

# Detection of endocrine disrupting and genotoxic compounds in waste water treatment plant (WWTP) effluents

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Institute affiliated with the

UNIVERSITÄT  
DUISBURG  
ESSEN

*Open-Minded*



## IWW in figures

**100 scientists, engineers, economists and technicians**

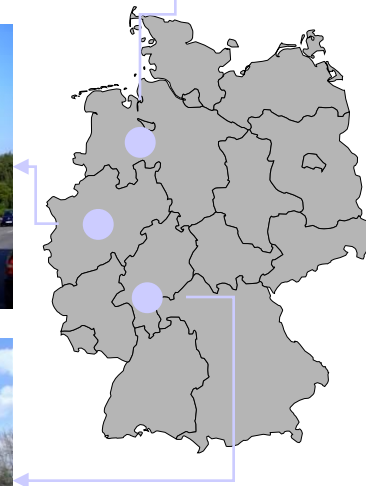
**45 % Research, 55 % Consulting**

## Customers

**Water supply ca. 50 %**

**Government (national/regional/local) ca. 20 %**

**Industry ca. 25 %**



## Regional branches

**Mülheim a.d. Ruhr, Nordrhein-Westfalen**

**IWW Rhein-Main: Biebesheim, Hessen**

**IWW Nord: Diepholz, Niedersachsen**

# Areas of Competence



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**Water Technology**  
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**Water Chemistry**  
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**Microbiology**  
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Resource Protection	Drinking Water Treatment	Corrosion Prevention	Inorganic Analysis	Biofilms Hygiene	Efficiency Consulting
Integrated Water Resources Management	Membrane Technology	Maintenance Strategies	Organic Analysis	<b>Toxicology</b>	Performance Indic. Benchmarking
Water Catchment	Process-engineering Analytics	Material Testing	Microbiological Analysis	Material Testing	Risk management
System Simulation			Radionuclide Analysis	Monitoring Industrial Systems	Software ADIS® TEIS®
Household Distribution Systems					



# Water Quality

## Inorganic Analysis

Extensive spectrum of inorganic routine parameter  
Customer-specific parameters: development on demand  
Investigation and evaluation of water treatment chemicals

## Organic Analysis

Routine- and special analysis on more than 600 organic substances  
New methods or parameters according to varying needs

## Microbiological and toxicological Analyses

All relevant parameters with various methods  
Problem-oriented evaluation of data  
Consulting on rehabilitation and support

## Radioactivity analysis

Relevant isotopes (tritium, uranium, radium, radon)  
( $\alpha$ ,  $\beta$ ,  $\gamma$ ) activities  
Treatment technology



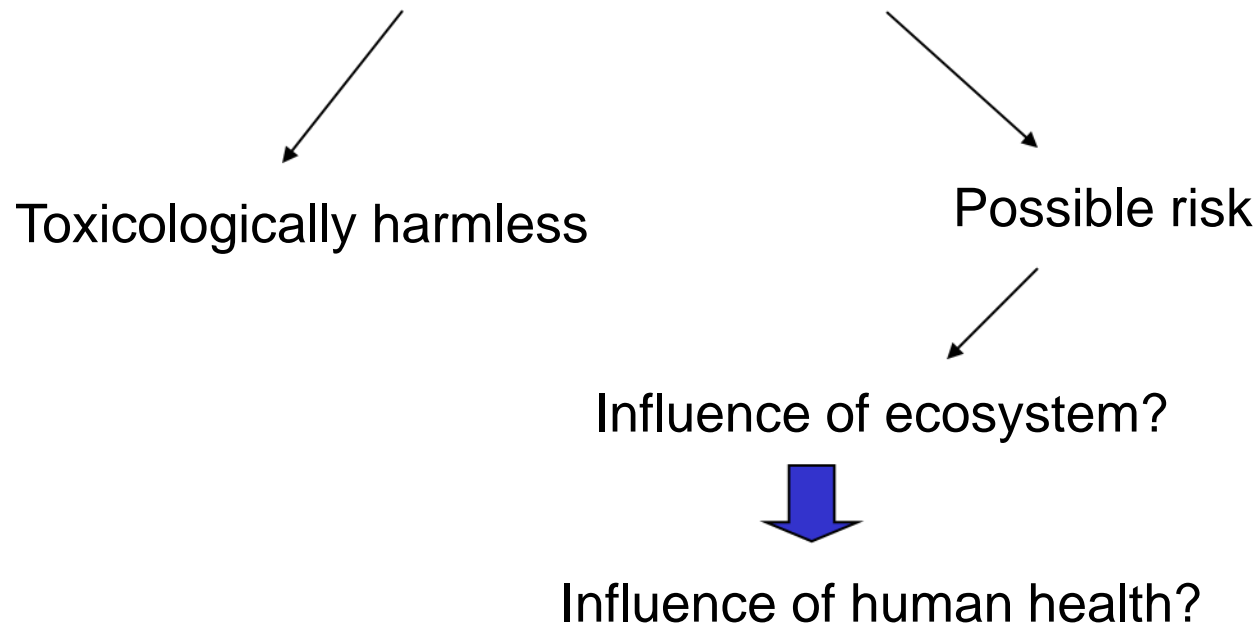
# Introduction

- Increased detection of micropollutants in municipal wastewater and surface waters
- The EU Water Framework Directive requires the achievement of good ecological status for all rivers until 2015
- About 5000 organic trace substances are relevant for 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](http://www.uba.de/uba-info-medien/4188.html)

