



The application of endocrine *in vitro* and *in situ* based bioassays


-

a comparison at a German WWTP



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- Large-scale ozonation plant for treatment of wastewater
- Evaluation of ozonation concerning influence on receiving stream
 - Improvement of water quality via reduction of anthropogenic residual contaminations
- Determination of the *status quo* before implementation of the large-scale ozonation plant (phase 1) ✓
- Experiments on half-scale ozonation plant ✓
- Construction of large-scale ozonation plant ✓
- Determination of the status after implementation of the large-scale ozonation plant (phase 2) 
- How is the water quality going to be improved by further reduction of trace contaminants?
 - Or do other factors have a higher impact on the water quality?

- Wurm River
 - W2: upstream ROB and WWTP
 - W3: downstream ROB, upstream WWTP
 - W4: directly downstream WWTP
 - W5: ~ 2,5 km downstream WWTP



ROB = rain overflow basin
WWTP = wastewater treatment plant

source: google.maps

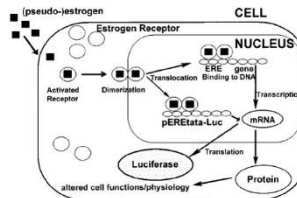
- Classical wastewater assessment
 - Algae growth inhibition assay (DIN EN ISO 8692/ DIN 38412-33)
 - Acute daphnia immobilization assay (DIN EN ISO 6341)
 - Luminescent bacteria assay (DIN EN ISO 11348-1)
 - Fish embryo toxicity assay (DIN EN ISO 15088, OECD 236)
- Mechanism specific assays
 - L-YES assay (ISO/FDIS 19040-1:2018(E))
 - ER α CALUX[®] assay (ISO/FDIS 19040-3:2018(E))
 - H295R-S assay (OECD 456)
 - Ames fluctuation assay (ISO 11350:2012)
 - Micronucleus assay (DIN EN ISO 21427-2, OECD 487)
- *In situ* experiments
 - Gammarid feeding experiments
 - QuEChERS with gammarids
 - Reproduction assay with *Potamopyrgus antipodarum* (OECD 242)
 - Caging experiments with *Oncorhynchus mykiss*

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- Before implementation of the large-scale ozonation plant

- ER α CALUX[®] Assay

- with extracts (0.1 % DMSO)
- According to ISO guideline



Legler et al. 1999

- Reproduction assay with *Potamopyrgus antipodarum*

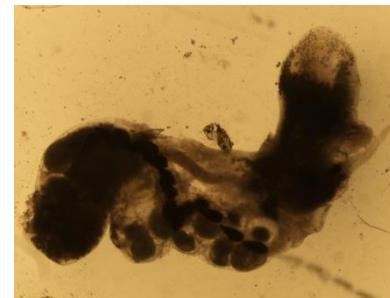
- Summer 2017
- Field study
- Temperature measurement in parallel
- PC: forskolin – reduction of reproduction
- Based on OECD guideline



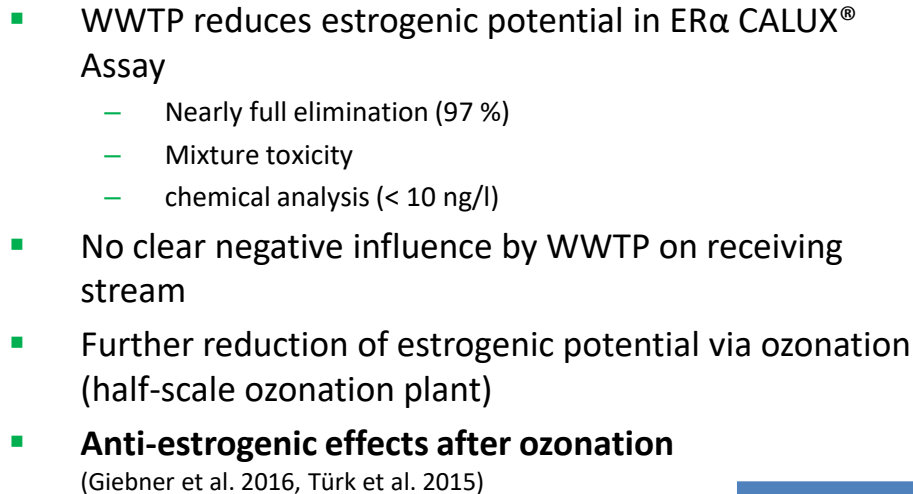
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Cage with snails inside

