

**INDUSTRIAL PRODUCTS OF MODERN BIOTECHNOLOGY INTENDED FOR RELEASE TO THE
ENVIRONMENT**

THE PROCEEDINGS OF THE FRIBOURG WORKSHOP

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 1996

31301

Document complet disponible sur OLIS dans son format d'origine

Complete document available on OLIS in its original format

**OECD
ENVIRONMENT
MONOGRAPH**

No. 117

**SERIES ON THE HARMONIZATION OF REGULATORY
OVERSIGHT IN BIOTECHNOLOGY**

No. 4

**INDUSTRIAL PRODUCTS OF MODERN
BIOTECHNOLOGY INTENDED FOR RELEASE
TO THE ENVIRONMENT**

THE PROCEEDINGS OF THE FRIBOURG WORKSHOP

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 1996

Also published in this series:

Environment Monograph No. 99, *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (1995)

Environment Monograph No. 100, *Comparative Analysis of Data Elements Used in the Assessment of Certain Products of Modern Biotechnology* (1995)

Environment Monograph No. 107, *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* (1995)

(See page 121 for a complete list of OECD Environmental Health and Safety publications)

© OECD 1996

*Applications for permission to reproduce or translate all or part of this material should be made to:
Head of Publications Service, OECD, 2 rue André-Pascal, 75775 Paris Cedex 16, France*

ENVIRONMENT MONOGRAPHS

The Environment Monograph series makes technical documents prepared by the OECD Environment Directorate available to the public. To obtain a complimentary copy of this publication, contact the Environmental Health and Safety Division, OECD Environment Directorate, 2 rue André-Pascal, 75775 Paris Cedex 16, France.

Fax: (33-1) 45 24 16 75

E-Mail: ehscont@oecd.org

<http://www.oecd.org/ehs/>

Foreword

The OECD Workshop on Industrial Products of Modern Biotechnology Intended for Release to the Environment was held in Fribourg, Switzerland, in May 1994. There were 53 participants from 14 countries, including representatives from the European Commission, Hungary and the Czech Republic (which became an OECD Member country in 1995).

The Fribourg Workshop was part of an OECD project entitled *Environmental Applications of Modern Biotechnology* (formerly *Industrial Products of Modern Biotechnology Intended for Release to the Environment*). The focus of this project is primarily on environmental applications of biotechnology, which involve living microorganisms, in uses such as bioremediation, bioleaching, biomining, or similar "industrial activities". In previous work, the **information elements** which national authorities address during the regulatory assessment of such applications had already been identified (see OECD Environment Monograph No. 100, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology*).

The primary objective of the Fribourg Workshop was to build on this previous work by identifying the **types of information** used to satisfy the **information elements** used in regulatory assessments: in other words, to identify whether the information was derived by reference to the scientific literature, from descriptive information, or using test methods. The Workshop presentations, which begin on page 41, were a major contribution to achieving this objective. The topics addressed included case studies (covering information used in risk assessments), as well as methods for predicting and evaluating environmental impacts.

The presentations were followed by three Working Group sessions (pages 20-31). The Working Groups found that much of the information used in regulatory assessments involves citations to the scientific literature. They suggested that one possible approach to harmonization could be the development of compendia of information for use during regulatory assessments.

A small Working Group also considered activities related to good laboratory practice (GLP) (page 32). It identified two types of information (that generated through field tests, or under conventional laboratory conditions) which could be generated under GLP. This Working Group suggested that the OECD's GLP Panel might wish to take this into account in their future work.

Derestriction of this Environment Monograph was recommended by the Joint Meeting of the Chemicals Group and the Management Committee of the Special Programme on the Control of Chemicals. It is being published on the responsibility of the Secretary-General of the OECD.

Table of Contents

Introductory Presentation <i>Bruno Milani, Switzerland</i>	11
Background and Overall Objectives of the Workshop <i>Larry Zeph (Workshop Chairman), United States</i>	15
Report on the Working Groups	20
Introduction	20
Working Groups I and II	20
Findings of Working Groups I and II	21
Suggestions for Future Work	26
Working Group III	27
Information Used in the Assessment of Microorganisms in Bioremediation	27
Suggestions for Future Work	30
Suggestions to the Tokyo Workshop Steering Group	30
Report on Activities Related to Good Laboratory Practice	32
Report on Activities of the OECD Panel on Good Laboratory Practice: Mutual Acceptance of Data <i>Hans Hosbach, Switzerland</i>	33
Presentations	41
The Current Status of Bioremediation Study in Japan <i>Tsugusyoshi Suzuki, Japan</i>	43
Report on a Feasibility Study of a Compendium of Common Answers to Specific Safety Questions <i>Penny Bramwell and Mark Bailey, United Kingdom</i>	45
Comparative Case Study: Release of a Genetically Modified Microorganism in the UK: <i>Pseudomonas aureofaciens</i> <i>Firoz Amijee, United Kingdom</i>	52

(continued on next page)

Comparative Case Study: Risk Assessment for a US Field Release of a Recombinant <i>Pseudomonad</i> <i>Philip G. Sayre, United States</i>	54
Findings of the 1993 US EPA-Environment Canada Bioremediation Risk Assessment Workshop <i>Philip G. Sayre, United States</i>	61
Partial Risk Assessment for a PCB-degrading <i>Pseudomonad</i> <i>Philip G. Sayre, United States</i>	71
Provisional Method for Evaluating Environmental Effects of Bioremediation <i>Hideto Yoshida and Osami Yagi, Japan</i>	79
A Method for Predicting Environmental Impacts of Field Releases of Effective Specific Microorganisms Using Microcosm Systems <i>Yuhei Inamori, Kazuhito Murakami, Ryuchi Sudo and Yasushi Kurihara, Japan</i>	93
Bioremediation of PAH-contaminated Soils from a Gasworks Site with the Ebiox Vacuum Heap™ System: A Swiss Case Study <i>Daniel Rolf Eiermann and Reinhard Bolliger, Switzerland</i>	105
Annex I Information Elements from Environment Monograph 100 (Tables I-III)	107
Annex II Workshop Participants	113
OECD Environmental Health and Safety Publications	121

Introductory Presentation

Bruno Milani

Vice-Director

**Federal Office of Environment, Forests and Landscape
Switzerland**

It is a pleasure for me to welcome you to Fribourg, in the name of the Swiss Government, for this OECD Workshop devoted to "Industrial Products of Modern Biotechnology Intended for Release to the Environment". And I would like to extend a special welcome on this occasion to your Chairman, Mr Larry Zeph, to your deputy Chairman, Mr Ryuichiro Kurane, and to the OECD Secretariat representative, Mr Peter Kearns. We are delighted to note what a success the meeting is, proof if any were needed of Member countries' interest in this OECD activity, which was arranged last year on the initiative of the European Commission and Canada.

The choice of Fribourg as the venue for this Workshop is an inspired one. Fribourg is a truly bilingual town, with a bilingual university, and as such is fairly representative of what we might call the "Swiss experience", which as you may know involves four national languages, two of which – German and French – are dominant. Swiss linguistic differences, which are also cultural differences, are more noticeable in some fields than in others. Genetic engineering is one such field. Here indeed we find a quite considerable difference between the German approach and the Latin approach, which has resulted in the spread, notably in German-speaking Switzerland, of a powerful movement opposed to genetic engineering, typified by an organisation which calls itself the "Genetic Technology Working Group".

The Swiss system of semi-direct democracy allows the electorate to express its view any time it feels so disposed, notably by means of the popular initiative. This instrument makes it possible to propose an amendment to the Swiss Constitution. Once such a proposal has gathered at least 100,000 signatures, which is not so many compared to our population of nearly 7 million, a popular referendum must be held. Such an initiative on the subject of genetic engineering has just been approved, and will be put to a referendum vote. This particular proposal is extremely restrictive in the use it would allow of biotechnology. For example, it would prohibit the use of genetically modified organisms in the environment. We in Switzerland thus find ourselves in an anachronistic situation, with on the one hand a highly active biotechnology industry and, on the other, a cross-section of public opinion which is extremely reluctant to see it used.

After this brief introduction, which I hope has given you a "taste" of the situation here in Switzerland, I would like to move on to the reasons for the Swiss decision to support this OECD work and, consequently, to host this Workshop.

As I have already mentioned, modern biotechnology, particularly through genetic engineering applications, is already making a considerable contribution to the Swiss economy, notably in the pharmaceuticals industry. There is every indication that in the very near future biotechnology will be equally important to other sectors of the economy – in agriculture, for example, and in the development of new environmental technologies. Our aim therefore is to put clear-cut framework conditions into place, in an effort to ensure that our economy remains competitive internationally, while at the same time providing enough guarantees for the safe utilisation of this technology to satisfy the public and protect the environment.

In the matter of regulation, however, Switzerland finds itself trailing the other industrial nations. We do not, for example, have any body of regulations to govern the use that may be made of genetically modified organisms within the environment. The government has nonetheless adopted the conclusions of a report produced by a working group inside the federal administration. This report, which unfortunately is available only in German and French,¹ provides a general regulatory framework and plugs the existing loopholes. The approach has been to integrate new regulations on genetically modified organisms into existing texts, rather than to create a totally new body of specific regulations, amounting to a law on genetic engineering. In the area of environmental protection, a draft amendment of the law on protection of the environment is currently being debated in the Swiss Parliament. This bill will serve as the basis for the adaptation into Swiss federal law of European Union Directives 90/219 and 90/220.

Because we are behind in this field, we Swiss are all the more interested in what is happening abroad, for it is clearly impossible to go very far down this road all by one's self. Only through closely following developments shall we be able, once the legal basis has been put in place, to rapidly adopt rules and regulations compatible with international practices. In this context, the OECD is for Switzerland an ideal forum. It enables us to participate actively in the work in hand, despite our lack of appropriate national regulations. Furthermore, OECD recommendations have the benefit of achieving international recognition, and indeed often serve as points of reference to guide the efforts of individual nations, even those outside the OECD itself. An instance of this is the well known "Blue Book", *Recombinant DNA Safety Considerations*. Published by the OECD in 1986, it has been the basis of most national regulations in the field of biotechnology.

Experience has shown that the development of new global technologies like biotechnology and genetic engineering inevitably leads to harmonization of the regulatory practices of the nations concerned. We have already more or less seen the completion of the first stage of this process: harmonization of legislative requirements. The majority of industrial nations accept that there is a need to provide legislative guarantees for the safe use of biotechnology and genetic engineering. In the European context, this principle is expressed in the Directives of the European Union to which I have already alluded.

¹ *Coordination de la législation sur le génie génétique et les méthodes médicales de procréation assistée*, Rapport IDAGEN, Rapport du groupe de travail interdépartemental en matière de génie génétique. Editeur: DFJP, January 1993.

The next step in this process of international cooperation calls for harmonization of data requirements. Despite very different approaches to regulation in this field at the national levels, the Brussels Workshop last year² showed clearly that there is nonetheless a high degree of similarity in the data requirements of these different national regulations. This is extremely encouraging. We may conclude that our hopes for this second stage are realistic.

Harmonization of data requirements and its corollary, harmonization of the methods used to generate the data with the introduction of a good laboratory practices type quality assurance programme – these are the fundamental requirements which must be met before we can think about introducing a system for the mutual acceptance of data. The OECD's experience in the chemical products sector has been very positive, demonstrating clearly the extent to which such an approach can simplify matters, while at the same time reducing administrative costs for both the industrial entrepreneur and supervisory authorities.

Here I would like to draw a parallel between the OECD Environmental Health and Safety Programme and the project which concerns us today, with regard to the regulatory situation in Switzerland. The OECD Principles of Good Laboratory Practice, in the context of the Environmental Health and Safety Programme, were elaborated at the international level. Switzerland played a significant role in this process, but the fact is that up to the present time it has not been necessary in Switzerland to observe GLP Principles in generating the data required for official registration of a product by the authorities. However, a programme of compliance with the GLP Principles was introduced in Switzerland in the mid 1980s as part of an effort to ensure the continued international competitiveness of Swiss labs. In view of the importance now given to GLP at the international level, Switzerland has decided to make the observation of GLP obligatory and to adapt our legislation accordingly. This is one field in which practice has preceded policy. We dare to hope that the Fribourg Workshop will have the same catalysing effect and contribute to the introduction of a nationwide regulatory system compatible with international practices in the field of biotechnology.