# Detection and effects of micropollutants

- Chemical analyses

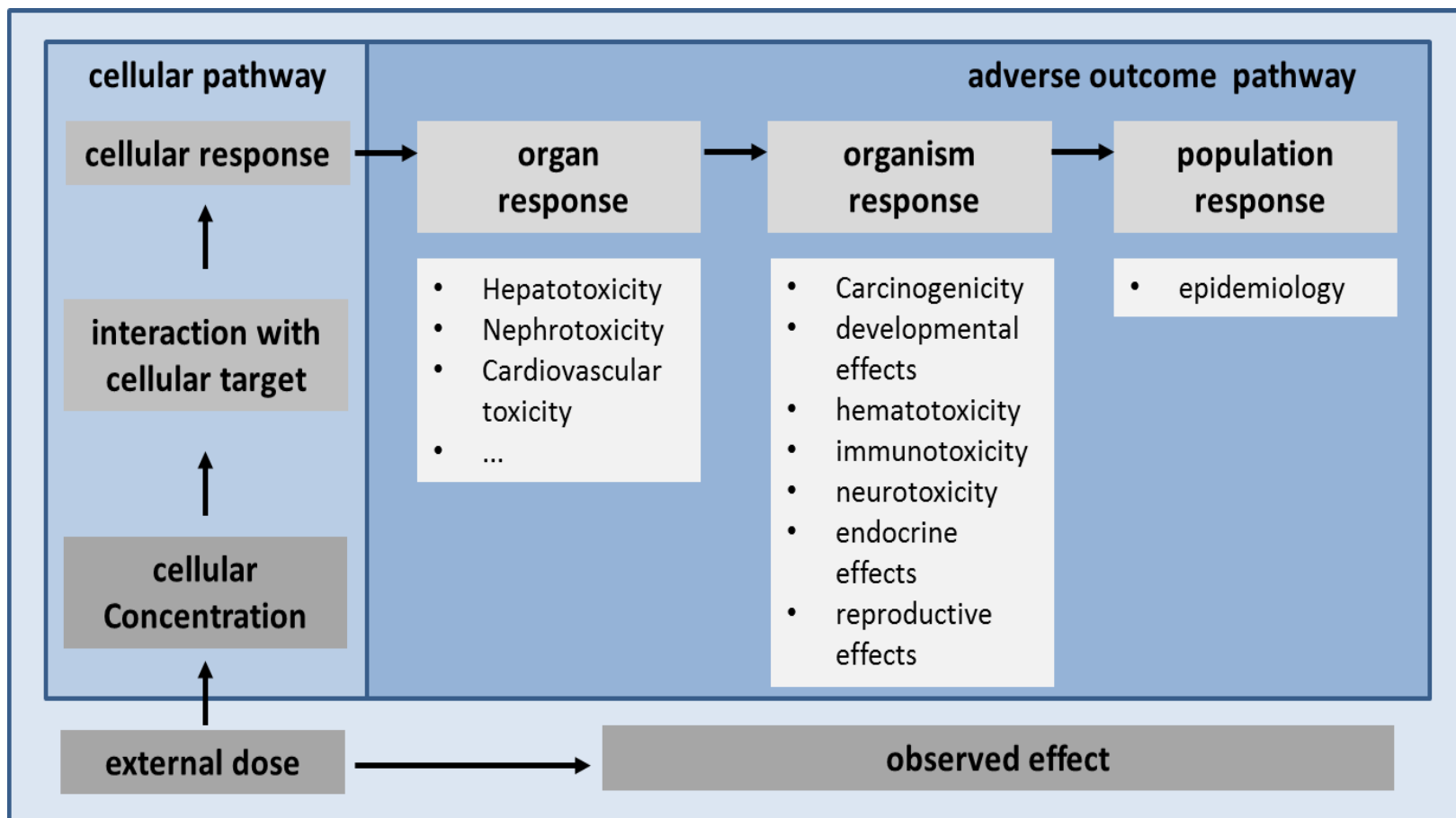
- Biological effects



# Bioassays: *in vivo* and *in vitro*

*In vitro*

*In vivo*

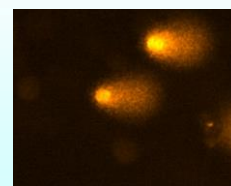
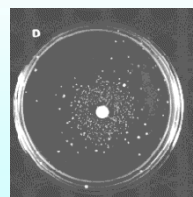
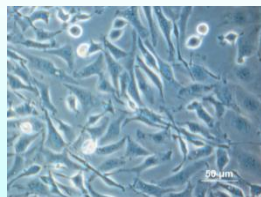


Escher et al. 2011

# Combination of various bioanalytical tests

## Cellular test systems

- Cytotoxicity
- Estrogenicity
- Genotoxicity



## In vivo test systems

- Growth inhibition
- Mortality
- Embryotoxicity



## Mass spectrometric detection and characterization

- LC-MS
- GC-MS
- LC-(HR)MS<sup>n</sup>
- structural characterization
- detection method



## Elimination of pharmaceutical residues in municipal wastewater treatment plants

On behalf of the: Ministerium für Klimaschutz, Umwelt,  
Landwirtschaft, Natur- und Verbraucherschutz  
des Landes Nordrhein-Westfalen



Project management : Ruhrverband, Essen



Institut für Siedlungswasserwirtschaft  
und Abfalltechnik



Lehrstuhl für Siedlungswasser-  
wirtschaft und Umwelttechnik



Abteilung für Hygiene,  
Sozial- und Umweltmedizin



# Investigated municipal WWTP

## WWTP Bad Sassendorf (Lippeverband)

- 12,000 PE.
- Post treatment dosing of ozone to the effluent of conventional biological treatment. Polishing pond.



