

# Meetings

## A national specimen bank for continental aquatic environments?

*A workshop organised by the Research centre for environmental quality in SW Europe (Orque Sudoe) and Onema.*

Last November, several of the leading international experts on environmental specimen banks (ESB) met in Pau for two days of discussions that highlighted the value of these tools in meeting the challenges raised by pollution management and biodiversity conservation. The idea of creating an ESB for continental aquatic environments in France was floated.

Between 1996 and 2006, concentrations of the flame retardant HBCDD doubled every two years on average in sea lions near San Francisco, a fact that confirmed the local dispersal of the emerging contaminant and proved that it bioaccumulates. On the eastern coast of Japan, mussels did not contain cesium-137 before March 2011, which means that the concentrations noted since are without any doubt due to the nuclear accident in Fukushima. In the Mulde River in Germany, mercury concentrations in the muscle tissue of common bream dropped by a factor of four between 1993 and 2009, documented proof of the reduction in the environmental exposure to the pollutant following the closing of an industrial source. These three examples illustrate the value of maintaining environmental specimen banks (ESB) in which items (sediment, plants, fish, etc.) are conditioned and archived, following in situ sampling according to long-standing, standardised procedures<sup>1</sup>. Organised on the national level in Germany, the U.S. and Japan, ESBs have for decades served as powerful tools for research, long-

term environmental monitoring and guidance for environmental policies to control pollutant emissions. In France, where the national authorities have expressed limited interest, the scientific community has nonetheless achieved some significant

successes with the Marine bivalve ESB launched in 1979 by Ifremer and the thousands of fish samples stored for the national PCB action plan and particularly for the plan's implementation in the Rhône-Méditerranée basin.



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<sup>1</sup> There are also "opportunistic ESBs", for example those containing biopsy material from stranded cetaceans.

How can these initiatives be sustained over time and a national network be promoted? Could France establish a new ESB devoted to continental aquatic environments? What would be the overall objectives, the technical options, the financial conditions? To launch the discussion on these topics, the scientific partners in the OrqueSudoe<sup>2</sup> project and Onema organised last November in Pau two days of discussions and debates, bringing together researchers and a number of institutional stakeholders from the field. Following the first day devoted to feedback from international projects, the workshop continued with a series of round tables to identify the issues and opportunities in France.

### 30 years of stored data in Germany

The German environmental specimen bank (GESB), considered a model worldwide, has archived since the middle of the 1980s a series of samples representing freshwater, marine, forest and land environments from a network of sites spread around the country. For continental surface waters, the work focusses essentially on 16 sites located on major rivers (Rhine, Danube, Elbe, Sarre) and deals primarily with two widely found, sedentary species known to accumulate contaminants, namely common bream (see Figure 1) and zebra mussels. Two sites on lakes fill out the network. The standard operating procedures (for sampling, preparation, conditioning, archiving and use), presented in detail by Heinz Rüdél from the Fraunhofer Institute, are laid out in methods guides that are regularly updated. The biological tissues are cryogenically ground to a fine, homogeneous powder, split into 10 g sub-samples (i.e. 200 units for a bream weighing 2 kg) and stored in liquid nitrogen at -150°C. Over the past three decades, the bank has collected over 2 100 bream specimens representing 320 000 samples. Under the responsibility of the Federal environmental agency and the Ecology ministry, the GESB is housed at the Fraunhofer

