European Science Foundation

Standing Committee for Life, Earth and Environmental Sciences (LESC)

## Scientific Report on

## ESF LESC EXPLORATORY WORKSHOP

# Dynamic bioavailability of pollutant species in aquatic ecosystems

**Hôtel du Signal de Chexbres, Switzerland, 16-18 October 2005**

**Convened by:**

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# EXECUTIVE SUMMARY

## Context and Objectives

Natural systems generally are subject to changing conditions and are not at equilibrium. The importance of a dynamic approach to interpretation of environmental processes is emerging as a key innovative concept essential for a more quantitative understanding and improved assessment of water quality and ecotoxicological risk posed by pollutants. The aim of this exploratory workshop was to discuss approaches to dynamic pollutant speciation analysis and ensuing impacts on ecodynamics of aquatic systems and to identify the research challenges involved. Key concepts included:

- the importance of fluxes at the microscale level, to/from and through biointerfaces, and timescales of processes, e.g. exposure time, internal organism processes/physiology, in determining bioavailability and ecotoxicological effects of pollutant species

*and*

- the inherent heterogeneity of the aquatic medium, e.g. distributed thermodynamic and kinetic features, and of organisms themselves, e.g. biofilm matrices, internal compartmentalisation

underpinned by:

- development of sensors for dynamic speciation analysis and ecotoxicological effects over a range of relevant spatial and temporal scales

*and*

- development of a quantitative generic dynamic framework for data interpretation that links chemical and physical exposure conditions to ecotoxicological effects from the cellular to the community level, and establishes the foundations for dynamic risk assessment.

## Structure and overview of scientific content

The framework for the discussions considered two key aspects that are fundamental properties of natural systems, yet to date have generally been ignored or oversimplified in interpretations of pollutant behaviour and impacts: (i) the importance of fluxes at the microscale level (to/from and through interfaces) and timescales of processes, e.g. exposure time, internal organism processes/physiology, and (ii) the inherently heterogeneous nature (physical and chemical) of both the external aquatic medium and of the organisms themselves, e.g. biofilm matrix, internal compartmentalisation. Both aspects must be underpinned by development of sensors capable of measuring the various phenomena over a range of relevant spatial and temporal scales and development of rigorous dynamic models for data interpretation and quantification of the links between the various processes. An overview of the various properties and processes that were addressed in the scientific sessions are shown in Fig. 1.

The program comprised five sessions covering (i) dynamic speciation in aquatic media, (ii) hydrogels and biofilms, (iii) biouptake, (iv) ecotoxicological effects from the molecular to the community level, and (v) risk assessment. The format consisted of 20 min presentations, each followed by a 20 min discussion. The overall context was development of a generic dynamic framework that links exposure conditions to ecotoxicological effects from the cellular to the community level, via quantitative understanding of the processes shown in Fig. 1. The workshop explored key knowledge gaps to development of such a framework.