## WWTP Schwerte (Ruhrverband)

- 50,000 PE.
- Consists of two separated lines. Ozone and/or powdered activated carbon are applied. Recirculation process can be operated.



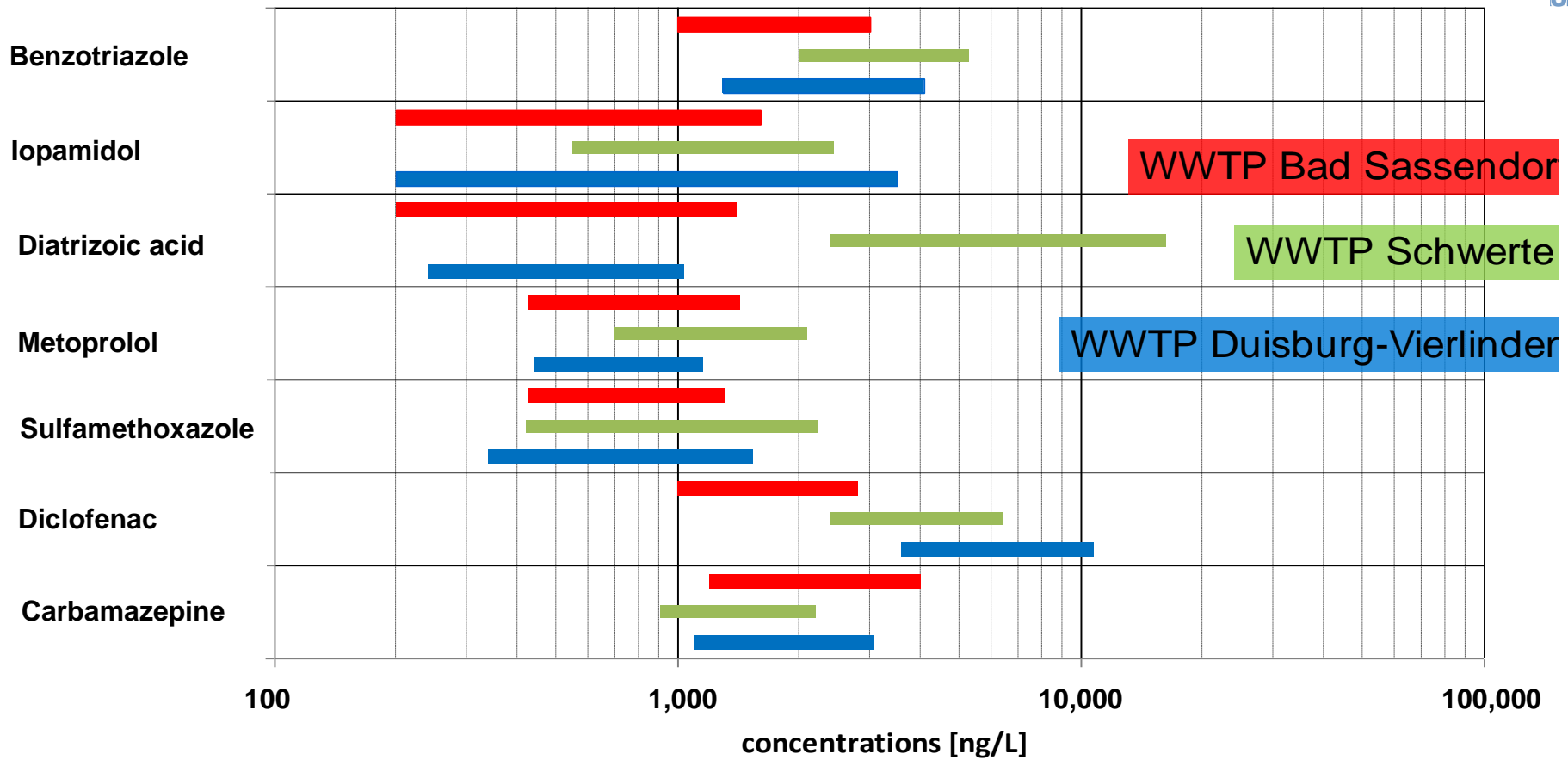
## WWTP Duisburg-Vierlinden (Wirtschaftsbetriebe Duisburg AöR)

- 30,000 PE.
- Two parallel lines have been installed to compare ozone dosage by diffuser or by injector. The wastewater outline is fed to an additional biological stage (fluidised bed reactor).



