# Work Area 4

## Implementation of novel rapid and quantitative bioassays for water quality monitoring

Ecotoxicological evaluations and examples for demonstration studies

This project has received funding from the European Union's Seventh Programme for Research, Technological Development and Demonstration under Grant Agreement no. 308339.

# Point of departure

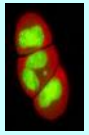
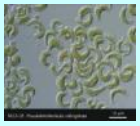
- Mostly *in vivo* bioassays applied for regulatory purposes
- Short-term assays with luminescent bacteria, algae, and water flea most common, partly also fish applied
- Chronic assays mostly conducted with duckweed and/or water fleas
- Apart from the umuC assay for genotoxicity (e.g. applied in Germany), the majority of *in vitro* bioassays was/is lacking regulatory acceptance to date

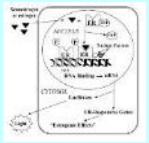
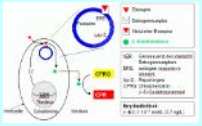
# WP 4.1 Selection and validation of bioassays

**Task 4.1.1: Establish selection criteria, e.g.:**

- Sensitivity
- Reproducibility
- Robustness
- Ease of use
- Cost Effectiveness

**Task 4.1.2: Select bioassays**

**Tasks 4.1.4 and 4.1.5: Develop bioassay guidelines and apply and validate selected (*in vitro*) bioassays**

- Laboratory studies with complementary chemical analytical analysis
- Validation of bioassays

# Bioassay validation

Demonstration of promising technologies to address emerging pollutants in water and waste water