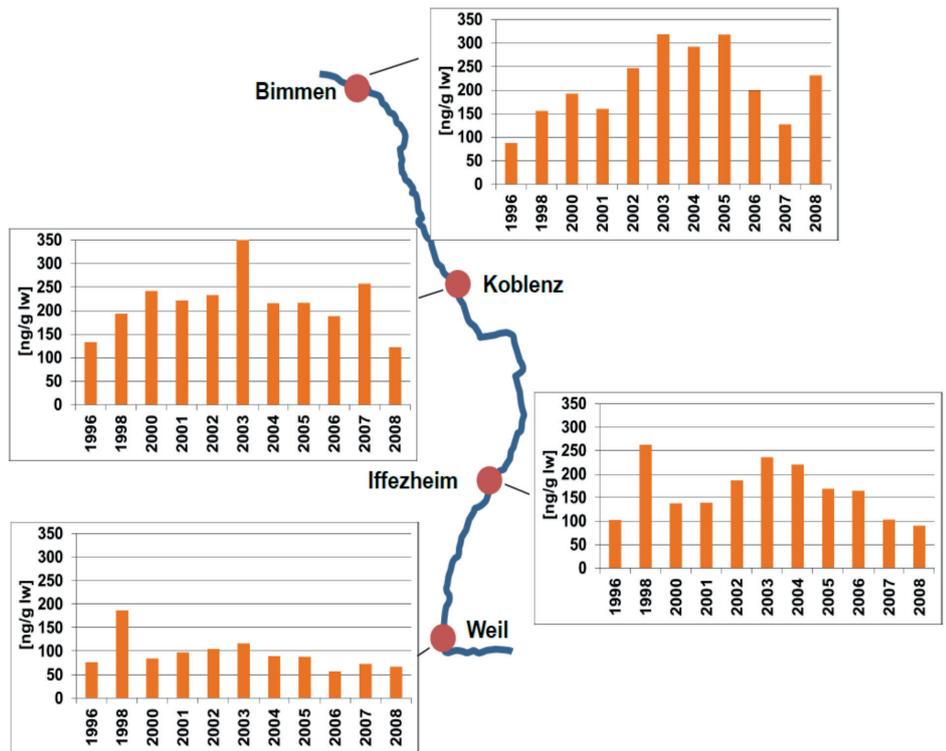


Figure 1. An example of the work made possible by the German specimen bank is the retrospective study on methyl-triclosan in the flesh of bream, at four monitoring points on the Rhine between 1996 and 2008 (Source: Fraunhofer Institute).

Institute in Schmallenberg where it has approximately 60 cryogenic storage containers representing over 80 cubic metres of storage space. A team of expert technicians works exclusively on system maintenance and operation.

The results of analyses on pollutants in the samples, carried out in real time for routinely monitored substances such as mercury and hexachlorobenzene, or retrospectively for emerging contaminants and for more in-depth environmental studies, may be easily accessed by the public at the GESB site ([www.umweltprobenbank.de](http://www.umweltprobenbank.de)). The data are highly useful for a number of applications, including research, monitoring of environmental quality and in support of regulations on hazardous substances. Examples were provided during the workshop by Jan Koschorreck from the Federal environmental agency, e.g. spatial-temporal analysis of wildlife exposure to pollutants, monitoring to determine the effectiveness of management work, data used to set maximum levels in foodstuffs, comparison of substance bioaccumulation profiles between species and between organs/tissues to optimise monitoring strategies for WFD priority

substances in fish, etc. An example of retrospective use of the data was presented during the workshop by Nicolas Estrade (University of British Columbia, CNRS). Isotopic analysis of the mercury in the muscle of bream stored in the GESB was used to interpret concentration trends of the contaminant between 1993 and 2009 in the Elbe and two of its tributaries, the Saale and Mulde Rivers, and to detect the main sources. The analysis produced valuable information on the formation and degradation of the form of mercury (methylmercury, MeHg) that can bioaccumulate, thus making it possible to anticipate the evolution over time of the contaminant in the hydrosystem once the direct sources had been eliminated.

### In Japan, the Time Capsule for post-Fukushima studies

Another outstanding project is the Environmental Time Capsule in Japan. It is the result of the development process initiated in 1979 by the National institute for environmental studies with the start of the first pilot specimen bank in the country.

<sup>2</sup> The project to create the Research centre for environmental quality in SW Europe, managed by the University of Pau, combines the work and know-how of a dozen partners to create innovative tools to monitor the quality of coastal marine environments over the long term.

Since 2004, it has been based near Tokyo in a high-tech storage centre for thousands of samples of atmospheric particles, sediment, fish and, above all, mussels systematically collected from all coastal regions around the island country. The bank also contains DNA samples of protected species (birds, mammals, fish, algae). Similar to Germany, this "time capsule" consists of samples stored for the long term in containers filled with liquid nitrogen. Two cold rooms (-60°C) are also used. In addition, the centre offers modern equipment for chemical analysis of the samples.

