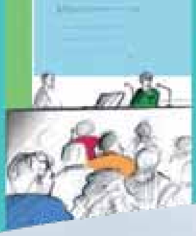

ONEMA Meetings

Recap

Mesocosms

Their value as tools for managing the quality of aquatic environments

ECOTOXICOLOGY SYMPOSIUM
14–16 OCTOBER 2009 IN LE CROISIC

Olivier Perceval – Thierry Caquet – Laurent Lagadic – Anne Bassères
Didier Azam – Gérard Lacroix – Véronique Poulsen

Mesocosms

Their value as tools for managing the quality of aquatic environments

Recap document
of the ecotoxicology symposium
14-16 October 2009 in Le Croisic

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The symposium titled "Mesocosms - Their value as tools for managing the quality of aquatic environments" was organised by Onema and INRA, in conjunction with Total and Ineris, at Le Croisic (France) from 14 to 16 October 2009.

This recap was prepared by Laurent Basilico on the basis of contributions from Gérard Lacroix, Thierry Caquet, Véronique Poulsen, Anne Bassères and Olivier Perceval, drawn from the minutes of the meeting (Laurent Lagadic, Olivier Perceval, Thierry Caquet, Anne Bassères and Didier Azam) and from the review of the literature prepared by Ineris on existing experimental platforms (Sandrine Joachim, Sandrine Andres, Eric Thybaud).

This recap version and the shorter version are available on the Onema site (www.onema.fr), in the Publications section, and at the national portal for "Water technical documents" (www.documentation.eaufrance.fr).

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Préambule

The protection and restoration of aquatic environments are a major ecological and social issue for the coming century. The European Union adopted a strong, pro-active policy, the Water framework directive (WFD), in 2000. Among other aspects, the directive created new obligations in terms of monitoring the chemical and ecological quality of aquatic environments. Implementation of the monitoring programmes by water managers represents a major scientific challenge requiring the development of new methods and the production of new knowledge.

Artificial, aquatic ecosystems, such as mesocosms, constitute an alternative approach to the traditional methods (in the lab or in the field) used to assess environmental risks. Their value as tools for managing the quality of aquatic environments must be studied.

To assist in launching the discussion on these issues in France, Onema and INRA, in conjunction with Total and Ineris, organised the national symposium on ecotoxicology at Le Croisic, on the Atlantic coast in France, from 14 to 16 October 2009.

Following a day of presentations by experts on the use of mesocosms in ecology, experimental ecotoxicology and risk assessment, the participants, primarily scientists and water managers, discussed the potential uses of mesocosms for managers in three specific fields.

This document recapitulates the information and ideas expressed during the three-day meeting.

Contents

Introduction

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Over 100 000 chemical substances have already been registered in the EU, of which 30 000 are used, imported or produced in quantities exceeding one ton per year. Many end up in aquatic environments where they may produce a toxic effect at very low concentrations, on the order of 1 microgram per litre, and directly or indirectly alter the quality of ecosystems. Awareness of these ecological impacts and the corresponding risks for human health has grown significantly since the 1970s and resulted in increasingly stringent regulations.

Since the 1990s, efforts to develop ex ante environmental risk assessment have resulted in European directives 91/414 and 98/8, on phytopharmaceuticals and biocides respectively, then the 2006 REACH regulation on chemical substances. In parallel, policies have addressed monitoring of waterbody quality. The Water framework directive (WFD), voted by the European parliament on 23 October 2000, set the goal of restoring all inland and coastal waters to good chemical and ecological status by 2015. Status is determined notably by reference concentrations for each micropollutant and biological-quality indices, many of which must still be formulated. In the years to come, water management will require improved understanding of the fate and the impacts of substances in aquatic ecosystems, i.e. progress in ecotoxicology.

The purpose of the symposium in Le Croisic was to determine the degree to which artificial, aquatic ecosystems, such as mesocosms, could meet these goals and thus serve for the management of aquatic environments.

In the first part, this document presents the current and past use of mesocosms for ecology, experimental ecotoxicology and risk assessment.

Then, the main issues involved in managing toxic micropollutants in aquatic environments are analysed, taking care to identify the needs and expectations of managers for whom mesocosms could provide useful results.

Finally, the third part, drawing on the contents of the workshops organised during the symposium, briefly presents the information provided by the participants concerning the basic issue, i.e. the value of mesocosms for quality management, divided into three subsections:

- value of mesocosms in defining reference thresholds for concentrations of chemical substances that are acceptable for aquatic environments;

- use of mesocosms (their representativeness, uncertainties) in setting regulations;

- use of mesocosms to develop and validate tools to assist in environmental monitoring.

What is a mesocosm?

Mesocosms have been used since the 1970s in ecology and ecotoxicology. According to the definition of Odum (1984), they are limited, more or less closed experimental systems at an intermediate scale between the microcosm of the lab and the full complexity of the real world, the ecosystem. Their volumes range from a few hundred litres to several hundred cubic metres and the design can vary widely, including polyethylene bags hanging from floats and installed in inland or coastal waters, areas enclosed by nets with variable-size mesh or with waterproof walls, experimental basins or ponds, artificial rivers, etc.

A number of classification systems, based notably on size (Bloesch *et al.* 1988, Heimbach 1994), have been proposed to distinguish different categories of "cosms" (microcosms, mesocosms, macrocosms). However, these distinctions would seem to be fairly irrelevant and often artificial. We prefer to define mesocosms as "artificial systems placed in natural environmental conditions and that are sufficiently complex and stable to achieve self-sustaining status" (Caquet *et al.* 1996).

The regulations governing toxic micropollutants and aquatic environments

European policy addressed toxic micropollutants as early as 1967 with the directive 67/548/EEC on substance classification and labelling, which made it possible to distinguish between toxic and non-toxic substances. Directive 76/464/EEC went further with two additional goals, a general reduction in pollutant releases and identification of substances requiring priority action. Initially, implementation of the directive suffered from a lack of tools for managers because reliable scientific data on risks was unavailable due to the number of contaminants in the environment, of exposure routes and the diversity of toxic effects (Lascombe *et al.* 2008).

Reinforcements in regulations subsequently addressed substance controls, i.e. uses were limited depending on the toxicity. Similar to the emblematic directive 91/414/EEC on pesticides, many regulations, following up on directive 76/769/EEC on "marketing and use of certain dangerous substances and preparations" (abrogated and replaced by the REACH regulation EC 1907/2006 on the registration, evaluation, authorisation and restriction of chemicals), have limited the use of toxic substances to specific sectors of activity, conditions of use or specific products.

In 2000, the Water framework directive (WFD) adopted the general approach of directive 76/464/EEC, but added monitoring of environments, notably with environmental quality standards (EQS) explicitly defined for water and biota (see daughter directive 2008/105/EEC).

The WFD, which concerns all aquatic environments including coastal and transitional waters, requires preservation of non-degraded aquatic environments (reference state) and the restoration of moderately or heavily degraded environments to good status by 2015, given that good status includes both the ecological and chemical status of a water body. The parallel implementation of the WFD and the Marine strategy framework directive (MSFD) will result in protection of all waters involved in the water cycle.

In France, the Grenelle environmental agreement emphasised the importance of pollutants and highlighted the need to restore a balance between the environment and human health. It set a number of goals with the corresponding policies, including:

- drastically reduce the release and dispersion in the environment of pollutants known for their adverse effect on health;
- prevent or manage the risks caused by products, techniques and changes in the environment;
- reinforce and share knowledge on the links between health and the environment. ■

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Mesocosms, a proven tool for ecology and ecotoxicology

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Bibliometric analysis can be used as a first step in tracking the progressive use of “cosm” experimental systems in ecology and ecotoxicology. The results of a search for the terms microcosm, mesocosm, macrocosm and enclosure in the articles in the “Web of Science” database can be used to draw initial conclusions (Lacroix 2009):

- the use of “cosm” terms started gradually in the 1970s and 1980s, then took off in the 1990s;
- as of the end of the 1980s, their use was much more frequent in ecotoxicology than in ecology;
- after 2000, their use stagnated and even dropped in ecotoxicology.

This numerical data reflects the changes in techniques and in topics pursued in each discipline. This first part proposes a retrospective and critical analysis of the use of mesocosms, first in ecology, then in ecotoxicology. It then presents the current situation for mesocosms in France in the field of ecotoxicological risk assessment.

1.1 – Écologie aquatique et mésocosmes : notions préliminaires

Source : Gérard Lacroix, CNRS, Le Croisic 2009

Many topics in the field of scientific ecology have benefitted from the use of mesocosms. Several decades of use have proven their relevance and advantages, but also raised many questions and criticisms. The table below lists the advantages and limits generally attributed to mesocosms.

It is interesting to note that what may appear to be a limiting factor is, in some cases, perceived as a quality in others. These contradictory judgements reveal the largely subjective nature of opinions on mesocosms within the scientific community. Their advantages and limits depend above all on the subject studied. This leads to several general observations concerning their use in aquatic ecology.

Table 1. Advantages and disadvantages generally attributed to mesocosms.