The type of product chosen as a model for this programme, which concerns all those products used for bioremediation, is one which has real potential in our country. At tomorrow's session the representative of a Swiss firm which is very active in this field will give you a good idea of what is being accomplished here. Despite all its promises, however, bioremediation is still in the early stages of development and there are still a number of points which need clarification. I refer particularly to the need for measures to test its effectiveness and to related areas, as well as to the need for methodological reproducibility. Let me take this opportunity to salute the initiative of Japan, which plans to organise a scientific workshop in Tokyo in November of this year, again within the context of the OECD, in order to explore these fundamental questions in greater detail.³

² An OECD Workshop on "Industrial Products of Modern Biotechnology Intended for Release to the Environment" was held in Brussels in May 1993 (see the Introduction to Environment Monograph No. 100, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology*).

³ The OECD Workshop on Bioremediation was held 27-30 November 1994 at the Ministry of International Trade and Industry (MITI). See *Bioremediation: the Tokyo '94 Workshop*, published in the "OECD Documents" series in 1995 (ISBN 92-64-14634-2).

Our own feeling is that the meeting in Tokyo, and the meeting today, are highly complementary. It may well be that certain of the questions raised here in Fribourg will find answers in Tokyo. At least we may rest assured that the presence here of our Japanese colleagues is a guarantee of continuity between these two stages.

Last but not least, I would like to say that by organising a workshop like this one Switzerland hopes to present to all of you, to all our international partners, the image of a nation open to the world, of a nation quite unlike the Switzerland some people imagine when hearing about the popular vote which only just – by 51 per cent – defeated the federal government's proposal to join the European Economic Area.

Let me conclude my introduction with this reflection. At the end of last year's Brussels meeting, the European biotechnology magazine *Biofutur* featured the headline: "OECD: The Long Road to Harmonization". Ladies and gentlemen, the road now stretches before us. We still have far to go along our road, and the journey will be full of difficulties. At times it may seem to many of us unrewarding. But rest assured, this is a major branch of a worldwide network, a network which in the long term will make possible the development and sustainable exploitation of biotechnology, working towards the objectives contained in Agenda 21. I invite you to make excellent progress in these next three days, and at the same time to enjoy this particular stretch of the road which passes through Fribourg before following the rainbow to the pot of gold labelled "Harmonization".

Background and Overall Objectives of the Workshop

Larry Zeph (Workshop Chairman)

United States Environmental Protection Agency

Introduction

I would like to begin by thanking the Swiss authorities for offering to host this Workshop. In particular I would like, on behalf of all the participants, to thank Bruno Milani for his kind welcoming remarks.

I see on the Workshop agenda that we have a Welcome Drink this evening, hosted by the City Council of Fribourg and the cantonal government, and tomorrow evening we have an official dinner in Gruyères hosted by the Swiss Federal Office of Environment, Forest and Landscape. These extra activities, coupled with the excellent conference facilities, are going to make this a very pleasant Workshop, so I would like to thank our hosts for their hard work in making the arrangements.

Regarding the work we plan to do this week, I would like first to say something about the background to the OECD project on "Industrial Products of Modern Biotechnology Intended for Release to the Environment",¹ of which this Workshop is a part. This will serve as a reminder of the overall goals of the project and will help us meet the objectives of this important Workshop.

I think it will be useful during the Workshop to take the opportunity to reflect on these goals, perhaps returning to discuss them towards the end of the Workshop. And perhaps taking the opportunity to reaffirm, refine, or even redefine them if necessary in the light of our discussions this week.

After stating the goals of the project, I would like to describe the objectives of this Workshop and then make a first attempt at outlining the work of the Working Groups.

Background

As I think everybody here is aware, the project on "Industrial Products of Modern Biotechnology Intended for Release to the Environment" was initiated by the 19th Session of the Joint Meeting in November 1992.² When the focus of the project was originally elaborated by

¹ The name of this project was subsequently changed to "Environmental Applications of Modern Biotechnology".

² The Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals is the lead policy body for OECD's Environmental Health and Safety Programme.

the Joint Meeting, foods, fertilizers, pesticides and pharmaceuticals were excluded. The goals of the project were expressed as the development of practical tools which would assist regulators in assessing new environmental products or applications. It was thought that this could include the development of guidance for the assessment of data as well as the testing methods necessary to collect the data. At that time it was agreed to hold an initial Workshop, which took place in Brussels in May 1993.

Let me briefly review the purpose and results of the Brussels Workshop. One of its main objectives was to examine the feasibility of undertaking this work. As a first task, there was agreement that the Brussels Workshop should make a preliminary assessment of whether there was commonality among the various national oversight systems in terms of the individual information and data elements which would be used when such products are assessed in Member countries. The logic behind this approach was that a reasonable level of commonality among Member countries would be necessary to carry the work forward.

At Brussels this preliminary analysis was undertaken within three Working Groups. These three groups analysed and compared information and data elements identified from national legislation, regulations, and associated guidance or interpretative documents from Australia, Canada, New Zealand, the United States and the European Commission (EC). *The Workshop concluded that there was a high degree of commonality in the national documentation in terms of the information and data elements identified.*

Consequently, the Brussels Workshop concluded that work of the type originally envisaged by the Joint Meeting was feasible. It also concluded that future work leading to the exchange of information and data, or even the mutual acceptance of data, was feasible as part of the development of practical tools which would assist regulators in the job of assessing new products.

The Brussels Workshop also confirmed that the primary focus of the project would be products intended as bioremediation agents, bioleaching agents, agents intended as biosensors, or agents involved in biomining or environmental activities. In addition, it was agreed that the focus would be confined to products containing living organisms.

Since the Brussels Workshop, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology* (OECD Environment Monograph No. 100) has been published. An earlier version of this Environment Monograph was one of the background documents for the Fribourg Workshop. In summary, it completes the task of the Brussels Workshop by demonstrating a high level of commonality across a wider group of Member countries than were compared at Brussels, providing further incentive for moving forward with the work.

Goals of the OECD Project

Having provided you with this brief historical background, I would like to return to the goals of the project as originally elaborated by the Joint Meeting. As I mentioned earlier, the primary objective was described as the development of practical tools which would assist regulators in assessing new products and would at the same time facilitate international harmonization.

There are a number of possible steps in the process of harmonizing the regulatory oversight of industrial products of modern biotechnology:

- i) harmonization of data generation;
- ii) mutual acceptance of data;
- iii) harmonization of assessments reports:
- iv) mutual acceptance of assessments;
- v) mutual recognition of products.

The harmonization of data generation would include the identification of important information and data elements to be addressed during the assessment process; the identification of appropriate approaches which will generate the data necessary to allow an evaluation of those information and data elements; and the development of agreed common approaches for describing information and collecting data. This is the stage we are at today.

The next step, which was envisaged by the Brussels Workshop, is the mutual acceptance of data, which is an agreement to accept one another's data for purposes of assessment. As I see it, the main objective of the mutual acceptance of data is to ensure that data generated in one of our Member countries as part of a product assessment may be accepted in other countries and need not be developed a second time.

I would like to remind you of two very important reasons for pursuing mutual acceptance of data. Firstly, it would save both regulators and industry time and effort. Secondly, it would help protect human health and the environment through harmonization of product assessments. The goal of mutual acceptance of data should allow us to develop practical tools to assist us as regulators and, at the same time, hopefully reflect the current "state-of-the-art" in terms of safety testing of these products.

As regards the harmonization of assessment reports, this would include an agreed format to display information and data elements, and an agreed procedure to evaluate data and information (assessment methods and practices). This is something very much for the future, but is perhaps important to keep in mind as we undertake our work.

The notions of the mutual acceptance of assessments, as well as mutual recognition of products, are probably concepts to be considered far in the future.

Let me now mention some of the ideas which were generated in Steering Group discussions to assist the work over the next few days. I hope the discussions we will be having will generate more ideas, as your input and experience are what is needed.

As a first example, we might wish to begin by thinking of ways of sharing experience in the assessment of products. This would be of particular value given that none of our countries has yet had a lot of experience assessing the types of products which fall within the focus of this activity.

Another idea is to look at ways to reach agreement on how to collect the data necessary for the assessment of products. In this context, Penny Bramwell's presentation on the feasibility study of a compendium of common answers to specific safety questions will be important because it could begin to point the way forward to the types of information which would satisfy certain data or information requirements.

Another important process the Steering Group discussed was the need to consider the quality assurance of data. In this context, we will have an important presentation on Good Laboratory Practice (GLP) and mutual assessment of data from Mr Hosbach of the Swiss Federal Office of Environment, Forests and Landscape.³

Introduction to the Working Groups

In turning to the specific objectives and expected output of this Workshop, I would first draw your attention to the Chairman's Report of the Washington Steering Group meeting, the planning meeting for this Workshop. The Chairman's Report was included as part of the background documentation. In particular, it mentions the role of the Working Groups which are going to be at the heart of our work this week.

Working Group I will discuss information needs in relation to basic organism characteristics such as taxonomy, details of genetic constructs, and pathogenicity. This Working Group will be chaired by Desmond Mahon of Canada.

Working Group II will discuss information needs in relation to the environmental fate of organisms, for example information on survival, dispersal, and methods for detection and monitoring in the environment. This Working Group will be chaired by Iain Gillespie of the United Kingdom.

The objectives of Working Groups I and II are essentially the same – that is, to identify the sources of information or data required to satisfy the various information needs identified. In other words, these groups are to establish whether a specific information need is typically satisfied by a test method, by information derived from an experiment in a laboratory microcosm or field trial, by a simple description (e.g. of a DNA sequence), or by a simple citation of a reference in the scientific literature.

Having established the sources of the needed information, we can begin to discuss the level of detail of information typically required for each information element. We can then begin to consider common ways of collecting this information, given that our main goal is mutual acceptance of data). If the information need for a given element is to be satisfied by a test method, for example, then we can begin to think about mechanisms for developing test methods in common. If, on the other hand, the information need is best satisfied by information derived from an experiment in a microcosm or field trial, how can we describe, in common, the conditions under which the data should be collected and presented? If we are thinking about descriptive information such as a DNA sequence, how can we develop criteria for establishing the level of detail required?

³ A small group of participants met to discuss the potential role of the OECD Good Laboratory Practice (GLP) Principles in the assessment of the types of products falling within the scope of the Workshop. Their report is included on page 32.

Perhaps we can even go on to begin considering the minimum information needed for an assessment, taking into consideration the case studies we will be hearing later in the Workshop.

The objectives of **Working Group III** are slightly different from those of the other two groups. It will identify questions specific to the regulation of bioremediation. In other words, it will begin to identify those regulatory issues with which we have had little experience, such as the toxicity of intermediate by-products. The main aim of Working Group III, therefore, will be to identify questions arising from, and commonality among, existing regulatory approaches in OECD countries that are specifically related to the issue of bioremediation.

Report on the Working Groups

Introduction

In previous work carried out as part of this OECD project, the information elements national authorities use when they undertake regulatory assessments were identified.¹ The results of this work, which were used as background material at the Fribourg Workshop, are presented in *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology* (Environment Monograph No. 100).

As described in the foregoing presentation by the Workshop Chairman, Larry Zeph, the primary purpose of the Fribourg Workshop was to build on this earlier work by **identifying the types of information used to satisfy these information elements, as a prerequisite for future work on the harmonization of approaches to the collection, exchange and assessment of information or data used in regulatory assessments.**

The presentations given at the Workshop made an important contribution to fulfilling this purpose. They are published beginning on page 41. Following the presentations, Workshop participants took part in one of three Working Groups. Their discussions, which included suggestions for future work, are reported below.

Working Groups I and II

Working Group I discussed information types in relation to the **basic characteristics of organisms** (for example, taxonomic information, details of genetic constructs, and information on pathogenicity). These basic characteristics correspond to the information elements in Environment Monograph No. 100, Tables I (General Scientific Considerations) and II (Human Health Considerations). The information elements which are the subject of these two tables are listed in Annex I of this document.

Working Group II discussed information types in relation to the **environmental fate of organisms** (for example, information on their survival and dispersal, as well as information concerning environmental detection and monitoring methods). These information types, which correspond to the information elements in Table III (Environmental and Agricultural Considerations) of Environment Monograph 100, are also listed in Annex I.

¹ The OECD's "Environmental Applications of Modern Biotechnology" project was formerly called "Industrial Products of Modern Biotechnology Intended for Release to the Environment". The project is focused on environmental applications of biotechnology involving living microorganisms, for uses such as bioremediation, bioleaching, biomining and similar "industrial activities". The information elements identified in this OECD work were those used in assessments of the applications which are the focus of the project.