During the workshop, Akinori Takeushi (NIES) presented several examples of how the data are used, e.g. a study on the spatial-temporal distribution of emerging contaminants such as perfluorooctane sulfonate (PFOS) or "historic" contaminants such as DDT in bivalve molluscs. However, the most impressive use was the retrospective study on mussel samples carried out following the Fukushima nuclear accident in March 2011. The absence of cesium-137 and strontium-90 in the mussels collected along the Japanese coasts prior to the accident meant that 100% of the radioactive contaminants subsequently found could be attributed to leaks from the nuclear reactors caused by the earthquake. The analysis also revealed a major increase in PAH concentrations in coastal environments at the same time, due to the accidental combustion of petroleum products. The Japanese time capsule thus demonstrated its outstanding value in quantifying the damage inflicted on the environment by the accident and in the efforts to manage the ecological consequences and those concerning human health.

## **An archive for U.S. federal administrations**

Via a video-conference line from the U.S., Pr. Paul Becker (National Institute of Standards and Technology) continued the international panorama with a presentation of the Marine Environmental Specimen Bank (MESB). Established in 2002 in Charleston (S.C.), the bank makes its equipment and know-how available to various governmental agencies, ranging from the Fish and Wildlife Service to the Defense department

and the Food and Drug Administration. Via the different research programmes launched by the agencies, the MESB has acquired 85 000 samples from over 10 000 specimens, including 68% from marine mammals, 24% from sea birds and many from molluscs, sediment, coral and fish. Heavily focussed on biology, the bank takes part in the international Arctic monitoring and assessment programme (AMAP) via two programmes to archive the tissue of Alaskan marine mammals and the eggs of wild sea birds. More generally, it assists many research projects in the fields of ecology and ecotoxicology, and serves as a prime component for the management of aquatic resources and the preservation of biodiversity.

## **Synergies required in France**

Elsewhere in the world, a growing number of countries are establishing specimen banks. In Europe (see Table 1), specimen banks are already operational in Norway and the U.K. (fish samples from the Thames and rivers in the East of England). Other major national or regional projects have been announced. In the Spanish Basque country, a scientific partnership (I. Marigomez, Orque-Sudoe) is currently developing the Biscay Bay Environmental Biospecimen Bank (BBEBB), a structure intended to assist in implementing European directives (Marine strategy framework directive, Water framework directive, etc.) along the Biscay coast. The bank already has a dry-storage unit for DNA samples, mollusc shells and fish otoliths. It is also equipped with high-capacity freezers (-40°C and -80°C) and an initial cryogenic storage unit. On the other side of the Pyrenees, the Equipex project (Olivier Donard, CNRS-Orque-Sudoe) will take form in 2015 with the construction of a new building that will house similar equipment and an advanced analytical platform that will collaborate with the BBEBB.

Another important project was launched in 2014 by Andra (National nuclear-waste agency) on the site preselected for the storage of radioactive waste (Cigéo project) in Bure. A 900 square kilometre zone as been established for the Long-term environmental observatory (OPE) that will monitor local eco-

**Catherine Galy, Andra :**  
**Designed for the long term**

The sizing studies for the Andra specimen bank, launched last year in Bure (Meuse department), were initiated in 2008 with our scientific partners (INRA, Ademe, University of Lorraine, etc.). Once the overall objectives had been set, it was necessary to provide the facility with enough capacity for specimens collected over 20 years. For example, we estimate that we will fill 3% of the dry-storage section on average each year. For the longer term, there are plans to double the storage capacity. In designing the facility, we benefited extensively from the experience acquired by the Fraunhofer institute and INRA in Orléans. Networking of the available know-how is essential because the French ESB community is still not well organised. The meeting in Pau was an excellent occasion to meet and talk shop. That will also be the case at the upcoming international conference that will be held in Nancy (France) on 30 June and 1 July 2015.