# Pollution concentrations

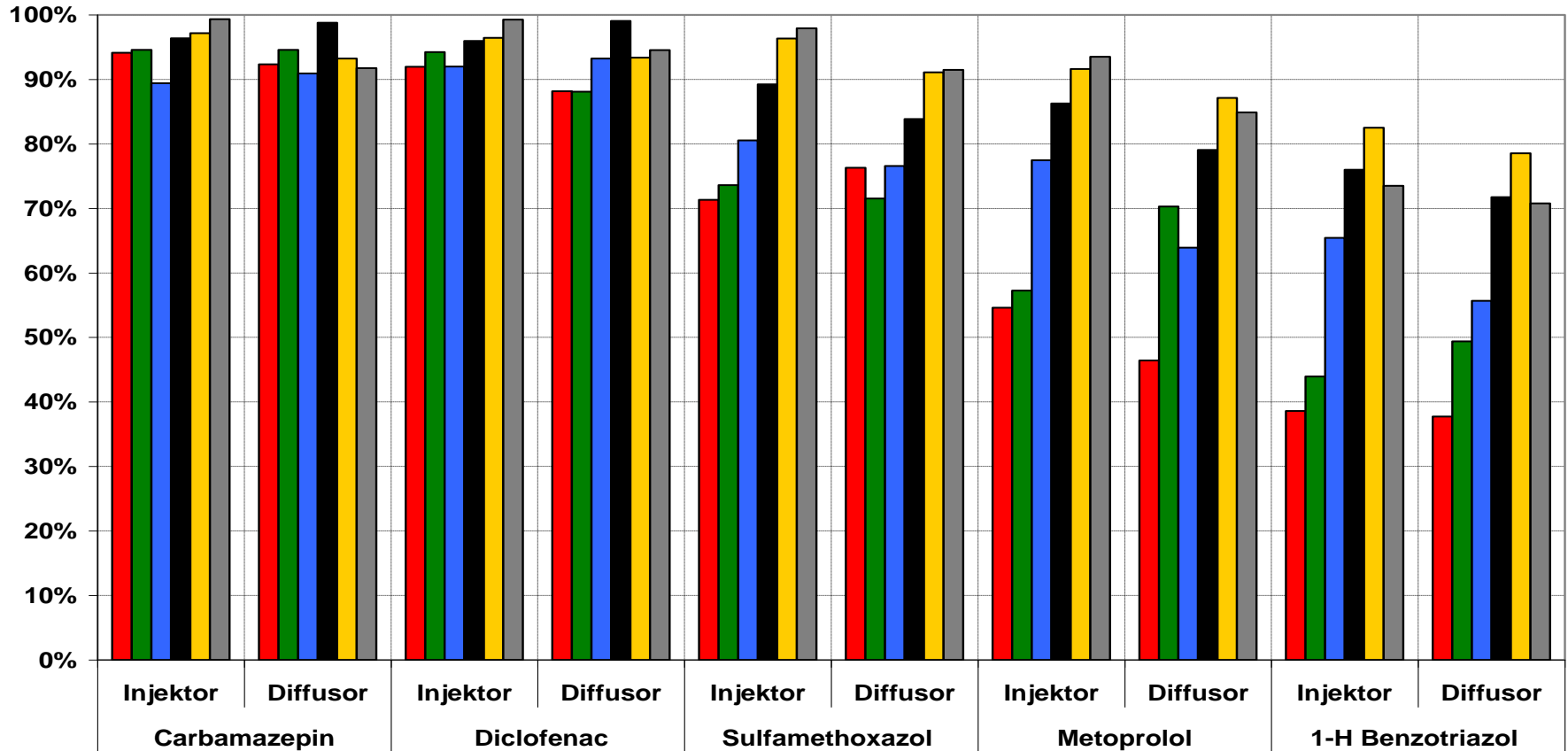
## Effluent concentrations of conventional biological treatment



# Removal efficiency

## Elimination of micropollutants in WWTP Duisburg-Vierlinden after ozonation

■ 3 mgO<sub>3</sub>/l ■ 4 mgO<sub>3</sub>/l ■ 5 mgO<sub>3</sub>/l ■ 6 mgO<sub>3</sub>/l ■ 7 mgO<sub>3</sub>/l ■ 10 mgO<sub>3</sub>/l