Advantages	Disadvantages and limits
- Capacity to simulate fairly realistic environmental conditions	- Artificial nature
- Simultaneously address different food-chain levels	- Too small in size
- Sufficient complexity for long-term functional communities	- Importance of wall effects (adsorption of contaminants and development of periphyton)
- Capacity to reveal fairly tenuous mechanisms	- Importance of sedimentation processes
- Management of complex factorial plans and analysis of multi-factorial effects	- Lack of ecological realism
- Reproducible treatments	- Low degree of representativeness
- Sampling of the same populations over time	- Only fractions of ecosystems taken into account
- Ease of setting up experimental systems	- Low signal-to-noise ratio
- Rapid acquisition of results	- Short duration of experiments
- Easy to publish results	

Realism and reproducibility

The recurrent issue concerning the realism and reproducibility of mesocosms should be analysed in terms of the necessary compromise between attempts to reproduce the full complexity of the natural environment and the capacity to analyse the processes involved, i.e. to reveal an effect.

Mesocosm studies suffer above all from significant variability within each treatment and the low number of replicates (Eberhardt & Thomas 1991, Caquet *et al.* 2001). This difficulty can be avoided by:

- increasing the amplitude of the effect by exceeding the natural range of variation of the studied factor, however, caution is then required in analysing the results;

- attempting to reduce the variability within each treatment, but this effort to standardise replicates quickly reaches certain limits.

The other and more reasonable solution from the statistical point of view is to increase the number of replicates. It is also more expensive in terms of funding and resources.

Enclosure bias

Effects caused by enclosures, e.g. periphyton development on walls, impacts on the spatial distribution of organisms, increased sedimentation of particulates, etc. constitute a frequently mentioned weak point of mesocosms (Bloesch *et al.* 1988, Carpenter 1996). This criticism must be tempered by noting that the purpose of researchers is rarely to simulate a given natural ecosystem, but rather to understand the processes involved in the studied ecosystems. A mesocosm is in itself an ecosystem. To avoid enclosure bias, it is possible to consider sedimentation or periphyton development as properties of the ecosystem. In addition, some mesocosms have mixing systems to limit these effects. Finally, an increase in the size of the mesocosm can significantly reduce the enclosure effect.

Size of mesocosms

There is no ideal size for a mesocosm. Everything depends on the topic studied and “bigger is not always better”. A mesocosm must be large enough to operate over time without external inputs (other than natural) and to have sufficient diversity of organisms so that the fundamental ecological processes start up quickly. Practically speaking, for an identical amount of resources, there is often a compromise between the number of mesocosms and their size.

A meta-analysis of over 150 types of experimental lotic ecosystems (Belanger 1997) concluded that, in general, the size of a mesocosm had no significant effect on the diversity, abundance or richness of algae or invertebrate communities. In most cases, enclosures of a few cubic metres up to tens of cubic metres made possible considerable progress in knowledge. On the other hand, analysis of certain processes such as movements in water bodies or the regulation of fish communities is not compatible with small sizes and short time steps. For such studies, very large experimental systems (several thousand cubic metres) are the best solution, though the cost becomes a limiting factor.

Effects of isolation and the duration of experiments

Mesocosms are by definition closed systems, which may in itself represent a limit to their use over time. That is particularly the case for artificial rivers, which generally lack any inputs from upstream, downstream or from the banks. In the absence of regular external inputs, the productivity and diversity of the communities in these systems drops rapidly, which limits the duration of experiments. In experimental ponds, a gradual drop in biological diversity and productivity, as well as an increase in the inbreeding of certain species may also occur.

Finally, in enclosures, reduced turbulence and isolation from the rest of the ecosystem lead to a deficiency of nutrients and a drop in the abundance and diversity of communities, particularly for plankton. The isolated environment diverges in its evolution from the surrounding environment.

From mesocosms to natural ecosystems

Following this line of reasoning on integrating complexity and ecological realism leads to the idea of manipulating natural ecosystems. Though of obvious scientific value, such experiments are extremely regulated (and correctly so), particularly if they are likely to result in ecosystem degradation. Another approach that could be highly useful would be ecological-engineering projects in highly deteriorated ecosystems. They would be a means to test theories in the real world while directly targeting improvements in ecosystems.

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Three examples of mesocosm contributions to ecology

- Various lab experiments have shown that certain algae react to the presence of herbivorous zooplanktonic organisms with changes in their morphology (gathering in colonies, appearance of spines, etc.). Experiments to characterise these modifications, often difficult under breeding conditions very dissimilar to natural conditions, are a typical application for mesocosms. For example, it has been shown (Hamlaoui-Rézig 2001) that the chlorophycean *Desmodesmus quadricauda* forms colonies due to increases in the abundance of herbivorous microcrustaceans. Similarly, the dinoflagellate *Ceratium hurundella*, which is very difficult to breed in the lab, responds to structural variations in food webs with a change in the number of its caudal spines. Mesocosms were the means to clarify the factors determining these changes by placing the micro-organisms in different environmental conditions (Hamlaoui *et al.* 1998).

- The trophic-cascade hypothesis (Carpenter *et al.* 1985, Carpenter & Kitchell 1993) stipulates that for a given level of nutrient inputs, an increase in planktivorous fish should result in a reduction in large herbivorous zooplankton and an increase in algal biomass. Long contested (De Melo *et al.* 1992), this theory received strong support from an in-depth meta-analysis (Brett & Goldman 1996), based on 54 experiments using mesocosms and macrocosms.

- Studies based on overly simplified functional networks are limited by the complexity of trophic interaction, the richness of species and the major importance of omnivory in natural ecosystems. An alternative is to consider all species via a topological analysis of the food webs based on the existence or absence of a eater-eaten relationship between the various taxa. This approach can use mesocosms to compare the effects of different treatments on the food webs. For example, this technique was used to demonstrate the high impact of the behaviour of two planktivorous fish (*Lepomis macrochirus* and *Dorosoma cepedianum*) on the structure of food webs, the degree of connectivity, omnivory and the length of food chains (Lazzaro *et al.* 2009).

1.2 – Mesocosms, a proven tool for ecotoxicology

Source: Thierry Caquet, INRA, Le Croisic 2009

Though originally used for ecological research, artificial aquatic ecosystems were rapidly seen as prime experimental tools for evaluating the fate and effects of chemicals in aquatic environments. This section will present a typology of these tools and their uses for ecotoxicology, then discuss certain practical aspects of their use that are specific to the discipline. It ends with observations on the role and contributions of mesocosms to aquatic ecotoxicology.

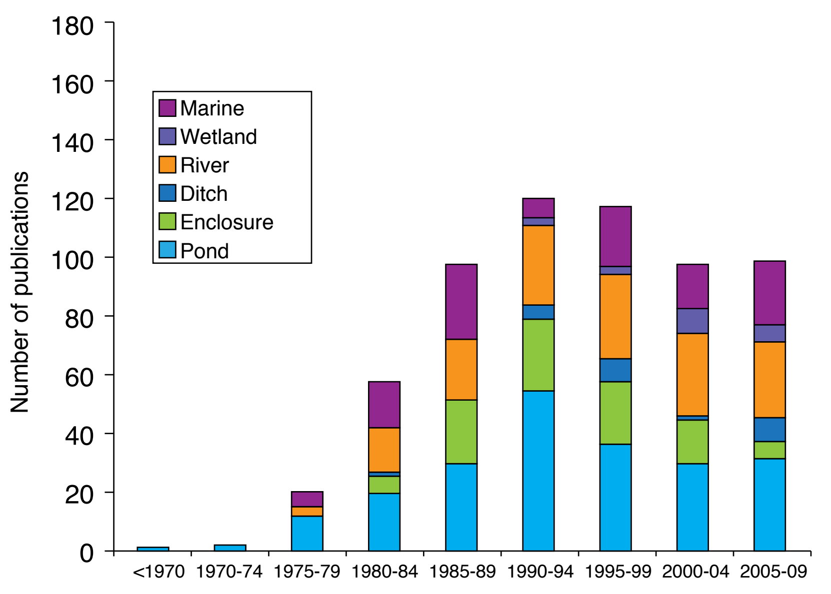
Typology of tools

The international literature on ecotoxicological experiments using mesocosms was analysed via queries to the *CAB Abstracts* and *Web of Science* databases spanning the years 1975 to 2009. Hits concerning indoor studies or ecological research were subtracted from the total results obtained for keywords (mesocosm, artificial stream, enclosure, ditch, etc.). The resulting list contained 769 documents, including a majority of original publications presenting new data. Though not absolutely complete, this list may be considered a good starting point for statistical analysis of mesocosm use in aquatic ecotoxicology.

Figure 1 (next page) shows the changes over time in the number of ecotoxicological publications mentioning mesocosms and the breakdown in the different types of system.

Starting in the 1980s and above all in the 1990-1995 period, mesocosms were used extensively in ecotoxicology. Since then, publications have continued regularly at an average rate of 20 per year, down slightly, which may be due to the very common use of mesocosms in ecotoxicology and consequently less

Figure 1. Typology of mesocosms (T. Caquet).



frequent use of the term "mesocosm" or similar terms in titles.

