Comparison of different genotoxicity tests *in vitro* for assessment of surface water quality

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Introduction

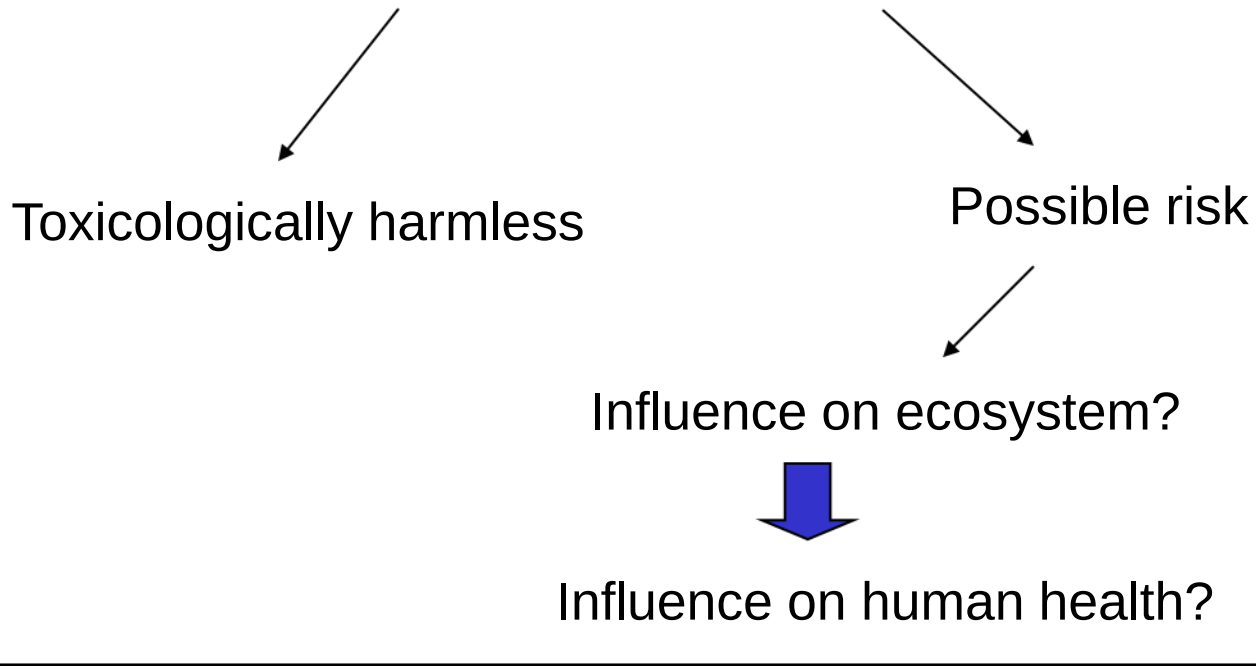
- Increased detection of micropollutants in municipal waste water and surface waters

- The EU Water Framework Directive requires the achievement of a good ecological status for all water bodies until 2015

- About 5000 organic trace substances are relevant for the aquatic environment
  - Substances which are: deposited in organisms and the environment, persistent, bioaccumulative, toxic and / or cause an endocrine effect
  - Pharmaceutical residues: 156 agents with positive results (literature review German and European monitoring data; www.uba.de/uba-info-medien/4188.html)

Detection and effects of micropollutants

- Chemical analyses
- Biological effects



Possible endpoints for biological effects

In vivo

Algae growth (*Desmodesmus Subspicatus*)

