Network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental pollutants

SUB-PROJECT CASE

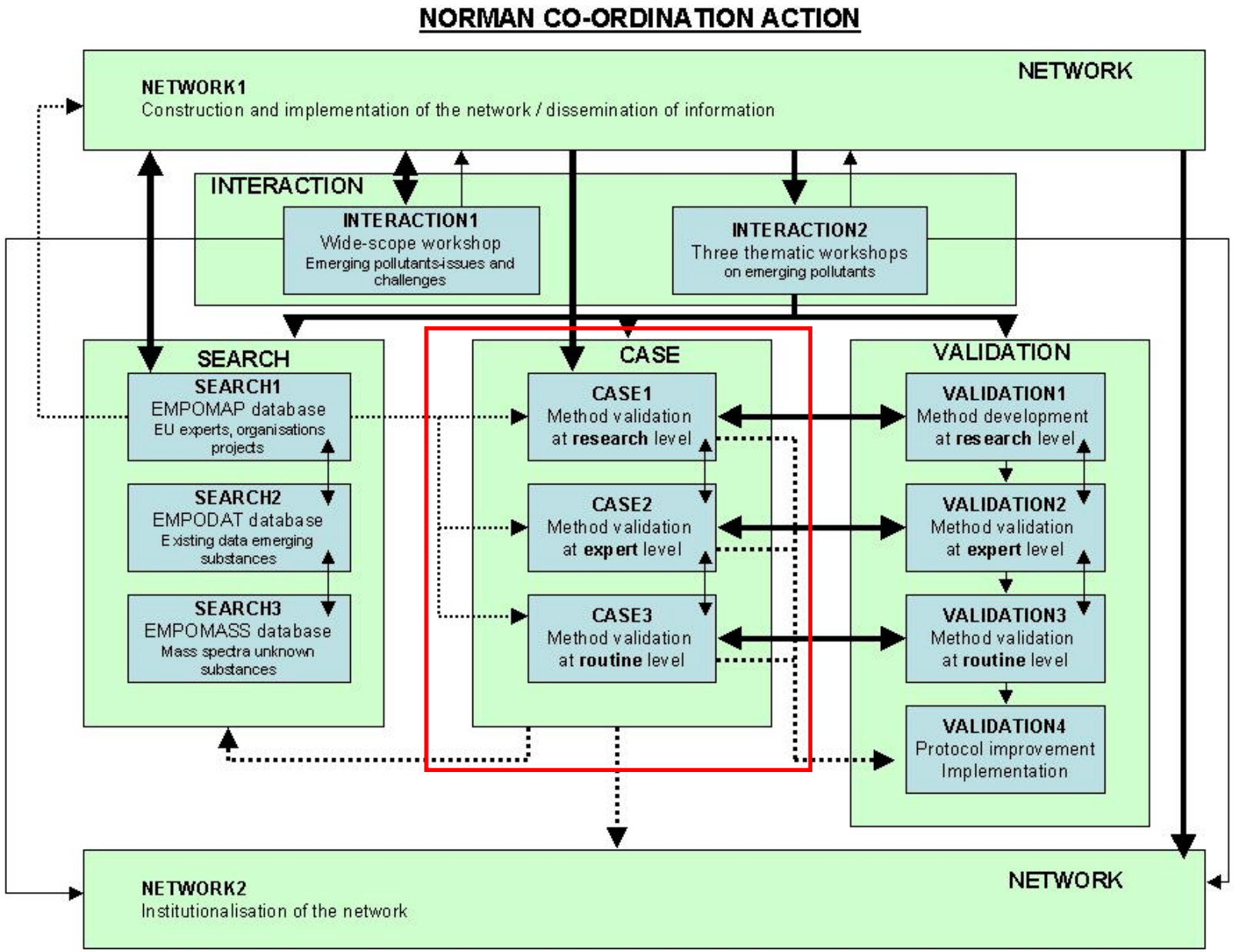
Three case studies of emerging pollutants to test the functioning of the network

WP-C1: Oestrogens in sewage treatment effluents

WP-C2: NSAIDs

WP-C3: brominated flame retardants (DecaBDE)

Sixth Framework Programme



Three case studies matching the three scenarios addressed by VALIDATION

To test the functioning of the network of reference laboratories and its ability to produce validated data on emerging pollutants, a logical sequence of steps is followed for all three case studies:

- review the existing methods, including the use of the SEARCH databases (S1, S2) and apply the criteria defined in the protocols developed in VALIDATION (V1, V2, V3),
- select laboratories that are involved in the analysis of the specific emerging pollutant, using the SEARCH database (S1),
- select representative sites for collection of samples using existing results from EU RTD projects (e.g. SWIFT for aquatic environment),
- organise expert meetings to discuss the methods with the selected laboratories and prepare inter-laboratory studies,
- evaluate the results of the meetings and inter-laboratory studies,
- harmonise and test the methodology,
- give feedback to VALIDATION (V1, V2, V3, V4) and to SEARCH, drawing conclusions about the protocol and its applicability (in order to enable improvement of the protocol) and about possible upgrading of the SEARCH databases,
- give feedback to NETWORK (WP-N2) on the functioning, design and structure of the network and the JPA proposal.

