Endocrine disrupting potential of relevant exposure mixtures and prioritized pollutants

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INTRODUCTION

Water polluted with highly complex mixtures of chemicals causing endocrine disruption.

- **incomplete removal** of organic micropollutants by conventional waste-water treatment plants (WWTPs)
- **more toxic/potent metabolites** created by microbial transformation of parent compounds
- **unknown drivers** of possible adverse biological effects
- **unknown** adverse effect **pathways**
- monitoring of a **few priority substances**

AIMS

Development of innovative approaches for the identification of compounds responsible for endocrine disruption in water ecosystems.

- novel method **pull-down assay:** development and optimization

Design of ligand binding domain (LBD) of hormone receptor into a plasmid construct, its expression, following extraction and purification. Assay is used to determine drivers of adverse effects by interaction between LBD and pollutant mixture.

- **effect-directed analysis (EDA) + pull-down assay:** identification of the effect drivers

METHODS

- **Battery of *in vitro* bioassays**

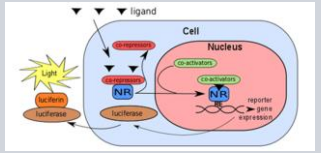
Evaluation of the endocrine-disruptive potential of environmental water samples

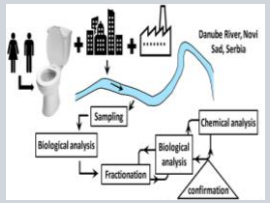
- **Effect directed analysis (EDA)**

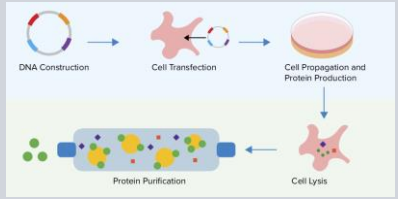
Repeated biotesting, HPLC fractionation and chemical analysis of active fractions

- **Pull-down assays**

Novel complementary high-throughput tools for identification of endocrine disruptive effect drivers







RESULTS

- **anti-androgenicity: Danube River long-term passive samples at all sites (Fig.3)**

highest at Side 6-Pančevo (SRB), (Fig.4a)), high dilution of contaminants

- **androgenicity: smaller streams (Živný potok, CZ),**

highest at WWTP effluent (Fig. 4b)), lower dilution of contaminants

- **LBD of human androgen receptor (AR-LBD) designed into plasmid for heterologous expression in *E. coli* for pull-down analyses of active samples (Fig.1)**
- **Similarity of AR-LBD in human, mammals, fish and amphibians was assessed using MeGaX software and SeqAPASS tool (level 2) → AR-LBD is relatively well-conserved → further assessments need to be done for individual ligands (level 3) (Fig.2)**

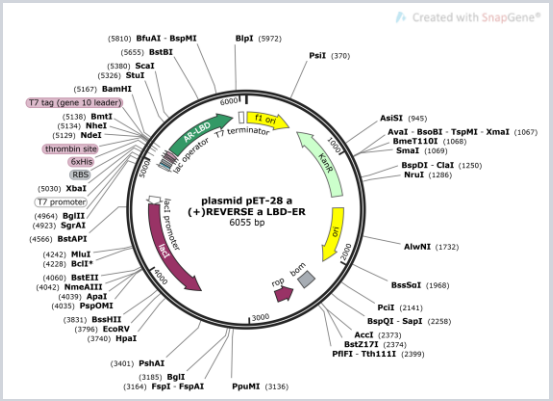


Fig.1: Plasmid design (pET28) for heterologous expression of AR-LBD in E. coli

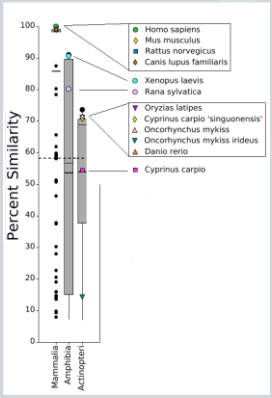
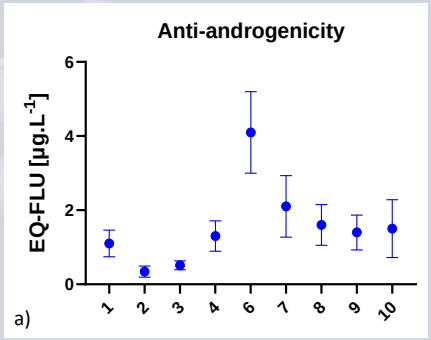


Fig.2: Alignment of protein sequences of AR-LBD with SeqAPASS tool (% similarity)

Fig.3: Sampling sites on the Danube River – JDS4



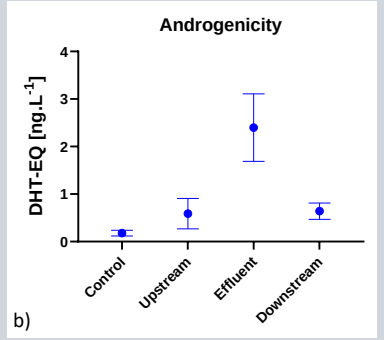


Fig.4: Biological effect equivalents (BEQs) of passive sampler's extracts collected in a) Danube River and b) Živný potok (CZ)

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FUTURE PROSPECTS

- **Development and optimization of pull-down assays for TTR, PPARγ, AR and their application for environmental samples**
- **EDA of highly active samples from Živný potok to identify effect drivers (AR, TR, TTR)**