&

**Reconstituted samples**

**Surface water:**
0.13-0.43 ng EEQ/L

**Effluent receiving water:**
1.28-4.3 ng EEQ/L

## INTRA-DAY AND INTER-DAY VARIABILITY

**Reconstituted extracts**

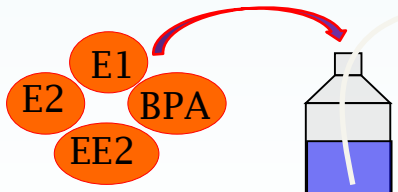
**5x**

**5x**

- Intra-day variability
- Inter-day variability
- Recovery

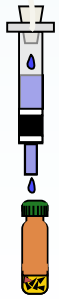
**Testing: ER-TA assays**

## OVERALL VARIABILITY




**Reconstituted water samples**

**Solid Phase Extraction**



**5x**

**5x**

- Overall variability
- Recovery

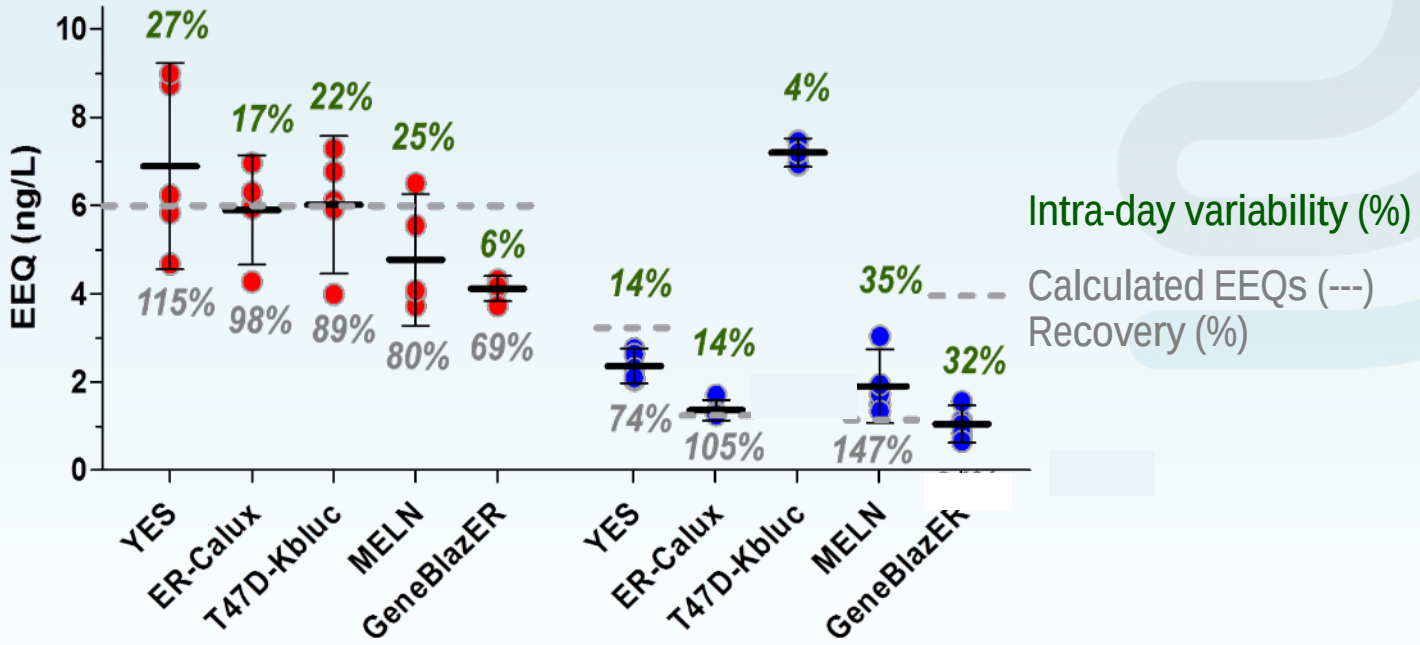
**Testing: ER-TA assays**

*Kunz et al., Ecotox Centre, CH*

# Bioassay variability and recovery

**Variability range: 16 -21 %** **Recovery range: 75 – 102 %**

17β-Estradiol „Effluent-receiving water“




**Are *in vitro* bioassays suitable for monitoring waste and surface waters *?***

***Yes, but…***

**I. Suitable assays have to be standardised (ISO)**
**II. Clear, assay specific guidance on EEQ derivation has to be provided.**

*Kunz et al., Ecotox Centre, CH*

# WP 4.2 Implementation of bioassays for water quality monitoring

## Task 4.2.1: Promotion of regulatory acceptance

ISO

OECD
BETTER POLICIES FOR BETTER LIVES

DIN

## Task 4.2.2: Establishment of a Testing Framework

Establish a concept on how to evaluate bioassay results for risk assessment.

## Task 4.2.3: Introduce bioassays at labs and water utilities

- Great interest in bioassays in Switzerland
- A cost-effective and easy to use set of tests would be very much appreciated

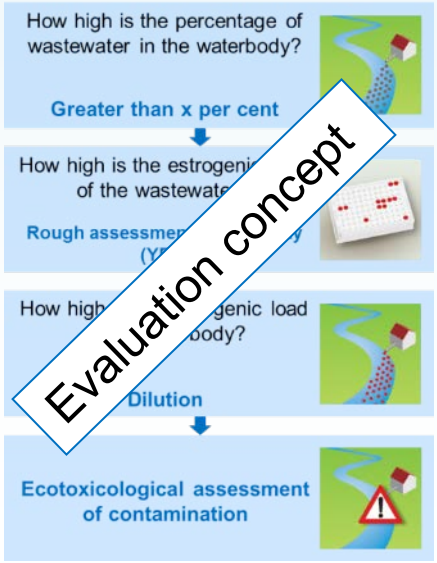
## Task 4.2.4: Demonstration in pilot studies

