Rovaltain science and technology centre

Centre for Ecotoxicology and Environmental Toxicology

(provisional title)

I – Scientific project brief

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With the collaboration of CNRS and Cemagref

Contents

Project outline

- ***Challenges***

Human activity has resulted in voluntary and involuntary changes and disturbances to the environment. Among these changes, actual and hypothetical stressors of physical origin (ionising and non-ionising radiation, construction), chemical origin (agricultural products, residue from combustion and industrial activity, household products and medicines etc.) and biological origin (proteins, DNA fragments, plant and animal toxins, and even potentially pathogenic microorganisms), can affect both human health and that of other living organisms inhabiting our planet. The problem—which has been detected on a local scale by inhabitants, formed the subject of scientific inquiry, and largely adopted by society—is to identify these stressors, detect and understand their effects on biosystems and ecosystems, above all on humans, to assess the level of risk they present and to class them according to their gravity. Scientific disciplines, largely from the field of toxicology, have thus emerged: environmental toxicology and ecotoxicology or, to use more recent expressions, stress biology and stress ecology. Natural occurrences can also have significant adverse effects, primarily serious pollution—the history of the planet serves as a reminder of this. Studying the effects of these occurrences from a toxicological and ecotoxicological perspective is a desirable, natural progression because the amplitude of these rare occurrences is several orders of magnitude larger than what we can observe today. Today's living organisms have descended from ancestors who were exposed to the stresses from which selected mechanisms of resistance have evolved. Identifying and assessing the effectiveness of these mechanisms is an important and innovative area of research which will lead to a better understanding of certain sensitivities and resistances.

In face of these facts and speculation, society's concerns have translated into, and will continue to translate into, political action through the implementation of standards and regulations at national, European and international levels. It is necessary to assess the real risks, and to organise them into a hierarchy before expressing them in operational terms—providing the necessary knowledge and techniques for applying these standards and regulations; or sometimes simply analysing their pertinence, since the "precautionary principle" is increasingly applied. The knowledge and techniques must be communicated to the various sectors concerned, especially to industry, users, managers, and politicians, and moreover must be developed together with them.

- ***Purpose***

In order to develop the disciplines in question, the scientific community will need to be broadened and strengthened. This can be done by proposing stimulating projects and developing sophisticated experimental facilities. It is important to note that a considerable amount of fundamental research needs to be carried out within a clearly defined framework and with practical objectives. Its results will yield technological, economic, cultural and social benefits, and will naturally form a basis for further training. The Centre for Ecotoxicology and Environmental Toxicology in Rovaltain ***(CETER – provisional title and acronym)***'s project adheres to this model.

- ***Facilities***

A Large Scale Facility is planned for the research, enabling work to be carried out that would otherwise be impossible in normal laboratory conditions (spatial and temporal scales too large: long-term experiments; volumes of several cubic metres; difficult to implement in a laboratory setting due to complexity of experiments; low doses; a combination of stressors; animal, vegetable and microbial populations and communities). The facility would function as a *"hôtel à projet"*[1] with a basic management, technical and scientific team of 20-30 people—just enough to manage the site. The facility will be able to welcome around forty researchers, engineers and technicians for varying lengths of time. Projects will be selected on an evaluation by the International Scientific Council of proposals received in response to calls for research projects based on the technological and scientific topics it has defined. The final selection will be made by CETER's Steering Committee. The facility will be located in Rovaltain, a highly accessible location, already partly developed and served by the high-speed TGV train which stops on site at Valence-Nord.

A particular focus will be placed on developing the methodological and technological aspects, such as modelling, compiling a large database and knowledge bank on ecotoxicology, developing specific instruments and remote management of experiments.

A training scheme at masters and PhD level will be implemented on site in conjunction with initiatives of the universities and higher education institutions in the Region. Naturally, ongoing training is a priority.

An expertise service shall be set up. This expertise could extend to providing the necessary resources for performing experiments.

The structural and functional safety of the centre is of utmost importance. This includes waste and effluent management aiming at causing no impact on health and the environment, protection and supervision of the zone, and complete confidentiality of subjects and data.

- ***Key players***

Four higher education and research institutions in the Rhône-Alpes region will participate in the project: University Claude Bernard (Lyon 1), University Joseph Fourier (Grenoble 1), Grenoble Institute of Technology and INSA Lyon. The project will be financed to a sum of € 40 million by the Rhône-Alpes Region and the local authorities involved (the Drôme department and the mixed syndicate of Rovaltain). Two scientific and technological public institutions are project partners: CNRS and Cemagref. Others have already expressed an interest in participating.

Partners from the economic sector are being sourced. Two competitive clusters—AXELERA and LyonBioPôle—have already expressed their interest in this project.

- ***Advance timetable for the implementation of the project***

The initial scientific project—the subject of this document—will be completed and assessed at the end of 2009. A call for research projects will be launched in January 2010, with proposals expected by 1 March 2010. The technical report will be completed by the end of March 2010. The necessary calls for tenders are drawn up and published immediately, with a 3-month timeframe for receiving proposals. The proposals will be analysed at the beginning of summer 2010 and construction work is scheduled to begin from October 2010. The facility should open its doors during 2011, when the first permanent members of staff and research teams arrive. In addition to the initial investment, the region and the local authorities will contribute towards operating costs during the first years to ensure that the project gets off the ground. Eventually, the Centre should be able to finance itself (using funds from private and public contracts). This original—and we believe very relevant—concept may spark interest at a European and international level, and in the long run could initiate a network of similar experimental systems. In fact, the idea has already attracted interest, in particular on a European level.

[1] This original idea can be related to the concept of an "Innovation centre", but with a strong component of basic research, or to "Large scale facility".

Technology and science centre in Rovaltain

Centre for Ecotoxicology and Environmental Toxicology in Rovaltain: CETER

Scientific and technological project

Alain Pavé[2], Marc Babut, René Bally, Pierre Caumette, Sandrine Charles, Michel Sèves, Andreas Riel.

"Stress is life and life is stress"[3]

Introduction

The impact of human activity on our health and the environment has long been a source of speculation and concern. First concerns were expressed about the impact on natural resources due to their exploitation, then about incidences of pollution—which were further provoked by the devastating effects observed, for example to certain aquatic ecosystems, which have been destroyed as a result of accidental leakages or even due to a constant release of pollutants. Moreover, employees exposed to contamination in their workplace have suffered from health problems. Epidemiological studies bring to light the health consequences for human populations.

Identifying and assessing risks

Nevertheless, recent history has shown that scares can lead to a better appreciation of the impacts, and to a renewed effort to understand the real causes. This was the case with "acid rain" and its effects on vegetation, in particular on the forest trees in the East of France, which were hardly affected compared to the trees in Germany. The differentiating factor lay in the silvicultural system, which is exposed to greater stresses in Germany than in France. That being the case, limiting industrial and transport off-gases still remains a sensible precaution. We could quote numerous examples of suspected or actual effects, sources of concern, of which many have proven to be

[2] Coordinating editor. Most of the footnotes provide additional information and are therefore "neutral" in the sense that they do not express a particular opinion. Others are not so neutral. They are therefore initialled AP, and only commit the signer.

[3] Chrousos G., Gold P. 1995. Introduction. In: Stress, Basic Mechanisms and Clinical Implications. New York Acad. Sci., 771, XV-XVI (quoted by M. Bounias in "Treatise of General Toxicology - from Molecular Level to Planetary Scale" Springer, 1999, 804p.). This quote places us in an evolutionary perspective: current living systems are the result of almost 4 billion years of biological evolution during which life as a whole has suffered considerable stresses. We think that around 99% of species that have lived at one point in time have disappeared, but that biodiversity has not stopped increasing, in particular since the Cambrian era. Living systems are resilient and have not stopped evolving and transforming to adapt to a changing environment.

founded, although certain concerns have also been unfounded. However, an increase in stressors increases the potential risks. Questions and concerns are therefore understandable and should be taken seriously. Technological and regulatory measures have already been taken, but they are not enough as the process continually evolves: the sources of potential risks multiply faster than the rate at which decisions are taken. Conversely, being over precautious does not necessarily eliminate all risks, especially economic and even health risks.

Nature is not without its own dangers either, and that includes chemical dangers. An infinite number of natural substances can be very toxic—volcanic gas is a major pollutant, and certain organisms are pathogenic and very harmful. Living organisms, including humans, that inhabit the surface layer of the earth have come into contact with them and are exposed to them. These living organisms have evolved over almost 4 billion years, punctuated by major catastrophes and constant exposure to "pollution", the main source of which is an accumulation of oxygen in the atmosphere. They have built up tolerance, adapted, and developed unanticipated mechanisms of resistance, such as immunoresistance. The notion that stress, especially in low doses, can have positive effects has also been put forward. We should never lose sight of this background.

Nevertheless, it is necessary to assess the risks, to organise them into a hierarchy and to implement measures to avoid them, or at least deal with them in a fully knowledgeable manner. Besides the acute effects, the **consequences on living organisms**, including humans, **of exposure to small doses of a combination of chemical, physical and biological risk factors over the long term needs to be assessed.** This translates to the most common **practical situations** which are often a **source of concern for society.**

There is no longer the need to for the scientific community to identify and verbalise these problems. On the contrary, it needs to join forces to tackle the problems and find solutions. But as it happens, there are few specialists in the relevant disciplines—in particular in ecotoxicology and environmental toxicology—and the testing facilities available are predominantly laboratory-based. It has now become an urgent priority to increase human talent and to design both a scientific framework and adequate testing facilities for furthering research in these fields. The Rovaltain project subscribes to this philosophy, although it cannot accomplish these goals alone. It must form part of a collective national and international effort. This document presents an argued approach to this ambitious project.

Necessary scientific development

The development of a new scientific field is primarily driven by three forces: natural developments within science itself; endeavours to provide responses to questions raised or to society's expectations; and economic reasons. The development can also be due to political motivation to develop a certain region. Depending on the circumstances, one or other force is predominant. Ecotoxicology and environmental toxicology both have equal weighting in terms of logic, yet there is a difference between the two fields: if we are to use the number of publications in each scientific field as an indicator (see I.1., figure 1) then the development of environmental toxicology—a direct descendant of toxicology—has outpaced the development of ecotoxicology, an interdisciplinary science formed chiefly from chemistry, biology and ecology. Although our project proposal does include environmental toxicology, our focus shall be on ecotoxicology, which we feel merits more attention because the situation is more complex and because it is increasingly in demand.

At a very basic level, the main difference between toxicology and environmental toxicology lies in the problems caused by exposure. The overall goal and objectives of both disciplines remain the same: preserving human health. Although there is still a lot of groundwork to be done—particularly on identifying the far greater number of risk factors which are, to date, less detected than those in medical toxicology, food toxicology and even occupational toxicology—the common goal and profound knowledge gained from animal models enables us to apply methods from toxicology to environmental toxicology. We feel that environmental toxicology not only provides answers to current issues concerning the identification of risks and risk factors to human health, but that it bridges the gap between toxicology and ecotoxicology.

On the other hand, ecotoxicology is an infinitely vaster discipline; it deals with problems due to exposure, but also has a great variety of target organisms (broadly defined as living systems[4], ranging from cells to populations and communities), it encompasses different environments, for example, aerial, aquatic and edaphic environments, it is interested in the potential "health" effects on the target organisms, and the direct and indirect consequences on human health. The scope of the field can be overwhelming, and there is a real risk of combinatorial explosion due to the sheer number of factors to be studied. Nevertheless, it is worth noting that the same problem emerged during the dawn of biology, but this did not prevent considerable developments in the science over the past two centuries, including identifying the main invariants common to all life forms, and the fundamental processes and "biological models" on which these processes were built by creating a methodology in order to study them. We should remember that we owe the "scientific method" to Claude Bernard, and that statistics as well as mathematical and computational modelling were largely developed upon the basis of both fundamental biological problems (see, for example, biometry's role in the theory of evolution and genetics) and applied or solved problems (see biometry's contribution to agronomic and medical science, as well as its everyday use in laboratories). We therefore have access to a rich arsenal of methodologies. Lastly, population biology and ecology have provided us with the theoretical and conceptual foundations upon which

[4] The term living system (or biological system) is more generic than that of an organism or life form; it includes them, but it can also be used to describe more complex groups of organisms, such as populations and communities, and interactions between these organisms, such as symbiosis. The term is used to describe independent entities which are subject to evolutionary processes. The simplest living system is the monocellular organism. Cells making up a multicellular organism are also included in the definition of this term in the sense that the organism to which they belong is affected by biological, ecological, and more generally, evolutionary processes.

we can build. It should, however, be noted that ecotoxicology can also play a part in establishing fundamental results in ecology, but above all, it needs to build a generic knowledge base in which concepts are defined, mechanisms are better understood and relevant experiments are designed, thus avoiding a combinatorial explosion—a risk previously alluded to and one which is due to the choice of an empirical strategy. Modelling is one of the key tools for optimising experiments; it could even become a common objective, similar to the U.S. Environmental Protection Agency's NCCT (Virtual Liver and Virtual Embryo)[5]. At any rate, it will take time for models to replace reality, as was the case for "digital wind tunnels" in aerodynamics. For the time being, such an objective should be seen as a means of synthesising and formalising knowledge, with models fulfilling modest ambitions, such as supporting and complementing experiments.

The Rovaltain project

The aim of the Rovaltain project is therefore to promote the development of environmental toxicology and ecotoxicology. So how does this fit in with other existing initiatives? First, it responds to three concerns: (1) scientific, as a developing field of research with promising potential for scientific advances; (2) technological and economic concerns expressed by the production industry (industry and product consumers); (3) social and political concerns illustrated by European directives such as the Water Framework Directive and the REACH Directive, and debated in France at the Grenelle Environment Forum. The project also forms part of a range of solutions for developing a scientific field, including actions to increase awareness among the scientific community, creation of networks to structure the community around common objects and objectives, a research programme highlighting thematic and operational priorities, creation of specialised centres which enable investment in shared equipment to be used by all the on- or off-site teams as part of a *"hôtel à projet*" set-up (see footnote 1). These solutions and resources are not technocratic inventions; they have been built up over time by the scientific community itself. For this reason, a “top-down” hierarchical approach should be avoided: inventiveness and the capacity to innovate largely depend on the initiative of the researchers, either in groups, or more or less spontaneously. Accordingly, regardless of the chosen solution, this principle should be applied, for instance by according great importance to calls for tenders and the spontaneous and inventive expression of the scientists.