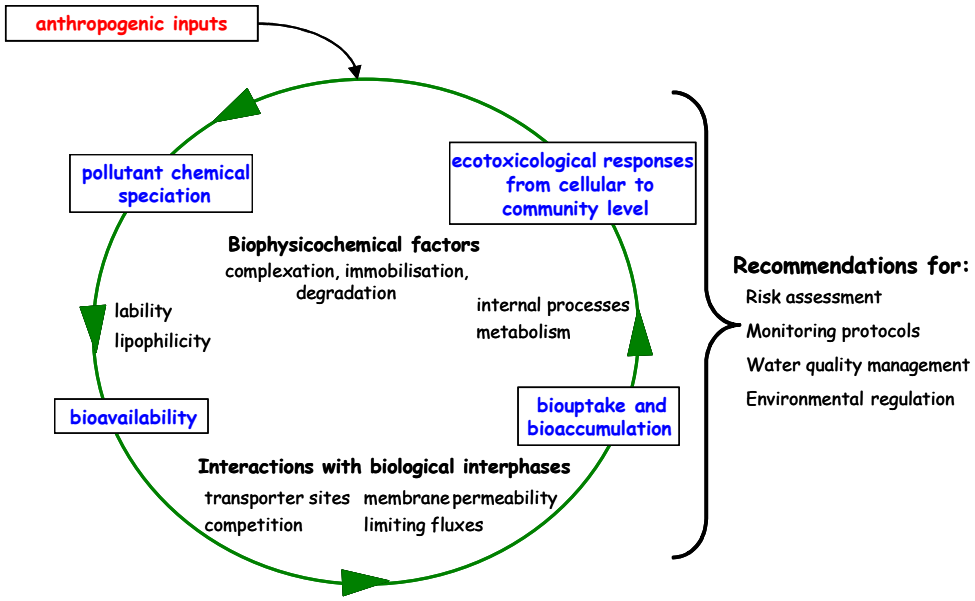


**Fig. 1.** Schematic diagram of the scope of the exploratory workshop, showing the interrelationships between, and selected factors determining, the speciation of pollutants and their ensuing ecotoxicological impacts in aquatic ecosystems

## Summary of main outcomes

The range of expertise of the participants brought a multidisciplinary perspective to each session. This feature was key to focusing on research challenges, and the most appropriate manner in which they can be addressed, e.g. biological mechanisms can override kinetic chemical considerations. Common challenges identified were: mixtures of chemicals, mixtures of organisms, distributed physicochemical properties, the significance of fluxes versus the integral thereof, identification of appropriate spatial and timescales for various processes, coupling of microscale processes and macroscale properties such as hydrodynamic flow, and translation of data, e.g. from speciation to biouptake. Key points are outlined below and elaborated in the following section.

*Dynamic speciation in aquatic media.* The significance of pollutant speciation for bioavailability and toxicity must be understood within a generic dynamic framework. Numerical simulation facilitates optimisation of sensor design to maximise sensitivity for target species with given kinetic properties. The most useful approach is to measure a kinetic speciation spectrum via a suite of dynamic sensors.

*Hydrogels and biofilms.* Biofilms are a ubiquitous interface in aquatic systems. Their structure and composition is dynamic; the significance of convection vs diffusion depends on the architecture and thus also age of the biofilm. Fundamental studies on biogels as a soft interface provides useful insights. Further characterisation is needed at the level of microenvironmental flux profiles.

*Biouptake.* The dynamic features of mechanisms of biouptake require further characterisation. Theoretical work shows that the transient can give useful information, but it is difficult to obtain experimental data at very short times. For organics, the lipophilicity, charge, and size impact on membrane partitioning and permeability. Biological mechanisms can overcome kinetic limitations.

*Ecotoxicology effects.* Internal speciation dynamics (concentration and residence time in bioactive and bioinactive pools) govern the eventual toxicological effect which depends on pollutant speciation in the exposure medium, exposure time, uptake route, and organism sensitivity. Both the uptake flux of pollutant species and the integral thereof are important parameters.

*Risk assessment.* A dynamic risk assessment approach must account for organism sensitivity, the time dependence of exposure profiles, adaptation and acclimation of organisms. A multiexposure-multieffect framework is necessary. It is a challenge to relate molecular level changes (e.g. genomics) to ecosystem level impacts.

# DETAILED SCIENTIFIC CONTENT

The ultimate biological impact of a given pollutant in the water body is a consequence of a chain of events that may involve (i) mobility (physical transport in the bulk medium and across interfaces) and reactivity of the pollutant species in the medium (encompassing sorption on particles, surfaces and biofilms, and chemical reactivity, including e.g. complex formation, hydrolysis, oxidation/reduction, degradation), (ii) chemical conversion of pollutant species into bioactive species in the local environment external to the organism, e.g. dissociation of lipophobic complexes into the free metal, (iii) transport of the bioactive species through the biomembrane, and (iv) bioreactivity of the pollutant within the organism. A rigorous understanding of the overall process involves quantification of all the steps involved at the relevant spatial scales and timescales, both external and internal to the organism; Fig. 2. During the various scientific sessions of the workshop, the dynamics of the different processes involved were discussed and research challenges were identified. The detailed contents of each session are elaborated below.