- ARA Neugut Dübendorf, Switzerland
- Assess samples from other pilot plants

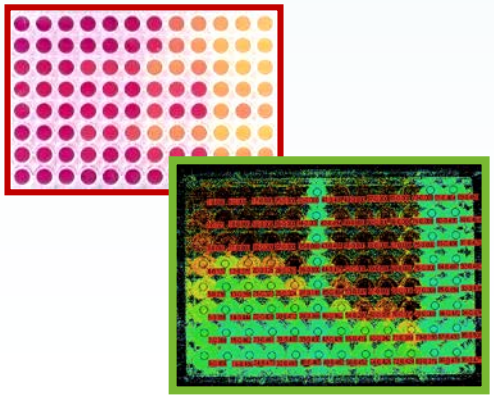
# Water quality monitoring

- Develop a routine concept for water quality assessment in Switzerland

**Recommendation of promising methods** to assess surface water quality




Estrogenic and photosystem II-inhibiting effects



**Evaluation of surface water quality** based on ecotoxicological bioassays

*Kienle et al., Ecotox Centre, CH*

# *In vitro* bioassays to screen for endocrine active pharmaceuticals in context of the EU Water framework directive

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## *In vitro* bioassays to screen for endocrine active pharmaceuticals in surface and waste waters

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ABSTRACT

In the context of the European Water Framework Directive (WFD) it is fully recognized that pharmaceuticals can represent a relevant issue for the achievement of the good chemical and ecological status of European surface water bodies. The recent European Directive on the review of priority substances in surface water bodies has included three pharmaceuticals of widespread use (diclofenac, 17α-ethinylestradiol (EE2), 17β-estradiol (E2)) in the European monitoring list, the so-called watch list. Endocrine active pharmaceuticals such as EE2 and E2 (also occurring as natural hormone) can cause adverse effects on aquatic ecosystems at very low levels. However, monitoring of these pharmaceuticals within the watch list mechanism of the WFD and national monitoring programs can be difficult because of detection problems of most routine analytical methods. With proposed annual average Environmental Quality Standards (AA-EQS) of 0.035 ng/L and 0.4 ng/L, respectively, the estrogenic pharmaceutical EE2 and the natural hormone E2 are among those substances. Sensitive *in vitro* bioassays could reduce the current detection problems by measuring the estrogenic activity of environmental samples. In a short review article the application of this approach to screen and assess the risks of endocrine active pharmaceuticals with a focus on estrogenic pharmaceuticals in environmental waters is discussed.