Concerning studies on freshwater ecosystems, ponds (pre-existing or created) are the most commonly used type of mesocosm (43.2%), followed by artificial rivers (29.4%). These running-water systems, whose ecological representativeness is limited when they are not connected to other elements in the river basin, are nonetheless well suited to monitoring the response of different organisms to pollutants.

Among enclosures, it is necessary to distinguish limnocorrals, i.e. enclosures set up in larger water bodies and possibly in contact with sediment, and enclosures in the littoral zone of a lake, with a maximum depth of two to four metres, that close off a section along the shore. Experimental ditches are extensively used in the Netherlands and may be lentic or lotic systems, as the case may be. Finally, the progressive emergence of artificial wetlands in ecotoxicological research should be noted.

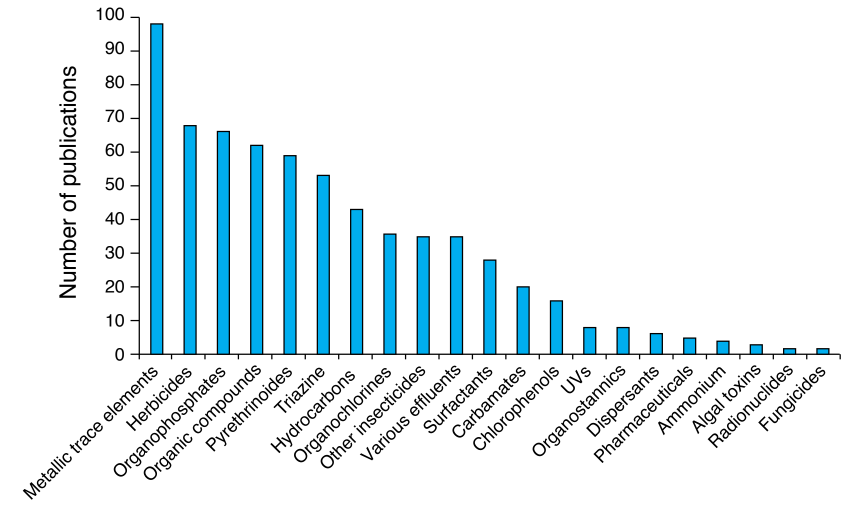
Typology of the toxic substances studied

Using the same bibliographical basis mentioned above, it is also possible to determine mesocosm use in ecotoxicology as a function of the substances studied (figure 2).

The results reveal a clear prevalence of studies on pesticides (46.1% of publications for both inland and marine waters), which may be explained by the work that made the use of mesocosms in substance-approval procedures a routine matter. Studies on metallic trace elements (15%) were the second largest group.

Principal-component analysis (PCA) on the same bibliographical data made it possible to link the studied

Figure 2. Frequency of mesocosm studies for different categories of environmental contaminants (T. Caquet).



toxic substances with the types of mesocosm used, thus revealing the specificity of certain uses:

- dispersants, organostannics and algal toxins with marine experimental ecosystems;
- effluents, ammonium and surfactants with artificial rivers;
- organochlorines, carbamates, organophosphates, metallic trace elements (MTE), triazine and pyrethrinoides with lentic mesocosms (ponds, ditches, enclosures, etc.).

Practical questions on size, species and effect criteria

A great deal of work has been devoted to analysing the various steps in using mesocosms for aquatic ecotoxicology (e.g. Graney *et al.* 1994, Hill *et al.* 1994, Campbell *et al.* 1999, Caquet *et al.* 2000, Giddings *et al.* 2002). A few observations on the size of mesocosms, their biological characteristics and the effect criteria used are presented here.

What size?

The size can vary considerably for ecotoxicology studies, with artificial rivers ranging from less than one metre to over one kilometre in length, ponds from two to 1 000 cubic metres and limnocorrals from two litres to 2 500 cubic metres. The observations made in the previous section on mesocosm use for ecological studies remain valid. An additional factor is that the impact of the measurement and sampling systems, and of the experimental work on the structure and dynamics of the mesocosms must remain negligible. However, very large units (several hundred cubic metres) make it more difficult to input contaminants and also run the risk of seeing different parts of the system diverge, which would result in greater variability in many parameters and more complicated sampling.

Which organisms?

The vascular aquatic plants play a major role in structuring mesocosms. They serve as supports for periphyton development as well as refuges and supports for egg laying by different animal species. Structure is also provided by phytoplankton, whose distribution influences that of the herbivorous zooplankton, and by certain predators that can influence the size and distribution of their prey.

Operation of mesocosms is significantly conditioned by the primary producers (phytoplankton, periphyton) and the detritivores (micro-organisms and invertebrates). The consumers, e.g. fish, produce direct (selective predation) and indirect (reduction in the pressure of zooplankton on phytoplankton) effects on operation.

Which criteria should be measured?

Mesocosms enable simultaneous use of different descriptors. By comparing them, it is possible to characterise cause and effect relations that are difficult to detect in the natural environment. The first type of useable descriptor corresponds to ecological parameters (dissolved oxygen, pH, etc.) which inform on the effects of pollutants on the ecosystem as a whole. Other descriptors are provided by the organisms, in terms of populations (abundance, size frequency distribution, etc.) or communities (diversity, dominance, etc.). Macro-invertebrates and plankton, plus periphyton in running-water systems, are the most studied groups.

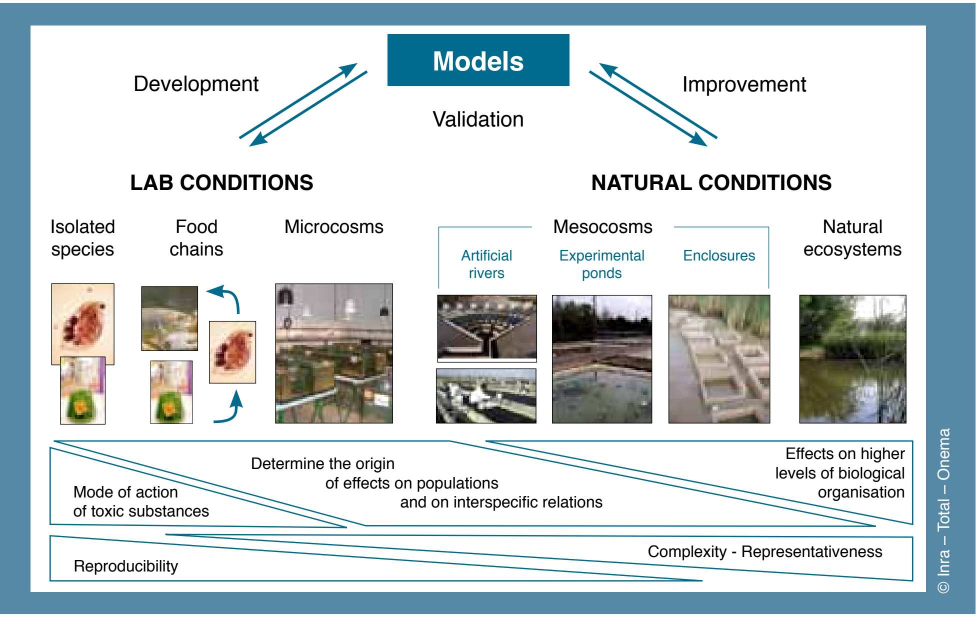
Role and contribution of mesocosms in aquatic ecotoxicology

Mesocosms are part of a wide range of tools used in ecotoxicology, from toxicity tests in the lab to studies in the natural environment (figure 3). Generally speaking, they constitute a good compromise between realism (better than in the lab) and ease of implementation (compared to studies in the natural environment).

A fundamental characteristic of mesocosms is their capacity to reveal the response of communities comprising different types of organisms (micro-algae, invertebrates, etc.) exposed to one or more contaminants, something that is not possible using monospecific toxicity tests in the lab.

A second essential feature of mesocosms is the possibility to simultaneously analyse the fate and the effects of toxic substances. They make it possible to take into account phenomena that reduce (e.g. adsorption by suspended matter) or increase (e.g. bioturbation) the bioavailability of contaminants and thus their effects. This is essential, particularly when assessing environmental risks. An example is pyrethrenoide-based insecticides. As shown in mesocosm studies, the acute toxicity (96 h LC50 < 1 µg/l in a lab) produces only limited ecotoxicological risks for fish due to the rapid adsorption of these substances by particles suspended in water and sediment, and then their degradation into nontoxic substances.

Figure 3. Position of mesocosms in the range of analysis tools used to measure effects at different levels of biological organisation (Caquet *et al.* 2000).



Another major advantage of mesocosms is that they make it possible to identify and study the indirect effects of toxic substances. Such effects take place when a substance directly affects certain key species in an ecosystem (vascular plants, dominant herbivores, predators, etc.), thus leading to various consequences, e.g. changes in abiotic parameters (e.g. pH), selection of tolerant genotypes leading to a loss of genetic diversity, proliferation of tolerant species at the expense of more sensitive species, or increased vulnerability of certain prey due to alterations in their behaviour or habitat.

