

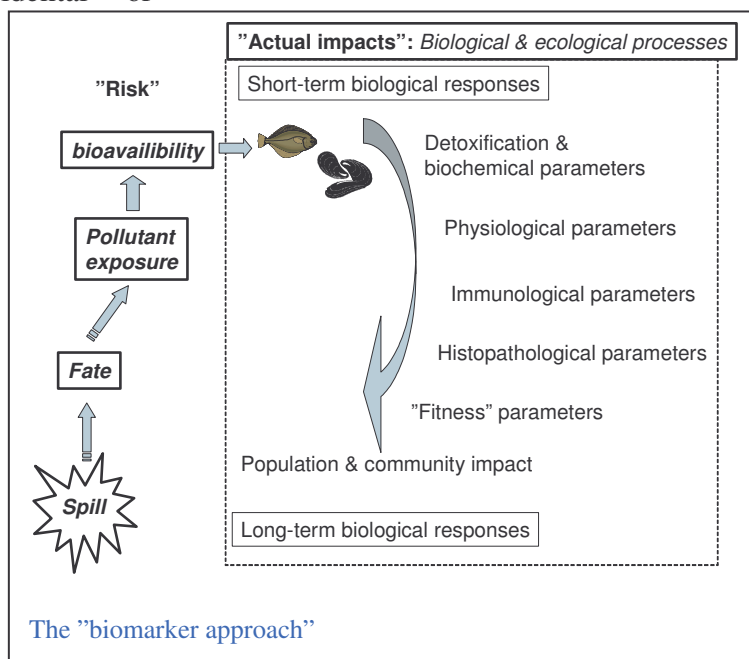
PRAGMA - A pragmatic and integrated approach for the evaluation of environmental impact of oil and chemicals spilled at sea: input to European guidelines

1. Project description

In the frame of the “Call for proposals 2005 in the field of Community Cooperation against accidental or deliberate marine pollution”, the European Commission DG Environment has retained the PRAGMA project which addresses priority field n°4 “*Environmental impact of oil spills and other harmful substances*” of the community framework. PRAGMA will address several issues related to that theme but more specifically related to the long term impact.

Beyond the immediate catastrophic effect of spill causing death of thousands of marine organisms, a major environmental risk to consider is the long term impact of oil and chemical spills on the local ecosystem. Biodiversity study of benthic organisms or/and the comparison between contaminant levels measured in water, sediment and biota to threshold levels established by international (e.g. the ecotoxicological assessment criteria – EAC - defined by OSPAR in 1997) or national agencies have traditionally been used as the main decision criteria. Biodiversity is ecologically very relevant but this requires years of observation because significant changes in population might only be visible on a long temporal scale. Also, it is not easily applicable for organisms living in

the water column. The chemical data include sensitive and accurate measurements that provide both qualitative and quantitative figures of

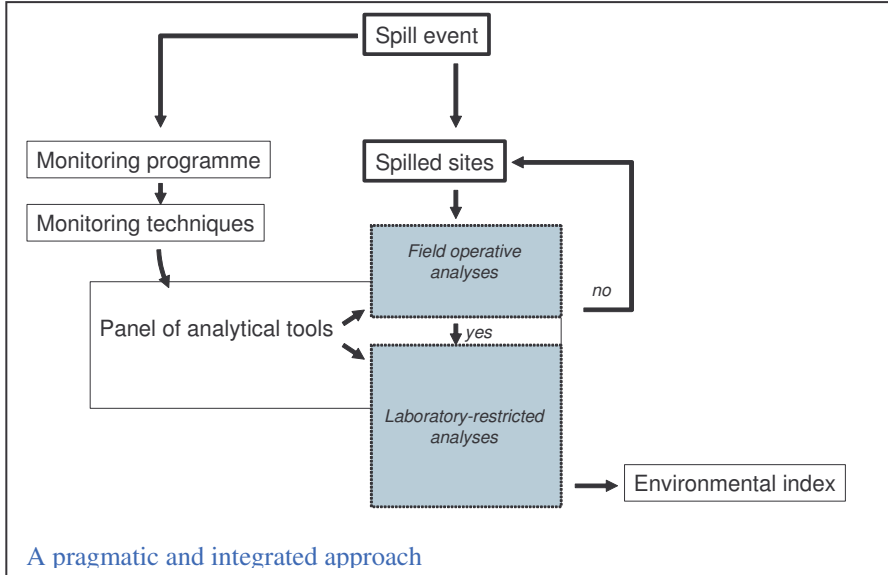


the presence of toxic substances and hence assess the hazard of exposure to the marine biota. Yet, the threshold levels which are defined according to the EU-Technical Guidance Document (EC, 2003) allow defining the risk based on the PEC/PNEC ratio but are not an expression of the actual impacts. In recent years, new criteria based on the measurements of biological markers (“biomarkers”) capable of identifying subtle changes with possible important consequences for the organisms sampled in polluted zone have been proposed. Compared to chemical analysis, a biomarker response will indicate that the pollutant has induced a biological response. Eventually, that response may persist over long time



even though the presence of the pollutant is no longer detectable. The ultimate goal with biomarkers is to evaluate the general health of individuals following exposure. Parameters related

monitoring programmes. This approach ought to be harmonised at the EU level in order to implement these techniques in current assessment and monitoring guidelines. Based on this concept, the