Findings of Working Groups I and II

Working Groups I and II found that four main types of information are used to satisfy these information elements:

- 1) information which is purely descriptive (**D**) (for example, the name of the organism, the site where work was undertaken, or a DNA sequence);
- 2) information cited in the scientific literature (**L**), which need not have been generated by the applicant;
- 3) information derived from experimental data generated by the applicant (or a contract laboratory) using a test method (**T**), including microcosm studies or field tests;
- 4) information generated through a modelling approach (**M**).

As can be seen in **Tables 1-3**, the Working Groups noted that a substantial amount of the information used in regulatory assessments is of a descriptive nature (D) and/or available in the scientific literature (L). Only some information elements require information derived using test methods (T) or modelling (M).

Tables 1-3 also indicate that the information needed to satisfy an information element will not necessarily come from a single information type (D, L, T or M). In Table 3, for example, those elements satisfied by a single type of information are mostly found in the first part of the table (items 1a to 4c). They refer to the ecological traits of recipient or donor organisms, site characterisation, or details of the release. This information is mostly descriptive (D) and/or found in the scientific literature (L). For the elements concerned with monitoring the organism in the environment (3a to 3c), some testing (T) may be needed.²

As the elements in Table 3 become more complex and increasingly refer to the attributes of the genetically modified organism and its ecological effects, the information types become correspondingly complex. The scientific literature is typically used as a basis, providing information on, for example, parental organisms. For the *predicted* behaviour of the organism in the environment, it is likely that a combination of information from the scientific literature and information obtained through testing (LT) or modelling (LM) will be needed.

Comparatively more complex information elements referring, for example, to the stability of the organism or to potential environmental impacts could require even more complex information types (e.g. LTM).

The Working Groups noted that the types of information needed for complex applications, such as those involving a consortium of microorganisms, might need to be addressed on a case-by-case basis.

² In this context, it was noted that the activities of organisations like the European Committee for Standardization (CEN) and the International Organization for Standardization (ISO), aimed at establishing guidance for the validity of monitoring programmes, might be helpful for future work.

Table 1

Information Elements – General Scientific Considerations

	1a	1b	1c	1d	1e	1f	1g	2a	2b	2c
Descriptive (D)	X	X	X	X	X	X	X	X		
Literature (L)			X	X		X				
Test Method (T)			X	X						
Modelling Approach (M)			X	X						
Review (R)	X	X	X	X			X		X	X

	3a	3b	3c	3d	4a	4b	4c	4d	4e	4f	4g	4h	4i
Descriptive (D)	X	X	X		X	X	X	X	X	X	X	X	
Literature (L)	X				X	X	X	X	X	X	X	X	
Test Method (T)	X				X	X	X	X	X	X	X	X	
Modelling Approach (M)					X	X	X	X	X	X	X	X	
Review (R)				X		X	X	X	X	X	X	X	X

(For a list of these information elements, see Annex I)

Table 2

Information Elements – Human Health Considerations

	1	2	3	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	4	5	6	7
Descriptive (D)	X	<-	-	-	-	-	-	-	-	->	X	X	X				
Literature (L)	X	<-	-	-	-	-	-	-	-	->	X	X	X				
Test Method (T)	X	<-	-	-	-	-	-	-	-	->	X	X	X				
Modelling Approach (M)	X	<-	-	-	-	-	-	-	-	->	X	X	X				
Review (R)	X	<-	-	-	-	-	-	-	-	->				X	X	X	X

<- -> = These information elements are interrelated:

The information needed for item 1 is descriptive or derived from testing, depending on the scale of use. Where genetically modified microorganisms are to be used, the level of testing will be based partially on the acceptability of the identification criteria in Table 1. If an organism is known to be pathogenic to humans (or animals) (3), Working Group I believed that priority should be given to items 3h (antibiotic-resistance patterns), 3i (toxicogenicity) and 3j (allergenicity). Results obtained for these information elements should *trigger* the need for information for item 2 (capacity for colonisation) and the rest of the items under 3.

(For a list of these information elements, see Annex I)

Table 3
Information Elements -
Environmental and Agricultural Considerations

	1a	1b	1c	1d	1e	1f	1g	1h	2a	2b	2c
Descriptive (D)	X								X	X	
Literature (L)		X	X	X	X			X			
Test Method (T)				X	X						
Modelling Approach (M)				X				X			
Review (R)						X	X				X

	2d	2e	2f	2g	2h	3a	3b	3c	4a	4b	4c
Descriptive (D)	X	X	X	X	X	X					
Literature Review (L)									X		X
Test Method (T)							X	X	X	X	
Modelling Approach (M)											X
Review (R)											

(continued next page)

(For a list of these information elements, see Annex I)

Table 3 (continued)
Information Elements –
Environmental and Agricultural Considerations

	5a	5b	5c	5d	5e	6a	6b	6c	6d	6e	7a	7b	8a	8b	8c	9a	9b
Descriptive (D)											X	X					
Literature (L)	X	X			X	X	X	X	X	X	X		X	X	X	X	X
Test Method (T)					X	X	X	X	X	X			X	X	X	X	X
Modelling Approach (M)	X	X					X	X	X	X	X				X	X	X
Review (R)			X	X								X					

(For a list of these information elements, see Annex I)

Suggestions for Future Work

Once Working Groups I and II had identified the types of information needed during regulatory assessments, they went on to consider specific future work that might help realize the overall goal of the OECD project (i.e. to develop practical tools which would assist regulators in assessing new products, while at the same time facilitating international harmonization).

In this context, the Working Groups found that much of the information used in regulatory assessments is not case-specific, but could apply to a number of different assessments concerning the same or similar host organisms. They also found that this information (for example, information related to the biological properties of the host organism) is largely available in the scientific literature.³

The Working Groups considered it feasible to develop "compendia" of such information for use during regulatory assessments, in respect of commonly used organisms. In particular, they noted the Workshop presentation by Penny Bramwell and Mark Bailey, "Report on a Feasibility Study of a Compendium of Common Answers to Specific Safety Questions" (see page 45). The Working Groups suggested that activities might be developed using a lead country approach, in which a Member country would assume the responsibility for compiling a compendium for a specific organism.

The Working Groups also noted that a number of elements (for example, those concerning stability, potential effects on organisms, and ecosystem effects) could be satisfied by a combination of information types. The type of information would sometimes be determined by a form of *trigger*. For example, in Table 2, if an organism is known *not* to be a pathogen (as documented in the literature), it is unlikely that there would be a need for additional information or tests related to human health considerations. Reference to the literature would suffice. However, if an organism is known to be pathogenic, or if there is some doubt regarding its pathogenicity, further information would need to be provided, possibly through testing.

The Working Groups suggested that future work could involve analyses for the purpose of identifying such triggers, carried out by small working groups.

Finally, the Working Groups noted that Tables 1-3 had been of value in demonstrating commonality among OECD countries in terms of the information elements addressed in regulatory assessments. These information elements, developed in previous OECD work, had in turn been the basis for identifying the information types indicated in the tables. However, a number of these elements, as indicated in Tables 1-3, would need to be reviewed (**R**) if used in future work, either because their meaning was unclear or because of redundancy in relation to other information elements.

³ Where there might be a need for information developed through testing, it was suggested that there could be a need in the future for mutually acceptable test methods.

Working Group III

Working Group III focused on the information that might be used by regulatory authorities in addressing the use of microorganisms in bioremediation. Where possible, the Group took into account countries' practical experience.

Information Used in the Assessment of Microorganisms in Bioremediation

This section of the report highlights information which, in the opinion of the Working Group, would be relevant to the regulatory assessment of microorganisms in bioremediation. Four categories of application were recognized: genetically modified organisms; naturally occurring but non-indigenous organisms; indigenous organisms; and nutrient and electron acceptors. Not all the information below will apply to every case or category. It is expected, therefore, that individual assessments will address only the particular subset of relevant considerations.

The information identified by the Group may be organized under six major headings:

- A) Site characterisation;
- B) Microbial interactions;
- C) Additives (nutrients, etc.);
- D) Technology/engineering;
- E) Monitoring/control/measurement of efficiency;
- F) Emergency planning.

Each of these headings is elaborated below. It should be stressed that the descriptions under each heading will need further detail – perhaps a task for future work. Under each heading there is a need to identify all the protocols used when measurements are made, e.g. analytical procedures, data sampling, etc. Some items are relevant to more than one heading.

A. Site characterisation

Site characterisation was regarded by the Working Group as the initial task to be undertaken. It is important to present a full history of the site (*site* can mean contaminated land, rivers, lakes, estuaries, canals, or the open sea) and to indicate the potential site use.

Geological and geographic considerations need to be detailed, including:

- geographic considerations of the site location;
- geochemistry and mineral association;
- subsoil hydrogeology/geology;

- flow rate of groundwater;
- depth of vadose zone, etc.;
- characteristics of sediments (permeability, grain size, organic content, nutrient level, redox, natural bacteria counts).

The nature of all contaminants, including their distribution and concentrations, should be specified.

B. Microbial interactions

Data should be provided on the interaction between the microorganisms applied and the target and non-target contaminants. These data could include information on the metabolism of the contaminants (both primary and secondary and their degradation by naturally occurring organisms), on the metabolism of any additive, and on metabolite toxicity and ecotoxicity.

Also to be considered are data on interactions with consortia (natural and artificial constructs), bioavailability/bioaccumulation of contaminants and metabolites, and interactions with site flora and fauna, any modification of soil structure, etc.

C. Additives

The nature and detailed use of all additives should be set out. These additives may include nutrients, surfactants, other microorganisms, electron acceptors, soil structure improvers, and air.

When additives are applied, the rationale for using them should be presented, including, for example, considerations of nutrient balance or imbalance, toxicity, possibility of eutrophication, possibility of inhibition of natural activity, and any interaction with contaminants.

D. Technology/engineering

This section includes all initial documentation, description of the engineering systems to be used, and a rationale for the particular choice.

Initial documentation could include:

- issue management;
- degradation documentation;
- toxicity monitoring;
- safety data for final concentration (including ultimate use of cleaned material);
- all plans for publicity.

The description of the engineering systems to be used should be based on site simulation (model systems) and time considerations (e.g. seasonality, acute response, etc.). The rationale for the choice should answer the following questions:

- Why bioremediation?
- Why this type of bioremediation?
- Why *in situ* (reinjection, bioventing, biosparging) or *ex situ* (landfarming, heaps, slurry phase)?

Also to be identified are the techniques for the application of additives, monitoring, and equipment cleaning, as well as considerations of organism stimulation, organisms in run-off, etc.

E. Monitoring/control/measurement of efficacy

This section particularly applies to the organisms and pollutants involved. Protocols are especially relevant here: note should be taken of the EU document on protocols⁴ and OECD Environment Monographs No. 90 and 91.⁵ Before, during and after treatment, the timing and location of all measurements should be provided on-site and outside the site boundary.

It is necessary to have information on the identification of all relevant organisms, their tracking and counting, and the movement of genes (relevant to genetically modified organisms). This is also important in relation to worker safety and environmental spread.

The measurement and monitoring of both primary pollutants – the actual hazard – and secondary pollutants (secondary in time rather than importance) – which are a potential hazard – should be set out.

F. Emergency planning

All documentation should set out the plans for emergency response to:

- unexpected secondary hazards (including metabolites);
- stimulation of organism(s);
- a worker safety incident;
- an unexpected release.

⁴ *Methods for the Detection of Micro-organisms in the Environment* was the product of cooperative efforts by the European Commission (EC) and US in the Permanent Technical Working Group on Biotechnology and the Environment. This Working Group was established in the framework of the EC/US Bilateral Environmental Consultations.

⁵ Environment Monograph No. 90, *Ottawa '92: The OECD Workshop on Methods for Monitoring Organisms in the Environment*, and Environment Monograph No. 91, *Compendium of Methods for Monitoring Organisms in the Environment*, are companion documents (both published in Paris, 1994).

The potential wider relevance of any emergency to, for example, public health should be identified.

Suggestions for Future Work

The information regarded by Working Group III as relevant to the regulatory assessment of microorganisms in bioremediation has been described above. However, the Working Group was aware of the lack of experience in many countries with regulatory assessments of these microorganisms. It also recognized a need for further information gathering, and recommended that a questionnaire be prepared in order to meet this need. Some recommendations are presented here on the possible nature of any questionnaire sent to OECD countries.

The questionnaire's introduction should stress that current OECD activities are targeting the goal of mutual acceptance of data in relation to the use/release of microorganisms specific to bioremediation. However, because many countries do not have experience, information may currently be required on a "case-by-case" basis and may not yet have been laid down in regulatory or guidance documents.