# Arge Ozonation, Workpackage 10

## Study of metabolite formation during the use of ozone in municipal wastewater treatment plants

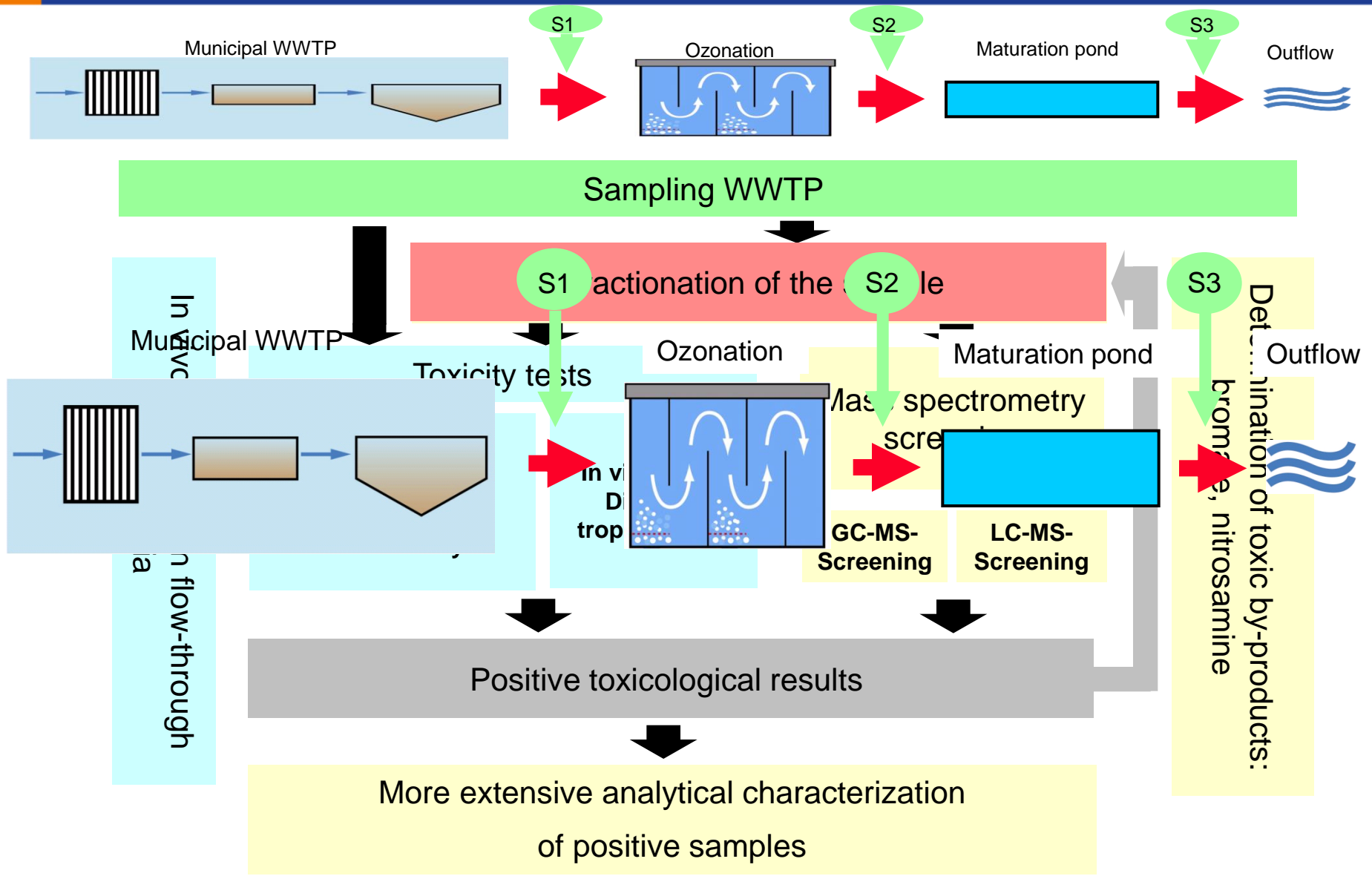
On behalf of the: Ministerium für Klimaschutz, Umwelt,  
Landwirtschaft, Natur- und Verbraucherschutz  
des Landes Nordrhein-Westfalen



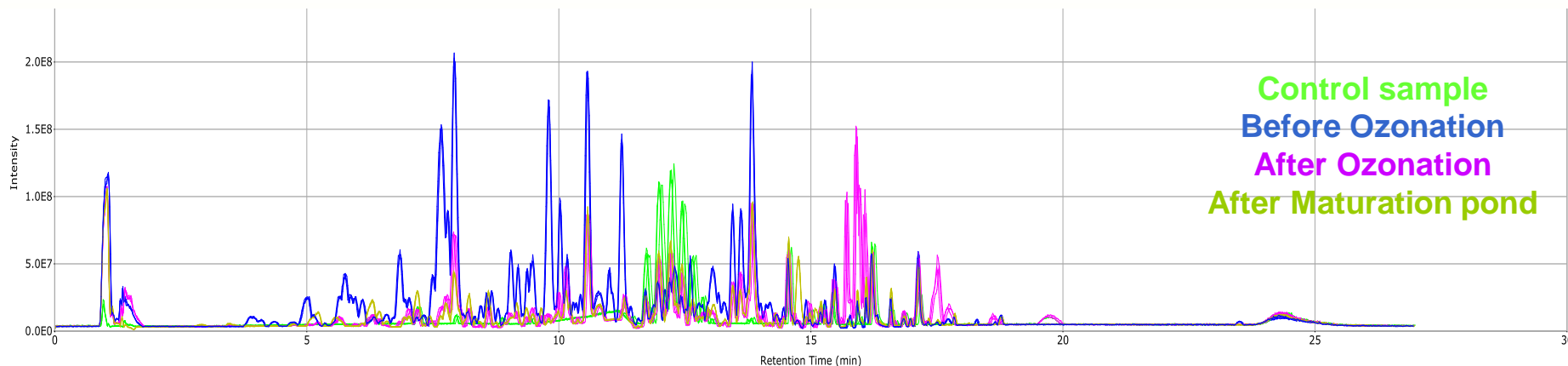
Project management : IWW, Muelheim an der Ruhr



