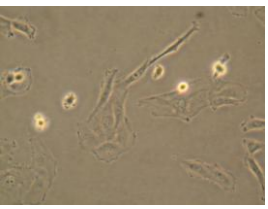
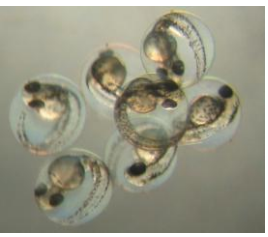



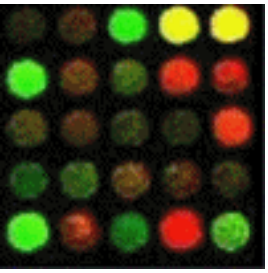

3rd NORMAN workshop

New tools for biomonitoring of emerging pollutants

Organised by
FP6 project NORMAN
IVM – Institute for Environmental Studies
VU University, Amsterdam
The Netherlands

29-30 October 2007

VU University
Amsterdam, The Netherlands

Meeting report

http://www.norman-network.net/

Vrije Universiteit amsterdam
Institute for Environmental Studies

norman

Sixth Framework Programme

New tools for biomonitoring of emerging pollutants

Report on the 3rd Workshop of the EU Project NORMAN, held 29–30 October 2007 at VU University, Amsterdam, The Netherlands

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1. Organisation

The third workshop of the European Union (EU) NORMAN project ‘‘Network of reference laboratories and related organizations for monitoring and biomonitoring of emerging environmental pollutants’’ was dedicated to the state-of-the-art of tools for biomonitoring of emerging pollutants, with special emphasis on effect directed identification of emerging pollutants by combining the results of bioassays and chemical analyses. The previous, second workshop in the framework of the Norman project was dedicated to the chemical analysis of emerging chemicals (Barcelo and Petrovic, 2007). One of the outcomes of that workshop was the observation of a gap between knowledge on toxic effects of emerging contaminants in laboratory tests and the effects occurring in the field; this topic was specifically highlighted in one of the sessions of the third thematic workshop.

Sixty-six participants from 14 countries attended the workshop. With 20 oral and 8 poster presentations (presentations and abstracts are available at the NORMAN workshop website http://norman.ineris.fr/public/workshops/ workshopss.htm), the program was structured into four sessions:

i) Current approaches to biomonitoring in the field
ii) Modern approaches for development of biomonitoring tools
iii) Biomonitoring: from lab to field
iv) Identification and measurement of emerging pollutants

2. Objectives and topics

The main focus of the workshop was on biomonitoring tools for detection, identification, quantification and effect assessment of emerging chemicals in the environment. Current approaches in biomonitoring in the lab and field as well as novel concepts for the development of biomonitoring tools (e.g. omics, lab-on-chip) were presented. The linkage of cause and effect between laboratory experiments, field observations and biomarker studies was discussed. The topics addressed during the workshop included:

- Opportunities and challenges of biomonitoring tools (e.g. omics, bioassays, biomarkers, community analyses) for risk assessment of both regulated and emerging pollutants.
- Bioassay-directed monitoring strategies based on a battery of bioassays representing different endpoints (e.g. genotoxic, carcinogenic, endocrine disruptive, immunotoxic, neurotoxic, reproductive and teratogenic effects).
- Developments in the field of new, high throughput *in vitro* assays for thyroid hormones, glucocorticoids, progestins, genotoxic compounds and steroidogenesis.

- Current advances and limitations of toxicogenomics, proteomics and lab-on-a-chip technologies for the biomonitoring of emerging pollutants.
- Discovery of new biomarkers is possible with a combined lab (*in vivo* and *in vitro*), field and statistical modelling approach to assess the health status of organisms.
- Biological and chemical identification techniques (e.g. Effect Directed Analysis (EDA), LC-Orbitrap, GC-high resolution ToF) to unravel the structure of unknown compounds.