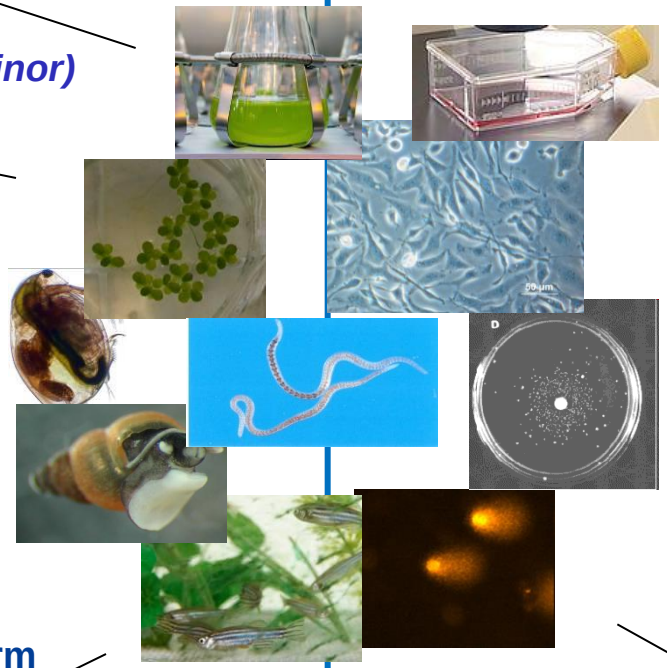
Duckweed growth *(lemna minor)*

Toxicity to water flea *(Daphnia magna)*

Estrogenicity to snails *(Potamopyrgus antipodarum)*

Growth inhibition to gloss worm *(Lumbriculus variegatus)*

Toxicity to fish eggs (Danio rerio)



In vitro

General cell damage (*Cytotoxicity*)

DNA damage (*Genotoxicity*)

Health oriented guided value:
0.3 µg/L if not genotoxic
0.1 µg/L if genotoxic

Inheritable DNA damage (*Mutagenicity*)

Estrogenic effects (*Estrogenicity*)

Genotoxicity tests *in vitro*

Description	Test Code
Bacterial Reverse Mutation Test (Ames)	OECD 471
In vitro Mammalian Chromosome Aberration Test	OECD 473
Mammalian Erythrocyte Micronucleus Test	OECD 474
In vitro Mammalian Cell Gene Mutation Assay	OECD 476
In vitro Sister Chromatid Exchange Assay in Mammalian Cells (SCE)	OECD 479
DNA Damage and Repair, in vitro Unscheduled DNA Synthesis (UDS) in Mammalian Cells	OECD 482
In vitro Mammalian Cell Micronucleus Test	OECD 487
The Alkaline Comet Assay	Not yet adopted (OECD)
Determination of the genotoxicity of water and waste water using the UMU test	DIN 38415-3
Evaluation of genotoxicity using amphibian larvae	ISO21427-1:2006
P53 CALUX	Not yet adopted