The questionnaire should therefore be formulated to encourage the maximum number of responses (not just from those countries with experience and/or relevant regulations). It should give as much background as possible to the questions, in order to help the reader understand their intent. Yes/no answers should be encouraged, with "comment" boxes to allow elaboration.

Where relevant, the terminology of the preamble to the OECD publication *Safety Considerations for Biotechnology: Scale-up of Micro-organisms as Biofertilizers* (OECD, 1995) should be used.

Respondents should be asked whether their existing regulations are covered by these questions or, if they do not have existing regulations, whether the data indicated by this questionnaire appear valid and relevant to their situation. They should also be asked if the questionnaire covers all the questions they would like to ask. All existing regulations should be quoted, and copies sent to the OECD Secretariat. Countries with experience should be asked about this experience, in order to document how it was achieved (i.e. use of test methods, etc.).

Suggestions to the Tokyo Workshop Steering Group

The Fribourg Workshop is one of a succession of OECD workshops. It was therefore considered appropriate that issues be raised relevant to the scientific workshop to be held in Tokyo, under the auspices of the OECD's Committee for Scientific and Technological Policy, in November 1994.⁶ The Working Group was concerned that scientific data were lacking which could be beneficial to the application of microorganisms to bioremediation. Four major subject areas were identified: identification of organisms; risk/benefit analysis; process mechanisms; and the role of higher organisms.

⁶ See the Introductory Presentation, note 3.

On this basis, the following issues were referred to the attention of the Tokyo steering group:

- 1) How can we deal with the identification of components of microbial consortia (not constructed but partially natural)? The Tokyo Workshop might like to consider the development of novel techniques for identification and characterisation, bringing experts together to discuss this.
- 2) Can scientific help be provided through better defining risks (e.g. non-target side effects) to permit a better risk/benefit analysis? We might make deductions from historical practice – do modern risks differ from historical ones?
- 3) On the benefit side, can we optimise the efficacy of microbial bioremediation practices by a better understanding either of consortia, or of microbial physiology and interactions in heterogeneous situations?
- 4) Is it possible to combine the interactions of microorganisms, plants and higher organisms to optimise bioremediation?

Report on Activities Related to Good Laboratory Practice

A small group of the Fribourg Workshop participants met to discuss the potential role of the OECD Principles of Good Laboratory Practice (GLP) in the assessment of the types of products falling within the scope of the Workshop.

The group noted that GLP is a managerial concept which covers the organisational process and the conditions under which studies are planned, performed, monitored, recorded and reported. The purpose of this concept is to promote the quality and validity of test data to be submitted to regulatory authorities for the assessment of the health effects and environmental impact of a test substance, which may be a chemical or an organism.

The group also noted that the role of the Fribourg Workshop was primarily to address the appropriate methods for addressing information elements associated with safety assessments. However, the group recognised that once there is a need for test data associated with safety assessments, it would be necessary for purposes of mutual acceptance of data to have a system to promote the quality and validity of test data, i.e. to require the application of the OECD Principles of GLP.

This would have a number of practical benefits: for example, to ensure that it is possible to partially or completely reconstruct the conduct of a specific study. In addition, this would harmonize the quality of test data requirements among Member countries, as well as over time.

The group identified several sets of information which could be amenable to GLP. However, two types were discussed which seemed to be of particular priority:

- 1) Data generated through field tests, for example, are often collected to examine environmental fate of microorganisms, including issues related to survival and dispersal. Given that data generated through field tests are a particularly important component of the assessment of "industrial products" in Member countries, it would appear to be a priority to examine how to apply GLP to microorganisms in field tests.
- 2) Data generated under conventional laboratory conditions, for example to determine pathogenicity (toxicological tests) or taxonomic identification of a microorganism, are also likely to be important in assessments.

Finally, the group discussed how work on GLP for "industrial products of modern biotechnology" may move forward. The most appropriate process would appear to be to convene a consensus workshop under the OECD's GLP Panel to address the application of the Principles of Good Laboratory Practice (published in OECD Environment Monograph No. 45) to biotechnology. In doing so, there may be points which require further elaboration to facilitate their application to "industrial products". This may require input, from the "industrial products" project, to the work of the GLP Panel. It would also be important to consider OECD work on pesticides, particularly with respect to the application of the Principles of GLP to microbial pesticides.

Report on Activities of the OECD Panel on Good Laboratory Practice: Mutual Acceptance of Data

Dr Hans Hosbach

**Federal Office of Environment, Forests and Landscape
Switzerland**

In the late 1970s and early 1980s, a number of OECD Member countries passed legislation to control chemical substances. This legislation usually required the manufacturer to perform laboratory and field studies, and to submit the results of these studies to governmental authorities for assessment of the potential hazard to human health and the environment.

Both government and industry were very concerned with the quality of the studies upon which such hazard assessments had to be based. As a consequence, several OECD countries began to establish national criteria and standards for the performance of these studies. Moreover, they introduced national procedures to control compliance with these criteria and standards.

However, the issue of data quality also has an international dimension and may impact considerably on international trade. To avoid different national standards and schemes of implementation that could impede international trade, OECD countries recognized the opportunity for international harmonization.

In 1981, the OECD Council adopted the Decision/Recommendation on Mutual Acceptance of Data. This Council Act recommends that, whenever tests are performed, the test data should be scientifically reliable and of highest quality, and that the means to provide this assurance are the use of OECD Test Guidelines and the application of the OECD Principles of Good Laboratory Practice (GLP). This Council Act also states that data generated in accordance with the OECD Test Guidelines and the OECD Principles of Good Laboratory Practice (GLP) shall be accepted by all Member countries for purposes of assessment and other uses relating to the protection of man and the environment. If individual countries can confidently rely on test data developed in other countries, duplicative testing can be avoided, thereby introducing economies in test costs and time.

OECD Test Guidelines

The first element of the OECD concept is the OECD Test Guidelines.

The supporting structure for developing new Test Guidelines or updating existing ones is provided by the OECD Test Guidelines Programme. A central position in this programme is assigned to "National Co-ordinators" from Member countries. National Co-ordinators make proposals for the work programme, and for priorities and review proposals for new or updated Test Guidelines. Scientific input, too, is obtained primarily through National Co-ordinators by

consulting experts nominated by Member countries. In addition, scientific societies may contribute either on their own initiative or by submitting proposals to any National Co-ordinator or, when requested formally by the OECD Secretariat, by contributing to the overall comments.

In order to achieve broad acceptance, the opinion of recognized experts and authorities in Member countries is requested at various stages of Test Guideline development. Depending on the extent and nature of the comments received from Member countries and the international scientific community, a formal OECD Workshop or an ad hoc Expert Meeting may be organized.

After final development, and subsequent editing and formatting by the OECD Secretariat, the Draft Test Guideline is submitted to the Group of National Co-ordinators and then to the Joint Meeting and the Environment Policy Committee for approval. Once these bodies have endorsed the proposal for a new Test Guideline, it is submitted to the Council with a request for adoption. The new or updated Test Guideline, once adopted, becomes an integral part of the Council Decision on Mutual Acceptance of Data.

For further information on this updating mechanism, I refer you to OECD Environment Monograph No. 76, *OECD Series on the Test Guidelines Programme No. 1: Guidance Document for the Development of OECD Guidelines for Testing of Chemicals* (Paris, 1993/1995).

Currently, 82 Test Guidelines have been approved by the Council. They cover four main sections:

- Physical-chemical properties, such as:

Melting and boiling points, water and fat solubility, adsorption/desorption, particle size distribution, partition coefficient octanol/water

- Effects on biotic systems, such as:

Ecotoxicity to algae and terrestrial plants, acute and reproduction toxicity to various aquatic organisms, effects on microorganisms, earthworms or birds.

- Degradation and accumulation

Biodegradability in water, sewage sludge and soil, bioaccumulation in aquatic organisms

- Health effects

Various tests in short- and long-term toxicology, genetic toxicology.

In addition to these 82 Test Guidelines, about 12 additional Draft Test Guidelines are in various stages of development.

Good Laboratory Practice

The second criterion for Mutual Acceptance of Data is adherence to the Principles of Good Laboratory Practice (GLP for short).

GLP is understood as a managerial concept which covers the organisational process and the conditions under which studies are planned, performed, monitored, recorded and reported. The purpose of this concept is to promote the quality and validity of test data to be submitted to regulatory authorities for the assessment of the health effects and the environmental impact of a test substance.

The GLP Principles are a set of approximately 100 principles. I will briefly describe what the different chapters require:

- Organisation of the facility

This aspect deals with the various responsibilities of management, which can and should delegate tasks. In GLP, however, management always remains fully responsible for the compliance of the facility with GLP.

- A key person in the GLP concept is the Study Director, who takes responsibility for an individual study by agreeing to and signing the study plan. The Study Director must ensure that the approved Study Plan is followed and that any deviations are fully documented.
- Personnel Management and the Study Director have to ensure that tasks are delegated only to appropriately trained and experienced people. An important issue for personnel is the exercise of safe working practices and appropriate health precautions to minimise risk to themselves and their surroundings, and to ensure the integrity of the study.
- Quality Assurance

The prime function of Quality Assurance in the GLP concept is to monitor the operations of the testing facilities, and to assess compliance with Study Plans and Standard Operating Procedures. QA, if functioning appropriately, will assure the credibility of the Testing Facility.

In GLP, Quality Assurance must be independent of the study. The optimal situation is that management establishes a QA Unit with dedicated staff. If this is not feasible, it is also possible to use a contract QA facility or an independent consultant, or to use staff from other units not involved in the study to perform QA functions.

- Facilities and equipment

Management must provide adequate facilities with respect to size, construction and location. The facilities must meet the requirements of the study. They must of course also be safe, and not pose a risk for the population and the environment. Facilities are not only laboratories but also field test sites. In GLP, the laboratory is where the study is being conducted.

- Test substances

Test substances must be well characterized and knowledge must exist on possible hazards. Records must be kept of the quantities received and used during the study, and of the fate of surplus test substance after termination of the study.

- Standard Operating Procedures

SOPs describe in writing all routine activities in the testing and the support areas. Management must sign and thus approve SOPs. SOPs must be directly available to all relevant personnel.

- Performance of the study

Before the start of a study, a written and authorized study plan must be available. Alterations to the study plan, as possibly needed during the course of the study, must be brought about by study plan amendments. All changes, modifications, revisions or additions to a study plan must always be agreed in writing by the Study Director.

The identification of the study is an absolute necessity and provides the key for data retrieval at any time during the study. For each study a Final Report must be prepared, which is signed and dated by the Study Director. He or she must also confirm in writing that the study was conducted in compliance with GLP. This implies that the conduct of the study was monitored and that the final report was audited by the QA Unit. For this purpose, the QA Unit issues a statement indicating the dates this QA work was done.

- Archives

The archives must be designed and equipped to ensure maximum security of the documentation. This includes documentation on removal and return of documents from and to the archive. All material retained in the archives should be indexed, so as to facilitate orderly storage and rapid retrieval.

The GLP Principles can be applied to a wide array of test substances. Typical test substances are pharmaceuticals, pesticides and industrial chemicals; but there are also other products tested under GLP such as cosmetics, veterinary drugs, food and feed additives.

The GLP Principles can of course be applied to the testing not only of chemical substances, but also of biological agents such as microorganisms used as pesticides or bioengineered plants released to the environment.

Despite its name, Good Laboratory Practice is not restricted to traditional laboratory tests such as toxicological or ecotoxicological studies or chemical analyses, but is also applied to field trials. Typical field studies include residue trials, metabolism studies in plants and animals, and studies on effects on mesocosm and ecosystems.

To improve and harmonize the implementation of GLP in Member countries, the OECD Council Act on Good Laboratory Practice was adopted in 1989. It consists of several recommendations to Member countries, namely:

- to establish national GLP compliance monitoring programmes;
- to designate GLP compliance monitoring authorities, charged with implementation of the programme;
- to exchange information internationally which is relevant to GLP Compliance.

Moreover, the Council instructed OECD subsidiary bodies to pursue a programme of work designed to facilitate implementation of this Act. This instruction has been and is the basis for the establishment of the OECD GLP Panel.

The recommendation of this Council Act to establish and implement national GLP compliance monitoring programmes means that national GLP compliance monitoring authorities must perform test facility inspections and study audits.