3. Summary of key points

- For risk assessment of both regulated and unregulated chemicals in the environment an integrated effect-based and chemical monitoring program is recommended, which was further discussed at the 4th NORMAN workshop (Lyon, France) on the integration of chemical and biomonitoring strategies for risk assessment of emerging substances (Tilghman et al., 2008). Biomonitoring tools like bioassays, biomarkers and community analysis tools have a great potential to reduce the uncertainty in risk assessment of emerging pollutants, and should be further incorporated in the Water Framework Directive (WFD). The alignment and harmonization of biological and chemical monitoring programs in the WFD (e.g. timing, locations, matrices) is then a crucial factor that should be accounted for.
- In a chemical's hazard assessment phase, the selection of bioassays, end-points, and biomarkers is a crucial step. Currently, environmental assays cover a wide range of end-points, however, neurotoxicity and immunotoxicity assays for environmental application need to be further developed in order to improve their performance. For these end-points, assays used in the medical field could provide good starting points for further study.
- Strategies for setting up a battery of bioassays to assess the risk of complex mixtures of pollutants in the environment are under development in the framework of water quality assessment programmes. Batteries of bioassays can be used as screening tools to provide toxicity profiles of samples and to select samples for further evaluation. Especially high-throughput *in vitro* assays with specific toxicological endpoints and on-line sensors are promising tools. The sample treatment steps (e.g. filtration, extraction, clean-up) before bioassays testing, should be optimised and validated for the relevant end-points. Often the sample treatment procedures is the speed limiting step in sample throughput. *In vitro* assays should be used as guidelines for further research in the fields such as *in vivo*, absorption, distribution, metabolism, and excretion studies (ADME) of the chemicals. The addition of a metabolic step (e.g. S9 mix) in bioassays testing to bioactivate compounds to reactive metabolites, possibly more toxic than their parent compounds, is highly recommended.
- Effect Directed Analysis (EDA) has great potential for the identification of compounds that are responsible for the observed effects in bioassays. EDA has primarily been used as a research tool until now. Especially high throughput *in vitro* assays with specific toxicological endpoints are suitable for inclusion in EDA studies. Despite the release of sophisticated hybrid mass spectrometric techniques (e.g. Orbitrap) capable of accurate mass measurement, chemical identification of the compounds causing an effect in the bioassays is the most

challenging step in EDA especially for unknown polar compounds. Yet, if the responsible compound(s) are not found, how should we assess the risk of known effects from unknown compounds in water quality programmes and risk assessment studies?

- Great progress has been made in the discovery of biomarkers of chemical exposure. A novel toxicoproteomic approach combining laboratory chemical exposure studies with biomarker identification techniques (e.g. Maldi-ToF, LC-MS/MS) and statistical models enables the discovery of new biomarkers. Future needs are towards more quantitative, reproducible and sensitive methods for the identification of candidate biomarkers. Attention should be paid to connect biomarkers to molecular functions and biological processes. For non-model species (wildlife) identification of proteins is a challenging task because of a lack of sequence information and relevant databases for these organisms.
- Due to the rapid progress in molecular biological techniques the genomics approach can help to unravel the mechanism of action of chemicals, while at the same time it can provide gene expression profiles of chemicals. This approach can be used to categorize chemicals on the basis of the mechanism of toxicity (e.g. hormone agonists and antagonists, inhibition transferring genes, etc.). In the future, gene expression profiles generated through exposure to environmental samples in combination with training sets of gene signatures of model compounds may be used as a diagnostic tool to unravel complex exposure of mixtures of chemicals. Currently, toxicogenomics can be applied to model species and a limited number of environmentally relevant species (e.g. Daphnia, zebra fish). There is a lack of commercial microarrays for non-model species, which, if they existed, would be of great help to further apply genomics in environmental risk assessment. A limited number of environmentally relevant genomic databases with DNA sequence information are available (e.g. wFleaBase, Collembase). Interpretation of the gene expression levels to physiological and population levels is a challenging task.
- To assess the effects of emerging pollutants on wildlife there is a need to develop suitable monitoring tools. Interpretation and extrapolation of biomarker responses from lab to field studies is a challenging task, as field studies often are based on associations between biomarkers and exposure. Defining the normal operating range of biomarkers and gene/protein expression responses of organisms both in the laboratory as well as under field conditions is a highly important aspect for the interpretation of gene, protein, and biomarker responses. Especially for the field situation it is difficult to define a “reference” population because many factors, beside chemical stress, can influence the gene, protein and biomarker responses.

4. Acknowledgements

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