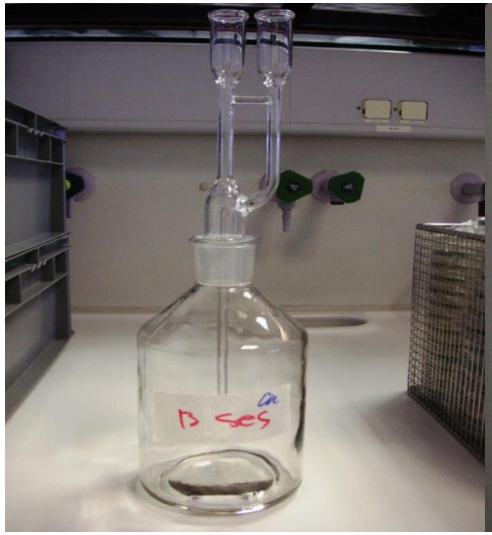
Aim of present study

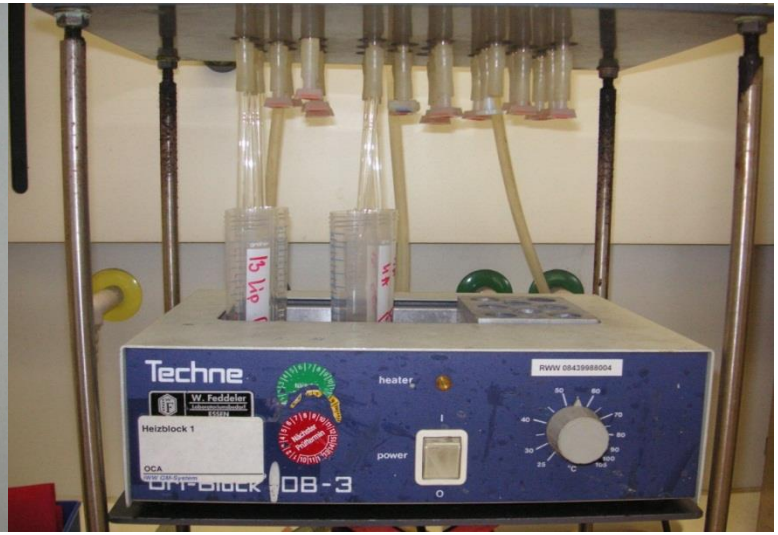
- Comparison of 4 different *in vitro* genotoxicity tests regarding their sensitivity
  - The **CBMN** Assay (OECD Guideline 487)
  - The **Alkaline Comet** Assay (OECD draft proposal)
  - The **UMU** Test (DIN 38415-3)
  - **P53 CALUX** (description of supplier)
- Genotoxicity assessment of **surface water** samples (original and 28x to 40x concentrated)
- Lowest detectable effect concentration of **2-Aminoanthracene (2-AA), N-Ethyl-N-Nitrosourea (ENU), 4-Nitroquinoline 1-oxide (4-NQO), Mitomycin C**

Material and Methods

Surface Water

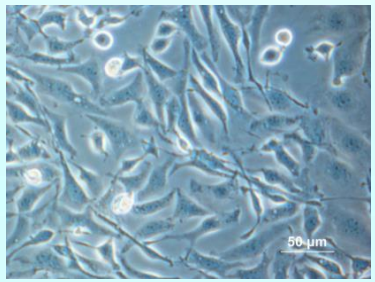
- Sampling sites: the rivers Lippe and Seseke in North-Rhine-Westfalia (GER)
- Provided by the “Kooperationslabor of Ruhrverband, Emschergenossenschaft and Lippeverband” in Essen
- Concentration procedure: n-Hexan (extracting solvent), concentration factor 28x and 40x





Material and Methods

Cell Line	Origin	Source
CHO	Chinese Hamster - Ovary Cells	European Collection of Cell Cultures (ECACC), (UK)
U2-OS	Human Osteosarcoma Cells	BDS (NL)
Salmonella enterica	Strain TA1535 (plasmid pSK1002)	DMSZ (GER)



Material and Methods

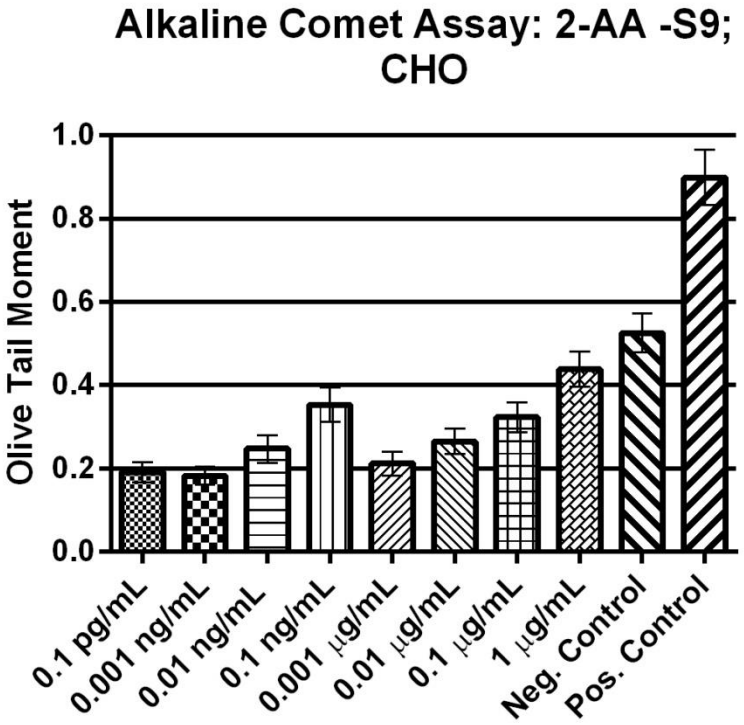
Cytotoxicity	Genotoxicity
MTT Test -2-Aminoanthracene (2-AA) without S9 (-S9); Two cell lines: CHO and U2-OS - Original Water Samples	**CBMN Test** - 2-AA - S9 - Original Water Samples
Chemicals: 2-AA, 2-Aminoanthracene ENU, *N*-ethyl-*N*-nitrosourea Mitomycin C 4-NQO, 4-Nitroquinoline 1-oxide	**The Alkaline Comet Assay** - 2-AA – S9; 2-AA + S9 - Original Water Samples
	The UMU Test - 2-AA – S9 - Original Water Samples - Concentrated Water Samples
	P53 CALUX - 2-AA –S9; 2-AA +S9 - Original Water Samples; Concentrated Water Samples