The Rovaltain Centre for Environmental Toxicology and Ecotoxicology (CETER) adheres to the principle that material and technical resources that are difficult—or even impossible—to implement in a laboratory setting should be made available to the scientific community. It also believes that the use and development of this equipment, although based on broad frameworks and priorities (calls for tenders), should largely be

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[5] NCCT : National Center for Computational Toxicology – Environmental protection agency (USA) : http://www.epa.gov/comptox/index.html
Virtual Liver Project : http://www.epa.gov/ncct/virtual_liver/
Virtual Embryo Project : http://www.epa.gov/ncct/v-Embryo/

left to the initiative of the researchers. The primary selection criteria will still be based on whether the project respects the terms outlined in the call for tenders, as well as the scientific quality of the research proposal. This project complements other countrywide initiatives undertaken by other clusters, networks and research programmes, and is in line with the sector's development policy at a European level. The Centre's long-term goal is to become an international reference centre.

In practice, the CETER will provide the chosen teams with both the resources for performing experiments and the technological and methodological expertise. It will function primarily on a *"hôtel à projet"* principle (see footnote 1), with a permanent team of around thirty people, and other teams for varying periods of time. The permanent scientific and technical teams will carry out fundamental research, oversee day-to-day operations, ensure the continuity of the work, develop the methodological assistance, and ensure that the research is applied, primarily in the form of training and expertise. The second research component will be made up of researchers and teams selected from responses to regular calls for tenders to design and perform experiments lasting from a few weeks to several years. In total, there should be a combined workforce of permanent staff and temporary teams of around 80 to 100 people at any given time.

Ecotoxicology and environmental toxicology both require specialists at all levels and with different skill sets. In terms of research, priority must be given to training PhD students, but we should also aim to broaden the community by attracting researchers from related disciplines, such as ecology, chemistry, biology, medicine, pharmaceutics, and even physics; and researchers from disciplines necessary to methodological developments, such as biometricians, computational biologists, computer scientists, automaticians and mathematicians. If we are to find solutions to existing problems in a timely manner, there are few alternatives. As a consequence, the project proposal must be attractive outside the current community. There is no mystery in what attracts researchers to respond to calls for proposals: offering attractive scientific prospects, providing them with the necessary resources for achieving their goals and creating an appealing framework and professional environment, not to mention the personal aspect. For example, ecotoxicology could open new horizons, beyond providing answers to concrete and practical questions, by embracing approaches that are likely to identify and study fundamental mechanisms (for instance by disturbing a system, we can obtain information on how it functions in a "normal" steady state).

I. The context

Let's revisit the conditions for developing a scientific field. As we previously mentioned, there are three main driving forces. The first is to do with the dynamics of the science itself, steering scientists towards new questions on subjects that they also consider in a new light. The second driving force comes from society's curiosity or wonderment (What is it? How does it work?) Or from a desire to answer existential questions and to fuel our imagination (understanding the beginnings and the ends, the capacity to dream, the need to position ourselves within a cosmos) and from concerns (the world is full of real and potential dangers, and it is better to understand them in order to avoid or control them. This, incidentally, was one of the first contributions of science). The third force is economic: knowledge must be generated in order to innovate, invent new products and markets, guarantee the survival of a market, generate profits, and above all, to provide work.

Although these three driving forces vary in their degree of importance, they are always present. And although the order listed above is debatable, we feel that it reflects reality. In ecotoxicology and environmental toxicology, contamination of the environment—which has largely been proven by scientists—has led to social motivations becoming the main driver, in the sense that society's concerns are legitimate. These concerns have resulted in a political and regulatory intervention (Grenelle Environment Forum, European directives etc.) These give rise to economic consequences such as the need to improve production quality and greatly reduce the hazards; although on the other hand, a thriving market has developed, including "green" chemistry, waste treatment and remediation etc. Scientific dynamics comes in third place, and as it is the crucial factor, whether we like it or not, we are confronted with a delicate issue: the weak position of this driving force has led to difficulties in its development within the scientific community. Its influence is still limited, especially for ecotoxicology, and human talent is still inadequate. The main challenge is therefore to strengthen the scientific dynamics so as to better identify, understand, compare and classify the risks according to their gravity.

This is naturally a very condensed analysis, but experience shows that this kind of analysis is necessary to develop a leading scientific field. Political and/or economic initiatives will have little impact if the scientific dynamics are not commensurate. It is what was achieved in environmental research in the 1980-1990s. And it’s what we must aim to achieve in ecotoxicology.

Lastly, we are also aware that if the science is to be "correctly applied", if it is to give rise to useful technological developments, and to prompt decisions which will have far-reaching and positive implications, it must be built upon a pre-existing solid, fundamental knowledge base. One of the greatest challenges is not only to create such a knowledge base, but to rally the necessary disciplines that already have such knowledge bases. For ecotoxicology and environmental toxicology, one of the primary objectives must be to further the modest contributions of ecology and biology (see references in note 14: Steinberg and Ade, 2005, Pelletier and Campbell, 2008). The idea proposed by Van Straalen in 2003[6] of progressively transforming *ecotoxicology* into *stress ecology* , thus integrating the discipline into ecological sciences, strikes us as being very pertinent, as long as it able to develop.

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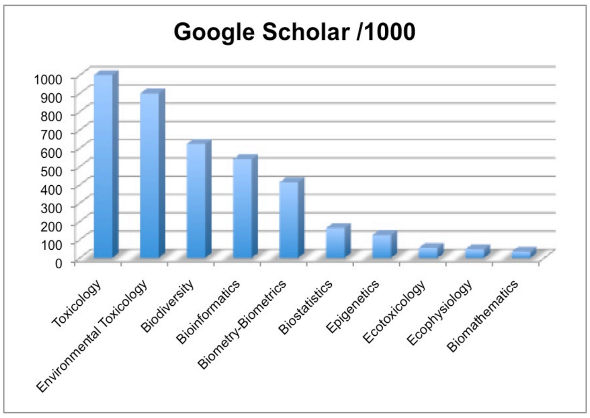
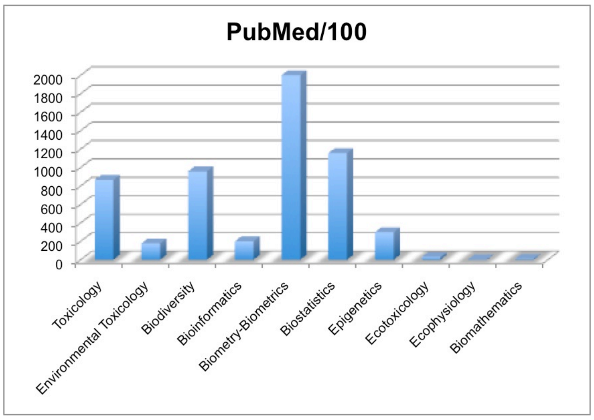
[6] Van Straalen N. Ecotoxicology Becomes Stress Ecology. Environmental Science & Technology, 2003, 37 (17), pp 324A–330A.

I.1. Scientific: ecotoxicology, an upcoming field

According to V. E. Forbes and T. L. Forbes (1997)[7], the word *ecotoxicology* was invented in the 1960s and defined in 1977 by the toxicologist René Truhaut[8] as the "branch of toxicology concerned with the study of toxic effects caused by natural or synthetic pollutants to the constituents of ecosystems, animal (including human), vegetable and microbial, in an integral context". This historic reference is useful for understanding the state of a discipline or a field of research at any given moment, especially if we compare it with closely related fields.

Accordingly, figure 1 shows that ecotoxicology is considerably less quoted than environmental toxicology, which is less quoted than toxicology. The introductory remark concerning the relationship between the two disciplines explains this observation, as does the relative age of each field, in particular that of toxicology, which was established from the 19th century[9]. On the other hand, a comparison with more recent fields is of interest. Bioinformatics appeared much later (it first featured in *Nature* in 1991) and epigenetics, an old discipline which was recently and actively revived[10]. The only similar field is ecophysiology, which exists for the benefit of ecotoxicology. However it has been noted that many of the publications which could be classed under *ecotoxicology* do not contain this key word, are not published in field-specific journals, or are labelled *environmental toxicology* due to the significant grey area which exists between the two fields.





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7 Forbes V.E., Forbes T.L. (1997). Ecotoxicology in theory and practice. Inra Ed., Paris, 253p.

8 Truhaut, R. Ecotoxicology: Objectives, principles and perspectives. Ecotoxicol. Env. Safety, 1, 1977, 151-73.

9 Its invention is generally attributed to the French doctor Mathieu Orfila (1787-1853).

10 When we consulted scientific or generic databases back in 2006, we were only able to identify a few references to epigenetics. In the autumn of that year, a Nobel Prize was awarded to Craig Mello and Andrew Fire for their work on RNA interference, which undoubtedly contributed greatly to the exponential growth of the field.

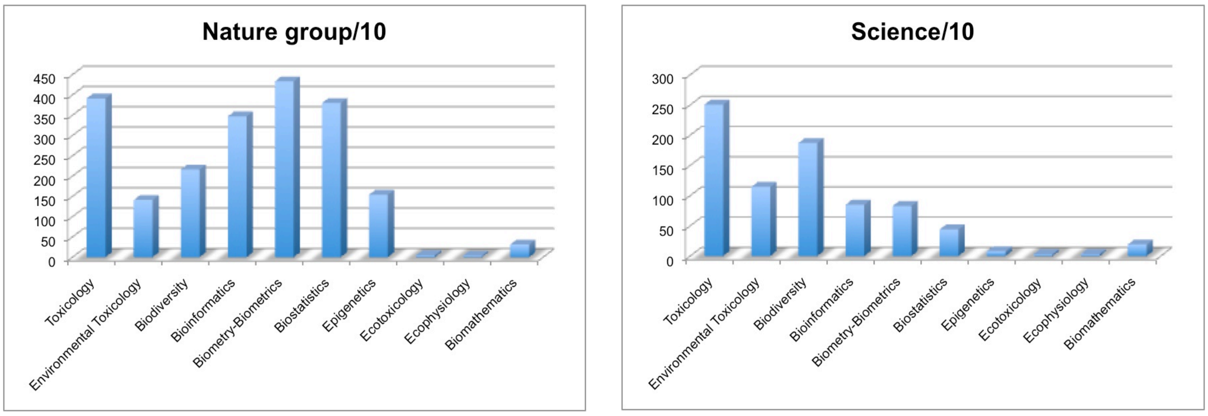

Figure 1. The number of occurrences of key terms in a small number of databases which characterise some of the scientific fields that have emerged or evolved during the 20th century, especially during the second half of the century.

The differences can be explained by the relative age of each discipline, but also by the difficulty in making scientific progress within the fields, which is partly explained by the complexity of the subject and the difficulty in defining the scope of the scientific field. Thus, the word *ecology* first appeared in 1874 in *Nature*, whereas between 1869 (when the journal was launched) and 1874, the term *biology* was used 157 times. At that time, the scope of ecology, its concepts and even its objects were less well defined than those of biology, whose origins, ascribed to J.B. Lamarck, date back to the beginning of the 19th century.

It is therefore not surprising that ecotoxicology, which is based upon at least three great disciplines: ecology, biology and chemistry, has had difficulty developing. Moreover, the current tendency is to extend scope of this field, therefore blurring the boundaries and subject areas of this discipline[11]. It should also be noted that the complexity of the subject, coupled with the pressing need to find solutions to practical problems, has not done much to increase its appeal, especially from an academic perspective.

I.2. Human talent at national and international levels

We will draw upon our own experience and the study commissioned by the *Bureau van Dijk*[12] in the report written under the responsibility of E. Couty[13].

[11] For example, as part of CNRS's Environment, Life and Society programme, naming the scientific activity, including ecotoxicology, "Transport, Transformations and Ecotoxicology of Contaminants" constituted a relatively limited definition. Indeed, we felt it appropriate to mention that in this context we are led to believe that the term "ecotoxicology" is limited to living beings, and does not include physical transport mechanisms, for example via liquids, or purely chemical transformations. On the other hand, biochemical transformation processes bioaccumulation, transport via living beings, and of course direct consequences on organisms and populations were considered as part of the scope. A lack of precision poses two dangers. The first is "nebulisation" or "dispersion" which involves incorporating contributions of the disciplines beyond what is reasonable. The second danger is an (often involuntary) misappropriation, whereby the core subject of the research becomes peripheral. This can happen for example by focusing on the non-biological transport and transformations of contaminants, thereby pushing living systems to the outer limits of the scope of ecotoxicology when in fact they constitute the main objects. By limiting, specifying and naming the contributions necessary for resolving real problems, we avoid these malfunctions.

[12] Study on France's position in the fields of toxicology and exotoxicology, Summary. Bureau van Dijk, Paris, 31/01/2008, 27p.

[13] Couty E., Bartoli F., Dalmas D., Verrel J.L. Interministerial mission for the creation of a national centre for toxicology and ecotoxicology in Rovaltain (2008). Report for the Prime Minister.

The scientific community in these two fields—in their strictest sense—is rather small. At least this is the understanding that prevails. Back in 1992, Claude Paoletti, the then director of the Life Sciences department at CNRS, had already remarked to us that this was the case for ecotoxicology.

However, a brief tour of the horizon shows that:

- Competencies in toxicology, in its strictest sense, come largely from the biomedical field and the medical industry; and public research competencies are primarily from universities, INSERM, the CEA and IRSN.
- Competencies in ecotoxicology are primarily found in the public sector. For instance, within CNRS, 17 units contain this key word. Cemagref has very talented teams, as does INERIS, as well as the CEA, INRA, IFREMER and IRD and of course at the universities (but for the latter, the "talented teams" work in collaboration with the CNRS and have already been identified).