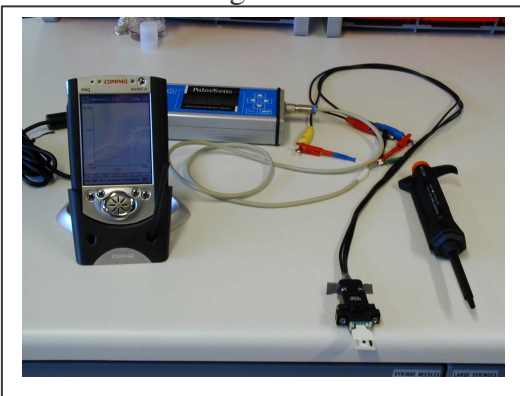
goal of PRAGMA will be to evaluate a battery of tools based on well-known biological methodologies and others new available tools based on biosensors for their possible incorporation in current EU guidelines related to spill

to growth and reproduction are particularly ecologically relevant since they can help to predict impairments beyond the individual level. Other analytical techniques related to developing cost-effective screening devices used at the desired temporal and spatial scale ought to be considered in the pragmatic assessment of environmental impact. Lately, arrays of sensors based on physical, chemical and biological signals have been proposed for the monitoring of the environment.

events and for the monitoring of the environmental effect of spills.

The following tasks will be aimed in the project:

- To evaluate existing methodologies based on biologically-important effect markers used in monitoring programmes and studies following some recent case studies with oil spill and chemical spill along the coastal zone of the Member States
- To propose the methodologies that can be used as a common platform in environmental monitoring of spills and contingency plans
- To incorporate simple, cost-efficient and manageable analytical tools in future pollution monitoring programme within the member states.
- To promote exchange of scientific and technical expertise in the field of environmental pollution monitoring
- To run pilot studies with key representative species (fish and mussel)
- To disseminate the protocols, research results at international/European level.



A combination of sensors as front line monitoring tools and both chemical and biological markers could be incorporated in future marine



IRIS (Norway) is the leader of the project and three other partners are involved, *Le Cedre* (France), University of Brest (France) and University of Bilbao (Spain). In addition, work related to biosensors will be contracted with the University of Florence (Italy).

2. Organisation of the work

PRAGMA is a pilot project and will be organized through four main work activities:

A. Evaluation and selection of analytical methods

Simple and easy-to-perform methodologies that can indicate general health status and impairments connected to reduced fitness of individuals will be prioritized. Other more in deep techniques will also be used. Biosensors will be evaluated for their cost-effective use in biomonitoring.

B. Pilot study experiments

Taking case studies in recent accidents (*Prestige, Erika, Ievoli Sun ...*) exposures with heavy oil and styrene will be simulated in the laboratory. The selected set of biomarkers will be tested using both fish and bivalves including sensitive stages in conjunction with analyses of chemical measurements. Test simple protocols, based on rapid low-cost analytical tools for routine application of biological and chemical monitoring applicable on field.

C. Analysis of results

The information coming from the pilot studies will be integrated into an environmental index used to environmental agencies and decision-makers in case of spill.

D. Dissemination of knowledge and input to EU guidelines

The project will contribute to technical and scientific exchanges between nations. At the community level, common future procedures will be identified enabling good operational practises for pollution monitoring. This initiative may help to define European research priorities and for example enhance the implementation of the water directive framework within EU members.

3. Project start and plan

The EU DG Environment has issued a contract to IRIS starting from the 14th February 2006. The project will be eligible for a period of 14 months. The

work will be distributed between the different partners. IRIS will be responsible for the experimental work with bivalves (*Mytilus edulis*). Le Cedre will be responsible for the experimental work with fish. Both the University of Brest and University of Bilbao will participate with their respective competence and experience in the field of biomarkers. The University of Florence will do the analytical work related to biosensors

Experiments are planned to run for several months including an exposure period (4/5 months) and a recovery period (1 month).

An update of events will be regularly made in the course of the project

4. The partners in brief

Project leader – IRIS (Stavanger, Norway).

IRIS is a joint newly formed research institute between Rogaland Research (RF) and University of Stavanger (UiS) The department “Marine Environment” of IRIS together with two affiliated companies - Akvamiljø and Akvamiljø Caspian - is specialized in marine environmental research. Together, this research centre provides unique expertise and laboratory facilities for testing and monitoring the biological effects of pollution in the aquatic environment. Totally the centre has 50 scientists and engineers with competence within marine environmental sciences. IRIS “Marine Environment” is a leading centre internationally within its field and can provide services and advanced research within ecotoxicology, environmental risk assessment, monitoring, field and laboratory experiments. Typically, the centre is performing research for oil companies, land-based industry, aquaculture industry and governmental institutions. Projects are also financed



by Norwegian Research Council, EU, and NORAD. IRIS and its two affiliated companies have an extended research network throughout Europe and North America.