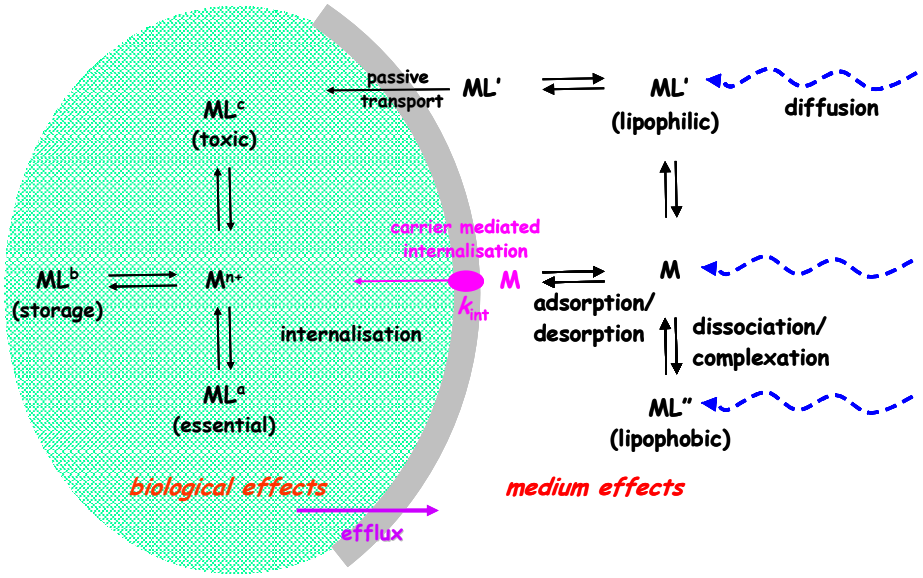


**Fig. 2**. Schematic illustration of the steps involved in the biouptake of a pollutant species by a microorganism. Simplified examples of a metal, M, forming complexes with a small number of ligands, L, and facilitated transport of M (supported by a supply via dissociation of ML″), and passive transport of a lipophilic compound, ML′, across the biological membrane are shown.

## Dynamic speciation in aquatic media

The concept of lability describes whether chemical species are able to maintain equilibrium with all other species in the medium over given spatial and timescales. All sensors used to probe chemical speciation and reactivity have typical methodological spatial and timescales, which can be compared to those of various environmental processes. Fig. 3 highlights that diffusion is a key transport process for both environmental systems and sensor functioning. The diffusion timescale is the one to compare to chemical reaction times, e.g. in discriminating between labile and nonlabile complexes.

The dynamic framework for the general case where the overall flux of metal, M, to a consuming analytical or biological interface results from the coupled diffusion and kinetics of interconversion between M and its various species in the complex system. For operationally dynamic systems, in which there is an interfacial flux of free metal species, metal complexes are labile if there is frequent interconversion between M and ML during their transport through the diffusion layer. The reaction layer concept lies at the heart of the concept of lability; the reaction layer thickness, $\mu$, is determined by the lifetime of free M and the distance it can travel within that time. Lability is conveniently expressed by $J_{kin}/J_{dif}$, where $J_{dif}$ is the diffusion-limited flux of M and ML, i.e. the flux in the limit of

infinitely fast dissociation of ML, and $J_{kin}$ is the kinetic flux, as ensuing from the rate of dissociation of ML into M on the basis of the reaction layer concept. Systems range from fully labile (for $J_{kin} >> J_{dif}$) to nonlabile (for $J_{kin} << J_{dif}$). The ratio $J_{kin}/J_{dif}$ is denoted as the lability parameter, $\mathcal{L}$. The conventional formulation of $\mathcal{L}$ uses the maximum kinetic flux, based on the bulk concentration of ML, and thus criterion for lability takes the form of an inequality, i.e. for labile species, $\mathcal{L} >> 1$. Recent work was presented on a more quantitative approach to kinetic responses that describes $J_{kin}$ in terms of the relevant species concentration in the reaction layer, in which case the lability criterion, $\overline{\mathcal{L}}, \rightarrow 1$ for labile species.