We have learnt from the survey that:

- The number of identified units appears to be higher than our estimates: 385 research units in the public sector, and 55 in the private sector. As a point of comparison for assessing the pertinence of the data, we know that the CNRS has 1200 units, which would be equivalent to a third of the potential within the CNRS, which seems a lot.
- What we believe to be an over-estimation is brought into perspective if we consider that there were only 56 replies to the survey. We are nonetheless surprised by the lack of feedback, for example in Lorraine, as we know that there is a well-known laboratory (established by Paule Vasseur) specialising in the field of ecotoxicology in Metz — UMR 7146 “Ecotoxicity, environmental health”, which runs a nationally recognised PhD programme. The authors of the study acknowledge that the shortage of responses to the survey has skewed the results, and the "one-man" reply from INERIS has given Picardy an undoubtedly disproportionate weighting.
- This being said, the survey is very useful for comparison purposes. Thus the leading position of the three regions: Aquitaine, Île-de-France, and Rhône-Alpes (in alphabetical order) is not surprising. Examining the results by heading reveals that, for both ecotoxicology and toxicology, each region’s position corresponds to its relative national ranking for research potential, irrespective of the field. However, Rhône-Alpes has a slightly stronger position if we take into account its full research potential for the two fields, in particular for ecotoxicology. For this latter field, we have taken into account projects backed by the National Programme for Research in Ecotoxicology since 1996 (PNETOX, initiated and sponsored by the Ministry of the Environment), with the distribution illustrated in table 1.

This data underscores Rhône-Alpes’ predominance in research in ecotoxicology.

	Aquitaine	Brittany	Île-de-France	Languedoc-Roussillon	Rhône-Alpes	Other Regions	Total
Number of projects sponsored by PNETOX	4	4	8	8	16	17	57
Percentage	7	7	14	14	28	30	100

Table 1 Analysis of the responses to calls for tenders launched by the National Programme for Ecotoxicology (1996-2003): geographical distribution of the coordinators for the selected projects.

From an overall perspective and with only slight variations, the bibliometric component suggests that the French scientific community largely mirrors scientific communities elsewhere. Although some countries have more dedicated centres, such as the Environmental Protection Agency (EPA) in the United States (as quoted in footnote 5), France's overall scientific potential is still within the average. It is a common observation that complaints of a weakness in a certain field in France often reflect a general weakness in the same field on a global scale. Nevertheless, France's performance in the field is merely judged "average, or below average", although we should treat these "observations" with a certain scepticism. Indeed, measuring the potential proves difficult because the majority of researchers within the ecotoxicology community fail to use the key word *ecotoxicology* in their own bios, laboratory profiles and publications. To give this situation some perspective, it is worth noting that 350 French people attended the SETAC Europe (Society of Environmental Toxicology and Chemistry) congress in 2005, which was held in Lille.

I.3. Social demand

These demands are reflected in 3 of the 268 Grenelle Environment Forum commitments, in which ecotoxicology and environmental toxicology appear under the umbrella themes "health and the environment" and biotechnology. For example, ecotoxicology, associated with toxicology appeared in the following three commitments:

- Commitment no. 132 **Strengthen the following disciplines:** ecotoxicology, toxicology, ecology, epidemiology, agronomy, microbial ecology, agricultural economics … by ensuring [over a 10-year period] the necessary funding of training and the consolidation of teams; the creation of an Advisory board focusing on the challenges within the Ministry of Research;
- Commitment no. 142: Creation of **inter-regional, multi-disciplinary health and environment centres, a centre of expertise in toxicology and ecotoxicology, and inter-University Hospital centres for treatment, prevention and clinical research**, all of which will create 400 new research jobs.
- Commitment no. 212 **Increase training of experts in subject fields that currently have limited expertise** (toxicology, ecotoxicology, epidemiology, ecology …)

This evidence may not seem compelling, but it should be put into perspective by comparing it to the number of appearances of key words for other disciplines: toxicology is also mentioned 3 times, as is genetics; biology is mentioned twice, one reference of which is to microbiology; ecology is more frequently mentioned, with 12 occurrences; and strangely enough, climatology isn't mentioned at all[14]. In fact, the primary focus of this forum was not on the sciences *per se*, but on general areas of concern, such as the climate, biodiversity, GMOs etc. These thematics were then translated into areas of scientific research by a specific operational committee.

I.4. The proposed policy

Environmental issues have been broadly adopted by the world of politics. In the 1970s, France set up state structures, firstly by introducing dedicated Secretaries of State and then Ministries. There has been a similar movement at an international level, with the creation of specific programmes such as the United Nations Environment Programme, which was established in 1972. Nevertheless, ecotoxicology has progressed more slowly than other fields, such as climatology. Moreover, the political influence is also reflected in the implementation of standards, rules, directives and laws, and the signing of international agreements. Ecology features on more than one account, for instance in the EU Water Framework Directive, or more recently, in the European REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances) directive.

Against this backdrop, the Rhône-Alpes region and local authorities' initiative is original. As part of a knowledge economy policy—which in practical terms relates to land planning—the first step involves quelling a national concern about the relocation of national structures, in this instance, IRSN (Institute of Radioprotection and Nuclear Safety). Following the failure of this relocation, the Region—backed by its proactive policy on research and further education—proposed to set up a toxicology and ecotoxicology centre which would tie in with the clearly stated environmental concerns. This initiative, which preceded the Grenelle Environment Forum, is perfectly in line with the conclusions of the national debate. The largely unfounded reluctance voiced by various state structures was therefore surprising, but seemed to indicate a resistance to an initiative that had not been fully understood. Indeed, more often than not, regional operations were until present deliberately managed at national level. This regional initiative was planned and financed by the Region and its local authorities, supported by the Region's major higher education and research institutions, and is present in the Region. Are we witnessing a shift from a *top-down* to a *bottom-up* approach? Actually, scientific activity has shown us that a subtle mix of the two appears to be the most effective method. Our conclusion has been drawn from 20 years' experience of this mixed approach, in particular as part of CNRS's interdisciplinary programmes.

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14 An analysis of the number of occurrences of certain words in a text and the contexts in which the words are used can be very informative. They are effective methods used by linguists to create indexes and concordances.

II. Scientific strategy

The issue at stake is ensuring the development of a scientific field which has been pushed to the forefront due to its ability to provide solutions to social problems and because it sets a real scientific and technological challenge, but which has not been adequately driven by its own momentum in relation to its objective importance. Although the scale is much larger, the situation is similar to that of research on the environment in the 1980s, or on a much smaller scale and more recently, that of bioinformatics. Let's take a moment to examine these two extreme cases. It took 30 years for research on the environment to evolve into a field of excellence, founded on interdisciplinary programmes and on the mobilisation of considerable means at both national and international level. Bioinformatics is generally thought to have originated in the 1960-1970s as a solution to biometricians' calculating problems which paved the way for the beginnings of this new discipline. The connection with computing occurred in the 1980s when the need arose for functions other than numerical analysis and the management of small groups of data—needs such as structured databases, algorithm databases, knowledge bases, interactive computer graphics and modelling assistance etc. It was thus necessary to encourage collaboration between biologists, ecologists, biometricians, computer scientists, automaticians, mathematicians and modellers. The task was facilitated by the biometricians who were able to bridge the gaps in understanding, thereby forming the basis of the dialogue. They were also amongst the first to benefit from this new science. Bioinformatics is in fact typically an interface discipline.

Ecotoxicology has a different status, even though it draws from a larger range of disciplines, in the sense that it needs to encourage collaboration between biologists, ecologists, biometricians, chemists and physicists. It also has more diverse objects of study, methodological needs and experimental needs. Ecotoxicology is therefore an interdisciplinary field of research. So how can we ensure its development with a small workforce and limited training opportunities?

Before we go on to examine the various strategic aspects, it is worth going over a few key terms, not for the specialists of course, but for the readers outside the field. We shall even quote a few examples of scientific questions.

II.1. Some prior definitions

Toxicology, environmental toxicology and ecotoxicology

Often, a distinction is only made between toxicology and ecotoxicology. In light of what we have observed, it seems of interest to distinguish a third, intermediary category: environmental toxicology. Thus, we shall limit our definitions to the following three terms:

Toxicology is the study of adverse effects on living organisms and their components due to ingesting, injecting or being exposed to toxic or pathogenic substances; the dose–

effect ratio plays a key role (pharmacology, agri-food industry, professional settings), but adheres to the precise objective of understanding the, mainly acute, toxic effects (i.e. high, or relatively high doses, short-term exposure). Humans are the focus of study, and many, if not the large majority, of toxicological studies, are carried out on animal "models" of humans. There is however a tendency to extend this definition to all organisms.

Environmental toxicology studies the effects on organisms, including humans, of low-dose, chronic (long-term) passive exposure to (usually a mix of) stressors that are prevalent in the environment. The challenge, therefore, is proving the actual toxicity or non-toxicity of these stressors, evaluating the dose and identifying the effects. Classic animal models, such as rats and mice, are used. The effects are both physiological and population-based—for instance, the effects of reproduction on mortality at a physiological and individual level are studied, as are the consequences on the reproduction rate and mortality rate at a population level, thus deriving demographic evaluations. In this sense, environmental toxicology can be seen as a particular case of ecotoxicology (see below).

Ecotoxicology is the study of the effects of toxic or pathogenic, or often a mix of the two, risk factors prevalent in the environment, on vegetal-, animal- and micro-organisms, populations, communities and the biodiversity of an ecosystem. The challenge, therefore, is proving the actual toxicity or non-toxicity of these risk factors, evaluating the doses, identifying the effects on the level(s) of biological organisation disturbed and gradually building up to the resistance of a community and an ecosystem. There is also the problem of understanding the normal functioning in reference conditions (a referential problem of knowing what to compare with what). The foundations for this comparison can be found in ecology, ecophysiology and even ethology.

Statistical population vs. biological population

It is necessary to mark the difference between the notion of *population* in a statistical sense, and as defined for the purposes of population biology and ecology.

In a statistical sense, a population is an aggregation of individuals among which differing characteristics can be studied and distributed according to a law, such as Gaussian distribution. The difficulty lies in taking into account all the variables, estimating the mean and the variations for the given characteristics of the population, and using these estimations, for example, for comparison purposes. These data analysis methods enable us to study potential connections between the characteristics, and thus to describe the statistical information contained in the data in a more succinct manner. In general, it is not possible to observe or measure the entire population of individuals, and statistics relies upon the random sampling approach. This approach involves randomly selecting, or selecting according to a sampling plan, a representative fraction of the population, and carrying out observations and measurements. Using the estimation theory (a basic concept in statistical inference), we can deduce from this random sample—allowing for a certain sampling error—the characteristics of the population. A population may also be virtual. This is often the approach adopted for performing experiments on what is considered to be a hypothetical, generally infinite, population of individuals. The limited number of observations or measurements actually made is considered as a sample of this population. Once we have established the identity of the real or virtual population, we can use the same methods. It is also worth noting that in statistics, the notion of a population is static, even for temporal analyses (the questions are similar: estimating the parameters and their variability, sample-population ratio).

Population as a biological and ecological concept is based on function. It is the "operational" unit of the notion of a species. An animal or plant population is made up of individual organisms of the same species that interact with each other, in particular during reproduction, and are subject to environmental factors. Various conditions change over time and space. Biological parameters, in particular genetic parameters, also change over successive generations. We generally look at the dynamics of a biological population, i.e. its demography, to observe the changes over time of its aggregate numbers and those of its various subpopulations (gender, age, phenotypes, genotypes etc.) and influences of this dynamic on its interactions with other populations of the same species (for example genetic changes in a metapopulation), or of interactions with other species (for example, competition and predation).

The two concepts complement each other harmoniously. Thus we use statistical methods and techniques to estimate demographic parameters, such as the rate of reproduction and mortality.

From both angles, the models play a central role. The statistical function of models is used for generating estimations and using these estimations, for example, to assess the accuracy of an estimation, to compare the values of one or more estimations, or to compare one or more parameters obtained in different contexts, such as for populations that have and have not been exposed to stressors. The models are dynamic for populations in their biological sense (for example models expressed in the form of differential, recurrent, deterministic and stochastic equations.

Statistics, modelling and "changes of scale"

At the core of methodological questions, there is what is known as a *change of scale*, which in practice refers to a change in the level of organisation: how can we determine facts for an entire biological population based on the observation of a limited number of organisms within this population? Statistics provides a good solution with its concept of a population if we rely on reasonable hypotheses. However, for populations in the biological sense, the situation is more delicate. Indeed, at this level, the relevant parameters are not those which are measured directly on individual organisms, or at any rate, the individual measure is not sufficient. For example, the fertility rate of an individual organism does not carry any meaning *per se*, and cannot be reduced to the number of offspring produced by the individual organism, at least in the same way that morphological or physiological parameters, such as height, body mass, lipid concentration in tissue etc. can. Moreover, as we have previously mentioned, the statistical approach is static, whereas the biological approach is function-based and dynamic. We are therefore obliged to rely more on concepts and methodologies. As the experiments will only involve a limited number of individuals, how should they be designed in order to apply the information obtained at population level, in its biological sense? In practice, the results can already be obtained by linking the two approaches together (for example, by studying the estimates of the parameters of dynamic models, their intra- and inter-individual variability, breaking up population-dynamic models etc.), but they are not sufficient, in particular because eventually we will have to make the transition from the population to the community.

II.2. Example statement and questions it raises

Environmental toxicology and ecotoxicology have arisen from observations and speculation on the **consequences of human activity** on **changes in the environment** due to:

1. The passive or active, voluntary or involuntary, dissemination of natural or synthetic substances, elements and compounds (such as synthetic products, xenobiotics, and heavy metals) on living organisms, (such as microorganisms) and on certain of their components (for example, genome elements and proteins);
2. The constant or intermittent exposure to physical factors, such as electromagnetic, ionising or non-ionising, waves and fields;
3. Physical changes, such as land improvements, which are likely to affect these factors, their dissemination, their diffusion, and to influence their transformation, especially their disintegration or their "extent of action".

Some of these stressors can present risks, or are suspected of presenting risks, to humans and living systems within our environment.