Network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental pollutants

SUB-PROJECT CASE: WP-C1

Validation and harmonisation of analytical methodology for research laboratories: Oestrogens in sewage treatment effluents

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WP-C1: Oestrogens in sewage treatment effluents

In the first case study the protocols for the research level developed in VALIDATION V1 will be applied for the biomonitoring and chemical analysis of a selected number of xeno-oestrogenic compounds (natural and synthetic oestrogens) in a range of samples.

These compounds have been selected as a class of emerging contaminants, as there is growing concern among policy-makers, producers and consumers in the EU about environmentally-related health problems.

WP-C1: Oestrogens in sewage treatment effluents

Three levels of methods are envisaged:

- *In vitro* bioassays that detect oestrogenic activity (for example, ER-CALUX)
- *In vivo* bioassays that detect oestrogenic activity, where the *biomarker product* of the bioassay is the measured parameter (for example vitellogenin, vtg mRNA, vitelline envelope protein) as opposed to somatic changes.
- *In vitro* direct measurement assays for measurement of *quantities* of target compounds, ethynylestradiol, 17b-estradiol, estriol, estrone, (for example ELISA for oestradiol-17B).

The program will be based on:

- The establishment of an integrated biological and chemical monitoring programme for environmental estrogens in sewage treatment effluents.
- Testing the operation of validated analytical and bio-analytical methodologies in a real-life case study.

WP-C1: Oestrogens in sewage treatment effluents

Task 1: Overview of the methods for estrogenic compounds, selection of laboratories and methods

-

Task 2: Prepare and organise an inter-laboratory study with the selected above methods

-

Task 3. Evaluation and assessment of the results, definition of a protocol for estrogenic compounds effects evaluation

-

Network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental pollutants

SUB-PROJECT CASE: WP-C2

Validation and harmonisation of analytical methodology for reference laboratories: Non-steroidal anti-inflammatory drugs (NSAID)

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Non-steroidal anti-inflammatory drugs (NSAID)

- Worldwide, frequent, and highly dispersed but cumulative usage by multitudes of individuals.
- Environmental occurrence result of manufacturing processes, the disposal of unused or expired products, and the excreta.
- Main source treated and untreated urban sewage effluent
- Due to their physico-chemical properties (high water solubility and often poor degradability) they are able to penetrate through all man-made treatments and natural filtration steps and enter surface water, groundwater and drinking water.

Objectives

- To create and have access to harmonised chemical methods validated at reference laboratory level for **non-steroidal anti-inflammatory drugs (NSAID).**
- To apply the protocol proposed by VALIDATION V2 to NSAIDs.
- To provide a validated harmonised protocol to be used in reference laboratories for NSAID analysis allowing a better monitoring of the studied compounds in the aquatic environment.
- To give feedback to the sub-projects NETWORK, VALIDATION and SEARCH

Task 1. Overview and preparation

- **Review of existing analytical methods for ibuprofen, naproxen, ketoprofen and diclofenac, degree of validation, domain of application, and performance**
- **Apply the criteria defined in WP VALIDATION V2 to select, within the methods which stem from the review, those which should be subjected to further optimisation and harmonisation**
- **Invite expert laboratories to agree upon the more promising methods.**