These advantages are of great value for ecotoxicological studies and complement the characteristics mentioned above for mesocosms, namely complexity, reproducibility, control over exposure conditions and comparisons with control systems. On the down side, their implementation must take into account the limitations also mentioned above, i.e. loss of productivity due to isolation, limited duration of experiments and variability within treatments. In addition, the cost is generally high. Use of mesocosms must be organised to optimise the cost-benefit (information) ratio.

On the whole, mesocosms are highly worthwhile tools to advance ecotoxicological knowledge and develop methods to characterise the quality of aquatic environments. However, their use must be carefully organised, which means, in general, integrated in an overall approach. Their cost makes it necessary to clearly pinpoint the questions to be answered. Once the questions have been formulated, it is possible to select the sampling and measurement methods, and the parameters to monitor.

1.3 – Current status and outlook for the use of mesocosms for ex ante risk assessments

Sources : Olivier Perceval, Onema, Le Croisic 2009, Véronique Poulsen, ANSES, Le Croisic 2009 ; Anne Bassères, Total, Le Croisic 2009

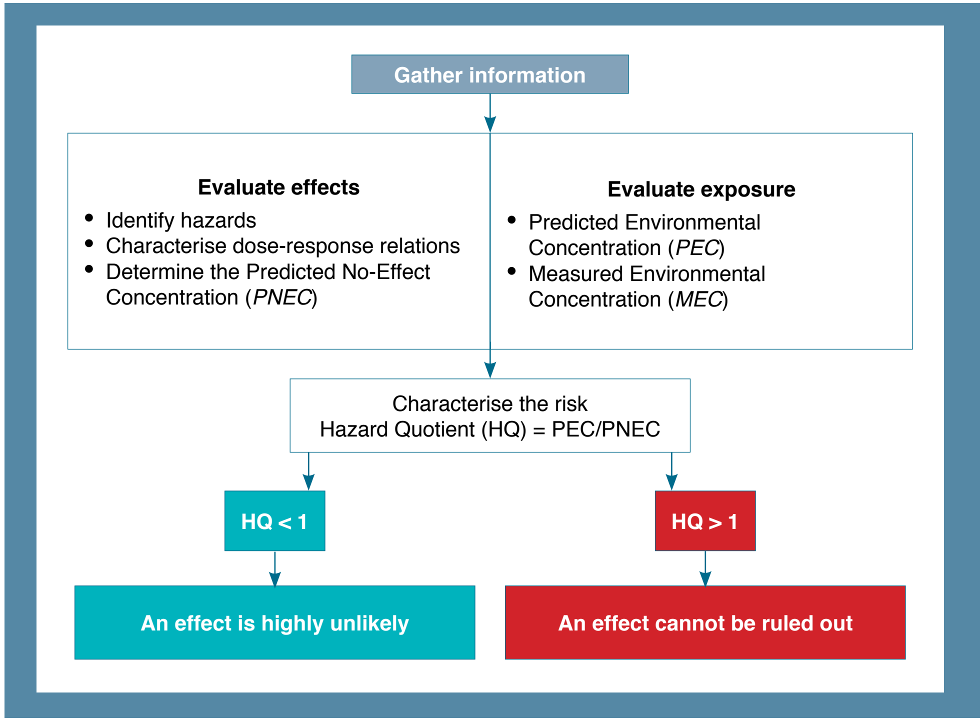
The field of ex ante ecotoxicological risk assessment came into being during the 1980s with the new collective awareness of the need to evaluate the impact of human activities on ecosystems. This resulted in European policies containing measures and legal stipulations intended to regulate the marketing of synthetic toxic substances. That has been the case since the 1990s for pharmaceutical products (directive 91/414) and for biocides (98/8), and more recently for chemicals with the REACH directive, which set up a registration system for chemicals that is not in itself an authorisation to market, but requires ex ante risk assessment similar to that established for pesticides. This section first presents the technical aspects of this risk assessment and the role that mesocosms can play. It illustrates this role with quantitative data on the contributions of mesocosms in the regulatory evaluations of phytosanitary (plant-protection) products, then with a spotlight on their use by Total Petrochemicals in another field. On the basis of this information on the current situation, the conclusion lists emerging needs in this field of ex ante risk assessment, for which mesocosms could represent a valid approach.

PNEC and mesocosms

Ecological risk assessment may be defined as “a process to evaluate the likelihood that adverse ecological impacts may occur or are occurring as a result of exposure to one or more stressors” (U.S. EPA 1992). For aquatic environments, it is based on determining the predicted or measured environmental concentration (PEC or MEC) of a contaminant in a precise water body and comparing that value to the predicted no-effect concentration (PNEC) of the contaminant. The PEC/PNEC ratio is the hazard quotient (HQ). If the HQ is less than 1, an effect is highly unlikely, if it is greater than 1, an effect on the environment cannot be ruled out (figure 4).

PECs, which represent the overall exposure of aquatic organisms to a substance in their environment, are generally estimated on the basis of more or less elaborate models. PNECs are determined on the basis of short and long-term ecotoxicity data for each substance.

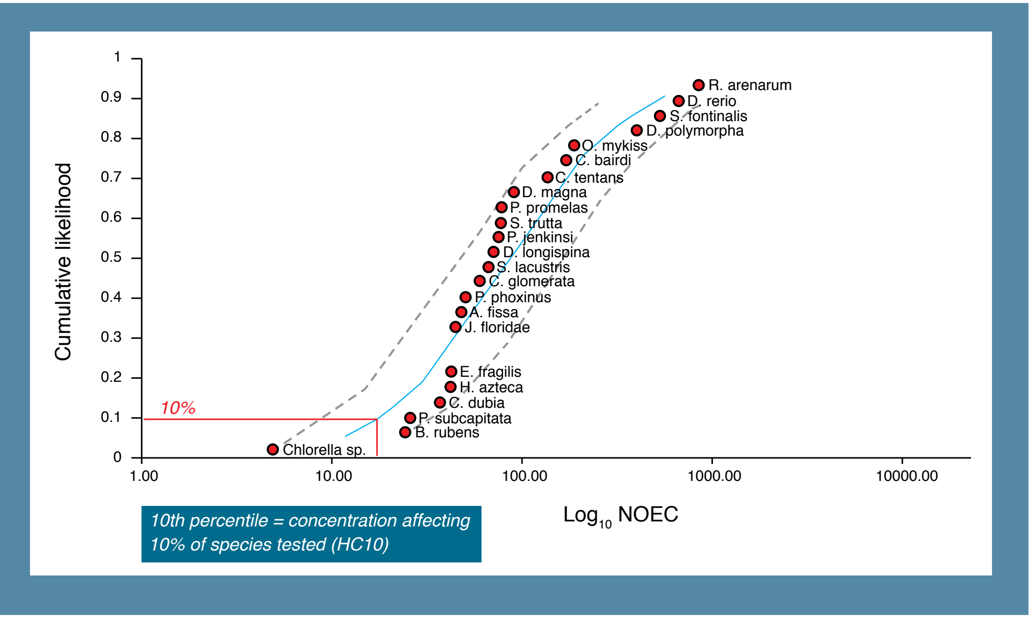
Figure 4. General principles behind environmental risk assessment (see EC 2003. Technical guidance document on risk assessment).



The most commonly used acute-toxicity parameters are LC50 (lethal concentration resulting in the death of 50% of exposed individuals) and EC50 (effective concentration inducing an effect on 50% of exposed individuals). Chronic effects are characterised by the No Observed Effect Concentration (NOEC) and/or EC10 (effective concentration inducing an effect on 10% of exposed individuals), and less frequently by the No Observed Ecologically Adverse Effect Concentration (NOEAEC), a concentration at which populations can return to a state comparable to the control group at the end of the experiment. The latter parameter is typically the result of experiments in a mesocosm.

If the data are sufficient, a probabilistic method based on an analysis of the species sensitivity distribution (SSD) for a given contaminant may be used, on the condition that the empirical data follow a specific theoretical distribution function (figure 5). If not, a deterministic approach using extrapolation factors (also known as assessment factors) is applied to the lowest NOEC or EC10 value. These safety factors compensate the lack of knowledge on the ecotoxicity of the considered substance, e.g. its toxic effects over the long term, toxic effects on other, potentially more sensitive species, extrapolation of effects to the natural environment based on data acquired in the lab, lack of information on the food web as a whole, etc. Efforts to gain more knowledge may be launched to reduce the degree of extrapolation, notably for substances with special economic value or representing a major environmental risk, by carrying out additional chronic-ecotoxicity tests or by running studies in mesocosms (e.g. Lepper 2005).

Figure 5. Species sensitivity distribution (SSD) for a given contaminant.



10th percentile = concentration affecting 10% of species tested (HC10)

Practically speaking, use of mesocosms in this specific regulatory situation requires that a number of conditions be met.

- Exposure to a given contaminant must be sufficiently characterised. A prerequisite to the use of mesocosm data is that the concentration of the "tested" pollutant be measured throughout the experiment to determine an average exposure level. The toxic effects observed must take into account the average exposure levels.

- The manner in which contaminants are introduced in the mesocosm must correspond to the likely modes of transfer of the studied substance in the environment. For pesticides for example, studies must use the products employed in agriculture, making every attempt to reproduce realistic doses, durations and frequencies of exposure, in compliance with technical guidance.