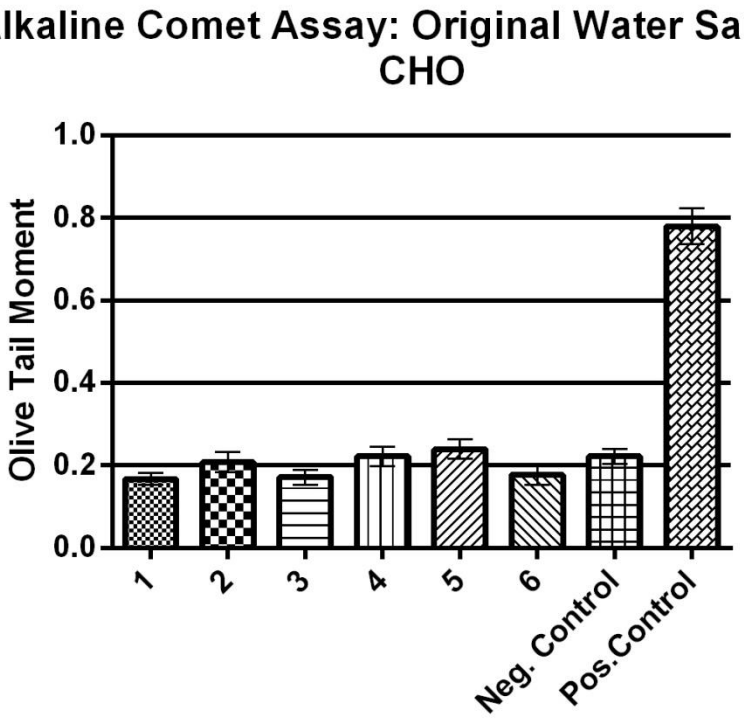
Comparison of the different test systems for their lowest detectable effect concentration

	MTT-Test	Comet-Assay	Umu-Test	MN-Test	P53-Calux®
ENU	100 µg/ml	100 µg/ml	-	No genotox up to 100 µg/ml	100 µg/ml
4-NQO	3 µg/ml	No genotox up to 0.3 µg/ml	-	No genotox up to 0.3 µg/ml	0.3 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

P53 Calux® and Umu-test are able to detect genotoxic effects at concentrations ≤ 0.5 µg/ml. Comet and MN assay require higher substance concentrations.