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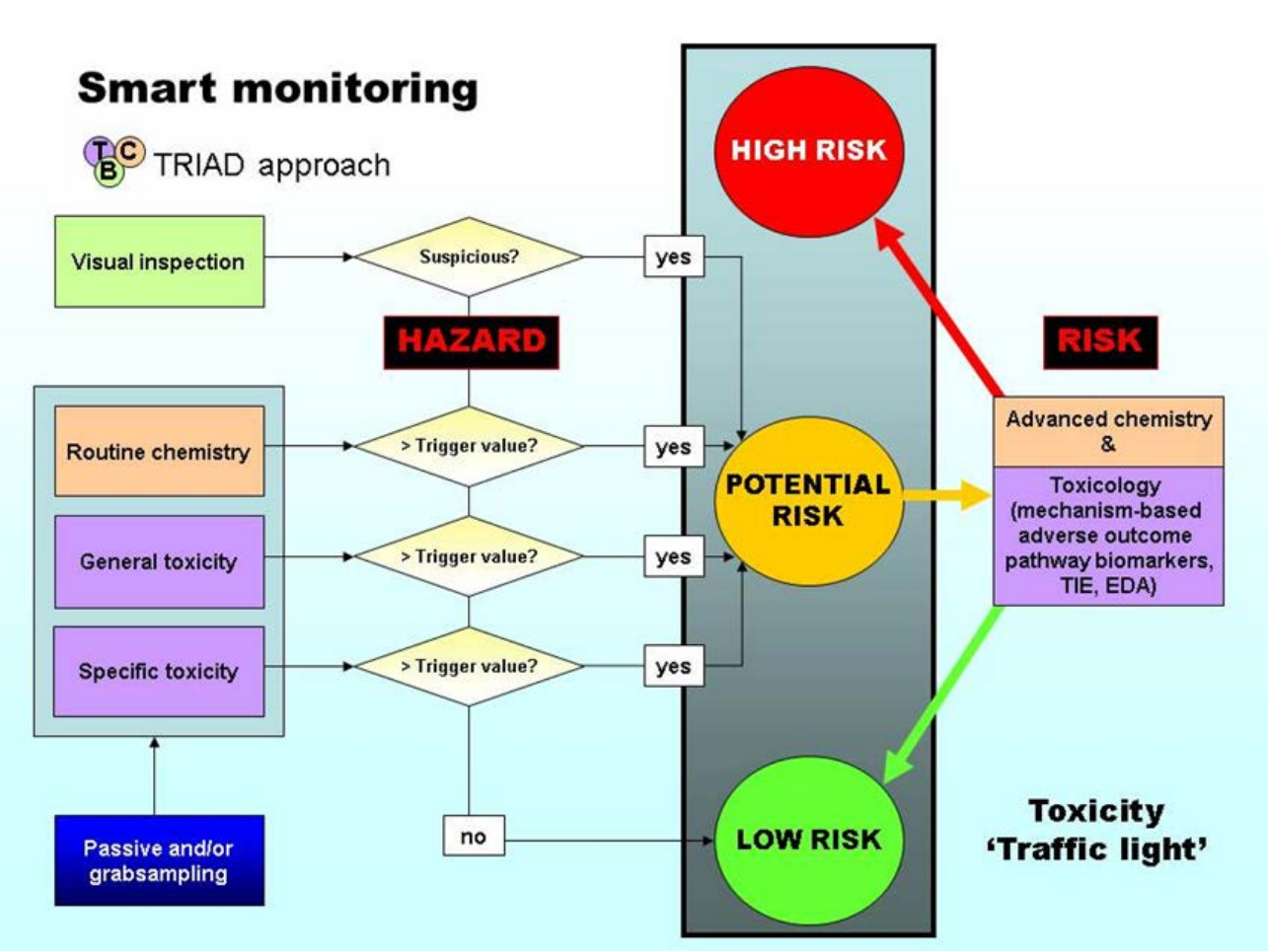
# SMART MONITORING

