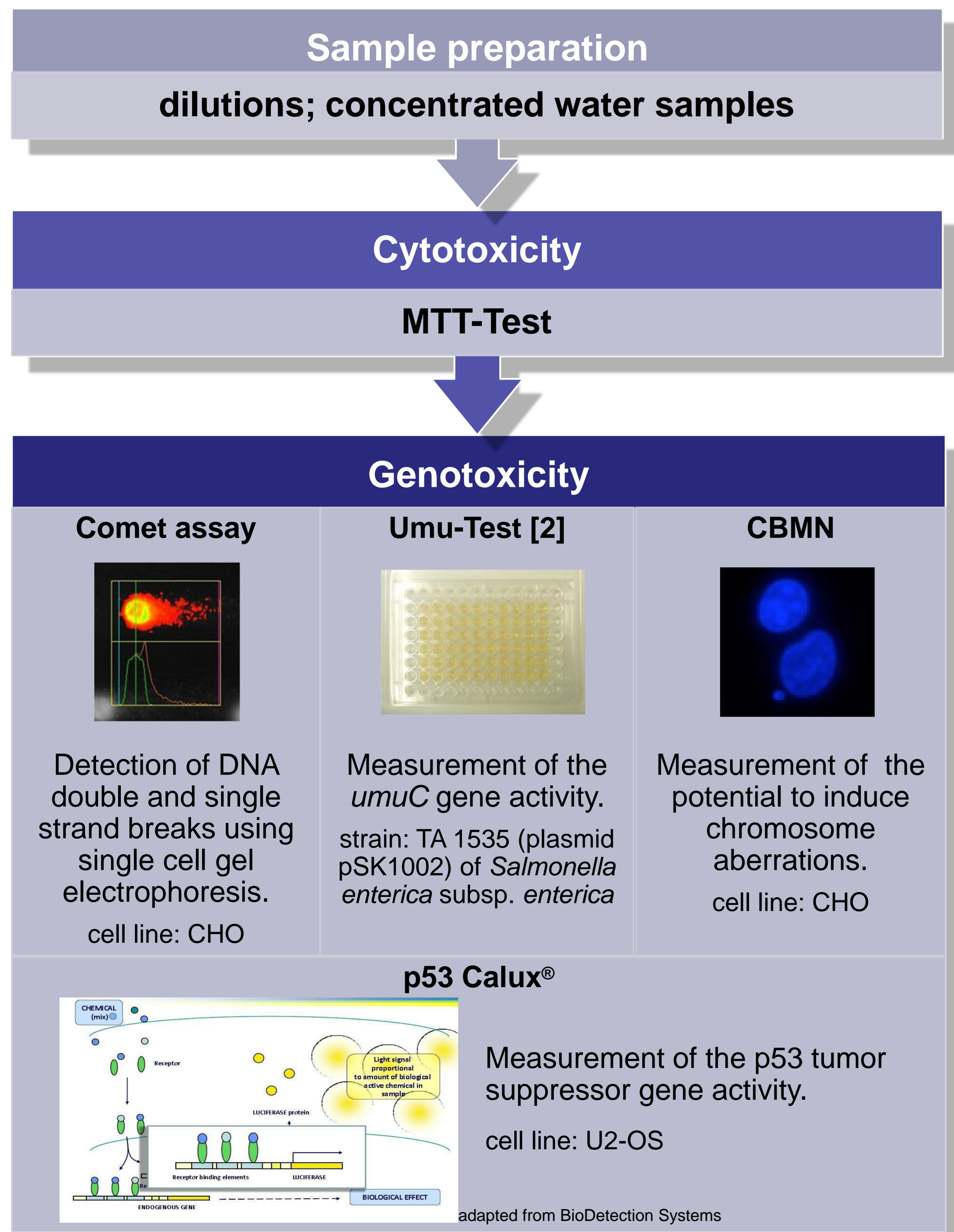
- Detection of micropollutants in water samples
- Toxicity evaluation using bioassays
- Comparison of four different genotoxicity tests for their sensitivities by testing chemicals that are commonly used as positive controls.
 - a) Alkaline Comet Assay
 - b) Umu-Test
 - c) Micronucleus-Test
 - d) p53 Calux®

MATERIALS AND METHODS

Exposition

- **Cell lines:** Chinese Hamster Ovary (CHO), Human Osteosarcoma Cells (U2-OS)
- **Time:** 24 h (ENU also 30 min)
- **Substances and concentrations:**
 - N-Ethyl-N-Nitrosourea (ENU): 0.01 – 1 mg/mL
 - 4-Nitroquinoline 1-oxide (4-NQO): 0.006 – 60 µg/mL
 - Mitomycin C: 0.002 – 20 µg/mL
 - 2-Aminoanthracene (2-AA): 0.01 ng/mL – 1 µg/mL
- Surface water samples from two rivers in NRW: Original, 2.8x and 4x concentrated with n-Hexane [1]

Genotoxicity testing



CONCLUSIONS

- Because of different endpoints there are different sensitivities for different genotoxicity tests
- Genotoxic effects were only detected in concentrated water samples (2.8x and 4x) by the receptor based p53 Calux®
- The p53 Calux® and Umu-test showed the lowest detectable effect concentrations
 - ➔ The p53 Calux® has been the most sensitive test to detect effects of micropollutants