This statement generates **major scientific questions.** As an example, we can quote:

1. What are the potential effects of risk factors on human health, both to individuals and to the entire population?
2. What are the potential physiological and genetic impacts on plant and animal organisms and microorganisms, and the demographic consequences on their populations?
3. How do ecological relationships change, leading to changes in the ecosystems, their functioning and their biodiversity?
4. How can we restore or rebuild a "damaged" environment, or more generally, how can we build a healthier environment for mankind and the living systems which inhabit it?
5. What are the short- and long-term effects of high and low doses of stress factors?
6. How can we evaluate the associated health and ecological risks using measurements and observations of the environment, in particular by using adapted and reliable indicators? What are the maximum doses permitted? The notion of *critical load*.

Following almost 4 billion years of biological evolution, during which living systems have been exposed to major stresses[15]:

1. To what extent can those who have survived and evolved resist new stressors, and how can they adapt to them and even evolve?
2. What are the associated processes and mechanisms likely to reduce these stresses, for example by destroying xenobiotics, or on the contrary, by increasing them, say, by transforming xenobiotics into toxic products for other organisms, in particular for humans?

Each of these questions can be **broken down** into more specific terms. For instance, the reference to biodiversity in the third question may give rise to the following questions: What are the direct effects on biodiversity (depletion, growth, change)? What are the consequences on the corresponding processes (erosion, diversification, substitution) and on the long-term effects? What are the associated qualitative and quantitative changes (replacement of species, introduction of new species, extinction of species, changes in the demographic relationships between populations and various species) and the effects on the functioning of the ecosystem? Could these changes provide reliable basic biological indicators?

II.3. Necessity of reinforcement

Data on researchers' reference works and demographic data do not tally with society's expectations and needs. For reference work data, such as can be interpreted by reading the graphs in figure 1, it is only worth noting total numbers, and not making a value judgement on the small number of publications in the "mainstream journals" whose publication policies limit comparisons. For reference articles of equal quality, articles on popular subjects are more readily published than those on the subject of ecotoxicology. Conversely, ecotoxicology has a duty not just to provide answers to practical questions, but to bring an "added value" to these answers by providing fundamental knowledge, and also drawing upon fundamental knowledge, especially in biology, ecology and ecophysiology. This is what toxicology succeeded in doing in a study on disturbances caused by exogenous substances. As well as providing solutions to the questions that led to the study being carried out, it also enabled us to understand certain mechanisms.

[15] This short section was written before reading the articles quoted in the reference below, but was inspired by an invested interest in evolutionary sciences and the articles published by J.C. Massabuau which support the theory of evolution. Not being an ecotoxicologist myself, and only having a superficial knowledge of the community, I chose not to criticise the weakness of the biological and ecological, and above all, evolutionary backgrounds, which apart from a few exceptions, such as this one listed above, I thought I could work out. This is not a criticism of ecotoxicologists, but of biologists and ecologists who haven't invested enough in the topic (notes AP).
Steinberg C.W., Ade M. Ecotoxicology, Where do you come from and Where do you go? Environmental Science and Pollution Research. 12, 2005, 245-246.
Pelletier E., Campbell P. Aquatic ecotoxicology - comparison between organic micropollutants and metals: current situation and future perspectives, 21, 2008, 173-197. Access to site members only:
http://id.erudit.org/iderudit/018465ar.
These references and the articles were kindly given by Peter Campbell, who I wish to thank for his interest in our project.
We should also recall the famous quote by Theodosius Dobzhansky: "Nothing in biology makes sense except in the light of evolution". (In *The American Biology Teacher*, 1973, 35: 125-129)

Quantitative reinforcement is therefore necessary. This, combined with a larger community, should lead to a broadening and deepening of the scope of the science.

However, due to the urgency of the situation, traditional methods of first setting up training will not be sufficient. Granted, it must be done, but it is not enough. It is therefore necessary to encourage a greater mobilisation. A facility such as Rovaltain may provide the catalyst for this mobilisation.

II.4. Resources

The necessary method is known. We need to set up an impressive research facility and offer a welcoming environment that is liable to draw in interested researchers. Focusing initially on the facility, it needs to offer a scientific programme and a robust organisation, as well as the necessary material resources. The organisation, for example a national network and centres such as Rovaltain, should offer the possibility of doing that which cannot be done elsewhere.

The commitment of 4 prominent further educations in the Region to the project constituted an important progress; University Claude Bernard (Lyon 1) and University Joseph Fourier (Grenoble1) will include the Rovaltain project in their strategy map and their future Objectives and Resources Contract. Two large Engineering schools: Grenoble Institute of Technology and INSA Lyon will also participate. It is also worth mentioning that in May 2008, UCBL appointed a professor (PREX2) to work part-time (75%), and created an assistant Professor post in "Ecotoxicology and Environmental Microbiology", a position which was announced and filled in 2009.

i. Mobilising researchers

The weakness of the field, although this can be seen as an opportunity, is its interdisciplinary nature. Chemists, biologists, doctors, pharmacists, ecologists, bioinformaticians, modellers, and even physicians and (applied) mathematicians are all likely to find some interest in ecotoxicology and environmental toxicology, as long as they don't mind engaging in the delights of modelling. The pool from which we can draw researchers is thus large.

The method is simple: researchers can be sourced through calls for tenders and subsequently integrated into an interdisciplinary team working on a common subject that requires input from all disciplines. Researchers will also have access to the necessary resources to achieve their ambitions. Both thematic and geographic mobility is necessary.

ii. Endeavours in initial and ongoing training

The **training component** must be bolstered on a national and regional level, and within the Rovaltain project itself. It should be developed upon Grenoble's experience, in particular its ISM Master programme on medical and health engineering, and Lyon's experience in Masters programmes in "Microbiology Ecology", "Health–Population", "Biosciences", "Urban and Industrial Environmental Sciences" with the option in "Bioinformatics and Modelling" at INSA Lyon. Teaching of ecotoxicology should also be consolidated in the lessons. In both instances we should preserve the strong “modelling" element, which is perfectly in line with the project's philosophy. However, it would be a good idea to consider implementing in the near future a "2nd Masters degree" sharing the common theme of Environmental Toxicology, and offering the option of specialising in either **ecotoxicology** or **epidemiology**.

We must once more insist on the necessity of a strong background education in biology and ecology, and at least a good grounding in evolutionary sciences. Correct application is only possible on the premise of a robust fundamental base. Modelling, which is gaining increasing importance, must also form an integral part of basic scientific training.

iii. Involving the community and developing specialised centres

The necessary foundations upon which an interdisciplinary subject is built have recently been examined. They rely largely upon mobilising the relevant scientific communities by supporting ongoing laboratory initiatives through the creation of specific programmes within research organisations and agencies such as ANR (French National Research Agency). By and large this is already the case, e.g. for PNETOX, the French national programme for Ecotoxicology, and today we can enjoy the positive consequences of this action[16]. These initiatives must now be strengthened initiatives by coordinating efforts, at least on a national level.

Creating specialised facilities is also an essential component. Indeed, there are problems of “scale” which, apart from the occasional exception, are difficult to solve in a laboratory setting:

- temporal scale: the ability to carry out experiments over a long period of time;
- spatial scale: the ability to provide an intermediary point between what can be carried out in a laboratory setting and that of the real world;
- dual scale of complexity: that of the multiplicity of stressors and the potential for mixing, as well as the biological subjects: diversity of the organisms, of course, but also of the levels of organisation (populations and communities), as well as the problems stemming from the relationships between the levels of organisation. To this we can add the diversity within the environment.

Moreover, in a specialised centre, researchers from a variety of specialist fields would have the opportunity to work together over a relatively long time span, which would facilitate the creation of a true interdisciplinary field. Likewise, it is preferable for “modellers” to be able to work on site and in close collaboration with experimenters. They should have access to specialised resources (a modelling and bioinformatics platform).

[16] http://www.ecologie.gouv.fr/-PNETOX-.html

III. Principles and definitions of the scientific and technical limits

The difficulties mentioned justify the need for a specialised facility, but there is a high risk of a combinatorial explosion. It is therefore important not to set the long-term plans for the facility in stone, thereby enabling it to adapt to new problems that arise. For this reason, we suggest limiting the stressors, biological objects and the mode of operation in order to define a preliminary working plan of the set-up of the facility.

III.1. Extent of the scope and demarcation of its boundaries

We have chosen to focus on **chemical, biotechnological, and physical risk factors of an electromagnetic origin**. Radioisotopes do not fall within the scope, so there will be no need to plan for special premises and equipment ("hot zones"). For ionising radiation, protection is kept to a confined area and does not pose any particular problems. Conversely, biotechnological risk factors will require corresponding safety zones, in particular a **P3 laboratory.** The same is true for the management of liquids and experiment environments. Here, we should point out that we will be using air and aerial environments, (fresh)water and freshwater environments, soil and sediments. The **exclusion of marine environments** can be explained by our geographic location, and more specifically because specialised institutes such as IFREMER, which have solid experience and strong teams in this area, already exist. The exclusion naturally only applies to conducting experiments in marine environments; interesting problematics in the field will be taken into account. The same argument partly applies to the reason for not taking radioisotopes into consideration. However there will be some overlap with ionising radiation. Our priorities respect the following order: research, training and expertise. The long-term goal of the planned Research Centre is to become an international benchmark in its field and to be an exportable concept (similar to CNRS's Central Analysis Service SCA). It could be the initiator of a European network of similar centres.

For this type of long-term project, it is best to avoid giving into perceived urgencies or to current trends. The chosen philosophy will leave the specific choices up to the scientific community, who will ensure the necessary intertwining of practical importance of the problem, its relevance to science and its feasibility. In this way, only the major risk factors are selected, and the facility can be configured accordingly. Nevertheless, to underscore these ideas and provide examples, we can mention:

(i) Chemical factors: pesticides from agricultural activity, pharmaceutical products in the environment, urban and industrial contaminants, such as PAHs (polycyclic aromatic hydrocarbon), heavy metals, xenobiotics.
(ii) Products of biotechnological origin, in particular antibiotics, GMOs, and products from living organisms such as toxins and pheromones.
(iii) Biotic factors, especially pathogenic microorganisms and opportunistic pathogens, which are stimulated in anthropogenically-impacted environments and are potentially dangerous.
(iv) Physicochemical factors, primarily certain nanoparticles.

(v) Physical factors: non-ionising radiation, weak electromagnetic fields, ionising radiation.

.The biological objects concerned are:

(i) Cell systems for detecting cytotoxicities and the study of molecular mechanisms of cross toxicities.
(ii) Animal and plant organisms and microorganisms chosen from the traditional biological, toxicological and ecotoxicological models.
(iii) Among these organisms, animal models for humans in environmental toxicology
(iv) Populations of these organisms
(v) Plant and animal communities and mixed communities that are limited, carefully established and controlled.

The world of microorganisms provides excellent models for genetic, physiological, population and ecological studies. For instance, using a metagenomic approach, we can study the diversity of and changes within microbial communities. We can also hope to learn a lot from a metabolomic approach, and more generally all the "-omic" approaches.

The objectives are:

(i) Understand the mechanisms of action and stressors on living organisms, both isolated and mixed, especially over the long term, and in particular over several generations, which thus implies envisaging genetic consequences, such as genotoxicity, genetic drift, and more generally, evaluative genetic drift, and its effects on the maintenance, erosion or diversification of biodiversity;
(ii) Develop an experimental specificity in mixed chemical and biological, physical and chemical, and biological and physical stressors. For example, by obtaining results on the biological–physical interaction, such as the effect of low doses of ionising radiation on organisms infected by the virus;
(iii) Focus on low doses which can have cumulative effects, but which can also give rise to reactions and adaptations (defence systems to stress, especially chemical stress), which in certain cases can have positive impacts (hormesis effect, see ref. footnote 15);
(iv) Promote comprehensive study methods of living systems, in particular metagenomics and metabolomics;
(v) Develop diagnosis methods and techniques, define bioindicators which can be used in practice;
(vi) Anticipate the effects on organisms, in particular pathological organisms, and the changes in populations (with a special focus on demographic effects) and communities (for instance, effects on their genetic and biological diversity);
(vii) Take into account the remediation process, and more specifically, bioremediation; and to assess the consequences of implementing these processes. Some companies are specialised in these technologies, especially the physical and chemical processes, and require a long-term evaluation of these consequences.

III.2. An adaptable and evolving system

Initially, an Ecotron-style facility[17] was envisaged, as we intended to draw upon the experience of existing facilities at both national and international level. A similar facility would solve many scale-based issues, but the initial framework was considered too restrictive and too binding (one type of experiment taking up the system for a long period of time). What's more, the validity of concept itself has not yet proved convincing in terms of its results or scientific production.

Also, a central facility such as the **technological hall** will be used. Housing "pilots", each designed and set up in order to perform a specific experiment, as inspired by process engineering. The facility will be built upon "high-tech" systems (acquiring local and remote data management, remotely controlled actuators, automation, and regulation). At least in the initial phase (and unless under very special circumstances) rather than developing instruments, priority will be given to setting up a robust process management system using the most recent industrial products. There will be a specialised laboratory for the design and management of this system, and for the development of specific instruments which do not have a market equivalent.

Among the tasks to be carried out, priority shall be given to automation and robotisation of experiments (multi-parameter sensor system, software sensors, remote control systems and monitoring systems, very high speed systems for acquiring and transferring data). These developments will require technological research which can already begin, in particular for the design and control of the overall system. Finally, a production facility for biological units (cells, cell systems, organisms) upon which experiments will be performed has also been included. Its specifications will be adapted to the needs. In the same vein we need to anticipate laboratories for ongoing analyses and analyses that cannot be relocated elsewhere.

As an example, we shall look at the requirements of an aquatic system. It would be necessary to set up one or several tanks, regulate the water quality, flow rate, control the surrounding air environment, regulate the physico–chemical factors (temperature, dissolved gases, pH etc.), the variations in lighting, the exact mix and the rate of flow of the stressors. Similar facilities will be set up for other environments of varying complexities.