Test facility inspections are examinations of active labs which allow a general determination of GLP compliance by the inspected facility. In most countries inspections are conducted periodically, usually every two to three years. Study audits are reviews of ongoing or completed studies. Their purpose is to reconstruct partially or completely the conduct of a specific study. Such audits may be part of an inspection, or may be performed at the specific request of a regulatory authority which has received a study and either has to base an important decision upon the result of the study or has good reasons to believe the study is not of highest quality. Indeed, our experience shows that up to 10 per cent of all studies show serious deviations from the GLP Principles and therefore must be used for assessment with reservations.

Another element of the 1989 Council Act on GLP is, as I mentioned before, the establishment of an international information exchange procedure. At least once a year, the Monitoring Authorities of all OECD Member countries provide a list of all facilities they have inspected and found to comply or not comply with GLP. In addition, there is an exchange of information on specific studies or facilities found to seriously deviate from GLP.

Finally, the 1989 Council Act is the basis for the GLP Panel. This Panel comprises people from the GLP monitoring authorities of the Member countries, i.e. those persons responsible for GLP compliance monitoring programmes. The Panel tries to promote a common understanding of the OECD Principles and facilitate harmonized approaches to technical and administrative matters of GLP compliance monitoring. Its activities are annual meetings, periodic exchange of information on compliance, the organisation of consensus workshops on issues of general interest (e.g. computer data), the organisation of training courses for national inspectors, and last but not least the evaluation of each other's monitoring programmes by joint inspection and audits.

Application to biotechnology

Can the OECD Principles of Good Laboratory Practice also be applied to biotechnology? The answer clearly is YES. In fact, they are already applied in some Member countries for some types of biotechnological products. However, the experience is still very limited and most GLP compliance monitoring authorities do not yet have inspectors with the appropriate training and experience. So far, the GLP Panel has not given much attention to potential problems with monitoring studies using organisms as test substances. In several countries, however, there are Working Groups dealing with these questions. The information I can provide you today is mainly based on these national experiences.

Most Principles of Good Laboratory Practice are written in such a way that they can easily be applied to any kind of test, including testing of bioengineered organisms. This holds true for aspects of facility organisation, quality assurance, Standard Operating Procedures, performance of the study, or archiving. The use of genetically engineered microorganisms or transgenic plants, on the other hand, requires some new interpretations of the GLP Principles in three fields:

- 1) Personnel;
- 2) Facilities and equipment; and
- 3) Test substance

Since the biotechnology field is very specialized, it is particularly important that qualified individuals hold the key positions on the team performing the biotechnology study. In particular, those in positions of responsibility such as the Study Director need to have the appropriate education, training and experience in the field of biotechnology.

Of course, any inspector will also be concerned with the safety of the working practice required for the type of work being done. To evaluate the laboratory's handling procedures, the inspector may interview laboratory personnel, examine SOPs, observe laboratory practices, and review control logbooks. He or she may also check the procedures for waste recovery and disposal and for decontamination.

The GLP Principles require that the test substance be identified and characterized, as appropriate for the type of study. A key issue with a bioengineered test substance is therefore the identification of the organism. The taxonomic identification of a microorganism involves the use of standard tests to determine individual characteristics of the microorganism which,

collectively, lead to exact identification. Identification may include genotypic and phenotypic characteristics. Laboratories would need a Standard Operating Procedure describing the fashion in which the taxonomic identification should be made.

Should the laboratory know the identity of the organism from external sources, through a letter of identification, then tests would only need to be done to verify the identification. Traits to verify taxonomic classification include morphological, biochemical, immunological and physiological characteristics.

The testing facility also has to assure that the cultures of microorganisms or cells are pure and that they are stable under current storage conditions. The GLP inspector would of course also verify whether the bioengineered test substance was tested for infectivity, pathogenicity or toxicity. All notebooks, worksheets, computer printouts, and calculations pertaining to such tests could be subject to examination. The inspector would try to find answers by interviewing people concerning how they selected the animal that was tested, what kind of technique was used for application, treatment or dosing, how long the observation period was, etc.

The characterization of plants as test substance is generally simpler. It would include information on the biology of reproductive potential and questions of persistence in an environment outside the test plot.

The facilities used for biotechnology studies must be adequate to enable the proper conduct of the study. The design of the facilities must provide appropriate space, environmental conditions, containment, decontamination areas and support systems for the study being conducted. The facility design should also take into account the biosafety controls required for the organisms used in the study and the need for maintaining a controlled environment.

The GLP inspector may evaluate both the laboratory facilities and any separate facilities, such as greenhouses and field sites. He or she may also examine the capacity and maintenance of equipment used during the development and processing of the microorganisms or plants, including bioreactors, sterilization equipment, and waste recovery, decontamination and safety equipment.

Ladies and gentlemen, I hope I have given you a sufficient overview of the activities of the OECD GLP Panel and how its activities fit into the concept of Mutual Acceptance of Data. As I said earlier, our experience in applying GLP to biotechnology is still very limited. Most of my information is based on documents I have received from the United States Environmental Protection Agency, which is one of the GLP compliance monitoring authorities active in the field of biotechnology. If you need more information on how biotechnological facilities are monitored in the United States, and what tests are needed for the identification of a biological agent as test substance, I'd like to refer you to the documentation of this agency.

Many national GLP authorities are aware, however, that there is a new field of work in front of them. At its last meeting the OECD GLP Panel also agreed that the time is ripe for the development of a guidance document on the application of the GLP Principles to the safety testing of biotechnology products, and that an OECD Consensus Workshop should be held on this issue. In my view it would be highly desirable to make these attempts in collaboration with experts from this Group.

PRESENTATIONS

The Current Status of Bioremediation Study in Japan

Dr Tsuguyoshi Suzuki

**The National Institute for Environmental Studies
Japan**

In Japan as well as in the other developed countries, the application of biotechnology has progressed to a remarkable extent in the medical, chemical, agrochemical, food, environmental and other fields. Among the various environmental reasons for using living organisms (bioremediation, bioleaching, biomining and others), bioremediation has been of particular concern recently since considerable soil contamination has been detected and environmental amelioration has become an area of environmental administration. As you may well know, atmospheric pollution problems and some surface water pollution problems have been improved significantly in recent years in Japan. However, the contamination of soil and ground water by heavy metals and organic chemicals has emerged as a new problem. To cope with this problem, the Japanese Environment Agency (JEA) established Environmental Quality Standards for Soil in 1991. These standards need to be supported by preventive and curative measures, of course; we need the new technology of countermeasures.

Our situation regarding soil and ground water pollution is going to become still worse. In 1993, according to a nationwide survey by the Japanese Environment Agency (JEA), 177 cases of soil/ground water contamination were reported. The relevant industries were chemicals, electroplating, electrical appliance manufacturing and others, and the major contaminants were heavy metals such as lead, mercury, hexavalent chromium, etc. and organochlorine compounds such as trichloroethylene and tetrachloroethylene.

A variety of countermeasures have been applied to reduce the burden of environmental contaminants. They mainly involve physico-chemical technologies, including containment using concrete pits or waterproof sheets in combination with pretreatment by stabilization or solidification.

In addition to these time- and money-consuming technologies, biotechnology use has been discussed but not yet actually applied. Many studies on the application of biotechnology to environmental remediation have been conducted at various institutions such as national research institutes, universities, and private industries.

Our institute, the National Institute for Environmental Studies (NIES), which belongs to JEA, has also been active in promoting studies on environmental remediation using microorganisms (i.e. bioremediation), including studies on air pollutant-resistant and nitrogen oxide-absorbing plants, as well as on the use of microcosms to evaluate the effects of recombinant microorganisms on ecosystems. It is also becoming crucial to study the environmental distribution and characteristics of microorganisms utilised for bioremediation in relation to human exposure and health.

In 1993, in order to promote studies on the environmental application of biotechnology and the evaluation of its environmental effects, the laboratory for environmental genetic engineering was built at the National Institute for Environmental Studies.

Along with these scientific activities at the NIES, JEA established a committee to study policy in regard to environmental aspects of biotechnology. Studies started in March 1994, related mainly to the evaluation of methods for assessing environmental effects necessary for the promotion of bioremediation. One of this committee's objectives is to achieve consensus on a procedure in the concerned population. Socio-psychological studies will be necessary on the information and actions required to obtain public acceptance.

In relation to public acceptance, we cannot forget the public's recognition or image of the role of microorganisms in daily life. Hitherto only the pathogenicity of microorganisms has been emphasised on every occasion during health education. Thus laypeople tend to consider any type of microorganism as dangerous. They also tend to feel that genetically modified microorganisms are more dangerous. Their anxiety will increase when they hear specialists articulating the uncertainties in a scientific manner. To change the public image, we need a comprehensive perspective of microorganisms in the environment. On that basis, we can extend educational activities to the public

Environmental microbiology is a new-born baby, and it will grow in the ecological context in various senses. To promote the application of modern biotechnology for environmental purposes, I have to say that there is a necessity to invest in fundamental environmental microbiology.

Anyway, I would like to express our expectation of a fruitful achievement of this Fribourg Workshop on Industrial Products of Modern Biotechnology Intended for Release to the Environment, and to add that we hope to cooperate with the other countries in the framework of OECD's Environment Policy Committee, which may make possible better information exchange for sustainable use of biotechnology.

Report on a Feasibility Study of a Compendium of Common Answers to Specific Safety Questions

Dr Penny Bramwell and Dr Mark Bailey

**NERC Institute of Virology and Environmental Microbiology
Oxford, United Kingdom**

This study represents an initial feasibility study to compare three different regulatory systems with a view to establishing the degree of commonality in information requirements for releasing genetically modified microorganisms into the natural environment. Time limitations have not permitted the comparison of regulations in all OECD countries; hence three contrasting systems representative of the UK, USA and Canada have been studied in detail. However, should further work be done in this area, it is recommended that the study be widened to include regulatory systems from other OECD countries. Investigations were made by speaking in person with members of the Biotechnology Unit, UK Department of the Environment; Toxic Substances and Chemical Act (TSCA), the Environmental Protection Agency (EPA), USA; and Environment Canada and Health Canada. The study will conclude in the production of a monograph containing generic answers to common safety questions on the release of genetically modified (GM) pseudomonads into the natural environment.

The documents that formed the basis for the comparison of information requirements were:

- 1) UK/EC 89 information requirements (EC directive 90/220);
- 2) USA Points for Consideration;
- 3) Canada Schedule XIV.

Superficially, the three regulatory systems appear very different. The UK regulates all genetically modified organisms planned for release into the environment. In contrast, the USA only regulates inter-generically modified microorganisms planned for release, while Canada regulates all microorganisms both modified and unmodified under both field and contained conditions.

The aims of the feasibility study are as follows:

- 1) to compare the UK, US and Canadian information requirements for release of a GMM into the environment;
- 2) to assess whether common safety questions require information of the same quality and quantity;
- 3) to identify common answers to key safety questions for GM pseudomonads.

Comparable experimental field releases of genetically modified *Pseudomonas aureofaciens* strains were carried out in the US and UK. The US strain was modified by inserting the chromogenic *LacZY* gene into the chromosome, while the UK strain was modified by the insertion of the *LacZY* gene and a *xylE*-kanamycin resistance cassette into two different sites in the chromosome. These studies allowed the comparison of the safety questions posed by the regulatory authorities and the information supplied. The Canadian system was assessed hypothetically to establish the information requirements for this type of case study.

OECD Environment Monograph No. 100 (*Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology*) highlighted significant commonality in information requirements between OECD Member countries. The appendices in the OECD "Blue Book", *Safety Considerations for Recombinant DNA Technology* (1986), detailing key "General Scientific, Human Health and Agricultural and Environmental" considerations formed the basis of the Environment Monograph and hence of this comparison. The accompanying tables offer a brief description of some of the information accepted by the UK and US, and that would also be acceptable to Canada for this type of case study. In general, good commonality was observed among the three regulatory systems (**Table 1**).

Given the high degree of commonality between information requirements, the next consideration was to identify those requirements that are universally required for an application in any country and which would be central to the Mutual Acceptance of Data (MAD) elements throughout OECD countries. Common safety questions were associated with the strain characteristics and identity, including its ecology, pathology and toxicity. The history of any genetic modifications, constructs, site(s) of insertion, etc. also comprises safety elements that would be common to all applications and central to the MAD. A subset of these information elements contains questions that might have generic answers, i.e. that are generic to a particular species, genus, etc. Areas that should be examined for the presence of generic answers include the geographical distribution and abundance of the strain, its ecological characteristics, phenotype, phenotypic and genotypic stability, and contingency plans for controlling the spread of the organism or decontamination of the site.