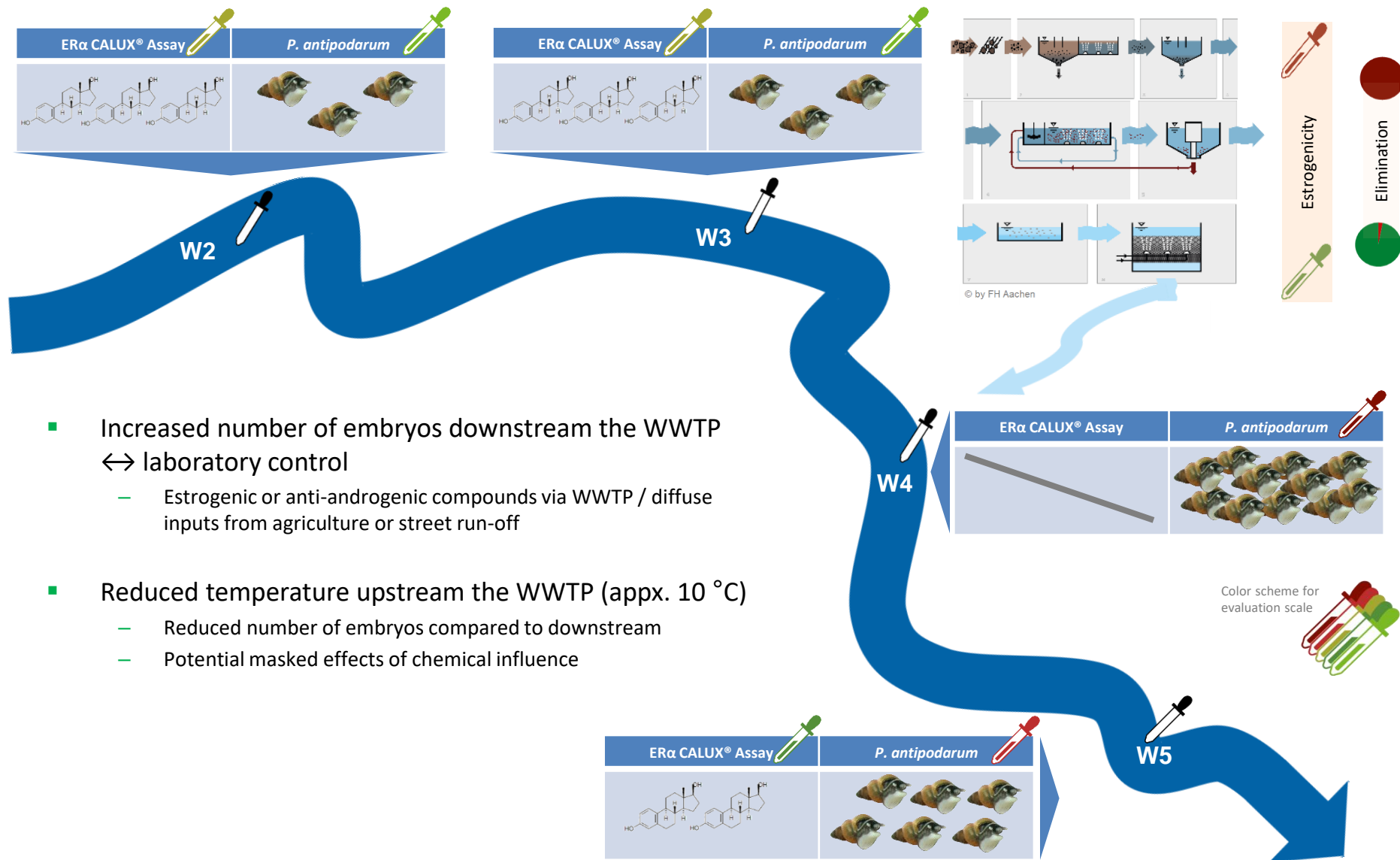


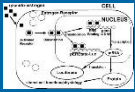
Snail without shell





Results and Discussion





Results and Discussion



- No clear relation between *in vitro* and *in situ* experiments
 - *In vitro* → reduction of estrogenic potential
 - *In situ* → (significant) increased number of embryos per snail
 - In alignment with literature (Galluba et al. 2012)

- While conducting *in situ* experiments a multitude of different **environmental factors** can have an influence on the reproduction of snails (e.g. temperature reduction leads to a decreased number of embryos → temperature experiments) (Sieratowicz et al. 2011)

- Different water composition

- Estrogenic compounds might not be responsible for the increased embryo number but **anti-androgenic compounds** (Weiss et al. 2009, Schmitt et al. 2011)

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Applicant:



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Thank you for your attention!



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