- Mesocosms must contain at least the most sensitive species identified in the lab during the ex ante ecotoxicity trials. They must also ensure sufficient biodiversity at each position in the food web.

- Mesocosms must be placed in environmental conditions comparable to those in the zone(s) where contamination is likely.

- Depending on the substance, the study must enable monitoring of contaminant concentrations in the various compartments of the mesocosm, including in the sediment. If no data are available on the latter compartment, it is difficult to interpret the results, notably when substances are rapidly adsorbed by the suspended particles or by the sediment.

Routine use of mesocosms for phytosanitary (plant-protection) studies

As noted above (section 1.2.), pesticides are by far the most studied toxic substances in mesocosms for ecotoxicology (42% of publications). This prevalence is due to the work that made the use of mesocosms in approval procedures for phytosanitary (plant-protection) products a routine matter.

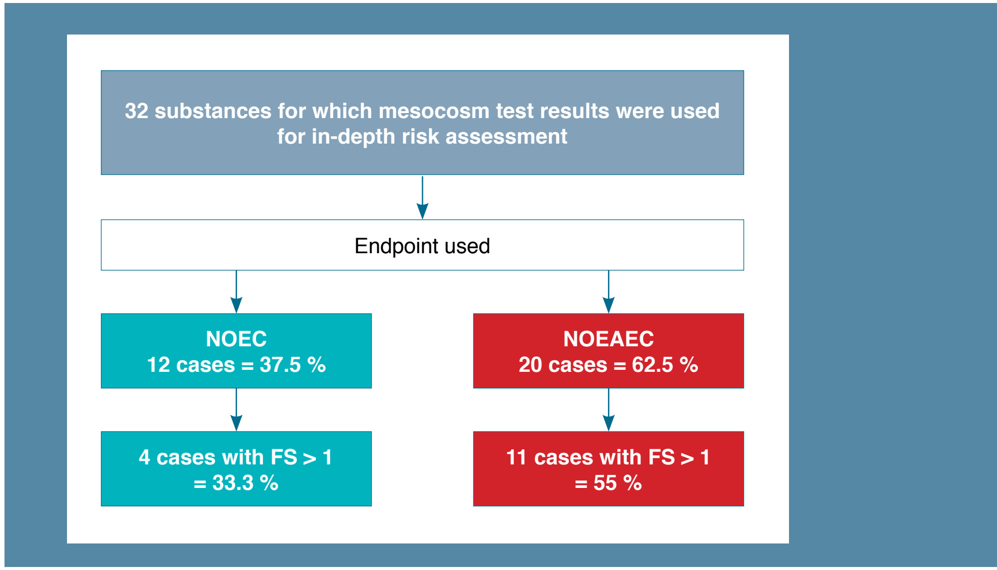
Quantitative analysis of mesocosm use in this field was carried out in 2007 by the AMPERE work group (*Aquatic Mesocosms in Pesticide Registration in Europe*, Alix *et al.* 2007). The group examined the files on the active substances listed in Annex 1 of directive 91/414 and checked, for each substance, if there were one or more mesocosm studies in the file and if they were used for risk assessment.

On the European level, of the 157 substances listed in Annex 1 of directive 91/414, 36 contained at least one mesocosm study in the approval file. Of these, 32 were used for risk assessment which thus constitutes by far the most common use of mesocosms for the studies listed in European files.

Examination of the measured biological effects (endpoints) proposed following these studies (figure 6) shows that in most cases (20 out of 32), NOEAEC was preferred to NOEC. However, selection of the less demanding reference value was accompanied by safety factors that were more often greater than 1.

The AMPERE work group also looked at how mesocosm studies are used by EU Member States for national market-authorisation procedures. In France, over half of the 36 substances in question were subjected to a mesocosm study. The percentages were 78% in Germany and 62% in the U.K. Analysis of the selected endpoints shows, in all cases, a clear preference for NOEAEC. Finally, concerning the safety factors, we note that they are more often greater than 1 in the national studies than in the studies for the European level.

Figure 6. Mesocosm studies for European risk-assessment procedures on pesticides listed in Annex 1 of directive 91/414 and selected endpoints and safety factors.



Total and its "model rivers", an example of industrial use

Regulatory changes (REACH regulation) have confronted industrial companies with new challenges for ex ante risk assessment of the substances they wish to market. In addition to these mandatory regulations, the new requirements (WFD) concerning monitoring of waterbody quality also constitute for industrial companies a strong motivation to play a proactive role in developing new methods to monitor and measure the impact of effluents.

It was in this context that the Total group set up in 2000, on its Mont-Lacq R&D site (SW France), 16 running-water channels (40 m long, 0.5 m wide and 0.5 m deep), supplied with water from the Gave de Pau river (see photo below).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These mesocosms or "model rivers" have since been used for various studies carried out with research organisations and water managers. Between 2000 and 2003, Total ran a programme with the Adour-Garonne Water agency to validate alternative monitoring methods for water bodies. This work, based on the use of exposure biomarkers in the freshwater clam *Corbicula fluminea*, contributed to a risk-assessment study on monochloroacetic acid (MCAA), the results of which were mentioned by the *European Chemical Bureau* in its risk-assessment file published in 2003.

Currently, this experimental site is also used by Total and INERIS for a risk assessment on xylene. This substance has already been assessed in lab tests, but the limited number of ecotoxicity tests led to application of an assessment factor of 100 for the PNEC. The mesocosm study, now underway, may provide useful data on the fate and impact of this substance at higher biological organisation levels and thus make it possible to reduce the assessment factor.

Emerging needs for risk assessment and outlook

Today, the biological test methods used to assess chemical risks are generally well established, codified on the international level and presented in a large number of technical guidance documents.

But many questions remain. For example, efforts to standardise ecotoxicity tests must be pursued (Breitholz *et al.* 2006) for substances that have low solubility and/or are instable in water (PCBs, PAHs) and that are often in contact with living organic matter or organic detritus. Interpretation of data from "standard" tests in the lab, using these substances in their dissolved phase, may be difficult because they often do not produce the desired toxic effect in an aqueous solution. In addition, the characteristics of these

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substances make it difficult to maintain organism exposure at a constant level throughout the experiment.

To counter these difficulties, different lab techniques have been proposed (use of alternate labware, preconditioning of glassware, a reduction in the ratio of the tested organism biomass to the volume of the exposure environment, etc., OECD 2000) and modelling techniques based on the equilibrium partitioning approach have been developed. In spite of this progress, most biological tests now used routinely would not seem to produce reliable results for hydrophobic substances and those instable in water.

Generally speaking, “exposure” remains the primary weak link in risk assessment, notably given the variety and heterogeneity of natural environments and the diversity of routes for contamination. Emerging substances (endocrine disruptors, pharmaceuticals, manufactured nanoparticles, etc.) will constitute a vast field of study for ecotoxicology and risk assessment.

The sections above indicated the degree to which mesocosms can serve as risk-assessment tools and illustrated their current use for regulatory work.

They have contributed to producing ecotoxicological knowledge, notably for the definition of reliable and realistic reference values (PNEC) for many products and they could also be used to better understand the impacts of emerging substances. ■

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2 Aquatic-environment managers and toxic micropollutants


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needs and expectations

The purpose of this second section is not to list all the needs and expectations of aquatic-environment managers concerning toxic micropollutants, but rather to evoke certain fields of work in which the R&D on mesocosms and more specifically in ecotoxicology could result in the creation of operational tools required for informed and acceptable management of these issues.

Taking into account environmental pollution by potentially toxic substances represents a major challenge for water managers and stakeholders. They must not only manage the status of environments and their malfunctions, but also take action to handle the consequences of contamination that are not yet even apparent.

A specific regulatory framework, outlined in the beginning of this document, exists for this work. In this context, the scope of action for managers includes ex ante assessment of chemical risks, evaluating and monitoring the status of aquatic environments and restoring environments with degraded ecological conditions (Pelte 2009).

The outlook presented in the conclusion of the previous section (1.3.) noted certain expectations of managers for ex ante assessment of chemical risks, which could potentially benefit from mesocosm studies, notably in view of:

- developing standardised methods to assess the chemical risk of substances that are only slightly soluble or instable in water;
- understanding uptake mechanisms for emerging contaminants and improving their environmental risk assessment, notably those representing a high risk of long-term effects.

The following sections pursue the discussion, presenting the expectations of managers concerning environmental quality standards, seen as the decisive element in determining the chemical status of water bodies (in the WFD sense), and concerning the development and validation of tools to monitor environments, e.g. biological indices, sentinel species, biomarkers of exposure and effects, etc.


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2.1 – Environmental quality standards

Environmental quality standards (EQS) set for all WFD priority substances are defined as “the concentration of a particular pollutant or of a group of pollutants in water, sediment or biota which should not be exceeded in order to protect human health and the environment”. These threshold values are essential elements in defining environmental goals and the corresponding management plans. They take into account the direct ecotoxicological effects (water, sediment) and the effects on human health through ingestion of food and/or water. (Indirect ecotoxicological effects take place via bioaccumulation (secondary poisoning) in biota.) Their formulation is based on risk-assessment methods used in regulating chemical substances (see the Technical Guidance Document (TGD) for industrial substances and directives 91/414/EEC and 97/57/EC). A new European guidance document on deriving environmental quality standards for the WFD is now being finalised.