systems. This multi-disciplinary project (C. Galy, Andra) involves a wide array of facilities, including an atmospheric station, monitoring points for water quality, an experimental forestry site, an experimental agricultural site, etc. The OPE also comprises an ESB for soil, plants, animals, local food products, in view of carrying out research and conserving a trace of the past environment (primarily the chemical diversity). The high-tech ESB facility has been operational for a few months. Several organisations contributed their know-how to its design, including the Fraunhofer institute, NIST/ IRD, INRA in Orléans and UPPA Pau. The initial sampling strategies have been established and storage started in 2014. The ESB, an integral part of the OPE, is housed at the Andra Meuse/ Haute-Marne centre, in a special building offering 280 square metres of floor space for dry storage (soil samples) and 470 m<sup>2</sup> for cryogenic storage. The

available space is sufficient for 20 years worth of specimens and may also be used in part for other projects.

To date however, the ESB in France that has made the most progress remains the Marine bivalve ESB that Ifremer has managed since 1979 as part of the ROCCH network (observation network for coastal chemical contamination that took over in 2008 the work that the RNO observation network initiated in 1974). The collection comprises over 8 000 specimens of mussels and oysters drawn yearly from a long-standing network of monitoring points along French coasts (English channel, Atlantic and Mediterranean) and is now stored in Nantes at a dry-storage centre (freeze-dried).

A number of telling examples on the value of the Marine bivalve ESB were presented (J.-F. Chiffolleau, Ifremer) during

the workshop. It has served to determine reference states prior to polluting events (e.g. the sinking of the Erica oil tanker at the end of 1999, where it was possible to prove the resulting increases in nickel and vanadium in mussels) and to monitor

the effects of political decisions, such as the halt in 1986 to cadmium mining in the Gironde basin, which led to a sharp reduction in cadmium concentrations in mussels in the Gironde estuary (see Figure 2).

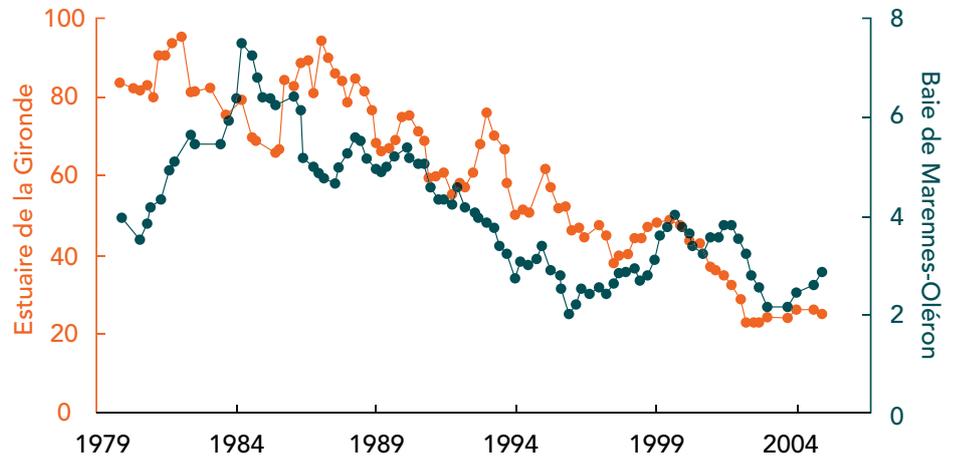


Figure 2. Example of the use of the Marine bivalve ESB. Concentrations (µg per gram dry weight) of cadmium in the flesh of mussels in the Gironde estuary (orange) and in the Marennes-Oléron bay (blue). (Source: Ifremer).

Table 1: The main environmental specimen banks in Europe (adapted from Koschorreck *et al.*, 2011, *Conference on European environmental specimen banks*).