Finally, another priority task is the design and implementation of **a large database and knowledge base** on ecotoxicology. To this end, we can draw upon the remarkable

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[17] A focus on Robert Escarpit: upon his return from Paris where he had raised several tens of thousands of francs for a project which was dear to him (the development of a new scientific field—information and communication sciences) explained that if he had called his project "Littératron" (in reference at the time to the sychrotron), he would have had have all the acclaim he could have hoped for. In the end he chose not to use this word, not for scientific reasons, but under the pressure of his friend Henri Flammarion, wrote a book which was hugely successful. We indeed saw the "–tron" trend (phytotron, ecotron and no doubt others). We shall not be following this trend, at least for the time being. You can read this anecdote, an extract from an interview with Robert Escarpit, at:
http://www.uni-bielefeld.de/lili/personen/rwolff/interview%20Escarpit.htm
(document in French) (Notes AP)

capacity of the Region in the bioinformatics field, in particular PRABI (Rhône-Alpes Bioinformatics Center). It is also worth noting that INSA Lyon was the first engineering school to offer a degree in this field (Bioinformatics and Modelling course in the Biosciences department). This IT resource would rapidly give CETER international visibility.

III.3. A shared methodology

In practice, the experiments carried out at Rovaltain are **initiated in a "traditional" laboratory setting**, based on the simplest of frameworks (for example a contaminant, an organism, a micropopulation over several generations, in a simple, monophasic environment) in order to prepare the model for a larger experiment. The proposed **large scale facility** must serve as **an intermediary between the laboratory and the real environment** (for example, a population, a mix of risk factors, a community limited to two or three populations, several generations, an experimental volume of 10 to 100 times larger than that which could be managed in a laboratory, a bi- or tri-phasic environment). Such scaling enables us to get closer to real-life situations while maintaining controlled conditions. By extension, the mesoscale experiment must result in a "model for the real environment" which can be tested in practice. So the major equipment envisaged does not substitute laboratories, but complements their experiments to better align with real-life situations and produce **realistic models**.

The development of quantitative approaches using statistical analysis and dynamic modelling of biological systems has enabled ecotoxicology and environmental toxicology to produce mechanistic models which can be experimentally tested. Through this, it has been possible to devise **tools predicting the effects** on different levels of biological organisation and to progress to an integrated vision of the impact of contaminants on organisms, populations, ecosystems and the environment[18]. In order to attain this objective which is directly linked to experimentation, **modelling has to be a core activity of the facility.** Moreover, **bioinformatics** provides tools and methods for implementing these models, in particular digital simulation, or even development of new models (for instance, integrated models) as well as the organisation and processing of information obtained or available and accessible (database and associated algorithms, knowledge bases) and lastly, assistance in designing experiments and experiment automation (see the Adam robot example used in molecular biology[19]). A collective effort must be made, similar to developments made in genomic information, to create such tools. Finally, specific methods should be developed to **avoid a combinatorial explosion**, which would occur if traditional approaches were taken, even if rigorous

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[18] We could draw inspiration from the work carried out at the NCCT (National Center for Computational Toxicology) for toxicology by envisaging similar approaches to environmental toxicology and ecotoxicology:
http://www.epa.gov/comptox/index.html
Virtual Liver Project : http://www.epa.gov/ncct/virtual_liver/
Virtual Embryo Project : http://www.epa.gov/ncct/v-Embryo/

[19] King et al., A robot scientist discovers orphan enzymes that take part in yeast metabolism. *Science*, 2009, 324: 85-89. Also quoted in *La Recherche* (questions to François Rechenmann, 431, June 2009, p27)

experimental designs were applied. This is precisely what largely justifies the dynamic model approach. Combined with experiments, it gives access to relevant information: that of the mechanisms and processes involved, and direct access to the biological variability and identification of what causes it.

III.4. Course of action and operating procedure

The facility will therefore be designed as a "shell" suited to hosting experiments, that is, experimental systems designed, similar to the pilots in process engineering. (i) containers (chambers, sensors and actuators), (ii) contents (environments and biological or ecological systems) and (iii) specific instruments (for example to take measurements and process them on site). The shell will provide resource management and basic services: liquids, energy, effluent treatment and information systems. Surrounding the experimental hall housing all the experimental systems, a star-shaped configuration of laboratories will accommodate the more general "services", such as producing living systems (animals, plants and microorganisms), the correct doses of simple or mixed stressors, in-house analysis and monitoring systems. Lastly, a there will be a biometrics unit where bioinformaticians, biostatisticians and modellers will design the shared models, set up the models to be used according to needs, design and manage the database, and analyse the statistical results of the experiments.

The experiments, which are foreseen to last at least one year, will be subject to calls for tenders. The responses are expected to cover two dimensions:

- a scientific dimension: the experiment itself (objectives, hypotheses to test, the *a priori* operating model, biological systems for experimenting upon—biological material and stressors, the environment(s), the design of the experiment, methods for collecting, organising and analysing data, using this data to improve the model etc.)
- a technological dimension: the containers, measurement and control systems (chambers, instruments and connections, remote controlled actuators and manipulators) designed in view of the limitations, especially size limitations, of the hall.

Remember that the experiments and models are designed on a laboratory scale and are implemented at Rovaltain. They therefore consist in projects designed to meet **thematic calls for tenders**, which leave a great deal of initiative to the teams. The terms of the calls for tenders and their responses are drawn up and evaluated by the International Scientific Council (CSI) together with the operations team managing the centre. The final selection is made by a steering committee based on this prior evaluation. Certain field projects could be accommodated outside the experimental hall (for example, “on the field” or in “greenhouses”), in a dedicated space surrounding CETER.

As the experiments are completed, new calls for tenders will be launched.

The aforementioned science and technology facility, CETER, will function as a *“hôtel à projet”* (see footnote 1) within the science and technology centre, which will have shared "hotel" facilities: accommodation, libraries, meeting rooms, network access, relaxation areas etc.).

IV- Science and technology project

IV.1. Main objectives

Let us now review and expand upon the objectives outlined above:

- impact studies: This descriptive approach still cannot be completely dismissed. This is especially true for low doses which we don't believe to be systematically toxic, and which can even stimulate the functioning of biological systems. Likewise, the impact of **mixed stresses**, as we have previously mentioned, should be developed, for example physical–biological or chemical–biological interactions, such as the impact of physical or chemical factors on the expression of pathogenicities (increased or decreased impact). Of course, this doesn't mean that stressors will be randomly combined, but based on initial theories, stressor combinations with likely outcomes will be mixed.
- understanding the underlying processes: this core component must be integrated into a general approach based on robust biological, ecological and evolutionary fundamentals, and the effects of physical, chemical and biological disturbances on living systems, as well as the solutions to these disturbances. It mainly involves understanding the whys and wherefores of the effects of low doses.
- production of biological indicators: as a result of the stressors, we can observe early changes in the structure and functioning of certain biological systems which can therefore serve as indicators. Finding such systems requires performing sensitivity analyses and identifying observation criteria of these systems and their changes (some may not be easy to observe).
- bioremediation: remediation must be one of the key concerns covered in ecotoxicological studies. However, there is a cause for limiting the scope to bioremediation, i.e. involving living systems. Identification of living systems and their actions respects the same thinking as ecotoxicological research, which is interested in studying living systems that resist stressors and that react when stressors are reduced or removed. However, as we have previously mentioned, there is a cause for seriously considering whether to become involved in a field which is already heavily invested in. A problem which has until now largely been neglected is the consequences of remediation. CETER could focus on this problem.
- Anticipation and prevention: another of the direct objectives of toxicological and ecotoxicological research involves anticipating and developing processes that will reduce or eliminate harmful effects before they occur.
- Setting up, organising and maintaining a large data base and knowledge base for ecotoxicology, and making documented data and models available for use on line.

Finally, and once more, an impact study of underlying processes on exposure to stressors is a means of better understanding fundamental biological and ecological mechanisms: by disturbing a system we learn more about it than by simply observing it in a steady state. Among the mechanisms directly involved, those which enable resistance to stress are particularly interesting.

IV.2. Two categories of objects for study

In response to the objectives of environmental toxicology, which focus primarily on humans and human health concerns, and ecotoxicology's objectives, which take an interest in other life forms at an organism level, and also other organised structures such as populations and communities, there is a cause for anticipating the use of, or even the production of adapted biological material. Moreover, cell culturing facilities should be envisaged for studying the mechanisms at a cellular and molecular level.

i. Animal models for humans

To study the potential effects on humans, laboratory mammals, particularly mice, rats and certain primates, are known. Given the type of studies planned and the restrictions on animal testing, we will limit ourselves to rodents. Primates are generally reserved for pharmacological studies, which do not form part of Rovaltain's objectives.

We can narrow down our focus by choosing not to locally breed laboratory animals. Therefore, the necessary breeding farm will be set up for temporarily looking after the animals.

Moreover, preference should be given to cellular models (human cells in culture) and in the long term, *in silico* models. There are two advantages of using cells in culture: tests would be carried out on real target organisms, as opposed to analogous target organisms, and it would also reduce testing on animals.

ii. Animal, plant and microbial populations

Although in the current state-of-the-art, the choices for environmental toxicology are relatively easy, ecotoxicology presents a more delicate issue. Reference animals do exist (for example, water fleas for aquatic ecotoxicology, whose genome is currently being sequenced), but there isn't an actual "biological model" as there is for all other life sciences (fruit flies, mice, zebrafish [*Danio rerio*] *E. Coli*, yeast, *Arabidopsis*, *Caenorhabditis elegans* etc., whose genomes are known), at both organism and population level. For communities, soil bacteria are also a good example. The question may arise as to why ecotoxicology does not rely more on these highly documented biological models, some for which the entire genome is known, and for which we are capable of producing transgenic varieties. "Ecological models", in particular plant and especially microbial[20] communities, are also starting to emerge.

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[20] The "microbial ecological model" is very useful, as it is in biology, both for studying the fundamental mechanisms at work and for advancing modelling in ecological systems.

The major difficulty is the great diversity of the living world, but conversely, we know that there are also major constants which unify it. Living environments are another difficulty. These include aerial, aquatic (freshwater and salt water), and terrestrial environments; environments ranging from the most standard and frequently encountered to the most extreme. These extreme environments are too specific to be used as a primary target. On the other hand, extremophile organisms must not be overlooked because they have mechanisms of resistance to stresses that are interesting to study.

iii. Initial choices

Without presupposing the responses to the calls for tenders, it could be considered necessary to envisage equipment for providing adapted biological material and enabling experiments on:

- Vertebrates:
    i. mammals (initially mice), especially for experimental toxicology;
    ii. fish for aquatic ecotoxicology (three-spined sticklebacks *Gasterosteus aculeatus L.*, zebrafish *Danio rerio*);
- Invertebrate models for ecotoxicology: water fleas, bloodworms, aquatic molluscs, nematodes and terrestrial arthropods etc. for population dynamics and choice model for genetics, in particular for inter-generations (organism genetics, population genetics, micro-evolutionary aspects).
- Microorganisms for studying biochemical, genetic and ecological mechanisms (studies on microbial communities) for bioremediation (ecotechnology). Along the same lines, we could also envisage building up and maintaining a collection of bacteria. The presence of actual or potential pathogens among the microorganisms used or stored requires specific P3 level equipment.
- Plants of agronomic interest could be considered, or conversely, we could choose the "model for plant biology": the a*rabidopsis*. Part of the experimental work could be carried out in greenhouses, some of which should be certified “GMO”.
- Finally, human, animal and plant cells in culture for sensitivity studies, for investigating cellular and molecular mechanisms, and for substituting to the greatest extent possible, the use of animals.

IV.3. Scientific approach

The general principles proposed and previously discussed are based on two principles: a strong modelling–experimentation coupling, developing the model and the experimental system on a laboratory scale, carrying out the practical work on an intermediary scale at Rovaltain, and finally, confronting real situations.

The proposed approach is a "hypothetico-deductive" method, led by the model. Based on *a priori* hypotheses on the possible effects of stressors, we simultaneously build a model and an experimental design to simulate the predicted effects, noting measurements and observations during the experimental process. The process is iterative: the model is gradually improved as more experimental data is acquired, and the experimental process is modified to acquire new data guided by the model. Of course, this design is idealised. In practice he possibilities of modifying the model are limited, if only by our imagination and by the difficulties in implementing the changes. The experimental system is also limited in its development. By and large, it will involve creating disturbances through carefully controlled actions, or taking further measurements and noting extra observations. The *a priori* introduction of biological and ecological knowledge will mean that the contributions of these disciplines to ecotoxicology will have to be reinforced (see above).

The second principle, which has also been previously mentioned, involves the joint development of the model and the experimental system on a laboratory scale, i.e. a microscale (short timeframe, limited biological complexity: an organism, a population, one or two stressors) and transferring it to a mesoscale (long timeframe, medium complexity: one, two or three simultaneous populations, mixes of two or three stressors at realistic doses). We will focus on closely monitoring the dynamics of the system (frequent temporal sampling, possibility for actions during the experiment, sensitivity and optimisation studies of temporal sampling, identification etc.) rather than on multiplying replicates, which reflects a more normative approach—that of statistical experimental designs. Although this approach is very useful for highlighting "significant" effects, it doesn't provide any information on the underlying mechanisms.

IV.4. Modelling

Modelling has become an almost indispensable component of the scientific method, including for ecotoxicology. The main merit of modelling, apart from its operational element, is that it forces us to conceptualise.

Most models require computer simulation which, for simple models, can be done on a PC, or can require substantial processing power. It is vital that, other than the model–experiment coupling described above, the centre can house a small number of ambitious, long-term projects such as those mentioned in the introduction. The projects would have a larger scale of complexity and, why not, a comparatively detailed digital ecosystem for testing virtual communities, whose behaviour has been rigorously validated on their reactions to stressors. In the long run, we envisage replacing testing on animals (for ethical reasons) with testing on digital animals. We could therefore conceive a large digital ecosystem[21] project, obviously for ethical reasons, but primarily

[21] In the 1970s, the *Grassland Biome* project had this objective. This programme was the United States' contribution to the International Biological Program launched by IUBS (International Union of Biological Sciences). It wasn't successful because we had neither enough knoweldge, nor enough processing power. However, very positive results were generated, in particular measuring our lack of understanding and attempting to remediate it. Envisaging such a project today seems to be within our capabilities, as long as

for practical reason that it is difficult, impossible even, to perform full-scale experiments at this scale. Moreover, having such an objective will once again require us to form the concept, identify the knowledge to be acquired, and to solve methodological problems.