Currently, the only proposed standards under discussion by the Member States concern the aqueous phase. The daughter directive establishing “environmental quality standards in the field of water policy” recommends using integrative compartments (sediment, biota), notably for hydrophobic substances having a high bioconcentration factor, but this possibility must be accompanied by the derivation, by the Member State, of EQSs specific to these matrices.

For example, for the sediment compartment, the proposed methods depend on the availability of ecotoxicity data on benthic organisms. In most cases, given the lack of such data, the partition-coefficient method is recommended. Based on the principle of an equilibrium of contaminant concentrations between the aqueous and solid phases, this method is used to calculate a standard for sediment, using the partition coefficient of the contaminant and the standard set for the organisms in the water column.

This approach is problematic (see Bonnomet & Alvarez 2006 for a critical analysis) in that it uses highly variable partition coefficients for a given substance, depending notably on the characteristics of the sediment, but above all it assumes that the only accumulation route for pollutants in benthic organisms is the dissolved phase.


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More generally, a majority of experts think that EQSs established for water, most of which are based on biological tests in the lab, are not very representative of ecological risks caused by environmental contamination, notably for substances likely to bioaccumulate and be biomagnified in aquatic food webs. The multiplication of safety factors at each step in the formulation process can result in unrealistic EQSs (Claisse 2009).

EQSs for biota and sediment are therefore absolutely necessary to ensure sufficient protection for predators at the top of food webs and for foraging organisms, and are even seen by some (e.g. Crane & Babut 2007) as one of the major issues in the WFD.

In this context, the needs and expectations of managers concern primarily rational and defensible EQSs for biota and sediment, based on either a critical analysis of the results obtained using the formula provided in the Technical Guidance Document, or exposure tests using contaminated sediment under standardised test conditions.

2.2 – Biological tools to assist in monitoring

Biological indices

Disturbances to environmental quality often result in a reduction of biodiversity in communities or a modification in the relative abundance of taxa (species, genus, family). Some taxa, termed “pollusensitive”, fall in numbers or disappear while other, more resistant taxa appear or multiply.

Consequently, evaluation of the ecological status of environments for the WFD must be based on an analysis of the resident communities and on measurement data with respect to a reference state. A number of indicators already exist (IBD, diatom biological index, IBGN, biotic index and IPR, river fish index), but they must be adapted to be fully compatible with the WFD (e.g. take into account the taxonomic or functional composition, abundances, the age structure of populations and communities). In addition, most bioindication tools developed to evaluate the quality of aquatic environments were not specifically designed to reveal the impact of toxic substances. For example, the IBGN biotic index is sensitive above all to variations in environmental oxygenation and to habitat modifications, but would not appear to provide useful data on micropollutants.

There would, however, appear to be other tools and methods better suited to micropollutants, for example the “Species at Risk” (SPEAR) approach based on an analysis of the benthic invertebrate community for pesticides in rivers (see Schäfer *et al.* 2007 for a large-scale example in Europe).

Sentinel species used for monitoring

Since the 1980s, the Water agencies have been responsible for monitoring waterbody quality. In the monitoring networks, chemical analyses are carried out on "standard" compartments such as untreated water, suspended matter and sediment, but also on biota such as bryophytes (primitive plants such as moss and sphagnum that easily accumulate certain pollutants). The latter compartment is a firmly established analytical tool for monitoring metallic contaminants in water (André & Lascombe 1987) and technical guides on their use for monitoring are available (Agences de l'Eau 1998). Aware of the importance of chemical monitoring using biota, the Water agencies with various research institutes have studied the possibility of using other sentinel species representing the different river basins. However, due to the excess costs incurred and the difficulties encountered during sampling, data analysis and use of the results, no generalised use of new sentinel species in the monitoring networks is currently planned. In addition, the fact that standardised chemical-analysis techniques do not generally exist for these matrices creates an obvious problem in terms of the quality of the data produced (Schiavone & Coquery 2009).

That could change. The daughter directive for EQSs defines biota environmental quality standards for at least three of the 33 priority substances (mercury, hexachlorobenzene, hexachlorobutadiene) and imposes monitoring of the temporal trends in contaminant concentrations in integrative compartments. These requirements should result in the increased use of aquatic organisms as an analytical means for monitoring of contaminants in the environment. Before implementing these EQSbiota, each Member State must set up monitoring strategies, notably for sampling and analysis techniques, to obtain consistent results that can be used for comparisons on the national and European levels.

To date, the sampling strategies and the selection methods for sentinel organisms have not yet been standardised for chemical monitoring of inland environments. There are many general criteria to define an "ideal" sentinel species (Phillips & Rainbow 1993; Beeby 2001). Selection of one species over another must take into account not only these criteria (table 2), but also the monitoring goals, the contaminants to be detected, the overall sampling strategy (passive monitoring/caging and translocation of organisms) and the sampling parameters (sample size, period and frequency of sampling, size/age of individuals, etc., Tilghman *et al.* 2009).

Table 2. Characteristics of an "ideal" sentinel species (Beeby 2001).

- Rapid reaction to variations in ambient environment (rapid balancing between external and internal media).
- Linear relation between contaminant concentration in ambient environment and that in the overall organism (or its tissues), for a wide range of contaminant concentrations.
- Relation between the quantity of bioaccumulated contaminants and the concentration of the same contaminants in the ambient environment must be the same for all sites studied.
- Abundant species from which large numbers can be taken without significant effect on the population.
- Ease of identifying the species and determining ages.
- Large body of knowledge on species physiology, including the effects of age, sex, season and reproduction on the content of bioaccumulated contaminants.
- Sufficient quantity of tissue for chemical analysis.
- Long-living species for integration of the pollutant over a sufficiently long time period.
- Sedentary species to assure sufficient data on contamination at the studied site.
- Exposure routes to contaminants well identified and understood.

Exposure and effect biomarkers

In terms of efforts to evaluate the effects of environmental contamination on organisms, biomarkers have been defined as observed or measured modifications on different levels of biological organisation lower than populations, that signal exposure of the organisms to at least one contaminant (Depledge 1993; Lagadic *et al.* 1997). However, to account for the diversity of contaminants in aquatic ecosystems and the multiplicity of their effects, it was rapidly deemed necessary to adopt a multi-biomarker approach based on measurements of additional biomarkers covering a wide range of effects on different levels of biological organisation (e.g. Galloway *et al.* 2004, Sanchez *et al.* 2008 and table 3).

Table 3. Examples of biochemical and cellular biomarkers used in ecotoxicology to detect exposure and/or effects (Sanchez 2008).

Biomarker	Description	Detected contaminants
EROD activity	Biotransformation enzyme induced by planar hydrocarbons	PCB, PAH and compounds such as dioxin
Acetylcholinesterase (AChE) activity	Neurotransmitter metabolism enzyme	Organophosphates, carbamates and similar molecules
Vitellogenin (VTG)	Precursor of the egg's nutritive reserves, normally synthesised by the female	Estrogen-mimetic endocrine disruptor
Metallothioneins (MT)	Chelating proteins involved in homeostasis of essential metals, protection against oxidative stress	Trace metals and inducers of oxidative stress
Delta-aminolevulinic acid dehydratase (ALAD) activity	Enzyme involved in metabolism of amino acids	Lead
Lysosomal stability	Test to determine lysosomal membrane integrity of the cell	General marker of health
DNA damage	Alteration in DNA structure	Genotoxic substances (PAH and other organic contaminants)
Lysozyme activity	Factor in resistance to illness	General marker of health
Analysis of macrophage aggregation	First line of defence in immune system	Multiple contaminants (metals, PAH)

Research on this topic revealed synergies between use of biomarkers and standard monitoring techniques for aquatic environments, i.e. chemical analyses on water, sediment and organisms, bioindicators (Lagadic *et al.*, 1997b). Biomarkers can detect early biological effects of contamination in organisms and, in some cases, the origin of the contamination. In addition, biomarkers measured on the lowest levels of biological organisation can assist in characterising mechanist relations linking exposure and effect (Caquet & Lagadic 1998, Lagadic 1999).

Finally, biomarkers measured on different levels of biological organisation can be used in a weight-of-evidence approach, combining chemical measurements in the environment and biota, biochemical measurements, histopathological analyses and studies on populations and communities, which would reinforce the environmental diagnosis (Sanchez 2008).

This type of approach could be implemented immediately for the WFD. It would be the means, in view of confirming study results or when chemical analysis has not revealed any degradation in the chemical quality of the environment, to assist in selecting additional chemical analyses to identify potential contamination by pollutants not initially looked for (Sanchez 2008).

However, very little use of these tools is made in monitoring networks. The U.S. BEST-LRMN network is the only one to employ them for inland environments. This situation may be explained by the difficulty of interpreting biomarker results for environmental managers and the lack of standardised methods for their large-scale implementation.

To solve these difficulties, work in a number of fields may be suggested.