RESULTS

Comparison of the different test systems

Table 1: Comparison of the different test systems for their lowest detectable effect concentration. The following chemicals, usually used as positive controls, were used for the study: ENU = N-Ethyl-N-Nitrosourea; 4-NQO = 4-Nitroquinoline 1-oxide; Mitomycin C, 2-AA = 2-Aminoanthracene. Exposure time: 24h

	MTT-Test	Comet-Assay	Umu-Test	CBMN	P53 Calux®
ENU	100 µg/mL	100 µg/mL	-	No genotox up to 100 µg/mL	100 µg/mL
4-NQO	0.6 µg/mL	No genotox up to 0.06 µg/mL	-	No genotox up to 0.06 µg/mL	0.06 µg/mL
Mitomycin C	No cytotox up to 20 µg/mL	20 µg/mL	-	2 µg/mL	0.5 µg/mL
2-AA	1 µg/mL	No genotox up to 1 µg/mL	<0.1 µg/mL	No genotox up to 1 µg/mL	No genotox up to 0.1 µg/mL

The p53 Calux® and the Umu-Test were the most sensitive systems in this study with detectable effect concentrations < 0.1 µg/mL.

Detection of genotoxicity in water samples

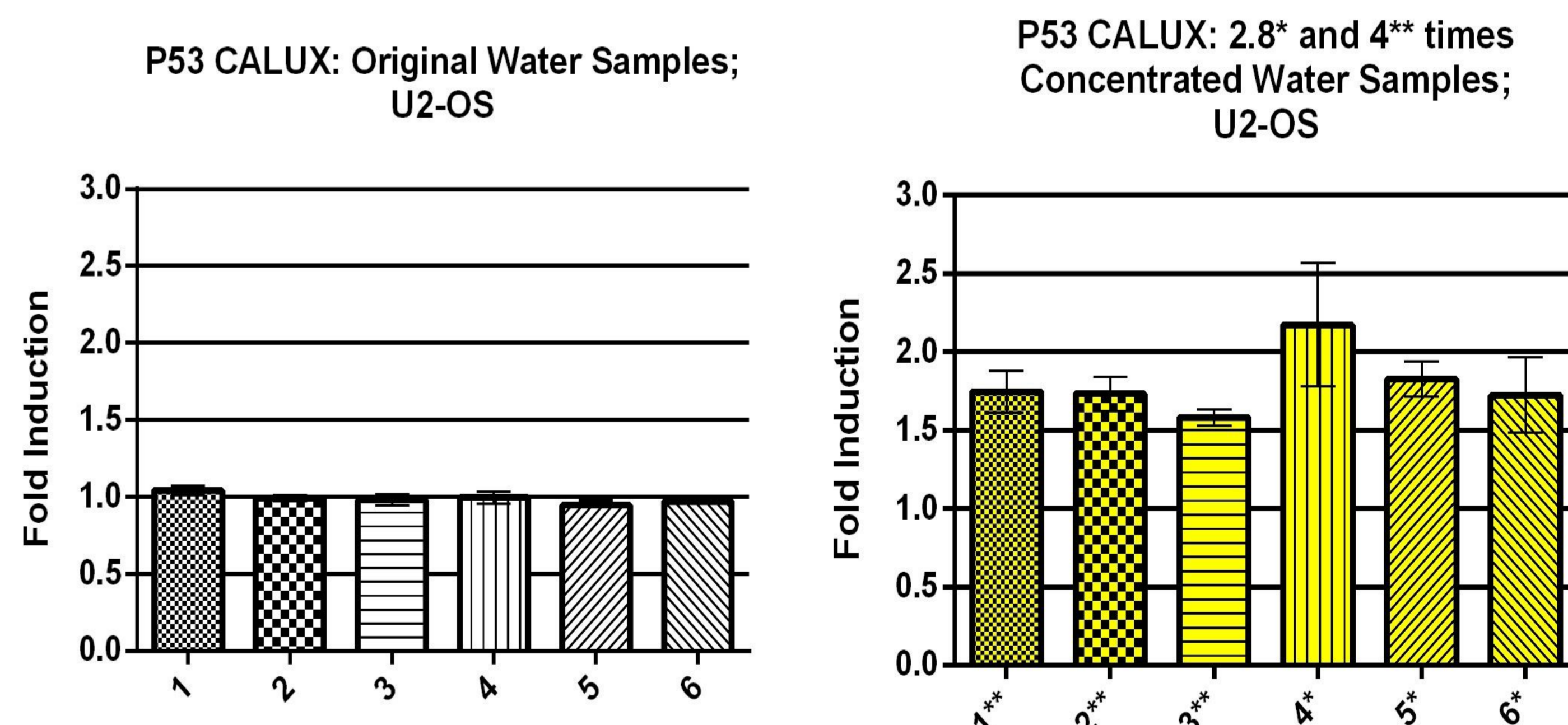


Fig. 1: Results of p53-Calux® with original water samples (left) and concentrated samples (right). Significant genotoxic effects are highlighted in yellow. [1]

No significant genotoxic effects of the tested water samples could be detected using the other test systems (data not shown).

References

- [1] I Curuia, *Master Thesis, University of Duisburg-Essen, 2012.*
- [2] Abwasserverordnung, *BGBl. I S. 2585, 2009.*

Contact

IWW Rheinisch-Westfälisches Institut für Wasserforschung gemeinnützige GmbH
 Moritzstrasse 26 • D-45476 Mülheim an der Ruhr
 Helena Bielak • Tel.: +49-(0)208-40303-224 • H.Bielak@iww-online.de

Partners