To undertake such a venture, appropriate computer equipment is necessary. Moreover, a specialised unit with the corresponding resources will be necessary for setting up a reliable and secure data and knowledge management system.

IV.5. Experimental challenges

The first experimental systems developed for ecotoxicological studies were based around aquatic organisms and focused on acute effects, notably mortality. Data from these systems is still widely used for regulatory purposes, and is prevalent in databases. Experimental systems then diversified, branching out in several directions, tackling other areas such as sediments and the ground, different effects, and longer periods of exposure (sub-lethal effects, chronic exposure). One of the systems involved associating several species, and sometimes several routes of exposure, within experimental systems: depending on the size and complexity, it involved micro- or mesocosms. These are also used for regulatory purposes, in particular for approving pesticides. In addition, society's approach to dealing with pollution flows and their effects has changed drastically. Although polluting substances that were targeted as priorities in the 1970s are still largely relevant today, other substances have now come to the forefront of concern. These changes are the result of advances in analytical resources, enabling us to detect a larger variety of substances and marginally reduce quantification limits. The most commonly observed situation in environment compartments is the mixing of varying, often moderate, concentrations of substances. Current challenges therefore involve compiling knowledge on the effects of these "emerging" pollutants and the manner in which they act, the effects of low dose exposure over long periods of time, and the effects of a mix of substances. The latter two challenges raise technical and methodological problems which are beyond the capacity of laboratories, and therefore their teams, in their normal configuration. This is what justifies setting up a specialised facility to study these types of problems.

IV.6. Special challenges

Among the more important challenges which have been identified, we wish to highlight the importance of five scientific and methodological challenges, which shall be considered as special challenges.

i. Change of scale (or level of organisation)

Although this term is of geometrical, or even cartographical origin, it is most often used, at least in life sciences, to indicate a change in the level of organisation. Living systems

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it is made clear that it must be a long-term project. The successful example of climate models, developed over a 30-year period, is a suitable reference, proving that we can have these kinds of ambitions..
Ref. US participation to the International Biological Program. Report n° 6. National Academy of Sciences, 1974, 166p.

are organised into a hierarchy of levels in which the entities of a given level, if they interact, form a group. The group's characteristics cannot be explained as the sum of the characteristics of its individual entities, nor as an average value of these characteristics. The values cannot be simplified into average values because the behaviour of the entities and their interactions is non-linear. The system is complex, in today's commonly accepted sense of the term. Naturally there is a connection with the notion of scale: a group is larger than each of the entities from which it is made up, but is not reducible. The most well-known example, because it is the most accessible example on our “scale” is that of a biological population made up of organisms. It should also be noted that there are no horizontal relationships between the entities, but that there are interactions between levels. For example, individual behaviour can influence group behaviour (this applies to socially stratified populations). Conversely, collective properties can act at an individual level (this is what is meant by the generic term "social pressure").

One of the questions raised is to try and understand the relationships between different levels of organisation: how can we deduce collective behaviour from our knowledge of behaviour of individuals and their interaction with others, and vice versa? For example, how can information on individual reactions to exposure to stressors further our understanding of the demography of a population? The situation becomes even more complicated if we consider the communities composed of a variety of different organisms, therefore different populations.

Finally, in a community, the dynamics of the populations in question depend on ecological relationships between the individuals of these populations: what effect do stressors have upon these relationships? What are the demographic consequences? What are the effects on the biodiversity (see below)?

Today we know that the route to solving these problems—which is crucial in ecotoxicology and already well-advanced in biology and ecology—is through a specific method largely reliant on modelling, hence the need to develop this area in the project.

ii. Modelling and model-based experiments

As we have previously mentioned, this involves closely combining modelling and experimentation, which in itself is hardly an original idea, even in the fields of research envisaged. But the process involves going one step further and designing an experimental system based on an *a priori* model built using available knowledge and the results of preliminary experiments carried out in the laboratory. Once more we are entering a new area for our particular field of research, although it is common practice, if not the general rule in many areas of physics. It should be noted that, as for all research activities, a certain adjustment period is necessary. We shouldn't expect that all the experiments set up for each project will immediately provide answers to this demand. It is, however, a focus which will become a necessity in the long term.

iii. Biodiversity The effect of the processes of erosion on diversification

Biodiversity, to take a specific example, is one of the measurable characteristics of a living community in an ecosystem, and, to a certain extent, it also characterises this ecosystem. This biodiversity changes over time, both in a qualitative and quantitative manner, from simple fluctuations of an average value (a steady state) to major spontaneous fluctuations caused by internal dynamics, or as a result of "forcing".

We could believe that studying biological diversity, or part of this diversity and its dynamics, would be a good indicator of the impacts suffered by forcing, in particular due to anthropological stressors. This is a core topic in ecotoxicology. Variations in biodiversity are a result of demographic impacts on the populations in question. The more immediately perceptible impact is that of a reduction in diversity, largely due to an increase in mortality in certain populations. The catastrophic dynamic process leads to the disappearance of all individuals in all populations for all the species present in a given area.

However, the dynamics of biodiversity do not boil down to catastrophes and irreversible erosion, otherwise life would have long disappeared off the face of the earth. Factors influencing biodiversity do not all give rise to negative or catastrophic dynamic processes. Spontaneous resistance and diversification processes also come into play. So when the factor(s) which have led to a local "catastrophe" disappear, and the area is left to the biological system's own dynamics, more often than not, the area is colonised by a rich diversity of living organisms. This is what happens to agricultural land which is left fallow. We are presented with an ecological diversification resulting from a process of colonisation. The species in question exist elsewhere, and colonisation is the result of their migration. We should also note that, more often than not, individuals from different species also mix haphazardly, to the point where the biological spatial structure is to a large extent random. This mixing which results in a diversified ecosystem ensures the survival of species present in large ecosystems.

Other processes of diversification exist at a biological level, and they are the processes that produce variants within populations of a species. They are of a genetic nature, relating to genetic diversity. This results in an increase in biodiversity. However, these various organisms are more or less well-adapted to the environments they inhabit, and to the variations within these environments. We therefore see a selection process taking place, resulting in a downward diversity trend. Gradually, new species are formed. This is known as speciation. We have just summarised the evolutionary process that has made today's biodiversity completely different to what it was thousands of years ago; a biodiversity that is probably very different to what it will be in a few thousand years' time.

From a global perspective, the history of the Earth, and from a localised perspective, current observations on the dynamics of land left fallow, both demonstrate that the processes of diversification are clearly very active. And they are very active because the biological and ecological mechanisms create "chance", and stochastic processes, not only which maintain it, but which secure it a long-term growth trend. By manipulating these mechanisms, we can alter the processes of diversification.

One of the priorities is the absolute necessity of studying the effects of "realistic" doses and combinations of stressors on these processes. It is vital both for the practical reason of developing indicators and changes in biodiversity, and for the fundamental reason of developing a clearer understanding of the ecological and biological processes of erosion and, above all, diversification. It is at least as important as the study of the impact of climate change. It is one of the current major focuses of biological, ecological and evolutionary sciences; a focus that will enable ecotoxicology to enter into a "sustainable" development phase, join the "big players", and lay the necessary biological, ecological and evolutionary foundations to ensure its development.

iv. Chemosensitivity *vs.* chemoresistance

“We weren’t born yesterday”: over the course of evolution, living organisms have been subject to various, significant physical and biological stresses. These stresses were selection factors. Those sensitive to the stresses were by and large eliminated. The remaining organisms produced mechanisms of resistance which enabled them to survive, or even develop. One of the most conspicuous examples on a paleontological scale is the implementation of mechanisms of resistance to oxygen and the use of the oxidative pathway (aerobic) to produce energy[22], and on a human scale, the emergence of resistance, followed by multiresistance to antibiotics.

Today we can see the results of this evolution, but to what extent have these mechanisms prevailed, and to what extent will they prevail? We still need to assess the effectiveness of the resistance acquired to these stressors, primarily chemoresistance, and conversely to assess the sensitivities, and then to identify the processes in action. For chemoresistance, it is highly likely that these processes are generic rather than specific (such as immunoresistance) and therefore they are expressed not by a precise molecule, but for a family of molecules, whether natural or not. This being said, living organisms will always be sensitive to molecules of natural origin since it is how they survive in relation to each other. This raises another question: how do gradual adjustments to resistance and defence occur?

We understand that without providing a biological, ecological and evolutionary backdrop, we will be condemned to chasing after the latest urgency and never producing what we most need: a theoretical and methodological framework which will

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[22] See for example the work of J.C. Massabuau and his team on ostracods:
Corbari L., Carbonnel P., Massabuau J.C. Des crustacés qui ont du souffle. La Recherche, 2006, 386, 58-61.
Massabuau J.-C. Primitive, and protective, our cellular oxygenation status? Mechanisms of Ageing and Development, 124 (2003), 857-863.
And more recently, there is a summary and references in the work of N. Pasteur, for example:
Pasteur N. Résistance aux médicaments et aux pesticides (biocides). In « Santé-Environnement et Santé-Travail. Nouvelles perspectives de recherche », Proceedings of the workshop on the Future of Science. French Ministry of Research and ANR, 2005. 6p

enable us to better identify the real risks, and tackle them by means of prevention or remediation.

v. Nonlinearity at low doses

The question of effects of low doses remains unanswered. For a start, the dose–effect relationship at conventional doses is not linear. In general, a sigmoid-type relationship can be observed, especially in terms of the mortality rate in a population, if only for the because for any dose higher than the "one hundred percent" lethal dose, a 100% mortality rate will always be observed. What may appear to be toxic at "regular" or high doses may not be toxic at all at low doses, even over the long term. It may even trigger a favourable reaction to certain biological parameters, including longevity (the hormesis phenomenon). Back in the 1960s, we observed that low doses of ionising radiation had a favourable impact on the demographics of the paramecium population; they showed better growth than those who were not exposed to this radiation. It seems to be the case in a number of situations, a lot of which still need to be analysed. At any rate, low dose mixes activate resistance, in particular to chemical agents. However, we have also observed the harmful effects of cumulative doses over the long term. This is an important field of research, with evident practical value. But even if there is no effect, or a slightly positive effect, this does not justify creating pollution, even in small quantities, as Steinberg and Ade emphasised (*op. cit.*).

Moreover, another area that needs to be developed is that of interactions between low doses of different types of stressors (for instance, the effect of low doses of ionising radiation on the process of cancerisation, and their interactions with biological factors, such as the expression of oncogenic viruses, and chemical factors).

vi. Expertise and confidentiality

The centre's goal is to mobilise experts in their fields of excellence to serve society and the industrial world. This expertise will be organised into several different levels:

- To provide a critical review of existing data from the database, to document or answer a precise question
- To draw up a summary of existing data by a group of specialists (board of expertise)
- To acquire new data using the research facility implemented

Responses will be guaranteed confidentiality according to the nature of the inquiries commissioned. Adequate safety measures are planned (computers, access to the premises etc.) for fresh data generated from new experiments in particular for industry.

vii. Quality and safety

The entire structure and its functioning must be designed to ensure an exceptionally high quality of data, producing exceptional research results and expertise. The degree of data confidentiality (including secrecy) requested or insisted upon by researchers, partners or clients, must be respected.

Moreover, the facility must ensure that its activities have no negative impact on the environment, nor on its staff, and it must ensure high security in the treatment of waste and effluents. Waste management could even form a subject of research. Experiments, products and living and non-living materials will be confined to appropriate chambers according to their nature and the danger they pose. Various certifications will be used to guarantee this quality. Controlled access to the individual zones will respect the most recent procedures. The entire centre will be enclosed and under constant surveillance.

These latter two points will be explored in further detail in the technical report.

V. First draft of the system architecture

At first, the centre was to be based upon the Ecotron, as we previously mentioned, and we envisaged using the neologism *ecotoxicotron*. It was an idea worth considering, but its disadvantage was that it was limited to one type of experiment which mobilised the system over the long term. Neither was it progressive. For this reason, the "technological hall" solution known to process engineering was chosen, and the specialists nominated to design this project seem to be in mutual agreement. Once this idea was chosen, it was obvious that not all tasks could be performed in this hall, but that surrounding systems were needed for ancillary services (analyses, preparation of the living and non-living material for experiments, sample conditioning, reprocessing and recycling, computing, modelling etc., as many tasks as are necessary, most of which are described in the appendix). A technical room is planned (but not yet represented) for finalising the pilots which will then be built, or simply installed, in the experimental field (similar to "satellite preparation" facilities in space centres before installation in the upper part of the launcher).

All these tasks are carried out in laboratories surrounding the central facility, resembling a daisy shape from a bird's eye perspective.

Funnily enough, the functional structure obtained is opposite to that of synchrotrons. At a synchotron, the resource (that is the light that is produced by the synchrotron) is accelerated up to high energies in a central position, and the experiments are performed in laboratories surrounding the synchrotron. In our facility, the resources are produced in the surrounding laboratories and the experiments are carried out in the central hall. We were careful not to use neologisms, but the comment made by Robert Escarpit deserves to be mentioned, especially if the concept is successful and exportable (see footnote 17).

On this basis, we have a first outline of the overall architecture of the facility.

Together, the central hall (figure 2) and the surrounding laboratories and services (figure 3) make up the CETER. Outside areas can be used for other field experiments or for setting up greenhouses. Once the "welcome services" and the premises for scientific activities are included, the centre's architecture is defined (figure 4).

Two key points need to be foreseen from the very beginning of the project:

1. The key motivation to create, upon which the architecture and the practical implementation must be developed, comes from the biological object being studied, and not the person studying it. This is not to say that ergonomic considerations for the experimenter should be neglected. We simply want to reiterate here that the conditions to which the experimental units (animals or plants) are subjected will have a considerable impact on the quality and significance of the results[23]. This is a core focus in ecotoxicology because the physiological state of the animals can have a profound influence on the effect of contaminants. An animal that is already stressed by poor experimental conditions will often react in a different manner than a "happy" animal.
2. The conditions under which an animal is tested must be such that the animal is not aware that it is being studied, i.e. the animal should not be "conscious" of the fact that it is part of an experiment. Such conditions may be the problems of vibrations and "noises" emitted by the materials, resulting from the general activity of the experimenters and their colleagues in the building, as well as the well-known weekend effect where the animal is more relaxed on Monday morning than on Friday evening because it hasn't stimulated by laboratory activity for two days. Isolation between experimental chambers (use of silent blocks and bridge breakers) must therefore be an immediate consideration in the design of the building.