The proposed UK DoE monograph will attempt to highlight where generic answers may be formulated, and what these are for pseudomonad GMMs. Two schemes are proposed for the incorporation of the MAD elements based on strain characteristics within regulatory systems. In the first scheme (**Figure 1**) the strain characteristics form a basic data package that is applicable to any application to release a GMM, whether it is considered agricultural, bioremediation, mineral mining or any other. Further information requirements will be required that are specific to the type of application, site of introduction, etc., which may be case- or application-specific.

The second scheme (**Figure 2**) acknowledges that many regulatory systems are divided (for example, into sectors such as agriculture, bioremediation, etc.) to reflect specialist expertise in each area. In these instances this core of data elements will be applicable to an application being presented at any sector with the sector requiring further case-specific details about the site and environment of introduction, etc. The final figure (**Figure 3**) graphically illustrates how common information elements/safety questions concerned with the characteristics of the strain may form the basis to a data set of mutually acceptable data elements amongst OECD countries. A subset of these elements contain information that is common to all applications involving that species/genus of microorganism and may have a generic answer.

Finally, we would like to thank the UK Department of the Environment (DoE) for funding this study and the UK, DoE, US EPA, TSCA and Health and Environment Canada for their help and co-operation in carrying out this study.

Table 1
OECD Recombinant DNA Safety Considerations
(Based on *Recombinant DNA Safety Considerations*, OECD 1986)

General Scientific Considerations	UK	USA	Canada
A. Characteristics of donor and recipient organisms			
1 a) names and designation	FAMES	Y	Y
	BIOLOG	API	Y
	LOPAT I & II tests	Y	Y
	Expert verification	Y	Y
1 c) Characteristics of the organism which permit identification and the methods used to identify the organisms	Antibiotic resistance	Y	Y
	Heavy metal resistance	N	Y
	FAMES/BIOLOG	Y	Y
	DNA fingerprint	Y	Y
1 d) Techniques employed in the laboratory and/or environment for detecting the presence of or for monitoring numbers of the organism	Selective plating	Y	Y
	FAMES	Y	Y
	DNA fingerprint	Y	Y
1 e) Source of the organism	Accession no., isolation details	Y	Y
		Y	Y
1 f) Information on the recipient organism's reproduction cycle (sexual/asexual)	Generation time	Y	Y
A 3) Pathogenic and physiological traits of donor and recipient organisms			
a) Nature of pathogenicity and virulence and infectivity	Literature search	Y	Y
	Growth at 37°C	Y	Y
	LOPAT I & II	Y	Y
Toxigenicity	N	N	N
B. Character of engineered organism			
a) Description of the modification	Phenotypic and genotypic characterisation	Y	Y
b) Description of the nature, function and source of inserted donor nucleic DNA, including regulatory or other elements affecting function of DNA and of the vector	Sequence/RFLP data	Y	Y
c) Description of the method(s) by which the vector with insert(s) was constructed	A description of the techniques used	Y	Y

d) Description of methods for introducing vector-insert into recipient organism and procedure for selecting modified organism	A description of the techniques used	Y	Y
f. i) Characterisation of the site of modification of the recipient genome; stability of the inserted DNA.	Sequence, hybridisation and PCR data	RFLP, hybridisation	
f. ii) Stability of the inserted DNA	Stable over 200 generations in liquid media	Stable over 110 generations in liquid media	Y
h) Rate and level of expression of the introduced genetic material; method and sensitivity of measurement.	Constitutive	Y	Y

Human Health Considerations

Characteristics of engineered organism

1. Comparison of the engineered organism to the recipient organism regarding pathogenicity	Competition experiments	Y	Y
2. Capacity for colonisation	Glasshouse/microcosm survival data	Y	Y
3 i) toxicity	Y	Y	Y
3 j) allergenicity	Y	Y	Y

Environmental and Agricultural Considerations

A. Ecological traits relating to donor and recipient

a) Natural habitat and geographic distribution; climate of original habitats	Y	Y	Y
b) Significant involvement in environmental processes	Saprophyte	Saprophyte	Y
d) Interactions with and effects on other organisms in the environment	Y	Y	Y
e) Ability to form survival structures	Y	Y	Y

B. Application of the engineered organism in the environment

A1) Geographical location of the site	Oxford/Littlehampton, UK	Clemson Univ., USA	Y
b) Site description including size, preparation, climate, etc.	Y	Y	Y
d) Introduction protocols including quantity and frequency of application	3 X 10 ¹⁰	4.8 X 10 ¹³	Y
e) Methods of site disturbance or cultivation	Normal agricultural practices	Agricultural practices/crop rotation	Y

C. Survival, multiplication and dissemination of the engineered organism in the environment

a) Description of detection, identification and monitoring techniques	<i>Pseudomonas</i> selective agar amended with X-gal and kanamycin	<i>Pseudomonas</i> selective agar amended with rifampicin and naladixic acid	Y
---	--	--	---

D. Interactions of engineered organism(s) with biological systems

1 e) Identification and description of non-target organism(s) which might be exposed	Other plant rhizospheres	N	Y
3 a) Routes of dissemination, physical or biological	Environmental (wind, rain, etc.), vertebrates, invertebrates	Y	Y

E. Potential environmental impacts

1 b) Known or predicted effects on other organisms in the environment	Data gathered on five other microbial communities	Data gathered on the effect of recombinant on mycorrhizas	Y
2 b) Potential for excessive population increase	Y (contingency plan)	Y	Y

Key: Y = a common data requirement; N = a difference in data requirements; X-gal = 5-bromo-4-chloro 3 indoyl β-0-galactopyranosidase; RFLP = restriction, fragment, length and polymorphism; LOPAT = a set of tests to differentiate the saprophytic fluorescent pseudomonads from the phytopathogens – they comprise levan formation, an oxidase test, potato rotting ability, arginine dihydrolase production and tobacco hypersensitivity; FAMES = fatty acid methyl esters; PCR = polymerase chain reaction; BIOLOG and API = commercially available identification kits.

Figure 1

Scheme to illustrate the inclusion of strain characteristics as a basic data package that is applicable to all GMM releases (regardless of application) and might form the basis of the MAD elements amongst OECD countries

Mutually Acceptable Data (MAD)

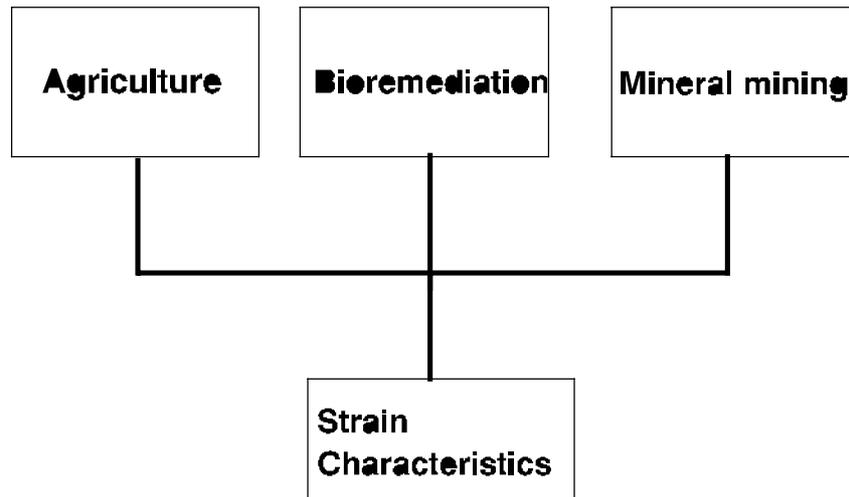


Figure 2

An alternative scheme for the mutual acceptance of data applicable to regulatory systems that have sectors that specialise in, for example, agricultural, bioremediation applications, etc.

Alternative MAD Scheme

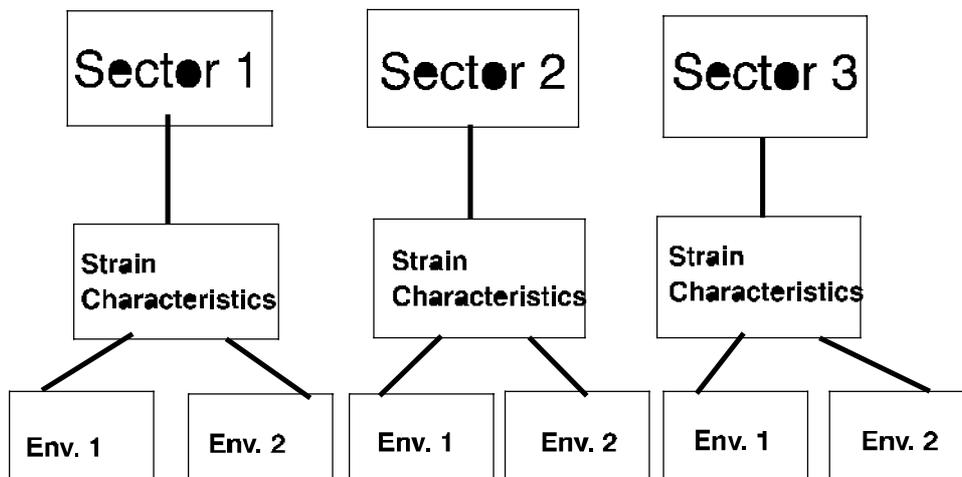
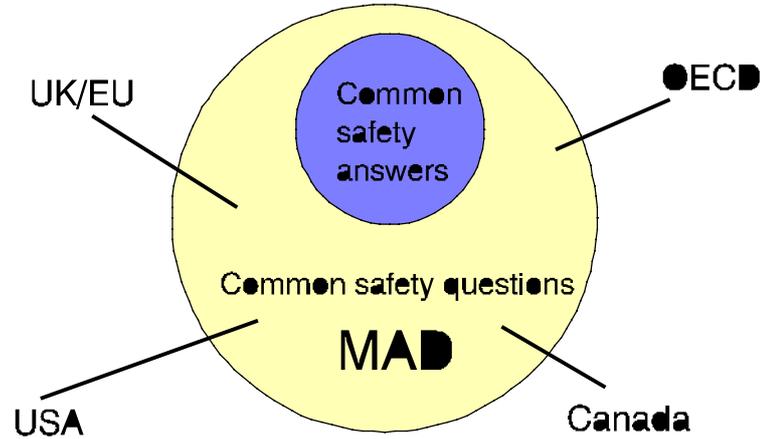


Figure 3

Scheme to illustrate that the mutually acceptable data package has a subset of data elements which will have generic answers at the species or genus level of the GMM

Common answers to safety questions



**Comparative Case Study:
Release of a Genetically Modified Microorganism in the UK:
*Pseudomonas aureofaciens***

Dr Firoz Amijee

**Department of the Environment
United Kingdom**

The experimental release of a genetically modified microorganism (GMM), *Pseudomonas aureofaciens*, containing selective marker genes was considered for this comparative case study because similar microorganisms were notified for release in the USA and UK. Also, pseudomonads are likely to be used for bioremediation purposes in the future. In this paper information supplied by the UK notifier, the Institute of Virology and Environmental Microbiology, Oxford, to assess the risk for the GMM release is outlined.

The purpose of the release was to assess the ability of the GMM to survive and spread in the phytosphere of wheat and sugar beet. Characteristics of the recipient, donors, vector and GMM, the release environment, and information on monitoring, control, waste treatment and emergency plans were supplied by the notifier.

The recipient was a natural component of the microbial community, as it was isolated from sugar beet leaves grown at the release site. The isolate was identified as *Pseudomonas aureofaciens* (SBW25): the genus by selective plating (utilisation of carbon source and fluorescence), species by chemotaxonomic methods (fatty acid methyl ester profile, FAME-MIS), and strain by genomic methods (restriction fragment length polymorphism). Tests were carried out to show that SBW25 was non-pathogenic to plants. A data base survey was used to show that *P. aureofaciens* was non-pathogenic to animals and plants. The antibiotic and heavy metal resistance profile for SBW25 was found to be similar as for other fluorescent pseudomonads.

The marker genes were obtained from two donor bacteria: LacZY gene from *Escherichia coli* for the GMM to grow on media containing lactose and to produce a blue product; Km gene also from *E. coli* for the GMM to grow on media containing the kanamycin; and XylE gene from *P. putida* for the GMM to convert catechol into a yellow pigment. A single copy of the marker genes was inserted at a non-essential chromosome locus by site-directed homologous recombination using a suicide integration vector. This modification was verified by Southern blotting. The genetic modification was designed to minimise the possibility of gene transfer and to minimise any metabolic disruption.