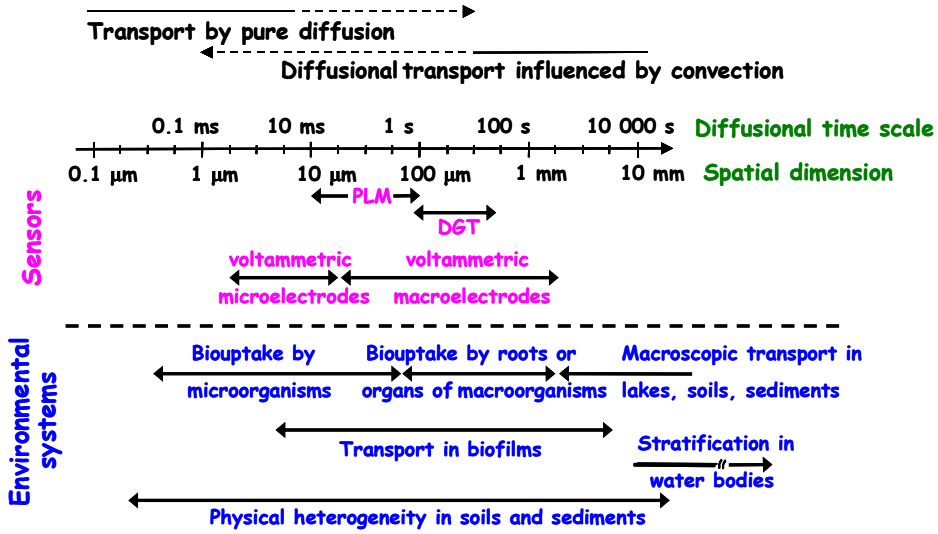


**Fig. 3.** Diffusional timescales and spatial dimensions for a range of environmental processes and analytical sensors. PLM = permeation liquid membrane; DGT = diffusive gradient in thin film

It is evident from Fig. 3 that species which are labile for one technique may not be for another. This feature is quantified by the lability criteria that are now available for a range of analytical techniques and for various complex stoichiometries, i.e. ML, … $ML_n$. Numerical simulations facilitate optimisation of sensors by identifying conditions under which the response to a given species is maximal.

Challenges for interpretation of dynamic pollutant speciation in aquatic ecosystems:

- mixtures of metals and ligands – even considering only 1:1 stoichiometries, numerical simulations indicate that interactive effects occur, such that a simple summation of individual lability criteria is not an appropriate descriptor of the system as a whole.
- the contribution of immobile complexes to speciation measurements needs to be ascertained. By definition, such species are labile, hence they contribute fully to the transient, but their low diffusion coefficient means their contribution to the steady-state flux may be negligible. Many dynamic sensors measure an integral of the flux over a given accumulation time, and thus inherently contain the initial transient component: in such cases the presence of immobile complexes can have a significant impact on the sensor signal.
- the theory for lability of soft particles, e.g. colloidal humic substances, needs to be further developed. Recent work shows that, considering ligands on the surface of a particle, the lability metal complexes with such entities is lower than that of the analogous homogenous ligand case. This needs to be extended to the permeable particle case, considering the effect of Donnan potential, etc..
- understanding of the properties of soft interfaces has wide significance for sensor functioning and interfacial processes in aquatic systems. The development of a Donnan potential within gels can significantly alter the speciation within such matrices.

- development of the theoretical background for lability of complexes in flowing systems, as relevant for biofilms, fish gills, sediment pore waters in which the hydrodynamics are not constant and the relevance of convection vs diffusion varies with spatial location and time.

For translation of speciation data to bioavailability and biouptake, the best approach is to measure a kinetic spectrum, via a suite of dynamic sensors, and to interpolate for the relevant dynamic conditions.

## Hydrogels and biofilms

Biofilms are ubiquitous interfaces in aquatic ecosystems and their impact on the dynamics of pollutant transport is yet to be fully characterised. They are comprised of communities of unicellular organisms supported and held together by extracellular polymeric substances (EPS); the water content is 90 – 95%. Biofilms themselves are inherently dynamic and heterogeneous in space and time. Their 3D physical architecture and porosity depends on hydrodynamic flow and on the age of the biofilm; the various voids and internal structures determine the relative importance of mass transport by convection or by diffusion. Within the biofilm, gradients in nutrients and redox conditions, and consequently in pollutant speciation, will develop. The composition of the biological community and the nature of EPS can change in response to exposure conditions. Dramatic changes may occur, e.g. sloughing of large sections.

