EDA-EMERGE Specialized Course 9:

"Hyphenation of cell-based assays with microfractionation procedures"

Venue: Institute for Environmental Studies, VU University, Amsterdam, Netherlands

Organizer: Dr. Pim Leonards, Dr. Marja Lamoree

Date: 21.01 to 22.01.2014

Time: 9h00 – 17h00; 09h00 – 16h00

Course description:

The SC9 was a 2 day EDA-EMERGE training course on hyphenation of cell-based assays with microfractionation procedures. Said training course focused on teaching the fundamentals of developing microfractionation procedures to obtain fractions containing relatively simple mixtures of compounds for *in vitro* testing and high resolution mass spectrometry.

The development of microfractionation procedures to obtain fractions containing relatively simple mixtures of compounds for *in vitro* testing and high resolution mass spectrometry is one of the major challenges of high throughput Effect-Directed Analysis. Microfractionation is expected to lead to an improved bioactivity-to-identity correlation of known and unknown compounds in environmental matrices, resulting in a wider acceptance of EDA as a tool for e.g. the investigative monitoring of water quality.

Therefore, the course included lectures on topics such as *in vitro* assays currently used in EDA, toxicity profiling, -omics approaches, DNA array fingerprinting as well as lectures on the use of LCxLC, combining fractionation with bioassays and chemical identification and on automatisation of the fractionation and bioassay axis. In an additional practical part of the course, the participants were introduced in LCxLC applications in the laboratory.

This amounted to a minimum total academic involvement of 15h hours (0.5 ECTS) for the participants.

AGENDA

Tuesday, 21.01.2014		
Time	**Title**	**Lecturer**
9:00 - 9:30	Walk in	
9:30 - 10:30	Welcome, introduction and EDA history	Marja Lamoree
10:45 - 11:30	Introduction to High Throughput Screening and novel approaches to bioactive mixture profiling	Jeroen Kool
11:45 - 12:30	High Throughput Effect Directed Analysis for the identification of estrogens in the aquatic environment	Willem Jonker
12:30 - 13:30	Lunch Break	
13:30 – 14:15	Automated bioassays for environmental analysis	Jean Froment
14.30 - 16:15	Challenges in toxicity profiling	Timo Hamers
16:15 - 17:00	General discussions and questions	All
18:00	Common dinner	All

Wednesday, 22.01.2014		
Time	**Title**	**Lecturer**
9:00 - 10:00	LCxLC application in separation of complex environmental samples; short lab demo	Xiyu Ouyang
10:15 - 12:00	Generation and analysis of transcriptomics data: Fishing for molecular mechanisms	Jessica Legradi
12:00 - 13:00	Lunch Break	
13:00 - 13:45	Advancement of effect-directed analysis by transcriptomics	Ana Catarina Almeida
14:00 - 14:45	Metabolomics - theory and examples	Sara Tufi
15:00 - 15:45	Applications of cell- and field-based metabolomics	Pim Leonards
15:45 - 16:00	Wrap up and goodbye	Pim Leonards

COURSE CONTENT

In detail the course covered the following topics:

- Introduction to High Throughput Screening and novel approaches to bioactive mixture profiling
  - Background and examples of EDA studies
  - Identification and Confirmations
  - LCxLC in and GC-fractionation in EDA
- High Throughput Effect Directed Analysis for the identification of estrogens in the aquatic environment
  - EDA versus HT-EDA
  - Fraction Collection Time and sensitivities
  - Anti-Estrogenicity
- Automated bioassays for environmental analysis
  - Achetylcholin esterase bioassay (bioassay; methods; results with compounds, environmental samples and fish extracts)
  - Automated bioassay in EDA study
  - Algae growth inhibition tests
- Challenges in toxicity profiling
  - What is toxicity profiling?
  - Toxicity profiling of individual compounds (parent compounds and meatbolites)
  - Toxicicity profiling of complex mixtures (from toxicity profile to (i) hazard profile, (ii) ecological risk, (iii) compound identification)
  - Challenges and case studies
- LCxLC application in separation of complex environmental samples
  - What is LCxLC
  - Why applying LCxLC in environmental research
  - How to achieve best separation of environmental samples
  - Generation and analysis of transcriptomics data: Fishing for molecular mechanisms
  - Lab demonstrations
- Advancement of effect-directed analysis by transcriptomics
  - What is transcriptomics?
  - Regulation of transcription
  - Microarrays (primary and secondary analysis)
  - Zebrafish as genetic model species
  - Ecotoxicogenomics in zebrafish embryos?

- Mechanisms and gene expression profiles
- Gene expression profiles and compounds
- From compounds to mechanisms

- Metabolomics - theory and examples
  - What is Metabolomics?
  - Workflow (study design, sample preparation, analytical methods, data analysis, identification, biological interpretation)
- Applications of cell- and field-based metabolomics
  - Cell-based metabolomics
    - Quenching and extraction approaches
    - In vitro systems: SH-SY5Y cells
    - Neurotransmitter, precursors, metabolites
    - Analysis of neurotransmitters
  - Field-based metabolomics
    - Biomarkers
    - Case studies