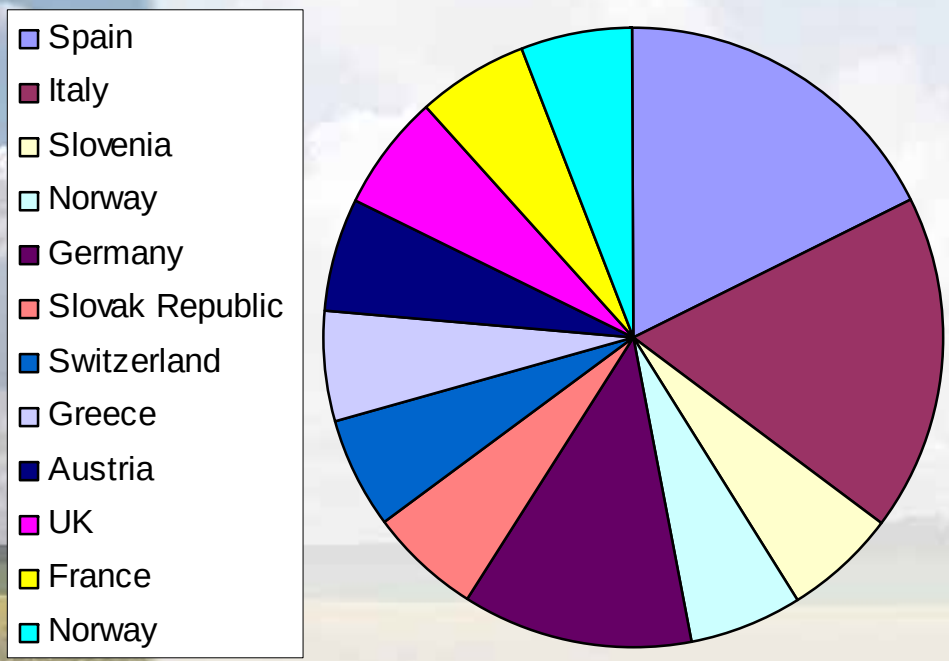
Task 2. Interlaboratory exercises

- **Two interlaboratory comparisons** will be conducted with expected participation of about 15 laboratories (from project partners and other EU laboratories).
- **Two series of real aqueous samples** (non-spiked and spiked) differ in complexity of matrix (ground water, river water and wastewater) will be prepared and distributed to the participating laboratories.
- In the **first interlaboratory exercise** all participating laboratories will be asked to use the method validated in their own laboratory and to report on the experimental conditions and method performance (recoveries, reproducibility, variability etc.).
- In the **second exercise** the agreed protocol (task 3) will be distributed to the participants.

First Interlaboratory exercise on Non steroidal anti-inflammatory drugs (NSAID)

17 participants – 12 EU countries

4 NORMAN partners



Participants

1- Environmental Institute, Kos, Slovak Republic
2- IES, JRC, Ispra, Italy
3- Jozef Stefan Institute, Ljubljana, Slovenia
4- Mario Negri Institute, Milan, Italy
5- Universita "La Sapienza" di Roma, Italy.
6- NIVA, Oslo, Norway
7- Universidade de Santiago de Compostela, Spain.
8- University of Almeria, Spain,
9- Europa Fachhochschule Fresenius, Idstein, Germany.
10-EPF Lausanne, Switzerland
11-General Chemical State Laboratory, Athens, Greece
12-Umweltbundesamt GmbH, Wien, Austria.
13-EcoChemistry,Science Laboratory, York, UK.
14-BFG, Koblenz, Germany
15-Eawag, Duebendorf, Switzerland,
16-CNRS, Bordeaux, France
17- IIQAB-CSIC, Barcelona, Spain

First Interlaboratory exercise on Non steroidal anti-inflammatory drugs (NSAID)

1st inter-laboratory – AGENDA

Kick-off meeting was held on 19 May in Barcelona

- Each participant presented the method and validation data
- The whole group discussed QA/QC issues
- Reporting format

Sample shipment, is planed for September 2006.

The results together with the report on experimental conditions and method performances 30 November 2006.

Report including statistical evaluation of the results and critical evaluation of the methodology used (Deliverable C2.2) – February 2007

2nd meeting of the first interlab (discussion of the results) and the Kick-off meeting of the 2nd interlab – April 2007

First Interlaboratory exercise on Non steroidal anti-inflammatory drugs (NSAID)

Methods used by the participants

50% Laboratories - LC-MS/MS
50% Laboratories - GC-MS

Different Solid phase extraction (SPE) procedures
- neutral or acidified samples
- Different SPE materials: RP-C18, OASIS HLB, STRATA…
- Different reconstitution of samples

LC-MS/MS different conditions (column, mobile phase, additives) most of them according to the chromatographic device

GC-MS/(MS) different derivatizations procedures including PFBBr + TEA, MSTFA, MTBSTFA, Diazomethane