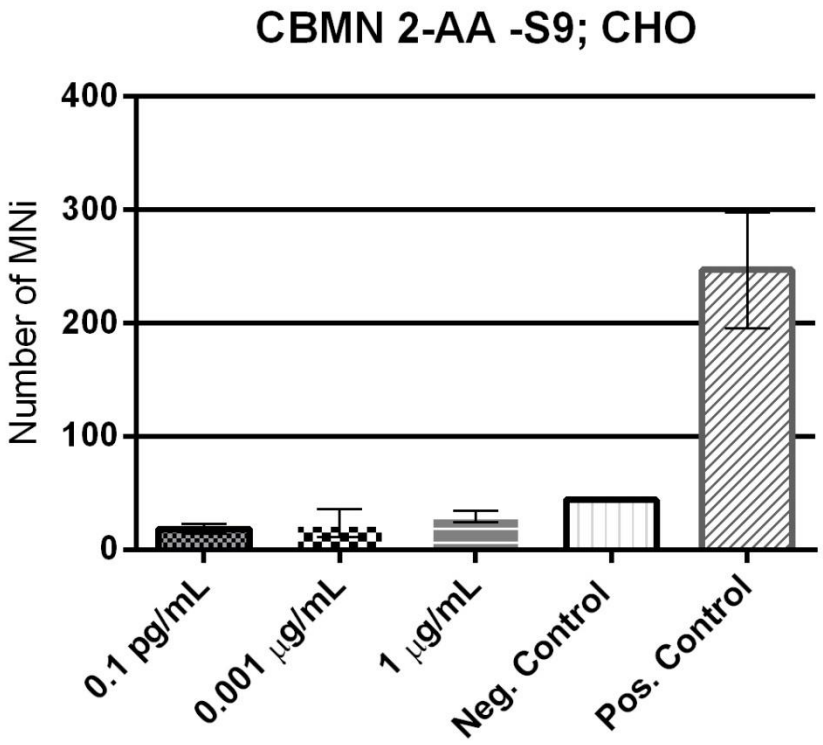
Results: Comet Assay

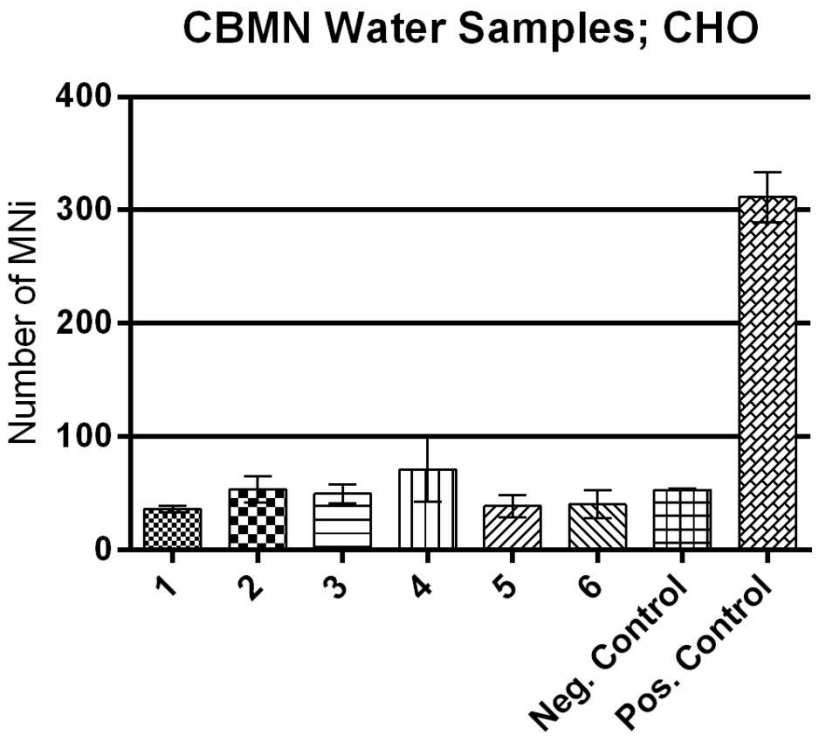




No genotoxic effects were detectable with the Comet Assay and the UMU-test (data not shown) in original and concentrated water samples (2.8x and 4x).