On-site training will be given, naturally at PhD level but also at Masters level, especially for the 2nd year of Masters in Research programme (MR2). Further details are provided in the appendix. However, we can already point out that this training will require dedicated training rooms and equipment: computer rooms, lab work and classrooms, video projector systems. Finally, full-scale experiments can be envisaged by using pilots towards the end of or at the end of research experiments.

[23] See for example: Calisi RM and Bentley GE (2009) Lab and field experiments: are they the same animal? *Hormones and Behavior*, 56: 1-10.

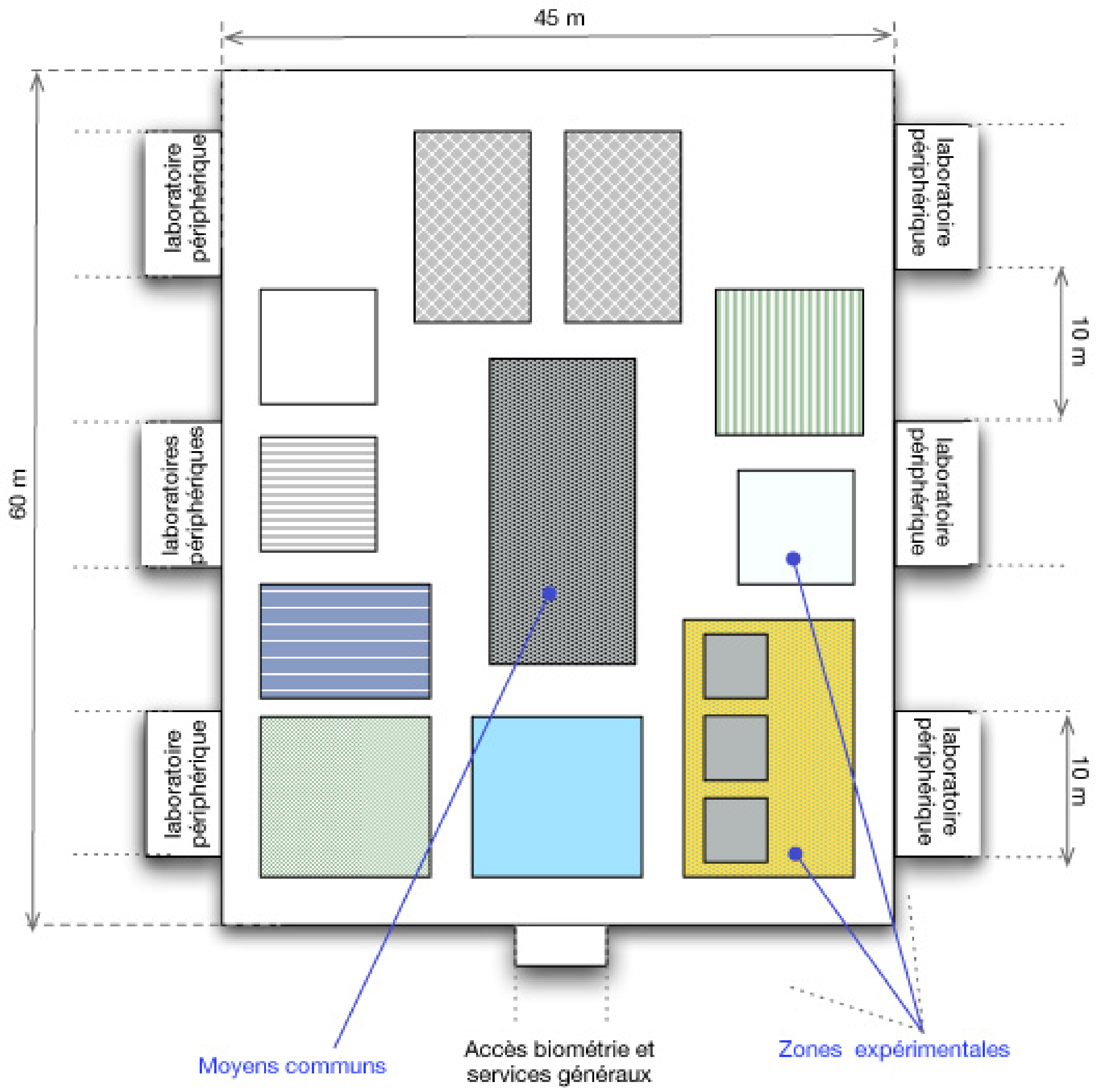


Figure 2. Experimental hall (2700 m^2) This hall can accommodate several experimental systems simultaneously. It is viable to have at least 8 systems operating simultaneously. An experimental system is the procedural basis for an experiment including the surrounding facilities, (for example measuring devices, specialised data acquisition and remote manipulation). Shared resources are located in the centre of the hall. The necessary resources such as living material, stressors, and analyses are produced and are made available in the peripheral area. A waste management system needs to be envisaged. This can itself form a subject of research.

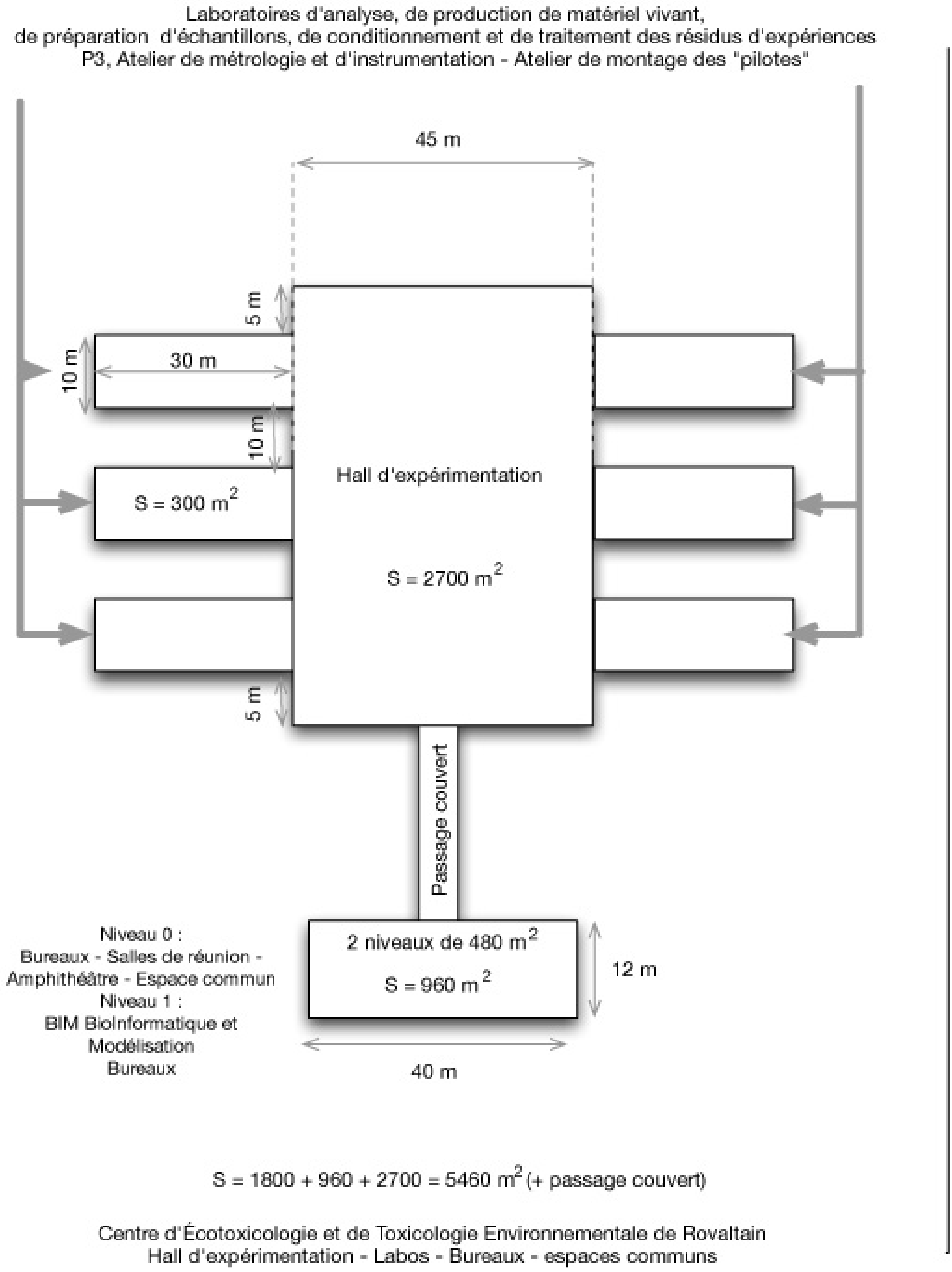
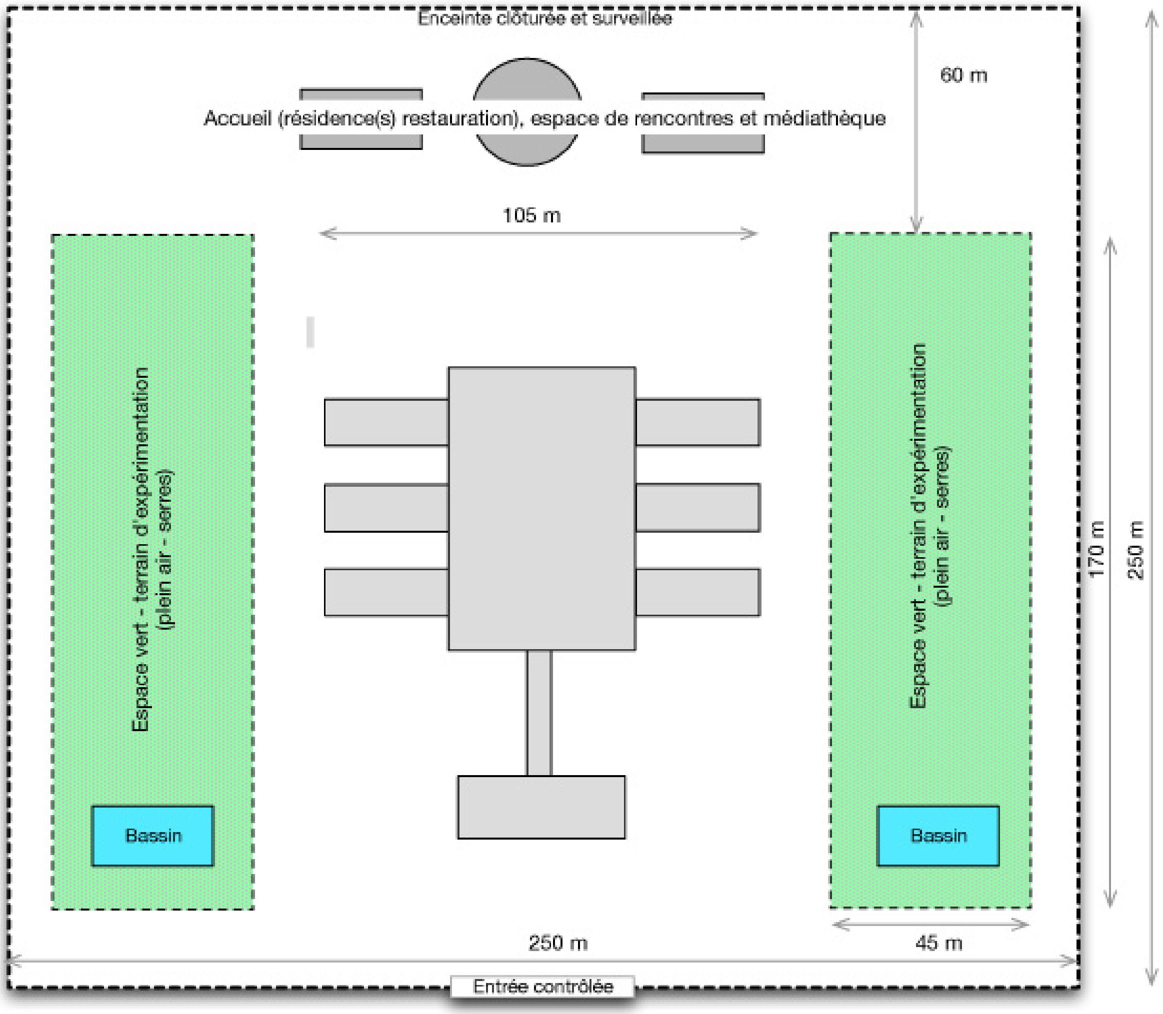


Figure 3 The CETER itself is made up of a central hall, where the experiments are performed (see figure 2) and the surrounding preparation, reprocessing, analysis and modelling laboratories.



Centre d'Écotoxicologie et de Toxicologie Environnementale de Rovaltain
Plan d'ensemble (hors voies de circulation)

Figure 4. General layout of the science and technology centre (possible names: Ecopro, Ecosafe …) with the CETER experimental facility in the centre (see figure 3). The adjoining land can be used for "open air" experiments, or for greenhouses. A scientific reception centre is planned from the beginning. Its dimensions (250x250 = 6.25 ha) are approximate, but give a good idea of its scale.