A number of studies were carried out in containment prior to the release. The results indicated that survival, spread, competition to test for fitness, and perturbation of indigenous microflora were similar for the GMM to those found for the recipient. The studies also showed that the GMM was stable over a period of 200 generations and that there was no evidence for gene transfer.

The GMM would be released through seed inoculation at the time of sowing to minimise dispersal. The numbers of GMMs released would be equivalent to approximately 10 per cent of the total pseudomonad population. An elaborate experimental design was used to allow for the extensive monitoring proposed for the release. Monitoring of the GMM would be in soil, roots and leaves of the host and any weeds growing at the release site. The monitoring would also include vertical and horizontal spread of the GMM. The frequency of the monitoring would be monthly in year 1, and bi-monthly in years 2 and 3 with review.

The risk to human health and the environment was assessed as low. The main considerations for this assessment were: the indigenous recipient, which was a phytosphere coloniser; the non-pathogenicity to plants, animals and humans; no evidence for antagonistic effects on microbial population; no increase in fitness and no selective advantage; stable insertion of the marker genes on to the chromosome with no detectable transfer frequency; no unknown sequence inserted; the phenotype from marker genes being common in the release environment; and the small scale of the release, in which limited numbers of GMMs were to be released.

Comparative Case Study: Risk Assessment for a US Field Release of a Recombinant Pseudomonad

Dr Philip G. Sayre

**Office of Pollution Prevention and Toxics
United States Environmental Protection Agency**

The first release of an intergeneric bacterium occurred at a Clemson University field site in South Carolina, and was followed over a period of approximately two years by Monsanto Inc. and Clemson researchers (**Figures 1 and 2**). Prior to this 1987 release, a risk assessment was conducted by the Environmental Protection Agency's Office of Toxic Substances (OTS). The objectives of Monsanto included determining whether the pseudomonad could be tracked over time in the wheat rhizosphere, and whether this pseudomonad exhibited any adverse effects. The OTS conducted a risk assessment prior to the release. The risk assessment for this release covered items listed in the OTS's "Points to Consider in the Preparation and Submission of TSCA Premanufacture Notices (PMNs) for Microorganisms" guidance document (**Figure 3**). These items included examination of the recipient taxonomy; the genetic manipulations, human health, ecological effects, and exposure considerations relevant to the recombinant; and a review of the field test protocol. Selected post-release data from the field test relevant to the risk assessment are also presented here.

The recipient was a soil isolate, obtained from the corn rhizosphere in Missouri, which was resistant to rifampacin and nalidixic acid. The taxonomic identity of the recipient was initially identified by Monsanto as a *Pseudomonas fluorescens*, based on an analysis conducted by the American Type Culture Collection using Analytab Products Inc. test strip phenotypic data. An additional fatty acid methyl ester analysis indicated that the isolate belonged to a related taxon, *P. chlororaphis*. Finally, a computerized numerical taxonomy method (MICRO-IS) indicated that the isolate was probably a *P. aureofaciens*. These three species are all in the same DNA homology group, and this group is one of three DNA homology groups in the *Pseudomonas* rRNA homology Group I. The DNA homology group in which the isolate was placed does not contain plant or opportunistic human pathogens, unlike the other two DNA homology groups. The genetic manipulations which resulted in the recombinant pseudomonad included addition of a well-characterized insert to the pseudomonad chromosome, using a plasmid that contained the insert between the Tn7R and Tn7L sequences (other plasmid-borne Tn7 transposition genes which were not integrated into the chromosome). The insert primarily contained the *lacZY* gene, driven by the *P_{aer}* promoter. Laboratory data confirmed the final construct.

Human health issues were considered to be of low concern for the field test. This conclusion (**Figure 4**) was based on literature for the recipient: of the three pseudomonad species, only *P. fluorescens* has rarely been isolated from compromised individuals. In addition, 28-day mouse toxicity tests indicated no toxic or pathogenic effects with the recipient, and the microorganism survived poorly at 37°C. The introduced DNA consists primarily of the

lac genes which are expressed by common human gut bacteria. Ecological concerns were also limited (**Figure 5**). Toxicity tests were performed with the recipient, including tests with quail, fish, *Daphnia* and corn (for effects on growth). The recombinant was tested for plant pathogenicity, and a similar recombinant pseudomonad was tested for its effects on soybean yields. After the initiation of the field test, tests to examine the effect of the recombinant on water quality testing and post-pasteurization spoilage of milk due to protease activity were also run. The survival profile of the recombinant microorganism in environmental media was estimated prior to release (**Figure 6**): its numbers underwent logarithmic decline in unfiltered lake water, and it survived no better than the recipient in corn and wheat rhizosphere tests. Tests on gene stability indicated the construct was stable after 110 generations under non-selective growth conditions. Review of the field test protocol (**Figure 7**) indicated that there was a low background of Rif^r and Nal^r native pseudomonads, RFLPs of native pseudomonads were unique compared to the recombinant, methods for application and decontamination were thoroughly described, and the field plot layout (**Figure 8**) and other characteristics were well described (plots and replicates, border areas, statistical design, slope, detection limits for recombinant in field samples, and soil type).

Given this information, it was concluded that the field test posed low risk to humans and the environment (**Figure 9**). This conclusion was reached by OTS and by an independent science review panel convened by EPA. Post-release information confirmed the findings of the risk assessment. The microorganism survived primarily in association with the wheat plant rhizosphere and declined to undetectable levels after 40 weeks. Very little horizontal or vertical movement of the pseudomonads was recorded, except for the populations associated with the wheat roots. There were no adverse effects of the recombinant on either wheat or soybean yields.

Figure 1
Risk Assessment for Field Release of a Recombinant Pseudomonad

- I. Introduction
 - a. Objectives of 1987 Field Test
 - b. TSCA Guidance – "Points to Consider"

- II. Pre-release Information Specific to this Release
 - a. Taxonomy
 - b. Construct
 - c. Human Health
 - d. Ecological Effects
 - e. Exposure Analysis
 - f. Field Protocol Review
 - g. Risk Assessment

- III. Post-release Data from Field Test
 - a. Exposure
 - b. Ecological Effects

Figure 2
Objectives of Field Test

- Monsanto – Monitoring and Field Performance
- EPA – Monitoring and Effects Analysis

Figure 3
Restructured TSCA Points to Consider

- Taxonomic Identification of Recipient and Recombinant Microorganisms
- Construction of Recombinant
- Health and Environmental Effects of Recombinant
- By-products, Production Volume, and Use Information
- Worker Exposure and Environmental Release
- Environmental Release Protocols

Figure 4
Were There Human Health Concerns?

Recipient

- Recipient belongs to group including *P. fluorescens*, *P. aureofaciens* and *P. chlororaphis*.
- Mouse 28-day tests (10^7 CFU/mouse via oral, ip., iv., it.).
No pathogenic or toxic effects (contract lab protocols).

Recombinant

- Construct information: *lacZY* genes from *E. coli* K-12, and *beta-gal* and lactose permease expressed in human intestine.

Figure 5
Were There Ecological Concerns?

Testing with Recipient

- 28-day quail tox/path (EPA GLP)
- 96-hour acute tox with fish and daphnia (EPA, APHA)
- 14-day *Daphnia magna* chronic tox (EPA, ASTM)
- 28-day earthworm tox

Testing with RN-3732-RN-LII

- Tobacco hypersensitivity
- Soft rot in potato (APS)
- Production of pectic enzymes (APS)

Milk (tests run in 1988)

- Proliferation in milk due to lactose utilization
- Spoilage of milk due to proteolysis

Water quality (tests run in 1988)

- Concern that lactose-utilizing pseudomonads would give false positives in standard water quality tests

Figure 6
Which Exposure Concerns Were Raised?

- Lactose utilization and increased survival/competitiveness
- Gene transfer
- Survival in wheat rhizosphere and fresh water

Figure 7
What Risk-related Information Was in the Field Protocol?

- Background Rif^r, Nal^r native pseudomonads
- RFLPs of native pseudomonads
- Method of application and decontamination
- Field plot layout and other field site characteristics

Figure 8
Field Plot Layout

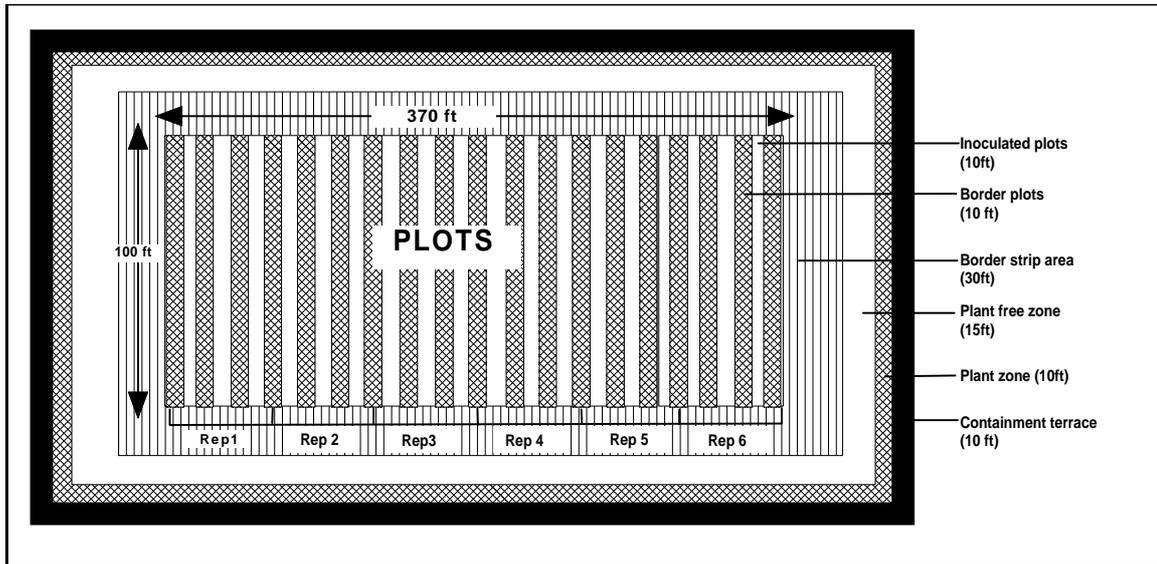


Figure 9
What Was the Overall Risk Posed by the Field Test?

- Low risk to human health
- Low risk to the environment
- Areas of uncertainty
- Testing prior to large-scale release
 - Water quality testing
 - Post-pasteurization spoilage of dairy products

Findings of the 1993 US EPA-Environment Canada Bioremediation Risk Assessment Workshop

Dr Philip G. Sayre

United States Environmental Protection Agency

The 1993 US EPA-Environment Canada Bioremediation Risk Assessment Workshop was composed of working groups investigating specific questions in five areas: 1) overall risk assessment schemes for assessing human and ecological health, 2) metabolic pathways and toxic metabolites resulting from microbial pollutant degradation, 3) ecological effects, 4) human health, and 5) fate considerations for microorganisms and metabolites.

The draft risk assessment scheme developed during the workshop (**Figures 1A and 1B**) is divided into four sequential steps. First, four different categories of pretest information should be available prior to the beginning of a field test. This pretest information is likely to be readily available since it is gathered to determine the success of the proposed test in degrading the target pollutant (efficacy studies), as opposed to determining the potential risk of the test. All four categories of information are described in Figure 1A, except for item IV which is directed towards the method by which the bioremediation microorganisms are applied to the site: land farming, *in situ* subsurface reinjection of ground water with introduced microorganisms, bioventing, etc. It is possible that, based on pretest information, a field test could be approved. If this is the case, then the assessment skips Steps 2 and 3, proceeding directly to Step 4. However, if concerns remain, then an exposure analysis should be done after Step 1 in order to lessen exposure to microorganisms and/or metabolites. This analysis, and subsequent analyses, may involve testing which goes beyond the information which is likely to be available as a result of efficacy studies already performed. If concerns cannot be mitigated through an exposure analysis, then a hazard assessment (Step 3) should be performed. Finally, a decision is made in Step 4 on whether to approve a given test. Approval may be obtained without conditions, or approval may be obtained with the stipulation that certain risk-oriented data be collected in the small-scale test (fate of the microorganism, generation of metabolites, etc.). After approval of the small-scale test, the process becomes iterative: additional field tests or commercial approval will be evaluated beginning again at Step 1 (changes in site characteristics, application techniques, etc. may change from site to site).