The EPS properties are fundamental to the overall biofilm functioning – organisms can modify the charge and hydrophobicity of EPS in response to environmental pressures, including pollutant exposure. EPS are typically negatively charged, and the concepts of Donnan potential and the properties of soft interfaces outlined in the previous section are pertinent in quantitatively describing their properties. There can be some highly specific binding sites for metal, but generally biofilms are not very selective in their sorption of compounds. Biofilms act as both a sink and a source of compounds.

The presence of biofilms at the sediment/water interface modifies the interfacial flux of pollutants and nutrients. This environment is highly dynamic with flows in the overlying water body setting up advection cells in sediment ripples that lead to changes on oxygen gradients over short timescales (minutes). Uptake of pollutants in to the biofilm is strongly influenced by the local pH, redox, and sulfide conditions. Whilst some aspects can be investigated in laboratory studies, *in situ* field studies are necessary to get the pertaining reaction rates.

Identified research challenges:

- characterisation of dynamic pollutant speciation with biogels. The physicochemical properties of the matrix, such as the presence of a Donnan potential and retarded mass transport, can modify the speciation as compared to the exposure medium. These features are just beginning to be explored in model gel systems.
- *in situ* microenvironmental profiling of pollutant speciation within biofilm matrices to determine spatial and temporal dynamics. Gradients of pollutant species will develop in response to e.g. gradients in redox conditions or bioactivity; local depletion may occur around clusters of cells.
- establishing relationships between pollutant speciation, biofilm composition (biodiversity, nature of EPS) and structure (convective versus diffusive mass transport)

## Biouptake

To date the majority of studies on predicting bioavailability have used simplified equilibrium models such as the free ion activity model (FIAM) or the biotic ligand model. These models are only applicable within a certain range of conditions. A dynamic analysis establishes the conditions under which complex species will contribute to biouptake, i.e. it identifies the domain of validity of equilibrium-based models. For example, the FIAM is restricted to cases where mass transfer to the biointerface is not flux-determining and the unsupported diffusion flux of the free metal alone is much larger than the maximum biouptake flux. The extent to which complex species in the medium contribute to biouptake, and thus the relevance of their lability, depends on the interplay between two fundamental quantities: the relative bioaffinity and the ratio between the limiting biouptake flux and the limiting supply flux from the medium. Until recently, most laboratory studies of metal biouptake were conducted under conditions where the flux of free metal ion alone is sufficient to satisfy the demands of the organism concerned, and the lability of any complexes present in the medium is consequently irrelevant. However, under environmentally relevant conditions, where essential trace metals may be limiting, or when depletion of the medium becomes significant, e.g. in biofilms, the contribution of complexes can be called into play to satisfy the organism.

There is a need to develop dynamic mechanistic understanding of the relationship between speciation in the exposure medium and biouptake processes, e.g. discriminating between adsorption vs internalisation. Membrane transporter dynamics play a key role, as does membrane permeability. Recent work on an 'instantaneous steady-state approximation' provides a good description of metal uptake by unicellular algae, so long as the internalisation rate is low, and the adsorption can be described by a linear or Langmuirian isotherm. However, experimental data at short times in the transient regime is required to discriminate parameters.

There needs to be close collaboration between chemists and biologists: whilst certain processes might appear to be chemically rate-limiting, biological processes may override such limitations, e.g. by increasing the number and/or nature of membrane transporters. The bioavailability of compounds that are taken up by active processes may exhibit a different dependence on speciation in the exposure medium than those whose biouptake involves facilitated diffusion. Organisms regulate uptake of certain compounds, e.g. Zn, to maintain homeostasis. For organic compounds, the classical octanol-water partitioning coefficient, $K_{ow}$, is not a reliable predictor of bioavailability. Charged organic compounds can also be transported across cell membranes, but the mechanistic details are not yet well characterised, i.e. it is not known whether the transport of a positively charged molecule into a cell occurs as an ion pair, or whether there is counter flow of anions out of the cell to maintain a constant potential difference across the membrane. Indeed, such mechanistic detail may be impossible to ascertain since organisms have independent means for regulating cell membrane potentials. Measurement of internal cellular concentrations would help support various models for membrane permeation.