- Research on biomarkers that recently emerged in ecotoxicology (immunotoxicity and genotoxicity markers) to prepare their use in monitoring programmes.

- Research on the effects of biotic and abiotic factors on the physiological levels of biomarkers and their response. This work should establish reference values for biomarkers and contribute to better interpretation of results in the field.

- Development and validation of data-interpretation tools that are compatible with WFD requirements and the needs of managers.

- Creation of a validation system for biomarkers, based on intercomparison tests and quality-assurance procedures. Such systems already exist for marine environments in the JAMP and MEDPOL programmes, and contribute to use of biomarkers over wide geographical zones (Sanchez 2008).

To sum up, the needs and expectations of managers concerning biological tools to assist in monitoring comprise the following list of priorities:

- modification, adaptation of existing biomonitoring tools to make them WFD compatible;

- development of specific bioindication tools to characterise the impact of certain toxic substances;

- research on the biological characteristics of sentinel species intended for environmental monitoring;

- validation of biomarkers identified in the lab and likely to be used in the natural environment;

- correlation of the response of certain biomarkers with longer-term modifications within populations in order to develop predictive tools.

Onema prepared a brief inventory of the data listed in this section, specifically for the symposium in Le Croisic, and presented it to the participants during a plenary session. By examining all the current needs and expectations of water managers, the goal was to reveal the various fields of study dealing with risk assessment and monitoring of aquatic environments for which the use of mesocosms in ecotoxicology could help to improve knowledge and methods.

This examination served to launch discussions on current possible uses of mesocosms by the people in charge of managing aquatic environments in a series of workshops whose results constitute the third part of this document. ▪

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3 The value of mesocosms for managers of aquatic environments

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The presentations at the symposium in Le Croisic were an occasion to discuss and analyse past and current uses of mesocosms in the fields of aquatic ecology, ecotoxicology and risk assessment. Following a precise examination of the new needs created by proactive public policies for monitoring of aquatic environments, the participants attempted to determine to what degree mesocosms, with their advantages and disadvantages, might serve to meet the challenges confronting the people managing aquatic environments.

This collective discussion, the first on the topic in France, was carried out simultaneously by three work groups, each comprising about ten people. Each group discussed in turn the three topics proposed by the organisers:

- value of mesocosms in defining reference thresholds for concentrations of chemical substances that are acceptable for aquatic environments;
- representativeness of mesocosms for use in setting regulations;
- use of mesocosms to develop and validate tools to assist in environmental monitoring.

The results of the work groups for the three topics are presented in the sections below.

3.1 – Definition of reference values for acceptable concentrations of chemical substances

For the WFD, robust environmental quality standards (EQS) would be a major plus for the management of aquatic environments. To set thresholds, the use of the results of mesocosm studies has been proposed as a parallel approach in a guidance document from the Frauenhofer Institute (Lepper 2005) and in the European technical guidance document on EQS derivation for WFD purposes, soon to be published. This contribution would above all reduce the uncertainty in calculating PNEC (*predicted no-effect concentration*) values for the environment and thus result in more reliable and realistic EQSs.

Though mesocosms can, theoretically, be used to evaluate the effects and fate of a wide range of chemicals in the environment, most studies using mesocosms focus on phytosanitary (plant-protection) products. This situation raises questions concerning the (economic) relevance and feasibility of using mesocosms to validate and/or set EQSs for chemicals other than phytosanitary (plant-protection) products, particularly for those:

- subject to high bioaccumulation and that are biomagnified in food webs (issue for EQS$_{biota}$);

- that link primarily to the sediment compartment once released to aquatic environments (issue for EQS$_{sediment}$);

- that have specific operating modes, such as medicinal residues whose concentrations in the environment are low (≈ ng/l) and that typically produce chronic effects on aquatic organisms.

Formulation of EQS$_{biota}$

Certain participants confirmed the relevance of mesocosms in determining PNECoral values that are essential reference data for setting the corresponding EQSs.

They would appear particularly well suited to determining bioaccumulation factors in organisms because they can take into account the various exposure routes under controlled conditions. For persistent substances, use of mesocosms to expose short-lived species in order to study the effects over several generations would appear feasible and useful.

Use of mesocosms may be limited for substances with high biomagnification potential in that the length of food chains, limited by the size of the mesocosm, makes it difficult to evaluate effects in fish-eating predators. Biomagnification in fish in particular is not easily observable in mesocosms. In this case, a two-pronged approach, in the field and the lab, would appear indispensable.

Selection of the species used in mesocosms to set the concentration thresholds will require study on the overriding purpose of the EQSs. Is the goal to limit the impact of contaminants on organisms in ecosystems or, in the final analysis, to protect human health? In the first case, the species most sensitive to a pollutant must be selected and mesocosms can help in identifying that species. In the second case, selection will turn first to fish consumed by humans. Due to variations in bioaccumulation within an organism (organotropism), it will also be necessary to decide which organ to study.

The choice of the organism to be studied would appear to be the critical issue. It must be selected taking into account the targeted substance and the goal of the protection measures.

Formulation of EQS$_{sediment}$

For the purpose of determining EQSsediment values, the capacity of mesocosms to take into account the different exposure routes of benthic organisms, the biodegradation of organic contaminants and to study simultaneously a wide variety of benthos taxa all constitute clear advantages. They could be particularly useful for hydrophobic substances that link significantly with particulate organic matter and end up, *in fine*, in the sediment compartment.

From the technical point on view, their use will require, however, research to precisely determine sediment-contamination processes.

Emerging contaminants

Emerging substances (endocrine disruptors, pharmaceuticals, GMOs, nanoparticles, etc.) listed among the priority emerging substances by the Scientific committee on emerging and newly identified health risks (SCENIHR) at the EU DG for Health and consumers (SANCO) constitute a vast field of study in ecotoxicology and risk assessment. It is today essential to progress in understanding their effects on aquatic environments and to provide them with threshold values in compliance with WFD goals.

To that end, mesocosms should fill out the range of available tools, given their capacity to integrate the “delayed”, i.e. transgenerational, effects that such substances are assumed to have and to take into account the degradation products, for which little ecotoxicological information is available.

For pharmaceuticals, as is already the case for pesticides and biocides, it would appear essential to study the parent substance, but also its metabolised by-products. A proposed solution is to test the impact of hospital or farm effluents in mesocosms.

Finally, the issue of the economic and political feasibility of mesocosm studies for emerging contaminants was emphasised as being very important.

Use of results

An increase in the use of mesocosms in defining new EQSs would thus appear possible and particularly relevant for certain substances. That implies, however, efforts to standardise methods and organise the presentation of results, similar to the work carried out by the AMPERE group for risk assessment of phytosanitary (plant-protection) products (Alix *et al.* 2007). A proposal has been made to set up a special work group to deal with these issues concerning methods and results.

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3.2 – Representativeness of mesocosms for use in setting regulations

As noted in the sections above, mesocosms may serve regulatory purposes in assessing risks for aquatic environments, prior to marketing of chemicals (phytosanitary products, biocides, etc.), or in setting reference values for ecosystems, such as predicted no-effect concentrations (PNEC), in the process of defining EQSs.

These values are estimated on the basis of data produced under experimental conditions that more or less represent natural conditions. Assessment factors are then applied, whose value depends on the quantity and type of data available.

When using this type of data, evaluators are confronted with a number of questions dealing with the "position" of mesocosm studies with respect to lab trials and the use of data obtained via systems that differ in terms of the experimental protocol (exposure duration, contamination mode, composition of living communities) and the environmental conditions (see table 4). By the "position" of mesocosm studies we mean their representativeness with respect to the natural environment.

The table below, submitted during the workshops in Le Croisic, compares the advantages and disadvantages of mesocosm data, compared to those from monospecific lab tests on chronic or acute exposure. This comparison illustrates the great informational value of mesocosm data, which are considered the most representative and are assigned the lowest safety factors.

The relevance of NOEAEC values must, however, be reinforced for EQS definition. This criterion, often produced in mesocosm studies, would not seem to be compatible

Table 4. Advantages and disadvantages of mesocosm data compared to lab exposure tests.

Parameter	Acute-exposure test	Chronic-exposure test	Mesocosm
Number of species	Algae, macrophytes, Daphnia, chironomids, fish	Algae, macrophytes, Daphnia, chironomids, fish	100-200 species (phytoplankton, macrophytes, zooplankton, macro-invertebrates, periphyton, juvenile fish, etc.)
Exposure	Permanent	Permanent	Depends on the degradation speed (DT50) used for degradation products
Variability of results	Limited	Limited	Significant (but close to natural variability)
Exposure information	Water	Water, sediment	Water, sediment, interstitial water, suspended mater, periphyton, etc.
Availability of toxic-effect criteria?	LC50, EC50	EC10 NOEC	NOEC NOEAEC
Information on food-chain levels	Primary producers Primary consumers (Daphnia) Secondary consumers (fish), taken individually	Primary producers Primary consumers (Daphnia) Secondary consumers (fish), taken individually	Same levels, but taken together (difficulty of maintaining piscivorous species)
Detection of indirect toxic effects	No	No	Yes
Information on population	No	No (perhaps Daphnia and Chaoborus test)	Yes
Information on living community	No	No	Yes (integrated approach possible, taking into account ecological aspects)

with goals to protect aquatic organisms against chronic exposure because the conditions required to restore environmental quality are never present (worst case). Conversely, greater use should be made of the capacity of mesocosms to inform on environmental restoration, e.g. to evaluate the ecological benefit of reducing, even partially, a pressure.

