

EDA-EMERGE Specialized Course 2:

“In vitro and in vivo assays of endocrine disrupting chemicals (EDCs) using fish models “

Venue: INERIS, Verneuil-en-Halatte, France

Organizer: Dr. Selim Aït-Aïssa

Date: 30.01 to 31.01.2013

Time: 8h00 – 19h00, 9h00 – 11h00

Course description

The SC2 was a 2 day EDA-EMERGE training course on *in vitro* and *in vivo* assays of endocrine disrupting chemicals (EDCs) using fish models.

The course aimed to provide theoretical background on EDC in the environment, considering molecular and cellular mechanisms of action and physiological impacts on fish. Further, the course provided theoretical and practical views of existing modelling approaches dealing with dose-responses curves obtained in bioassays as well as implications for TEQ calculation. During a practical laboratory training the participants were introduced *in vitro* and *in vivo* bioassays using reporter cell lines and transgenic zebrafish.

This amounted to a minimum total academic involvement of 15 hours and equivalent to 0.5 ECTS points for the participants.

AGENDA

Wednesday, 30.01.2013		
Time	Title	Lecturer
8:00	Welcome	
9:15-10:15	Introduction to EDCs in the aquatic environment : impacts on fish	François Brion
10:15-11:15	Cellular, molecular and structural views of ligand-nuclear receptor (NR) interactions	Patrick Balaguer
11:15-11:30	Coffee break	
11:30-11:45	In vitro cell-based assays for EDCs	Selim Aït-Aïssa
11:45-13:00	Statistical and mathematical models for bioassay data analysis	Cleo Tebby, Alexandre Péry
13:00-14:00	Lunch break	
14:00-19:00	Practical demonstrations 1) In vitro bioassays using reporter cell lines (2h) 2) In vivo bioassays using zebrafish embryos (2h)	

Thursday, 31.01.2013		
Time	Title	Lecturer
9:00-11:00	Bioassay readouts 1) Luciferase assay (micro-plates from in vitro bioassays) 2) Data analysis using "R" software	

COURSE CONTENT

The course consisted of lectures, workshops and practical demonstrations, covering the following topics:

- Introduction to EDCs in the aquatic environment and impacts on fish, covering:
 - Definitions
 - In situ effect of xeno-estrogens on fish at individual et population levels
 - Other modes of actions of EDCs: brain and gonad steroidogenesis, HPG axis disruption, impacts on reproductive status.
- Cellular, molecular and structural views of ligand-nuclear receptor (NR) interactions with regard to:
 - Structure and functioning of nuclear receptors : concept of antagonism, full and partial agonism, transcription co-regulators, activation of natural and reporter genes
 - NRs as targets of EDCs :
 - Interaction NR-EDCs : crystallization et modeling.
 - NRs profiling: Pan-(ant)agonism of environmental ligands towards different NRs;. Case of bisphenol A.
 - New strategy for establishment of reporter cell lines to study EDCs towards human and zebrafish NR by using the CMT platform.
- In vitro cell-based assays for EDCs, including the:
 - Sources of variation between existing in vitro bioassays for estrogenicity assessment: influence of the cell context, cross-species differences, cross-talks... Examples of fish versus human ER assays.
 - Assessment of complex environmental extracts: uses and pitfalls; confounding factors; establishment of dose-reponse curves.
- Statistical and mathematical models for bioassay data analysis, demonstrating:
 - Mathematical models for modelling of biological effects: existing models, how to deal with variability...
 - An application to dose-response modelling in in vitro bioassays; TEF/TEQ calculation.
- Practical demonstrations on in vitro bioassays using reporter cell lines and in vivo bioassays using zebrafish.
- Analyzing micro-plates from in vitro bioassays readouts on the example of the luciferase assay as well as data analysis using the “R” software