In natural waters, competitive interactions must be taken into account, and the various processes can be rather involved. Currently the Michelis-Menten approach is used, which assumes that the competing compounds have equal internalisation rates and that it is only the affinity of the biointerface for a given compound which is modified by the presence of another. However, the internalisation rate constants for the competing compounds may well be different, and each may be modified in the presence of the other.

Research challenges include:

- measurement of the transient. This can provide useful insights into the dynamics of mechanistic detail, but in practice measurements at very short times are difficult.
- characterisation of the impact of geometrical conditions, e.g. colonies of cells in biofilms in which the biouptake may be influenced by retarded mass transport in the EPS matrix.
- understanding the effects of depletion in the medium, as may arise e.g. for essential trace metals in marine waters (e.g. Fe), amongst cell clusters within a biofilm, and for plants. Data obtained under depletion conditions can provide useful mechanistic information.

- understanding of competition effects, i.e. mixtures of metals and/or ligands. For example, in response to a high concentration of one metal, certain membrane transporters may be shut down, thus impacting indirectly on uptake of another, perhaps essential, compound.
- determination of biouptake in natural water conditions. To facilitate interpretation, mechanistic biouptake studies are usually performed in well controlled, albeit environmentally relevant, laboratory conditions. In natural waters many other factors can affect results, e.g. the presence of organic compounds that may alter membrane permeability.
- linking mechanisms of biouptake to internal speciation dynamics.

## Ecotoxicological effects from the molecular to the community level

The impact of a pollutant on an organism depends on a range of factors including (i) the speciation in the exposure medium, (ii) the exposure time, (ii) the exposure route, (iii) the organism's assimilation efficiency, and (iv) the eventual internal compartmentalisation. Different organisms display different sensitivities to pollutants; environmental quality standards are typically set to protect the most sensitive biota. Both the biouptake flux and its integral are pertinent parameters: which is of greater significance depends on the question posed, e.g. acute vs chronic effects, and the nature of the exposure profile, e.g. whether there is a constant level of pollutant species or pulsed concentrations. The nature of the toxicological effects is also pertinent: for compounds which invoke reversible effects, the peak concentration is the most relevant, whilst the cumulative dose is the important parameter for compounds exerting irreversible effects.

Detailed dynamic mechanistic understanding is required to make relevant risk assessments for policy making. Traditionally, body burdens have been incorporated into risk assessments, but a high level of bioaccumulation of a toxicant does not necessarily result in high toxicological effects: the molecular handling during uptake (exposure route) and the ensuing internal speciation dynamics have a profound impact on the ultimate physiological effect. A model has recently been developed which differentiates bioaccumulated metal into bioactive and a bioinactive pools; the molecular pathways by which these pools are reached, and the concentration and residence time within them is being developed as a basis for predicting toxicology. It remains a challenge to link this model for internal compartmentalisation to speciation dynamics in the exposure medium, and to toxicological effects from the cellular to the whole body level.

Challenges identified for development of dynamic risk assessment:

- understanding of toxicological effects at the genomic level; toxicogenomics is emerging as a means to obtain a mechanistic understanding of dose-effect relationships.
- the role played by biological regulation of uptake and/or detoxification/elimination – for a given compound, one or both or neither may be regulated by the organism.
- competition effects arising in mixtures of pollutants.
- translation from effects at the cellular to the whole body to the community level – need to know about trophic transfer and adaptation/acclimation processes.
- characterisation of the relevant time constants to interpret pulsed pollutant exposure as may arise in disaster situations or from repeated flooding of contaminated areas. There is a need to relate changes in dynamic speciation in the exposure profile to variations in ecotoxicology, and to design appropriate measurement protocols for such situations. Typically there is a delay time between changes in the exposure profile and physiological effects; this timescale is organism dependent.
- determination of the best predictors of acute vs chronic effects.

# ASSESSMENT OF THE RESULTS, CONTRIBUTION TO THE FUTURE DIRECTION OF THE FIELD

The presentations and ensuing discussions identified common themes and challenges within each topic area. The need for a multidisciplinary focus was identified as key factor in developing a generic dynamic framework for relating speciation dynamics to ecotoxicological effects.