# Toxicity based identification scheme



## LC-Chromatogram of WWTP Bad Sassendorf 5 mg/L Ozone



- Analysis and comparison of samples:
  1. Suspected-Screening using MS-Databases (NIST, DAIOS, MassBank etc.)
  2. Non-Target-Analyses using multi-variate data analyses (PCA)
    - Ozone concentration
    - WWTP
    - Toxicological results



# Bioassays

## In vivo

Green algae (*Desmodesmus Subspicatus*)

Duckweed (*lemna minor*)

Water flea (*Daphnia magna*)

Draw snail (*Potamopyrgus antipodarum*)

Gloss worm (*Lumbriculus variegatus*)

Fish (*Danio rerio*)

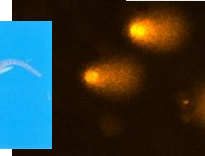
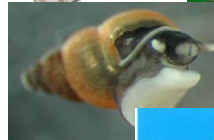
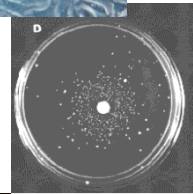
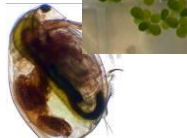
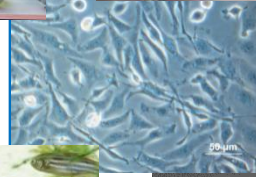
## In vitro

General cell damage (*Cytotoxicity*)

DNA damage (*Genotoxicity*)

Inheritable DNA damage (*Mutagenicity*)

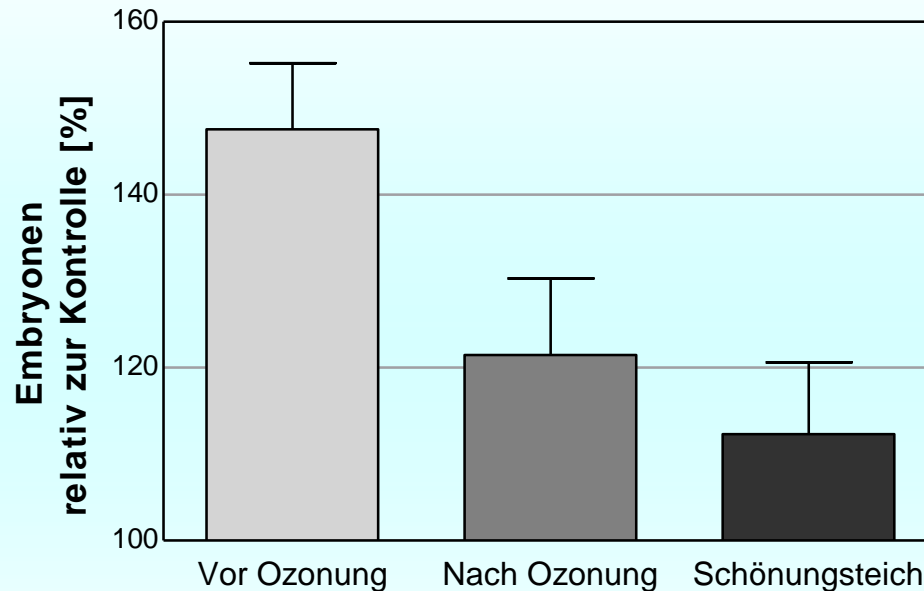
Estrogenic effects (*Estrogenicity*)