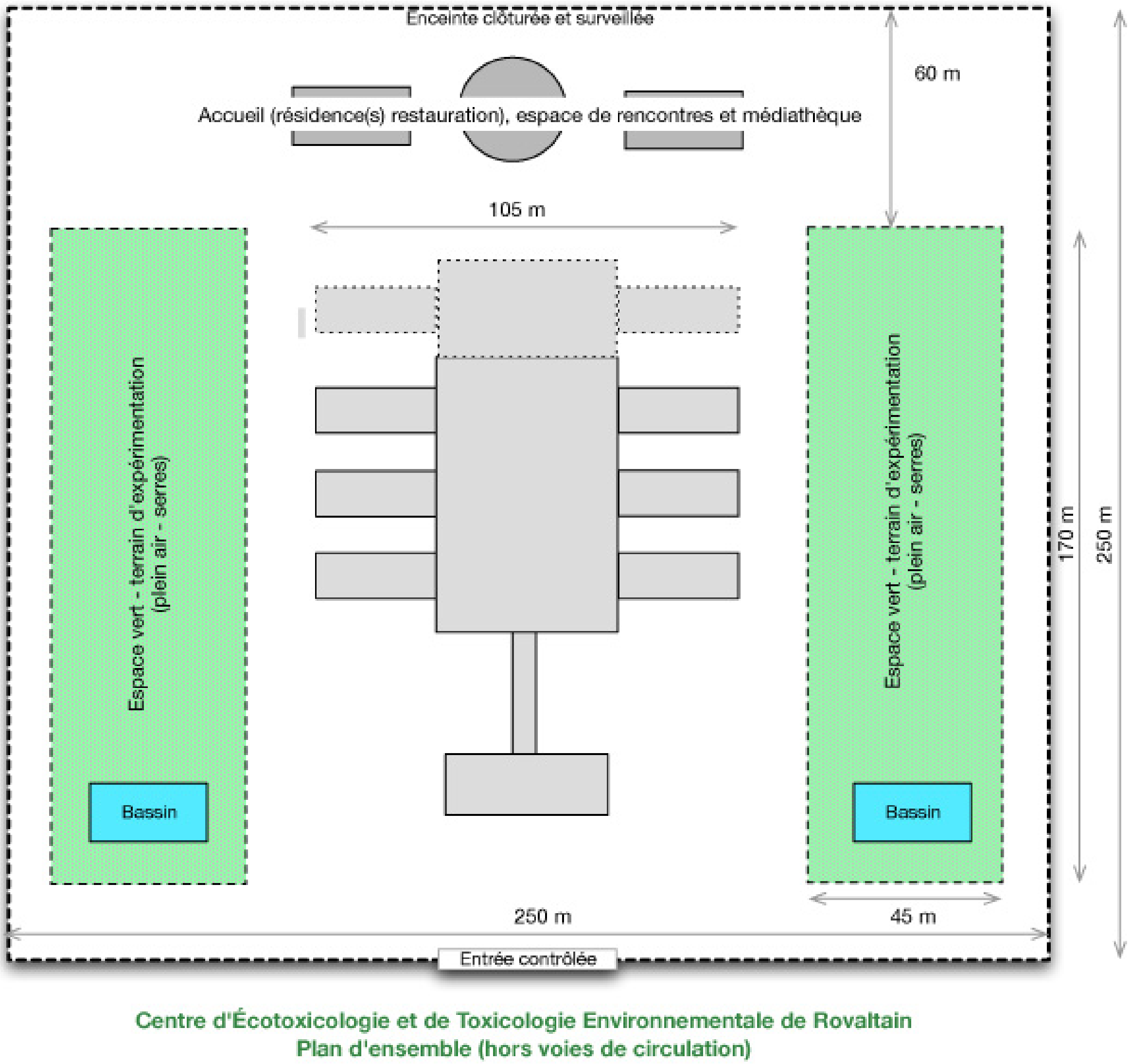


Figure 5. The facility can be extended. Sufficient available land should be factored in to the plans.

VI. Conclusion

This modern facility has thus been designed to house medium-scale experiments, functioning as an intermediary between field experiments and laboratory experiments. Experiments will run over the long term (several weeks to several years) and will be designed to study the effects of low-dose combinations on organisms, populations and communities. Preliminary technical details can be found in the appendix. However, in order to further develop the overall architecture and the more detailed specifications for the project to function successfully, it is necessary to call in project architecture and technical programming specialists. Their skills are necessary during the drawing up of the technical report, and at least during part of its implementation. Rather than recruiting for such specialised, short-term missions, we propose to use external expertise, at least for the project architecture. However, it is preferable to team up long-term administrative and management expertise as soon as possible in order to draw up the legal framework, negotiate with partners, write the framework agreements, study and implement administrative and financial management procedures etc. **This suggestion, which is an essential complement to the scientific component, has been inspired by the accumulated experience of previous projects of this type.**

Access to the centre's resources will be granted by calls for tenders (initially 2 over 4 years). The general objectives and the chosen thematics will be assigned at the centre: long-term effects on mixes of contaminants in realistic exposure conditions; the use of models prior to and following experiments. We will not require the knowledge produced to be immediately applied, or applied in the near term, for regulatory or other reasons. The first wave of responses at least should also include the pilots which will take up part of the hall (experimental chambers, connection devices and the necessary distribution). The scientific project text should be used as a basis for the thematic framework of the calls for tenders.

Key criteria for selecting projects are the originality of the subject, scientific excellence and the quality of the project. The selection will be made in two stages: (1) The centre's Scientific Committee will evaluate and prioritise the projects, then (2) a Steering Committee will make the final decision and attribute the necessary resources. It will also ensure an even balance between the various fields of study and the allocation of resources. Projects will benefit from resources from two sources:

- The teams' own resources, which can include external financing
- Resources allocated by the centre, including scientific and technical staff, and financial resources.

Project preparation must therefore be done in collaboration with the centre's team.

Appendix: Specific additional information

(to be completed)

A.1. Modelling

Ecotoxicology is increasingly focusing its efforts on measuring the effects of xenobiotics and other contaminants, not only on plant and animal organisms, but on whole populations and ecosystems, as well as on the dynamic equilibriums that characterise them. By using statistical approaches and/or developing mathematical models to provide a quantitative analysis of these relationships, ecotoxicology can now take on the challenge of producing tools that can predict effects on different levels of biological organisation, thus bringing us one step closer to an integral vision of the impact of xenobiotics on the environment.

However, even if the modelling approach has now made it is easier to extrapolate findings to different levels of biological organisation, it is important to focus on developing and integrating a series of *generic* modelling instruments for predictive ecotoxicology by better integrating the real conditions into the affected environment. Such conditions include: chronic/acute effects, exposure dynamics (continuous or intermittent), transfer of pollutants, synergies between the pollutants, interactions between the pollutants and the life history traits of organisms, population dynamics, variability, uncertainties etc. This would set the standard and consolidate the use of these tools as part of an evaluation of ecotoxicological risk. The expected genericity, with its inherent powers of prediction, should encourage us to develop models whose primary objective is to understand the functioning of the mechanisms and not an extensive description of the complex systems considered (mechanism before reality). Such models must respect the principle of parsimony, that is, they should remain simple enough to be analytically understood (inference, simulation), thus reducing the "black box" effect and ensuring the robustness and the general character of the knowledge produced. However, when reducing the description of the systems to produce relevant models, they must not conceal the elements essential to functioning, which will enable us to understand the underlying mechanisms involved in the contamination process, and the effects observed on organisms and populations. Therefore it is preferable to focus on mechanist models which integrate a crucial temporal dimension for anything that involves process dynamics. Moreover, because they complement each other, the cohabitation of both short-term and long-term effects criteria is necessary. They will not only contribute to answering problematics on restoring environments, but also improve the dynamic and integrated vision of effects over several generations.

We must therefore start with the designing of experiments and the collection of data (using statistical processing of the data and identifying the models) and work towards a validation protocol for the models, which will enable us to understand the geochemical and biological mechanisms involved, in order for them to be extrapolated to real-life situations (*in situ* or using hypothetical scenarios and multiple kinetics of contamination). This will mainly be done through *in silico* experiments, which is essential when real experiments are impossible or face too many constraints (e.g. long-term evolution). This exploratory approach may in return contribute to the design and planning of experiments.

Even if, in this context, modelling only appears to function as a simple generator of analyses and inferences, it should also solve real research questions, for instance taking into account factors such as variability and uncertainties when identifying models, predicting and extrapolating results, and defining risk factors at a population level. Indeed modelling should aim to define the variable characteristics in terms of demographic health of the populations, which are, unlike those traditionally routinely used on individual scale (e.g. EC_x, LOEC, NOEC), both continuous and independent of the experimental design used. Finally, developing stochastic models may also help to broaden the approaches that already exist in the field.

A.2. Experimental systems

Risk factors to be studied

Drawing up a list of factors (or priority substances) is a delicate exercise, and one which comes up against many hurdles, in particular that of typology. We should also avoid being influenced by trends, and should distinguish the risk factors representing relatively sustainable challenges. Even though we must state our priorities, limiting our ambitions to a definitive list of risk factors would appear to contradict the very purpose of research equipment. The following guidelines, which come from a seminar in Montélier, give a reasonable—and revisable—indication of areas that could be addressed using this equipment, especially in the first years:

- synthetic chemical substances produced by human activity;
- pesticides, which may or may not be from agricultural use;
- polyaromatic hydrocarbons (PAH), produced by transport activity, heating etc.;
- trace elements (metals and metalloids) in the environment from industrial or other sources (notably transport);
- medicines for human or veterinary use [1-6]. The former are found in urban waste water, and the latter in the ground or in livestock manure;
- other domestic substances or other substances (especially perfluoros [7-11]).

The different categories highlight the range of substances currently being studied—some of them for over a decade; others, such as perfluoros and medicines, have only emerged more recently. A certain number of these substances, regardless of their category, can disturb the endocrine system of many organisms [12-15]. Biological and biotechnological products:

- antibiotics

- genetically modified organisms (GMO)
- products of living organisms: Toxins, hormones …
- physical and physicochemical factors
- weak electromagnetic fields
- ionising and non-ionising radiation
- nanoparticles

Target organisms

In environmental toxicology, humans are the target organism, as well as "model" mammals, or other mammals of health or ecological interest.

In ecotoxicology, the horizon is larger. Among the aquatic invertebrates, species of interest include microcrustaceans (such as *Daphnia magna*, *pulex* and *Ceriodaphnia dubia*; gammarus, such as *Gammarus pulex*), molluscs (gastropods such as *Potamopyrgus antipodarum*, bivalves, such as *Dreissena polymorpha* and *Unio tumidus* etc.). The diversity of these model organisms can be explained by the diversity of routes of exposure, which depends in particular on the life-history traits of organisms (e.g. [16]), and on the course of action of substances, which can differ depending on functions of the phyla (e.g. [17]).

The stocks are introduced and kept on-site long enough for the experiments to be performed, without necessarily being permanent: this requires certain flexibility and good scheduling. The elements to be controlled are relatively standard: controlled temperature and lighting (light/dark cycle and intensity), water quality. The food provided may, however, be fairly specific.

A.3. Microbiological aspects

For ecotoxicology and toxicology studies in general, it seems necessary to better study:

- the impact, especially over the long term, of toxic substances on microorganisms both on a cellular scale (alteration of the integrity of cells and their functioning) and on population or community scale (impact on the structural and functional diversity) and the direct and indirect consequences of such potential modifications on human health.
- the role of microorganisms as bioindicators of the effect of toxic substances, and as a consequence, the quality of the ecosystems.
- the role of microorganisms in immobilising, breaking down and transforming toxic substances, and as a consequence, for conditioning the physical future (influence on availability and transfer between biosphere compartments) and chemical future (influence on speciation) of the elements. The role of bioremediation is to be optimised.

CETER's experimental proposals aim to **evaluate the impact of toxic metallic elements (metals and metalloids) and toxic organic elements (hydrocarbons, phytosanitaries) on microbial communities—including pathogenic agents and opportunistic pathogens—in the soil and aquatic environments, and in return, the impact of microorganisms on the future of contaminants, with a particular focus on their transformation or deterioration. This impact must be able to be evaluated in terms of dynamics and at different spatial scales.**

The working hypotheses are:

1. Introducing chemical contaminants into the environment affects the genetic and/or functional diversity of the microbial component. This component is an indicator of the healthy ecological state of an environment; in return, these changes are likely to affect the future of contaminants.

2. Chemical contaminants select bacterial populations which are dangerous to human health.

3. Chemical contaminants promote the emergence of new lines of bacteria which have altered resistance properties to antibiotics and modified virulence properties.

In order to carry out this research, a facility will be developed at Rovaltain (mesocosms made up of soils taken *in situ*, soil columns) which will enable researchers to i) control risks of exposure to pathogenic agents, ii) stabilise and monitor the physicochemical parameters (for long-term experiments), iii) be in a position to test the additive effect of certain parameters (for example, chemical exposure to several metals, or to a metal–organic compound) on the evolution of microbial populations and iv) adapt the level of complexity of the biological component of the ecosystem (for example, bacterial and fungal communities, predators (nematodes) and prey (bacteria)...)

These experiments require an analysis platform which will enable us to:

- isolate and cultivate microorganisms
- implement measures of metabolic activity, transformation capacity and the deterioration of contaminants
- determine the genetic diversity of communities and populations through dependent and independent approaches of the culture of microorganisms (genomic analyses)
- A ParMic-Rovaltain platform will be developed alongside the ParMic platform of Lyon1, ISA (Institute of Analytical Sciences) in Lyon, Cemagref and the Taberlet UMR platform for microbiology in Grenoble.

In these environments, the target microorganisms are either in an idle state, grouped around organic or mineral particles, or they are integrated into structured biofilms.

With regards to the size of the operation, **it is important to ensure the possibility of taking samples from the environment under investigation,** knowing that the quantity of the material sampled will be negligible compared to the size of the testing facilities.

Soil columns specifically designed for microbiologists will be introduced in order to enable sampling during the incubation period. These samples are necessary for measuring the impact on the genetic and functional diversity, and for displaying the potential of biotic transformations of contaminants, such "biological" sensors not existing…

A4. Animal and plant cells – human cells

As we have previously remarked, it is also important to further studies on animal and plant cells, and more especially on human cells. It is therefore necessary to foresee equipment for these cultures and to be able to maintain cell lines.

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Definitions of the French acronyms:

DR : Research director
PU : University professor
PH : Hospital practitioner
MCU : University senior lecturer
HDR : Authorised to carry out research

UCBL : University Claude Bernard (Lyon 1)
UJF : University Joseph Fourier (Grenoble 1)
INPG : Grenoble Institute of Technology

UMR : Joint Research Unit