- Change conventional monitoring practice

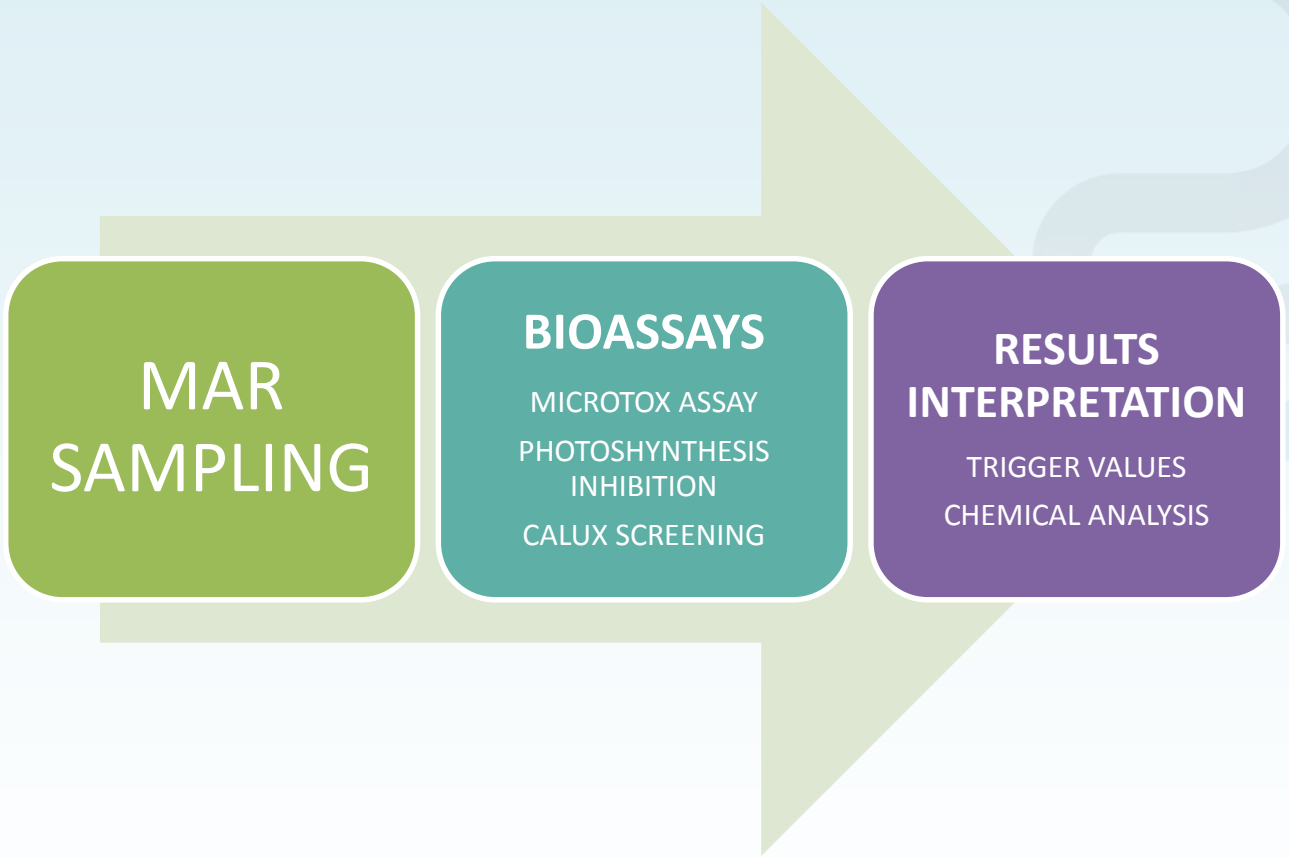



**Analytical chemistry alone might not cover the entire load of water pollution**

**Multidisciplinary quality assessment**

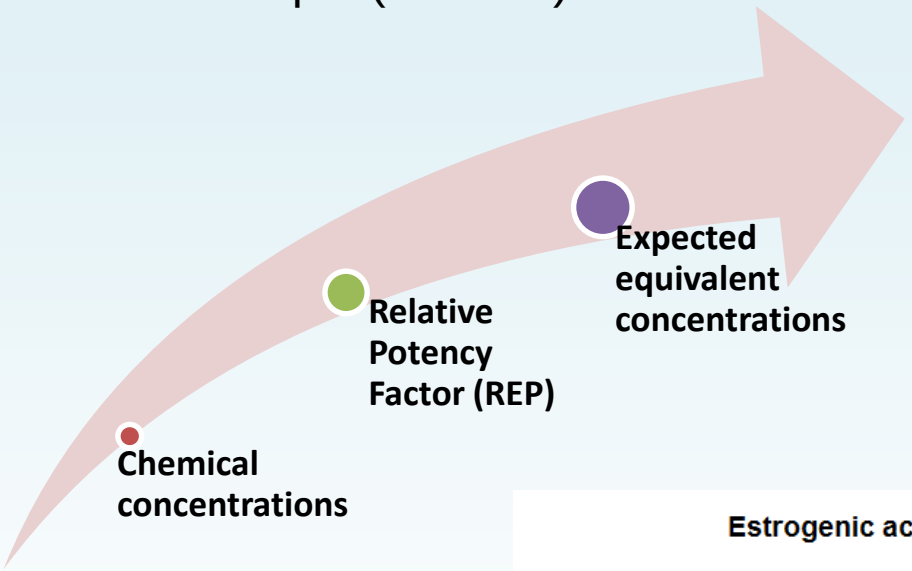
*Ron van der Oost, Waternet, NL*