The common key challenges across topic areas (speciation, bioavailability, bioaccumulation, ecotoxicology, risk assessment) are:

- how to interpret information for mixtures of pollutants.
- how to interpret information for mixtures of organisms, including sensitivity distributions and trophic transfer.
- determination of the significance of transient vs steady-state parameters, and the importance of fluxes versus the integral thereof.
- coupling of microscale processes to macroscale features such as hydrodynamic flow in the water body.
- determination of the relevant parameters for a given spatial and timescale.
- how to interpret distributed properties and effects (both chemical and biological).
- how to translate data, e.g. from speciation in the exposure profile to bioavailability to internal compartmentalisation to physiological effects.

There is evidently need for more fundamental research. Since the award of this workshop, the convenors have been active in making applications for funding to support such activities. An EU framework 5 project, ECODIS, (coordinated by Herman P. van Leeuwen) commenced on 1st October 2005 and it will address some of the issues raised herein, in particular the relationship between pulsed exposure profile dynamics and ecotoxicological effects. A stage one proposal has been made for a Marie Curie Research Training Network (coordinated by Raewyn M. Town) that will focus on dynamic metal speciation in biogels.

This workshop drew considerable interest from the scientific community, and there is a clear need to take these discussions to a broader audience. Accordingly an application has been submitted for an ESF-EMBO symposium (*ca*. 100 participants) on 'Dynamic metal speciation and toxicity in ecobiotic cycles' that would take place in 2007 (chair: Herman P. van Leeuwen, co-chairs: Ronny Blust and Raewyn M. Town). Furthermore, the workshop convenors, together with Ronny Blust have been invited to convene a special symposium on speciation-ecotoxicology relationships within the forthcoming SETAC meeting (May 2006, Den Hague, NL).

# FINAL PROGRAMME

## Sunday 16 October 2005

| | |
|---|---|
| Afternoon | *Arrival* |
| 15:30 – 16:00 | *Registration and coffee* |
| 16:00 - 16:20 | **Herman P. van Leeuwen**: Opening of the meeting, setting-the-stage |
| 16:20 – 16:30 | **Presentation of the European Science Foundation (ESF)**<br>**Sonja Lojen** (Standing Committee for the Life, Earth and Environmental Sciences) |
| | **Dynamic speciation in aquatic media** |
| | Chair: **Herman P. van Leeuwen** |
| 16:30 – 17:10 | **Jacques Buffle**: Key aspects of dynamic speciation and bioavailability in aquatic media |
| 17:10 – 17:50 | **David Turner**: Analytical dynamic modelling |
| 19:30 - | *Dinner* |

## Monday 17 October 2005

| | |
|---|---|
| | **Dynamic speciation in aquatic media, cont.** |
| | Chair: **Willem van Riemsdijk** |
| 09:00 – 09:40 | **Herman P. van Leeuwen**: Overview of dynamic speciation sensors |
| 09:40 – 10:20 | **Jaume Puy** and **Josep Galceran**: Numerical simulations of dynamic speciation and biouptake |
| 10:20 – 11:00 | *Coffee break* |
| 11:00 – 11:40 | **Bill Davison**: *In situ* techniques in practice |
| 11:40 – 12:20 | **Beate Escher**: Role of speciation for bioavailability of organic pollutants in aquatic systems |
| 12:30 – 14:00 | *Lunch* |
| | **Hydrogels and Biofilms** |
| | Chair: **Bill Davison** |
| 14:00 – 14:40 | **Hans-Curt Flemming**: Influence of biofilms on the dynamics of pollutant transport in aquatic ecosystems |
| 14:40 – 15:20 | **Dirk De Beer**: *In situ* measurements of microbial activities and transport phenomena in a deep sea cold seep |
| 15:20 – 16:00 | *Coffee break* |
| | Chair: **Laura Sigg** |
| 16:00 – 16:40 | **Jérôme Duval**: Electrohydrodynamics of soft interfaces |
| | **Biouptake** |
| 16.40 – 17:20 | **Kevin Wilkinson**: Bioavailability and biouptake by microorganisms: thermodynamic, dynamic, or biological control? |
| 19:30 | *Dinner* |