The main areas of activities are:

- *Controlled exposures of biota to pollution*
- *Environmental health status - new methods*
- *Environmental risk assessment*
- *Field monitoring*

Partner 1 – CEDRE (Brest, France).

Cedre (“Centre de Documentation de Recherche et d’Experimentations sur les pollutions accidentelles des Eaux”) is a non-profit making association that reports to the Ministry of the Ecology and Sustainable Development and has a Board of Directors from: •Government: represented by the Secretariat General of the Sea and the Ministries responsible for the Ecology Defence, Transports and Infrastructure, Interior, Research, Industry, Fisheries

The purpose of Cedre is to advise and assist the authorities in charge of accidental pollution response. Its role in the field of water pollution is specified in the Polmar circular of December 17th 1997 and the interministerial circular directive on accidental inland pollution of February 18th 1985. This includes a 24-hour operational availability to authorities in charge of pollution response. CEDRE provides assistance to the authorities as regards: design and update of response plans (e.g.: sea and land Polmar contingency plans); information on pollution response policies and techniques; improvement and evaluation of pollution response means and techniques; approval of pollution treatment agents; preparation of emergency guides (e.g. for assisting an oil tanker in distress, for response in case of a chemical spill); training of managers and response teams from

administrations, cities and local authorities and the industry; organization of pollution response drills.

During accident involving pollution, Cedre assists the authorities as regards to:

i) assessing risks related to pollution; ii) the choice of the best techniques and equipment to deal with the pollution and the organization of operations; assessing the consequences of both the pollution and the response.

Cedre also serves as an adviser to the European Commission’s Directorate of the Environment for pollution response to oil and other chemical spills.

Cedre has a pluridisciplinary team of 50 people (engineers, scientists, technicians)

Partner 2 – UBO/UPCI (Brest, France).

The University of Occidental Brittany (UBO) is the second French partner also located in Brest. UPCI (Unité de Physiologie Comparée et Intégrative), directed by Philippe Sébert, is composed of 35 researchers working on four main subjects: the endothelial function, the differentiation and structure of muscle and skin, the effects of high pressure on metabolism and the effects of osmotic pressure modifications. The last subject is studied by the team “cell volume regulation and signalling” who is directly involved in the PRAGMA project. The biological models we use are the turbot and the trout. The work carried out by UPCI is based on strong competences in physiology, and cellular physiology that allowed the team to work with a numbers of partners on fish biology and marine pollutions (CNRS, IFREMER, INRA, CEDRE...).

Our competence domain is the assessment of environmental effects on physiology and cyto-physiology and we are specialised in fish homeostasis and especially on hydromineral equilibrium,



a component of the homeostatic equilibrium of all animals. The capacity of all living organisms to maintain the composition of their internal medium is fundamental and any modification of the hydromineral equilibrium will induce important water movement between the different compartments of the organism and especially between extra and intra cellular medium. These water fluxes will induce modification of cellular volumes and modifications of the concentrations of all cellular constituent. The regulatory phenomenon allowing to keep the hydromineral equilibrium and the cell volume in normal state constitute a complex adaptative response. It depends on numbers of physiological mechanisms from the organisms to the sub cellular level of organisation and allow the animals to cope with the modification of their environment.

Partner 3 - UPV: Universidad del País Vasco (Bilbao, Spain).

Since 1985 the group of “Cell Biology in Environmental Toxicology” of the University of the Basque Country develops research and teaching activities within the Environment field (both aquatic and terrestrial ecosystems). The group, recognized since 2001 as consolidated research group, is composed of 7 university lecturers and researchers, 1 postdoc, 11 PhD students and 1 technician. The group’s lab contains facilities for histology, light, fluorescence and electron microscopy, image analysis, cell fractionation and biochemistry, cell culture and molecular biology. The main field of expertise of the group is in development of early warning cell and molecular markers of pollution exposure and effects. The group has developed a novel biomarker, peroxisome proliferation, useful as exposure biomarker of PAHs, PCBs, phthalate esters, pesticides etc, recently

adopted by different european labs and implemented in international programmes. The group also developed a battery of tests in vitro for immunotoxicity. The group measures routinely several well-established biomarkers of pollution (lysosomal stability, EROD, metallothionein-MT, vitellogenin-Vtg, micronucleus test, histopathology), participating in intercalibration trials for EROD, MT and Vtg (BEEP, BEQUALM). Recently we incorporated the proteomics and genomics approach with emphasis in the peroxisomal subproteome and in differential gene expression. Particularly important for this project is the involvement of the group in the management of the Prestige disaster through participation in national and regional expert committees and the short-, medium- and long-term assessment of the biological consequences of the disaster.