Different internal standards: d3-mecroprop, d3-ibuprofen, Cl-Br-diclofenac

Criteria (if applicable to the type of test method which has to be classified)	***Required at Endpoint of Validation level*** 1	2	3
Module A – Test method definition & documentation			
Definition of need	+	+	+
Purpose			
Development of knowledge	+	+	+
Regulatory purpose			(+)
Scientific basis			
Defined mechanism/effect	+	+	+
Scientific proof of relation between a measured signal or effect and a phenomenon in or property of the investigated system	+	+	+
Documentation (Protocol)			
sufficient information for a researcher with special expertise to "imitate" the method	+	+	+
with detailed information sufficient for a trained analyst		+	+
according to ISO 78-2 (standard-like)		(+)	+
with detailed QA/QC procedures and performance criteria		(+)	+
statistics available (record of performance characteristics)		(+)	+
Dissemination			
Grey literature	+	+	+
Peer-reviewed publication		(+)	+
National, European or International Standard			(+)
Module B – Applicability domain			
Applicability			
to the compound (class) or effect of interest	+	+	+
to the matrix of interest	+	+	+
to the compartment of interest	+	+	+
to the organism or cell type of interest	+	+	+
Modules C to E – Intra- and Inter laboratory Performance			
Matching the performance characteristics required from the regulator			
shown by one (research) laboratory only	+	+	+
shown by comparison study with at least 2 laboratories		+	+
by routine laboratories (proven by inter-laboratory study)			+

First Interlaboratory exercise on Non steroidal anti-inflammatory drugs (NSAID)

An agreement was reached:

2 analytical procedures (one based on SPE GC-MS and another based on SPE LC-MS-MS) will be proposed and used by at least 2 participants (NORMAN partners) (requirement for the fulfillment of VALIDATION criteria)

- Other participants wil used their own methods
- All methods will be used to select a method for the 2nd interlab

Task 3. Evaluation

Aims

- To evaluate the **variability** of results between different laboratories, the capacity and variability in front of complexity of the real samples, and to evaluate the rate at which participating laboratories successfully completed the exercise
- To evaluate the **applicability** of different analytical methodologies
- To give **feedback** to WP VALIDATION (V2 and V4) to draw conclusions about the protocol.
- To give feedback to SEARCH on the application of EMPOMAP (S1) and EMPODAT (S2).
- To give feedback to NETWORK (N1, N2) on the functioning, design and structure of the network.

Network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental pollutants

SUB-PROJECT CASE: WP-C3

Validation and harmonisation of analytical methodology for routine laboratories: brominated flame retardants (DecaBDE)

Sixth Framework Programme

Brominated flame retardants (DecaBDE)

- Taking into account both the need for monitoring and the difficulties in analysis, decaBDE seems to be **an ideal example for a case study in order to transfer knowledge from expert laboratories - which have to date been the only ones to produce satisfactory results in decaBDE analysis in interlaboratory exercises - to routine laboratories** (VALIDATION 3).
- In C3 a sequential approach will be followed, starting with the expert laboratories that will harmonise the methods, followed by a second round with routine laboratories to test the harmonised methods at the routine level. Samples of air (dust) and sediment/soil (sewage sludge) will be selected at different concentration levels.
- Until now laboratories were not very experienced in the analysis of this congener. As reflected in the results of interlaboratory studies the comparability of the participating laboratories for BDE 209 is not at the desired level. Although the within-laboratory variance is still not completely under control.
- BDE 209 is particularly expected to be present in sediment and suspended particulate matter.

Objectives

- To create and have access to a harmonised method validated at routine level to a family of selected emerging pollutants.
- To apply the protocol to be developed in WP VALIDATION V3 to an emerging pollutant belonging to the group of brominated flame retardants for which European risk assessment has revealed lack of monitoring data and extreme difficulties in analysis.
- To provide a validated harmonised protocol to be used in routine laboratories allowing an extended monitoring of decabromodiphenyl ether in the European environment by routine laboratory.
- To give feedback to the sub-projects NETWORK, VALIDATION and SEARCH

Tasks

- ***Task 1. Overview of methods for the analysis decaBDE, selection of reference laboratories and harmonization of procedures***
- ***Task 2: Organisation of intercomparison studies***
- ***Task 3. Evaluation and assessment of the results, definition of a protocol for decaBDE analysis, feedback to WP validation***

Work Progress

Overview of methods and list of laboratories participating in the interlaboratory study (UBA)	**Sep 05 - May 06**
Organisation of **1st interlaboratory study** (UBA) • preparation of the interlab study • 1st meeting of the participants (Stresa) • execution of the 1st interlaboratory study • preparation and publication of the *written report* • 2nd meeting of the participants *Feedback and recommendations to NETWORK, VALIDATION, SEARCH*	**Mar 06 - May 07** June 06 Oct 06-Dec06 May 07

Work Progress

Organisation of **2nd interlaboratory study** (RIVO)	**May 07 - Mar 08**
• 1st meeting of the participants	May 07
• execution of the 2nd interlaboratory study	Dec 07-Jan 08
• preparation and publication of written report	
• 2nd meeting of the participants	Mar 08
Evaluation of the results, definition of protocols for DecaBDE analysis (UBA) *Summary, feedback and recommendations to NETWORK, VALIDATION, SEARCH*	**Jan 08 - Mar 08**