Country	City	Year started	Coverage	Reference state / Polluted	Storage temperature	Ecosystem	Type of specimens
Sweden	Stockholm	1964	Entire country	R	-25°C -80°C Liquid nitrogen	Marine Limnic Land	Seals, fish, mussels, bird eggs Fish, sediment Mammals, birds, earthworms, moss, mud
Denmark	Aarhus	2000	Greenland	R	-21°C	Marine Land	Seals, bears, fish, birds Birds
Faroe islands	Tórshavn	1998	Entire country	R	-25°C	Marine Limnic Land	Cetaceans, seals, fish Seals Mammals, grass, soil
Finland	Paljakka/ Helsinki	1994	Entire country	R and P	Liquid nitrogen Ambient temp.	Marine Limnic Land	Fish Fish Moss, lichen, grains, pine needles and bark
Norway	Oslo	2005	Entire country	R and P	-25°C -80°C	Marine Limnic Land	Seals, bears, fish, mussels, bird eggs, sediment Fish Mammals, birds, moss, mud
Germany	Schmallenberg/ Munich	1985	Entire country	R and P	-80°C Liquid nitrogen	Marine Limnic Land	Plants, animals, sediment, human samples (blood, hair)
France	Nantes	1976	French coast		Ambient temp.	Marine	Mussels, oysters
France	Pau	2004	Gironde, Landes, Pyrenees		-80°C	Marine Land	Oysters, bivalves, eels, sediment Pine needles, leaves, lichen, soil, suspended matter
France	Bure	2009	Bure	R	-80°C Liquid nitrogen Ambient temp.	Land Limnic	Tree bark and leaves, soil, birds, earthworms, food products Fish
United Kingdom	Wallingford/ Lancaster	2007	Thames		-80°C	Limnic	Fish
United Kingdom	Cardiff	1992	England and Wales	R and P	-80°C	Land	Otter
Poland	Warsaw	Project	Entire country		-80°C	Marine Limnic Land	Several specimens representing each ecosystem
Portugal	Braga/Aveiro	2000	Mediterranean coast	R and P		Marine	Animal tissue
Spain	Pontevedra	1990		R and P		Marine	Animal tissue
Spain	Pentzia-Bizkaia	2007	Biscay bay	R and P	-40°C -80°C Liquid nitrogen	Marine Land	Oysters, bivalves, eels Earthworms
Italy	Padoue	2002	Mediterranean coast	R and P	-80°C	Marine	Animal tissue
Italy	Genes	1994	Antarctica	R	-40°C -80°C Liquid nitrogen	Marine Limnic Land	Seawater, sea ice, suspended matter, sediment, fish, molluscs, sponges Water, macroscopic algae, sediment Snow, soil, moss, atmospheric particles

## And continental aquatic environments?

This presentation of the situation in France, though not complete<sup>3</sup>, highlights the value, but also the relative paucity of specimen banks, whether operational or planned, in France. It makes clear the need to perpetuate the existing facilities and enhance networking to facilitate access to and use of the samples. It also reveals the current absence of a facility for continental aquatic environments. The fish samples collected from 2008 to 2010 for the national PCB action plan (see Onema Meetings no. 18) are currently stored in freeze-dried form by ADIV<sup>4</sup> thanks to a contract with Onema that is set to expire at the beginning of 2015. Above and beyond the need for a short-term solution for the samples, this situation raises the question of developing on the national level an ESB for freshwater environments (O. Perceval, Onema). In addition to the PCB plan, half a dozen research programmes and studies since 2008 have collected samples from aquatic environments over a wide range of sites and this material could constitute the starting point for a future national ESB for aquatic environments. The relevance of this type of facility has recently been enhanced by changes in the WFD,

which now requires routine monitoring of biota during the upcoming management cycle, and by the future launch of the French biodiversity agency, for which an ESB would be an outstanding asset.

## What are the technical options?

The plenary sessions and the round tables during the workshop identified features shared by the main specimen banks already operating worldwide and served to outline what a French bank could look like in terms of its objectives and the technological decisions. The first feature is that specimen banks were designed as national facilities in the public interest and public authorities were the driving force behind their establishment. Most banks are linked to long-term programmes to monitor environmental quality which include the regular collection of samples to supply the specimen bank. In France, the obvious solution would be to link the bank to the routine monitoring work of some of the monitoring points in the WFD surveillance-monitoring network (RCS), where the number of sampling points would depend on the available funds.