# Ecotoxicological studies

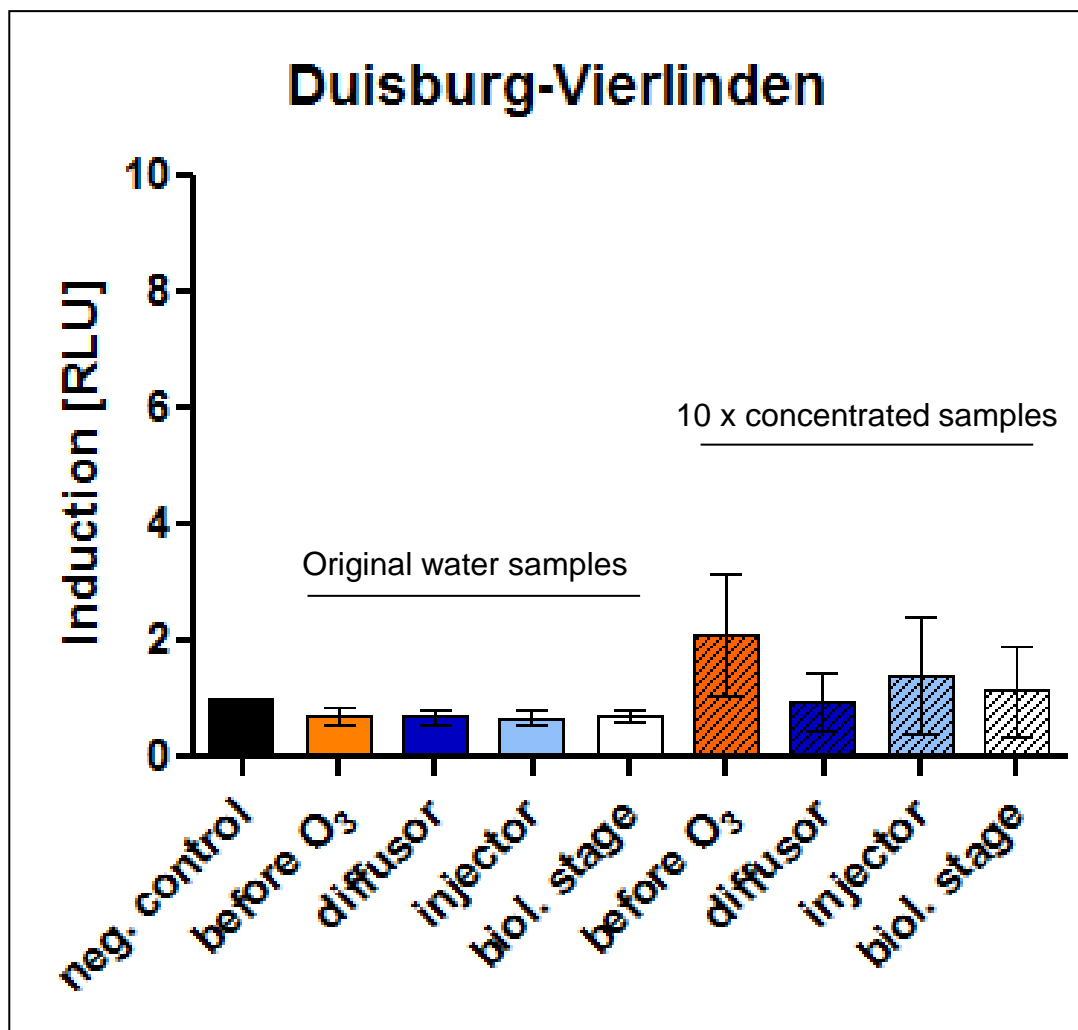
- Draw snail *Potamopyrgus antipodarum* Reproduction test (Estrogenicity)



WWTP Bad Sassendorf

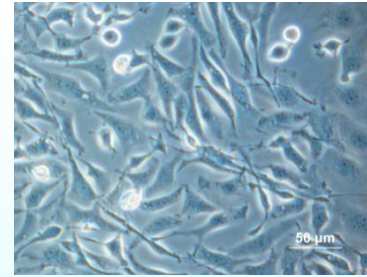
- ➔ Reduction of effects back to the control level after ozonation
- ➔ Effective elimination of estrogenic substances

# Estrogenicity *in vitro*: ER Calux



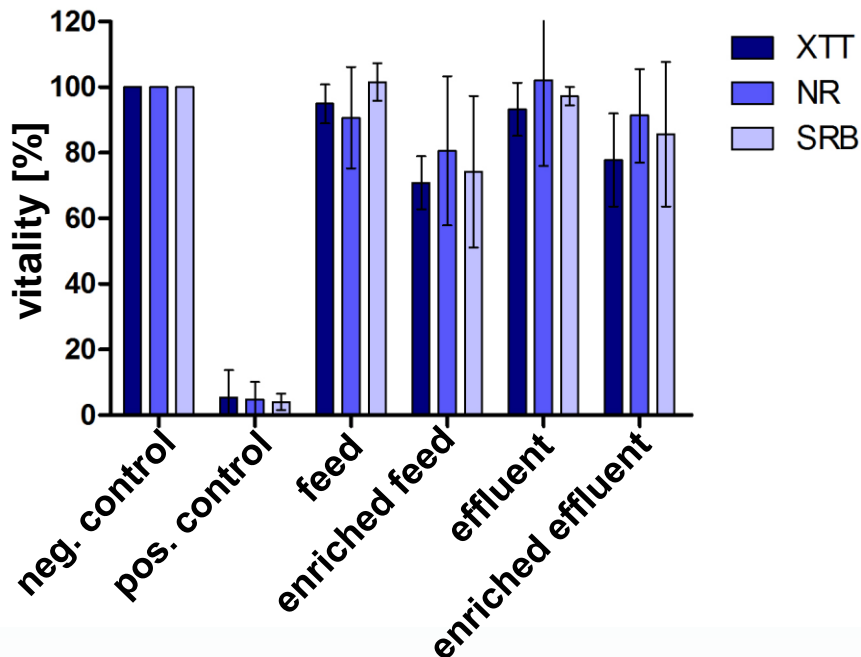
## ■ Cytotoxicity tests

- Multi-Tox-Test  
(general cell damage)



CHO = Chinese Hamster Ovary

### Cytotoxicity



▶ Direct feed and effluent showed no toxicologically relevant effects

▶ The enriched extracts of the feed and effluent after ozonation indicate cytotoxic effects (feed > effluent)