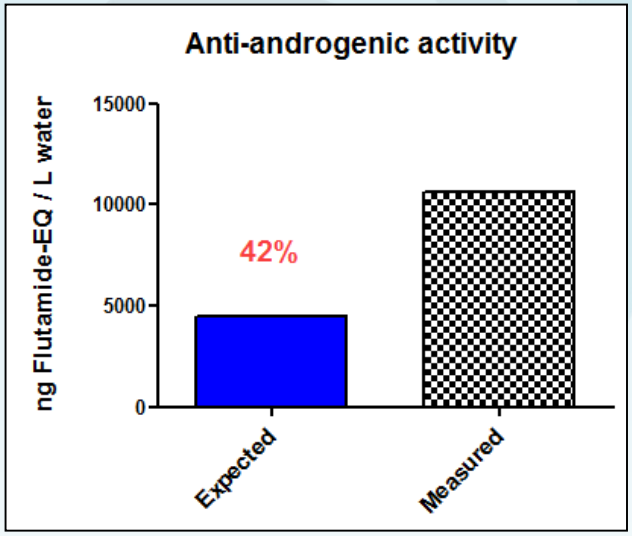
# Demonstration studies

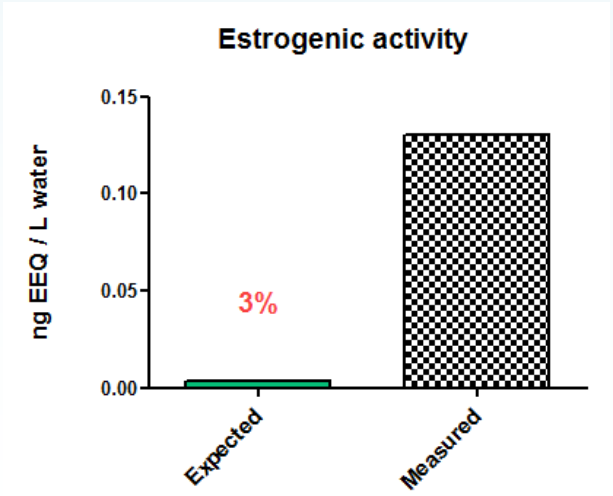


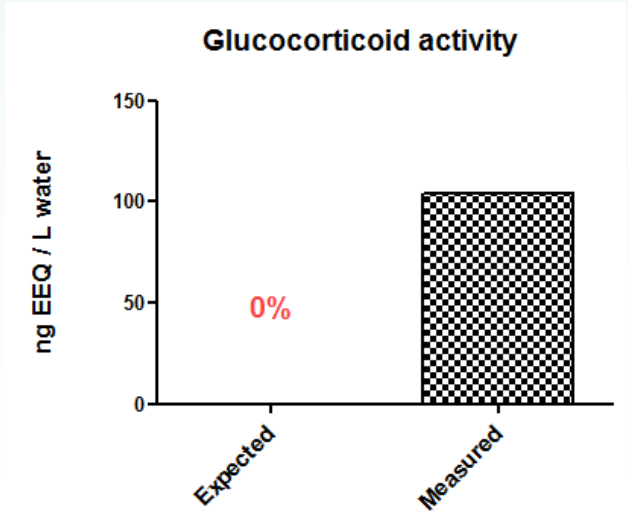
# Chemical analysis vs Bioassays

**Example**:
WWTP sample (CAS-01)

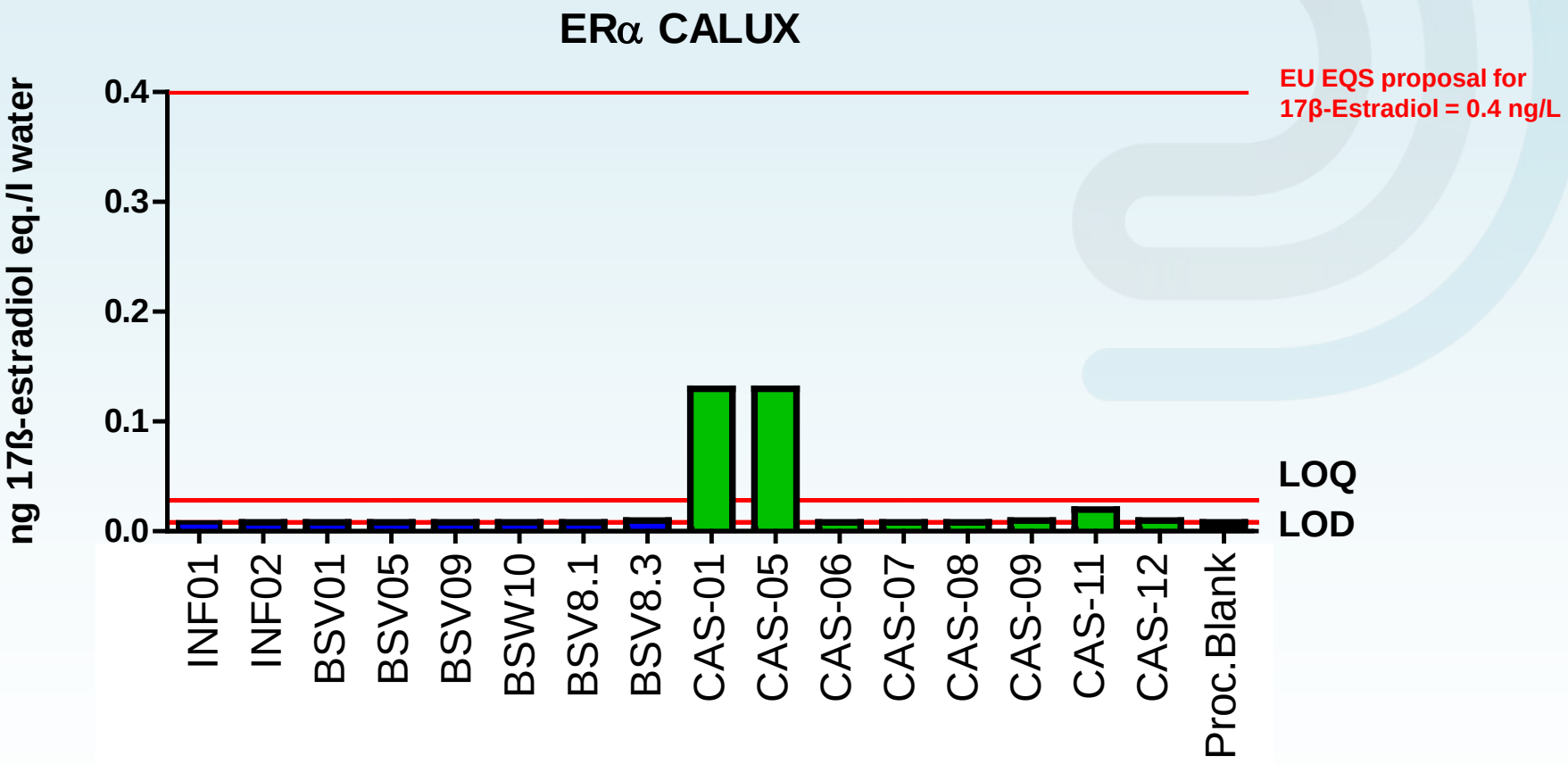


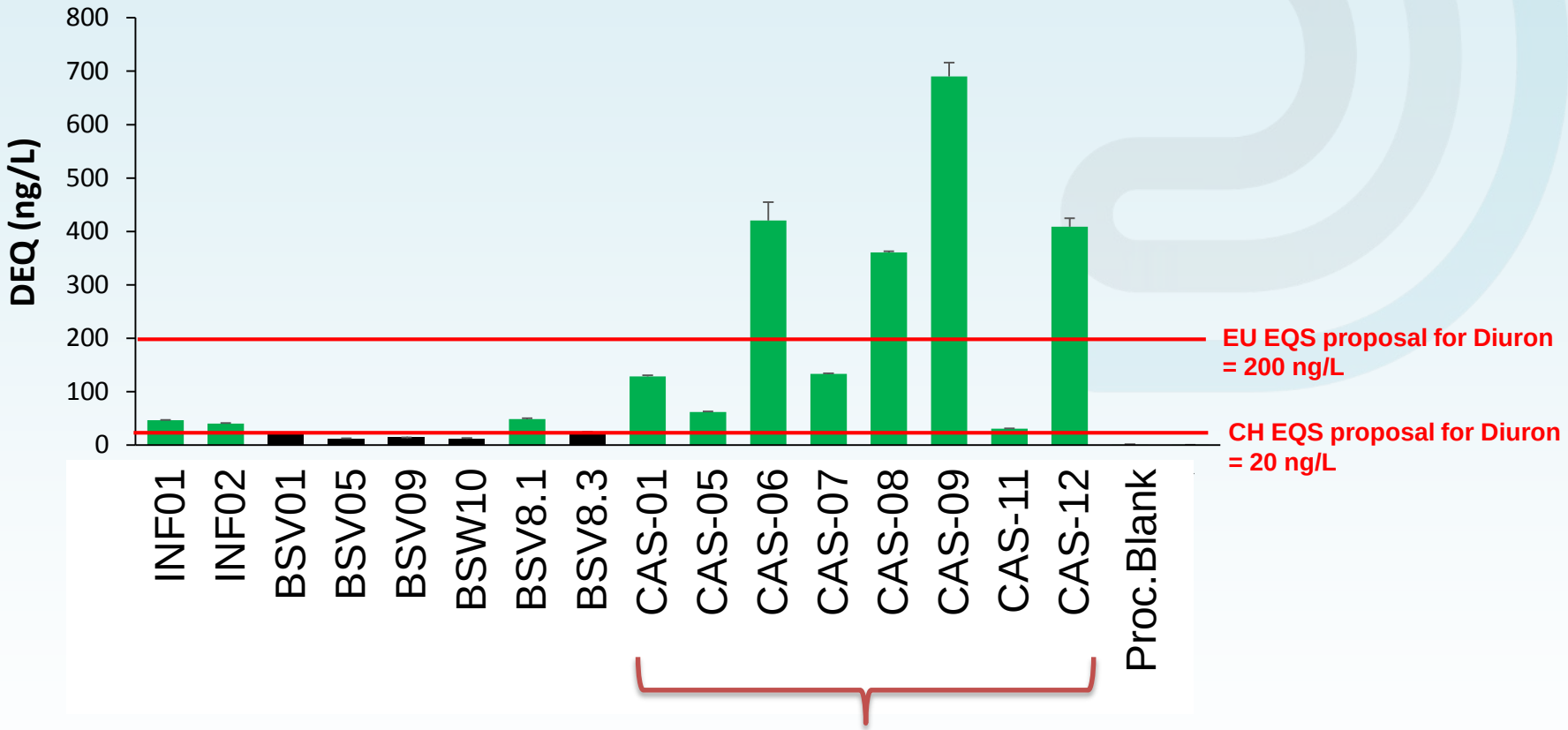



# Estrogenic Activity



# Photosynthesis Inhibition

## 1st campaign




**AGRICULTURAL ACTIVITIES**

**PESTICIDES**

DEQ = Diuron equivalent concentration

# Summary and Outlook

- In vitro bioassays can be applied for environmental monitoring of waste and surface water
- Monitoring concepts are developed
- The application of *in vitro* bioassays in the various demonstration studies has shown their added value.
- Further validation and promotion of regulatory acceptance is needed.

# Thank you for your attention!

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