## Tuesday 18 October 2005

### Ecotoxicological effects from the molecular to the community level, and biosensors

Chair: **Hao Zhang**

09:00 – 09:40 **Ronny Blust**: Biouptake by multicellular organisms and ensuing ecotoxicology

09:40 – 10:20 **Colin Janssen**: Bioavailability and ecotoxicity of metals: science vs. regulatory needs

10:20 – 11:00 *Coffee break*

### Risk assessment

Chair: **David Turner**

11:00 – 11:40 **Dimosthenis Sarigiannis**: Dynamic modelling of exposure and risk metrics

11:40 – 12:20 **Joop Hermens**: Dynamic risk assessment

12.30 – 14:00 *Lunch*

### Keynote Lecture

Chair: **Jacques Buffle**

14:00– 14:45 **François Morel**: Fe uptake by phytoplankton: the strength and limits of the Free Ion Model

### Overview and synthesis of workshop

14:45 – 15:25 **Raewyn M. Town**: Overview of key issues from all sessions and general open discussion

16:00 *Closure of meeting and departure*

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## Statistical information on participants

The home institutions of the participants were widely spread across the European Union and Associated States (Figs. 1 and 2), and there was one international participant from the United States of America. There was a good proportion (30%) of female participants (Fig. 3).

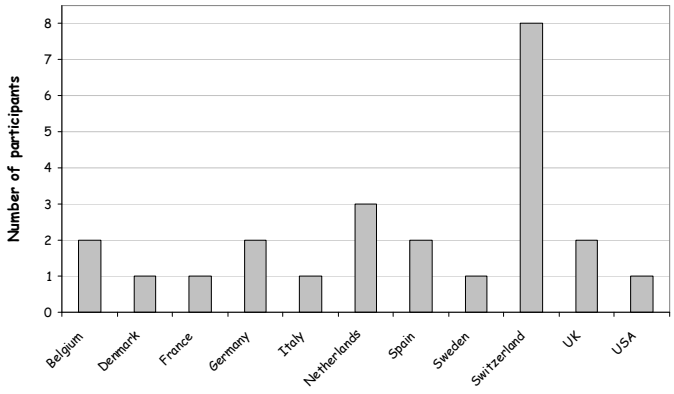


**Figure 1.** Geographical distribution of participants by country

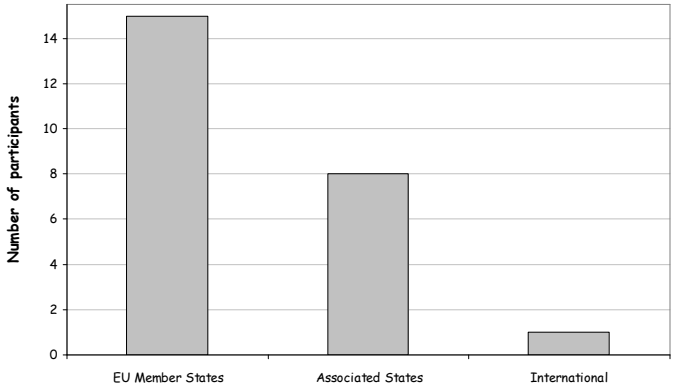


**Figure 2.** Distribution of partners across EU member and associated states, and internationally

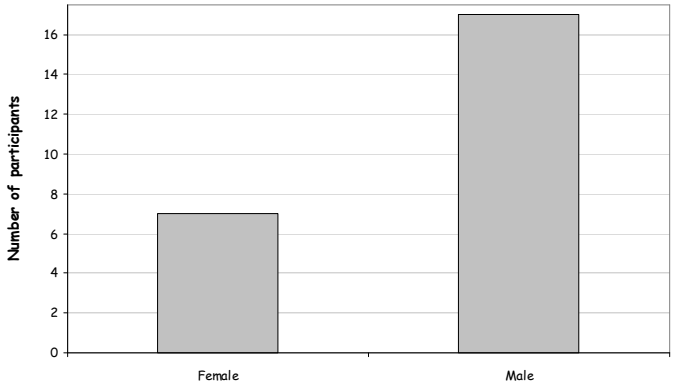


**Figure 3.** Gender balance of participants