The main difficulty in using mesocosm data for regulatory purposes lies in standardising the tools and methods for these approaches. Standardisation of mesocosm contents would not appear useful. A mesocosm cannot represent all ecosystems and must be designed to provide answers to a given question. This diversity in experimental systems is also a means to acquire information on the variability of responses. Though international comparisons between mesocosms would seem to indicate a certain stability in NOEC values, other toxic-effect criteria must still be evaluated.

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In this context, work must first address standardisation of measurement and sampling methods, the ranges of applied concentrations and optimisation of the number of replicates.

The importance of using mesocosm results in the framework of an integrated approach was reiterated. EQSs must be the result of data obtained from mesocosms, lab tests and field monitoring. It is the confrontation of these results that produces better understanding of any inconsistencies between the different approaches. Study is required, probably based on a meta-analysis, to meld the various levels of information and produce decisions, similar to the work done for pesticide risk assessments.

If carried out on the European level, this type of study would make it possible to organise feedback and could result in new versions of the guidance documents.

3.3 – Development and validation of tools to assist in environmental monitoring

The introduction of a toxic substance in an ecosystem may produce effects on different levels of biological organisation, ranging from individuals and populations, through groups of species and communities, up to entire ecosystems. The biological parameters measured on the various levels each constitute signals and can thus be used for bioindication. For an evaluation of natural environments, a number of biological techniques can be used together, e.g. measurement of biomarkers, analysis of sentinel species, study of groups of species and communities.

The use of mesocosms could optimise the potential of biomarkers by assisting in validating those identified in the lab and proposed for use in the natural environment, or even by identifying new biomarkers. In this context, mesocosms would facilitate the development of interpretative systems for field data, making it possible to detect "warning" messages, for example. In addition, mesocosms would appear to be capable of revealing the relations between certain biomarkers and longer-term modifications within populations in order to develop predictive tools.

The need to establish links between contamination and bioindicators was already mentioned in the second part of this document. During the workshops, the participants confirmed the value of mesocosm testing of bioindication tools based on an analysis of the structure of communities or certain functional parameters, prior to or in parallel with validation in the field. Similarly, mesocosms could be used to evaluate the impact of substances on biological indices that are new or already used in the field. An analysis of sensitivity to different groups of toxic substances would be a possibility, which could include an evaluation of confounding factors. In this context, mesocosms could contribute to the development of bioindication tools tailored for specific types of contaminants.

A number of other fields in which mesocosms could play a role were also mentioned:

- research on the biological characteristics of sentinel species intended for monitoring of aquatic environments;
- development of new tools, such as passive samplers, making use of the capacity of mesocosms to detect bioaccumulation phenomena, taking into account the bioavailability of substances;
- implementation of additional analytical methods to characterise exposure-effect relations;
- formulation of conceptual or predictive models linking toxic exposure to biological effects. It would appear necessary to draw the attention of managers to the potential here for decision making.

In addition, an emphasised strong point of mesocosms is the fact that the development of these various tools can be integrated in a single experimental study.

Finally, the capacity to test sediment and/or water that is representative of a given environment makes mesocosms a tool particularly well suited to environmental monitoring in the event of a specific problem in the field.

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Mesocosms would appear to have particularly strong potential for the development and validation of tools to assist in environmental monitoring. Studies of this type nonetheless continue to depend on a strategy capable of mobilising the necessary funding. A first step should be to encourage the use of the data already available, of which a part is not disseminated because it is confidential.

3.4 – Outlook for development and recommendations

The above analysis, based on the results of the workshops during the symposium in Le Croisic, confirmed the usefulness of mesocosms in light of current expectations of managers of aquatic environments and clarified their potential and relevance for each of the three proposed topics. Following the discussions at the end of the symposium, the points below were emphasised for future work:

- improvement of existing EQSs and development of EQSs for biota and sediment;

- PNEC validation;

- analysis of relations between contaminants and bioindicators;

- formulation of conceptual and predictive models linking exposure and effects;

- evaluation of the effects of emerging substances, particularly nanoparticles and endocrine disruptors;

- specific monitoring for a given ecosystem or effluent;

- impact of climate change and coupling with the effects of toxic substances;

- impact of invasive species on ecosystems and contaminant transfers.

The development of these tools and their use by managers, though feasible and clearly positive in light of the possibilities described above, will depend on the available resources. The creation of mesocosm experimental platforms must be designed to optimise the results with respect to the investment made. The use of mesocosms in networks by multi-disciplinary teams is highly recommended. That will make use of one of the advantage of mesocosms, i.e. all experiments can be designed to maximise the amount of information produced for a double goal, assist management work and improve scientific knowledge.

This approach includes the need to inform and communicate with managers. The relevance and feasibility of mesocosm studies must be reiterated and emphasised, as well as their limits, notably in terms of their capacity to represent natural ecosystems. The publication of the report by the AMPERE work group (Alix *et al.* 2007) contributed to the reputation of mesocosms in assessing pesticide risks. This type of initiative could be adapted to other groups of micropollutants. More generally, there is a proposal to create a consortium to set up a network between existing and future mesocosm platforms, to optimise the studies carried out on the platforms (including standardisation of sampling and measurement protocols) and to enhance dissemination of mesocosm information and their acceptance via the creation of a scientific work group. ■

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For more information...

Use of mesocosms in ecology studies

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Glossary

Biomarker

Biomarkers are "observable and/or measurable changes on the molecular, biochemical, cellular, physiological or behavioural level, that indicate present or past exposure of an individual to at least one chemical pollutant" (Lagadic *et al.*, 1997).

Ecologically acceptable concentration (EAC)

Concentration at which the ecological function and structure of the community are not disturbed (Workshop on Higher-tier Aquatic Risk Assessment for Pesticides - HARAP; Campbell *et al.*, 1999).

Predicted no-effect concentration (PNEC)

The predicted no-effect concentration is defined as the concentration of a chemical substance below which adverse effects in the environmental sphere of concern are not expected to occur (Annex I of the REACH regulation).

Hazard coefficient (HQ)

The hazard quotient of a contaminant in a given environment is defined as the ratio of the predicted or measured concentration (PEC, Predicted Environmental Concentration or MEC, Measured Environmental Concentration) in the environment to the PNEC. If the PEC/PNEC ratio is less than 1, an effect is highly unlikely, if it is greater than 1, an effect on the environment cannot be ruled out.

Indicator species (bioindicator)

The presence (or absence) and/or the abundance of certain organisms (species or groups of species) provide information on the status of ecosystems. Monitoring of bioindicators is often linked with analysis on the structure of the communities to which the indicator species belong. In most cases, disturbances in the environment are accompanied by changes in the number and type of taxa (species, genus, family).

Sentinel species

Sentinel species are of particular use for biological monitoring of environmental quality. They can be used to indicate the presence and toxicity of certain contaminants, or as a general indicator on the health of an ecosystem. Some sentinel species are bioaccumulators, i.e. they accumulate certain contaminants directly (bioconcentration) via their environment (soil, water, sediment, etc.) or via their food (biomagnification).

Lentic environment

A freshwater environment with very slow or no circulation (ponds, swamps, fluvial lakes, etc.).

Lotic environment

In the field of ecology, a freshwater environment characterised by running water (streams, rivers). The term "lotic" is used in expressions such as lotic ecosystems, lotic species or lotic mesocosms.

Environmental quality standard (EQS)

An environmental quality standard (EQS) is defined as "the concentration of a particular pollutant or of a group of pollutants in water, sediment or biota which should not be exceeded in order to protect human health and the environment" (Art. 2.35 of the 2000/60/EC directive).

Periphyton

A mixture of algae, bacteria and detritus that attach to submerged surfaces and develop in most aquatic environments. It serves as a food source for invertebrates and certain fish, it can also absorb certain contaminants.

This document is part of the “Meeting Recap” collection, intended
for technicians and interested persons. It presents the main
elements of meetings organised or co-organised by Onema.

Already published
Climate change. Impacts on aquatic environments and consequences for management (English edition, December 2011).
Economic instruments to support water policy in Europe: paving the way for research and future development (English edition, December 2011)
Drinking-water abstractions and nonpoint-source pollution: operational solutions for supply zones of priority water abstractions (English edition, December 2011)

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Acknowledgements
Our thanks go to the meeting participants
and Claire Roussel, Information and communication
department at Onema.

ISBN: 979-10-91047-04-3

Layout design: Inzemoon (+33 675 24 19 30)
Production: Bluelife (+33 467 91 33 55)

Printed in France by I.M.E. in December 2011.

This document was produced using plant-based inks and printed on 100% PEFC paper
from sustainably managed forests and verified sources, by a printer compliant
with all environmental standards.

ONEMA
Office national de l'eau
et des milieux aquatiques

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