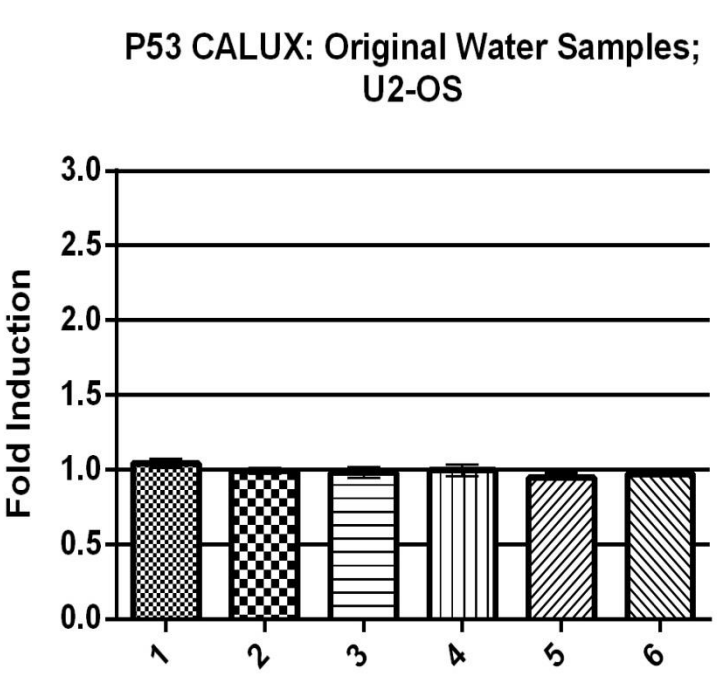
Results: Micronucleus Assay

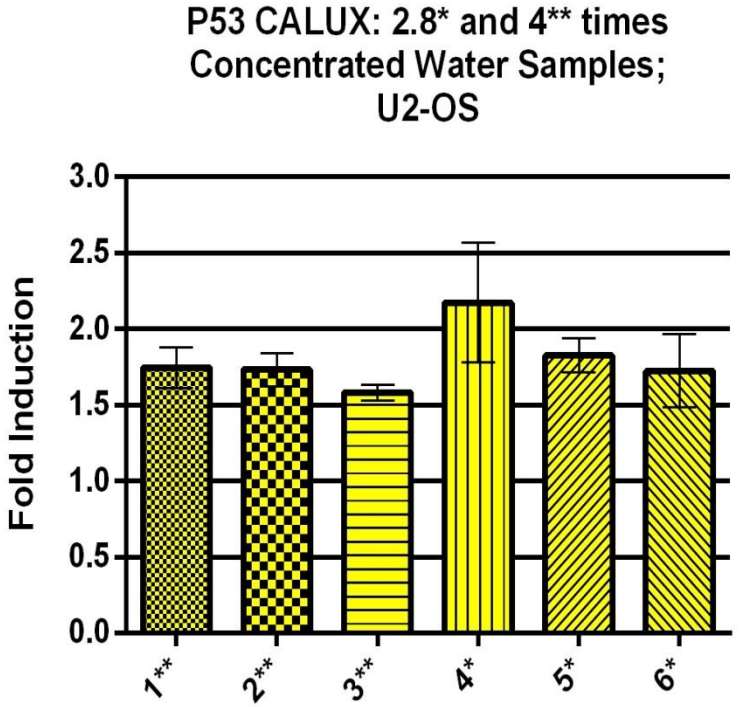




No genotoxic effects were detectable with the Micronucleus Assay in original and concentrated water samples (2.8x and 4x).

Results: p53 Calux®





Significant genotoxic effects were detected with the P53 Calux® in concentrated water samples (2.8x and 4x).

Conclusions

- The different test methods detect different endpoints with different sensitivity
- Neither cyto- nor genotoxic effects were detectable in original water samples
- In 2.8x and 4x concentrated water samples just the receptor based p53 Calux® was able to detect geno-toxic effects
- Higher concentration factors are necessary to apply the other test systems for original water samples
- Lowest necessary concentration factors will be investigated in further studies

Thank you very much for your attention

Project partners:

EMSCHER LIPPE GENOSSENSCHAFT EGLV.DE VERBAND

Ruhrverband WISSEN, WERTE, WASSER