

## **CEFIC Long-Range Research Initiative**

### **Request for Proposal (RfP)**

**LRI Project code: LRI-EMSG55**

**Title: Comparison of natural and synthetic endocrine active compounds -  
Assessment of potential combinatory effects**

**Deadline: 31 August 2010**

### **Background**

Exposure of humans and the environment to mixtures especially of endocrine active compounds (EAC) is of growing concern. Within the recently published EU Commission's *State-of-the-art Report on Mixtures Toxicity* it is stated that "more empirical evidence on the joint action of environmentally realistic mixtures, composed of members from different chemical and functional classes is needed" (Kortenkamp et al. 2009). The presence of naturally occurring compounds acting as endocrine active substances in the environment and their interference with monitoring procedures is to date poorly understood. Particularly concerning the antiandrogenic mode of action, there is a clear lack of information on background endocrine activities. In some cases, like with the River Lambro (Italy), endocrine activity of environmental samples (Urbatzka et al. 2007) in the scientific literature was immediately linked to potential chemical exposure without knowledge about possible natural sources of EACs.

Although there is evidence that many natural substances have endocrine effects (Barrett 1996.), little is known about the overall contribution of natural substances to environmental endocrine disruption and potential matrix or mixture effects with synthetic substances. The relevance for the environment can not be evaluated as most available studies are only *in vitro* studies. As leaf litter is one of the major input sources of organic carbon in freshwater systems and can contribute to about 30% of the total dissolved organic carbon (DOC) in surface waters (Meyer et al., 1998) the evaluation of this source is of major interest (Hermelink et al., in press).

### **Objectives**

The objective of the project is to characterize in a first step the potential endocrine activities of natural substances (leaf litter) concerning their endocrine modes of action (e.g. (anti)estrogenic (Denny et al. 2005), (anti)androgenic, and (anti)gestagenic), to suggest synthetic EAC for investigation and then to investigate the potential combinatory/mixture effects with natural endocrine active substances on the reproduction and development of fish. Thereby, the question whether natural and synthetic endocrine active substances act in a combinatory manner and how (additive, or inhibitory, or synergistic, or effect-triggered by the most potent endocrine substance) will be answered.

- Detailed endocrine characterization of natural substances (leaf litter) *in vitro* using yeast estrogen (YES), yeast androgen (YAS) and yeast progesterone (YPS) screen concerning their (anti)estrogenic, (anti)androgenic, and furthermore (anti)gestagenic activities to improve the significance of common screening procedures for environmental EACs.
- *In vivo* investigation of these extracts concerning their effects on the reproductive biology of fish, by using a well established ecotoxicological model (e.g. *Danio rerio* zebrafish) and a wildlife species (e.g. *Rutilus rutilus* roach).
- Further *in vitro* studies with the most relevant extracts, in combination with synthetic endocrine active substances showing combination and/or matrix effects of environmentally relevant mixtures.
- Investigation of mixture effects of natural and synthetic endocrine active substances concerning reproductive biology of fish by long and short term exposures using a well established ecotoxicological model and a wildlife species.
- Assessment of the contribution of synthetic substances in relation to, and in combination with, natural factors occurring already as background in the environment.

## Scope

The Principal investigator will be required to submit a yearly progress report during the course of the project. A detailed review of the project outcome should be published in a peer reviewed journal. The investigators are expected to present the project outcome at suitable scientific conferences as a contribution to the ongoing scientific discussion and the EU Commissions evaluation of the relevance of mixture effects.

## LRI Funding

Budget in the order of € 300,000

## Timing

Start mid 2010, duration up to 3 years

## References

- Barrett, J. (1996). Phytoestrogens. Friends or foes? *Environ. Health Perspect.* 104, 478-482.
- Denny, J., Tapper, M., Schmieder, P., Hornung, M., Jensen, K., Ankley, G., and Henry, T. (2005). Comparison of relative binding affinities of endocrine active compounds to fathead minnow and rainbow trout estrogen receptors. *Environ. Tox. Chem.* 24, 2948-2953.

Hermelink, B., Urbatzka, R., Wiegand, C., Pflugmacher, S., Lutz, I., and Kloas, W. (2010): Aqueous leaf extracts display endocrine activities *in vitro* and disrupt sexual differentiation of male *Xenopus laevis* tadpoles *in vivo*. *Gen. Comp. Endocrinol.* (in press) doi:10.1016/j.ygcen.2010.03.005

Kortenkamp, A., Backhaus, T., Faust, A. (2009). *State of the Art Report on Mixture Toxicity*.

Meyer, J.L., Wallace, J.B., and Eggert, S.L. (1998). Leaf litter as a source of dissolved organic carbon in streams. *Ecosystems* 1, 240-249.

Urbatzka, R., van Cauwenberge, A., Maggioni, S., Vigano, L., Mandich, A., Benfenati, E., Lutz, I., and Kloas, W. (2007). Androgenic and antiandrogenic activities in water and sediment samples from the river Lambro, Italy, detected by yeast androgen screen and chemical analyses. *Chemosphere* 67, 1080-1087.