The sampled species (fish, molluscs, invertebrates) must meet certain criteria, i.e. be common (and remain so) at all

sampling points and have documented bioaccumulation properties. The size of samples and sub-samples is another decisive aspect in planning for long-term operation. As noted for the German ESB, each fish specimen (bream weighing 2 kg on average) resulted in approximately 200 samples weighing 10 grams each for a given date. In Japan, composite mussel samples weighing 150 grams each are split between four or five tubes. It would thus appear very important to establish from the beginning a policy determining the access rights of the various ESB users.

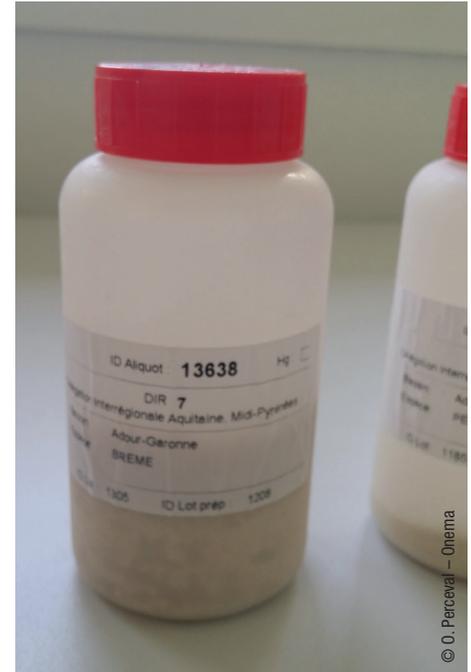
Finally, the preservation technique has a direct impact on the future use of the samples, notably the types of parameters measured and their integrity over time. Storage of freeze-dried samples at ambient temperature, the solution used by Ifremer for the Marine bivalve ESB, is the least expensive option, but it limits the possible analyses in the future. Freezing to -80°C ensures the preservation of samples without degradation of the chemical compounds for approximately ten years. Gas-phase (-150°C to -190°C) and liquid-phase (-196°C) cryogenic techniques, used by the German and Japanese ESBs, involve high initial investment and operating costs, but offer tremendous advantages in that they quickly block any degradation processes and ensure the integrity of the samples over several decades.



Zebra mussels (*Dreissena polymorpha*) and breams (*Abramis brama*) are two common freshwater species used in environmental specimen banks for long-term monitoring of chemical contaminants.

<sup>3</sup> A number of facilities recently created in France are not mentioned. That is the case of the environmental specimen banks working in conjunction with the SOERE units (long-term observation and experimentation systems for environmental research) approved by AllEnvi.

<sup>4</sup> Agro-industrial technical institute for the meat sector.



Cryopreservation and freezing to  $-20^{\circ}\text{C}$  of freeze-dried biological tissues are two techniques to store samples over long periods.

In addition, these techniques provide access to certain biological responses measured in the samples, e.g. biomarkers, thus making it possible to diagnose the health status of the organisms in response to exposure to one or more pollutants. And if the electrical supply is interrupted, the thermal inertia of the containers holding the liquid nitrogen is sufficient to maintain sample integrity for over ten days.

The above considerations are, of course, simply a starting point for a discussion on the national level concerning the

value of creating an ESB for the continental aquatic environments not covered by the existing facilities. The discussions on the project, approved by the Ecology ministry, will be part of the second National action plan on micropollutants, in line with the recommendations made by the assessment commission for the PCB action plan. In the meantime, the value of ESBs may be demonstrated in the framework of partnership projects on the practical implementation of specimen banks and of studies on the worldwide impact of certain major pollutants (mercury). ■

### Workshop organisation

- Orque-Sudoe/University of Pau project: Olivier Donard (director of the Institute for the physical-chemistry and analytical sciences of the environment and materials) and Christelle Bonnemason-Carrere (coordinator of the Orque-Sudoe project)
- Onema: Olivier Perceval, scientific officer for ecotoxicology, Research and development department

### Onema MEETINGS



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