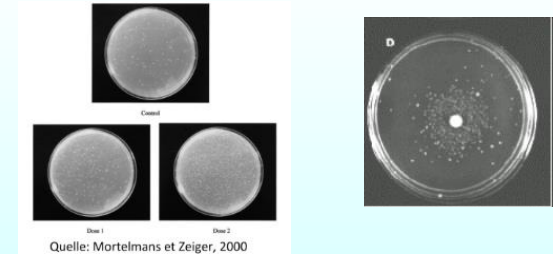
# Toxicological *in vitro* studies

RUB

RUHR-UNIVERSITÄT BOCHUM

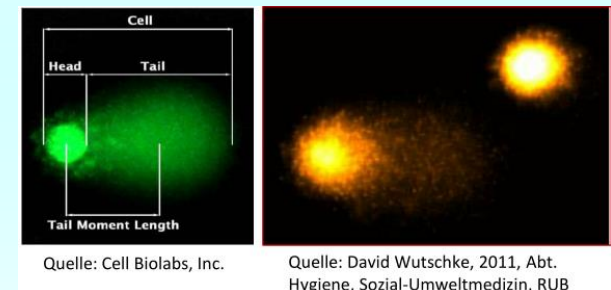
## ■ Mutagenicity (Ames-Test)

- no mutagenic effects
- no dose-response relationship



## ■ Single cell gel electrophoresis (Comet-Assay)

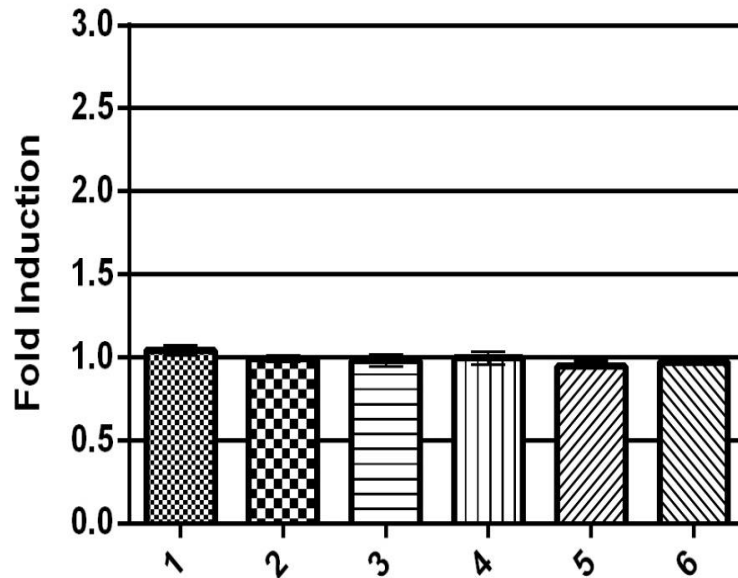
- no significant genotoxic effects



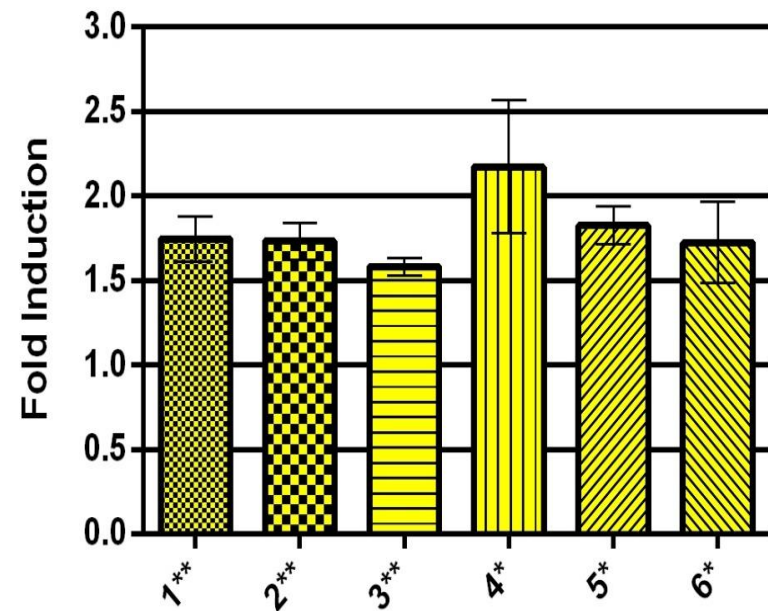
- No acute genotoxicity/mutagenicity in original waste water samples and extracts

# P53 Calux

P53 CALUX: Original Water Samples;  
U2-OS



P53 CALUX: 2.8\* and 4\*\* times  
Concentrated Water Samples;  
U2-OS



For details see poster H. Bielak

# Summary of results

Bioassay	Effect parameter	Effect before Ozonation	Effect after Ozonation	Effect after maturation pond
Cytotoxicity	Cell damage	+	+↓	—
Gentoxicity	DNA damage	—	—	—
Mutagenicity	Inheritable DNA damage	—	—	—
Estrogenicity	Estrogenic effects	+	+↓	n.a.
Green algae	Growth inhibition	+	+↑	+
Duckweed	Growth inhibition	+	+	n.a.
Water flea	Acute toxicity	—	—	—
Gloss worm	Mortality	—	+	n.a.
Draw snail	Estrogenicity	+	—	—
Fish egg	Embryotoxicity	—	—	—

n.a.: not analyzed yet

# Conclusions

- Biologically active substances as well as transformation products are detectable in waste water.
- Application of bioassays are useful for assessment of water quality. Sensitivity of bioassays is different.
- Ozonation is an effective treatment method for degradation of biologically active micropollutants. However, transformation by-products are formed.
- Post treatment steps (maturation pond) can be recommended for further reduction of bioreactivity

# Acknowledgement

## Our partners



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