The biodegradable pollutants most commonly found at US and Canadian dump sites were grouped for discussion, so that chemicals with similar microbial degradation pathways were in the same grouping. The groups were: metals and inorganics, aromatic hydrocarbons, phenolic compounds, halogenated aliphatics, pesticides, aliphatics and alicyclics (non-halogenated), and nitrogen/sulphur heterocyclics. Within each group, representative model compounds were discussed to determine the extent of pathway information known and the occurrence of metabolites which could be of concern. Conclusions on each class of chemicals (and on specific chemicals within a group) were reached, but are too lengthy to note here. General conclusions focused on the importance of site information to determine metabolites,

the complications of predicting the metabolites of complex mixtures, and the ability of quantitative structure activity relationships to predict the toxicity of metabolites.

An ecological effects test scheme was also developed during the Workshop (**Figure 2**). The test scheme first addresses pathogenicity concerns for the recipient genus, then examines the pathogenicity traits encoded by any introduced DNA sequences. If pathogenicity is unlikely, then metabolite toxicity (resulting from partial degradation of parent pollutant at a site) issues are examined and testing recommended based on most likely exposures. This ecological effects testing scheme was further developed in a 1994 US EPA-Environment Canada Workshop (**Figures 3A and 3B**).

The working group for human health issues divided concerns for bioremediation agents into those which centre on single bacterial species (**Figures 4A and 4B**), versus bioremediation agents which are composed of a consortium of microorganisms (**Figure 4C**).

Finally, the working group on fate concerns recognized the inadequacy of most mathematical models for tracking the fate of introduced microorganisms and their metabolites in the subsurface. They also noted the utility of laboratory bioremediation tests with field sediments. Although these tests are usually run to determine the efficacy of the bioremediation agent, they also can be used to determine microbial survival and likelihood for toxic metabolite production in the field. Subsequent to the Workshop, the different engineering techniques for *in situ* and *ex situ* remediation were examined, since the engineering technique will often determine likely exposure scenarios (**Figure 5**). Finally, the United States National Institutes of Occupational Safety and Health (NIOSH) has standards for hazardous waste site worker protection that apply to chemicals such as polychlorinated biphenyls (**Figure 6**). These standards, which are designed to limit worker exposure to hazardous pollutants, may also limit worker exposure to bioremediation microorganisms and their metabolites.

Figure 1A

1993 EPA/EC TIER TEST SCHEME FOR ECOLOGICAL AND HEALTH ASSESSMENT OF BIOREMEDIATION ORGANISMS

STEP 1 - RISK ASSESSMENT BASED ON PRETEST INFORMATION

- I. Organism Characterization
Species (Donor and recipient, consortia)
Pathogenicity associated with species
Metabolic pathway information
- II. Site Characterization
Differences between on- and off-site characteristics
Contaminants present
Modes of mobility
- III. Microbial Interaction with Contaminant
Likely transformation products
Aqueous solubility of contaminant
Interaction of contaminant with other contaminants on site
- IV. Application Characterization
Engineering design for bioremediation
Containment provided by design

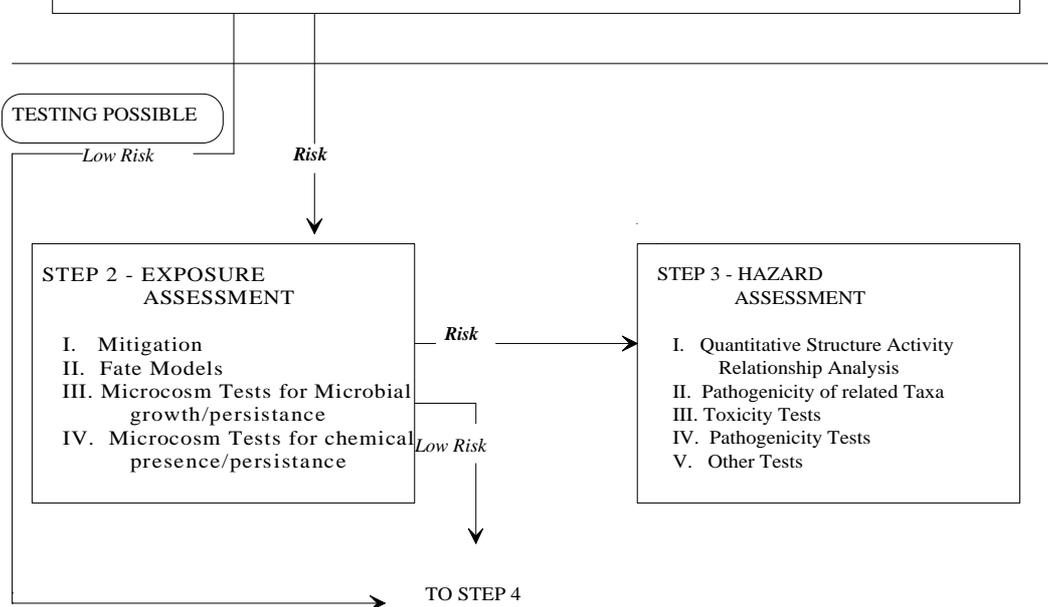
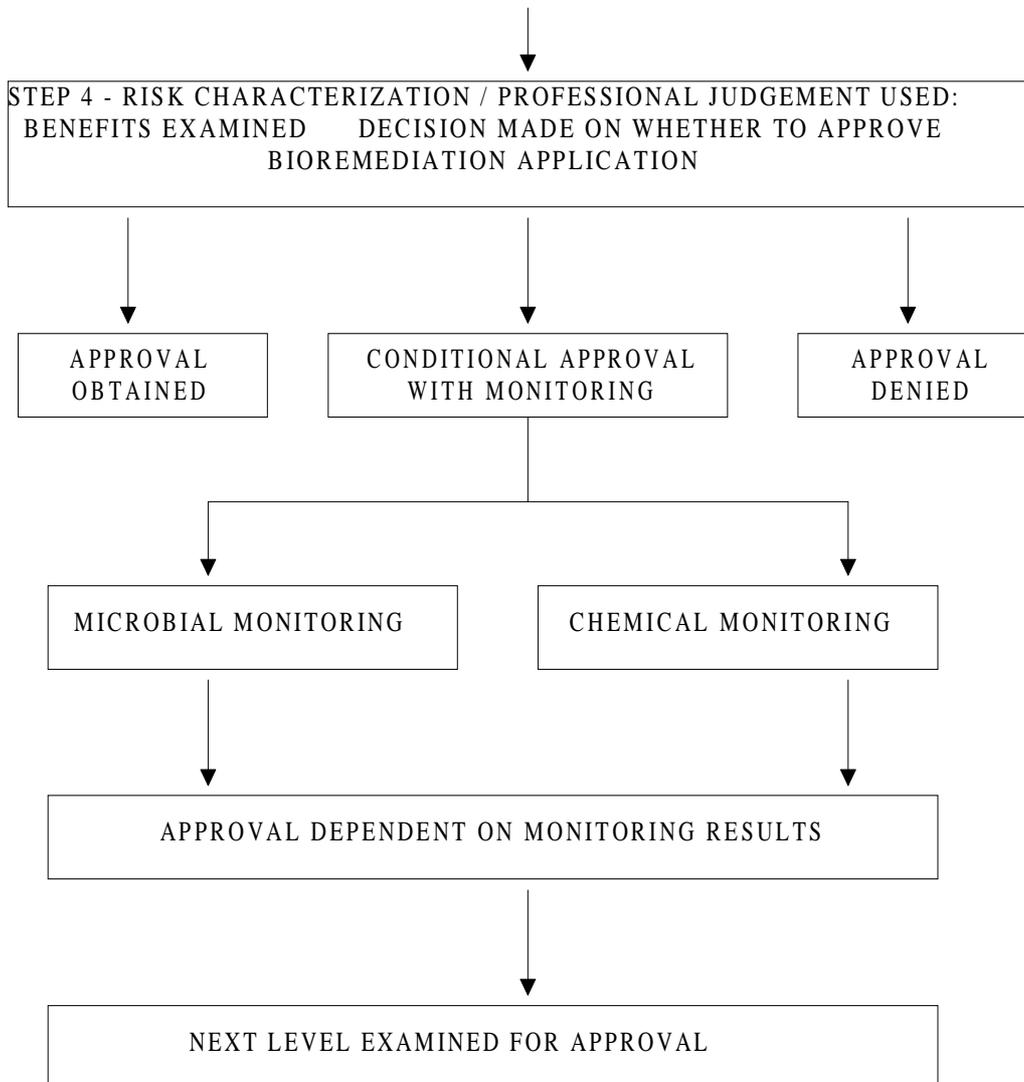


Figure 1B



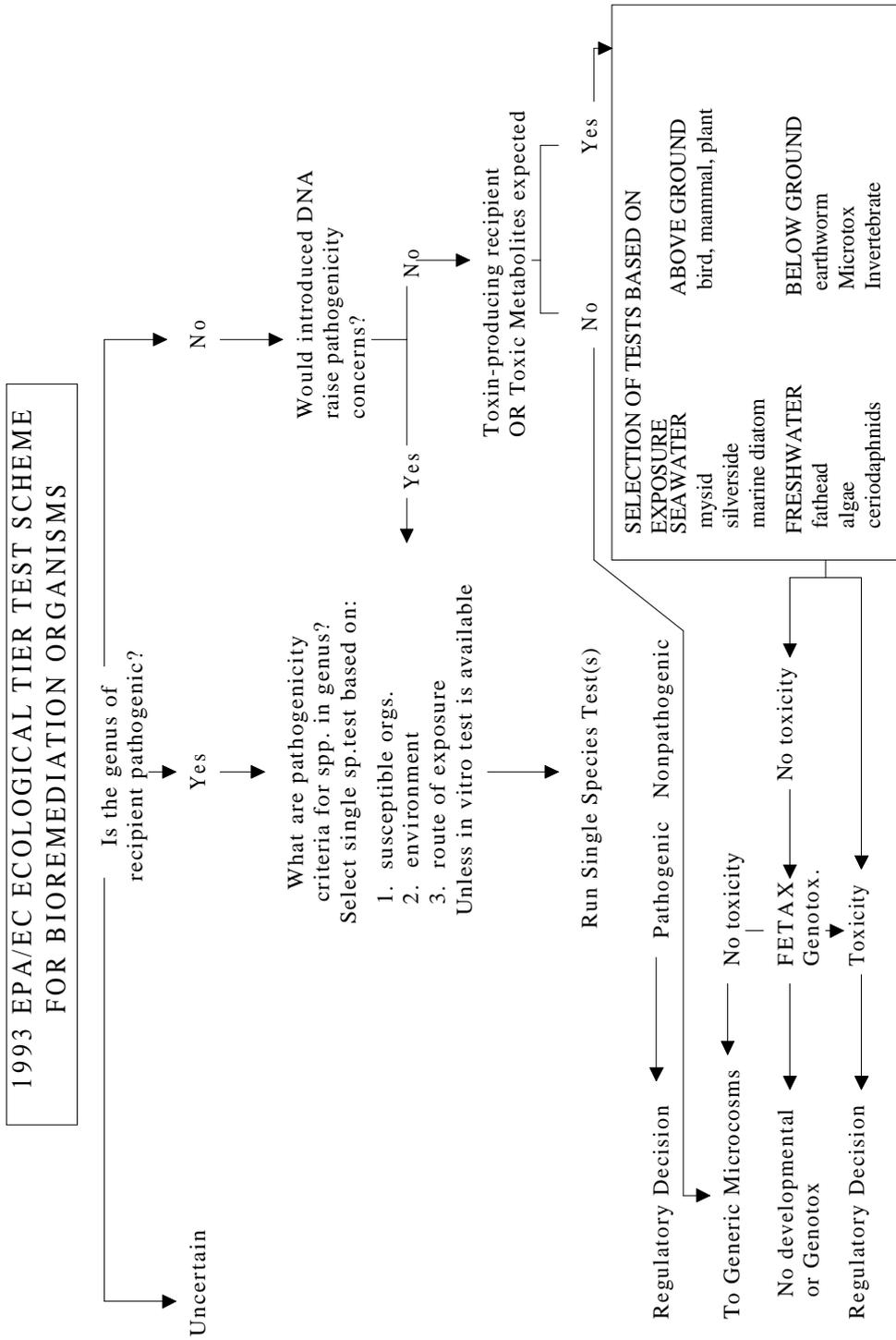


Figure 2

Figure 3A

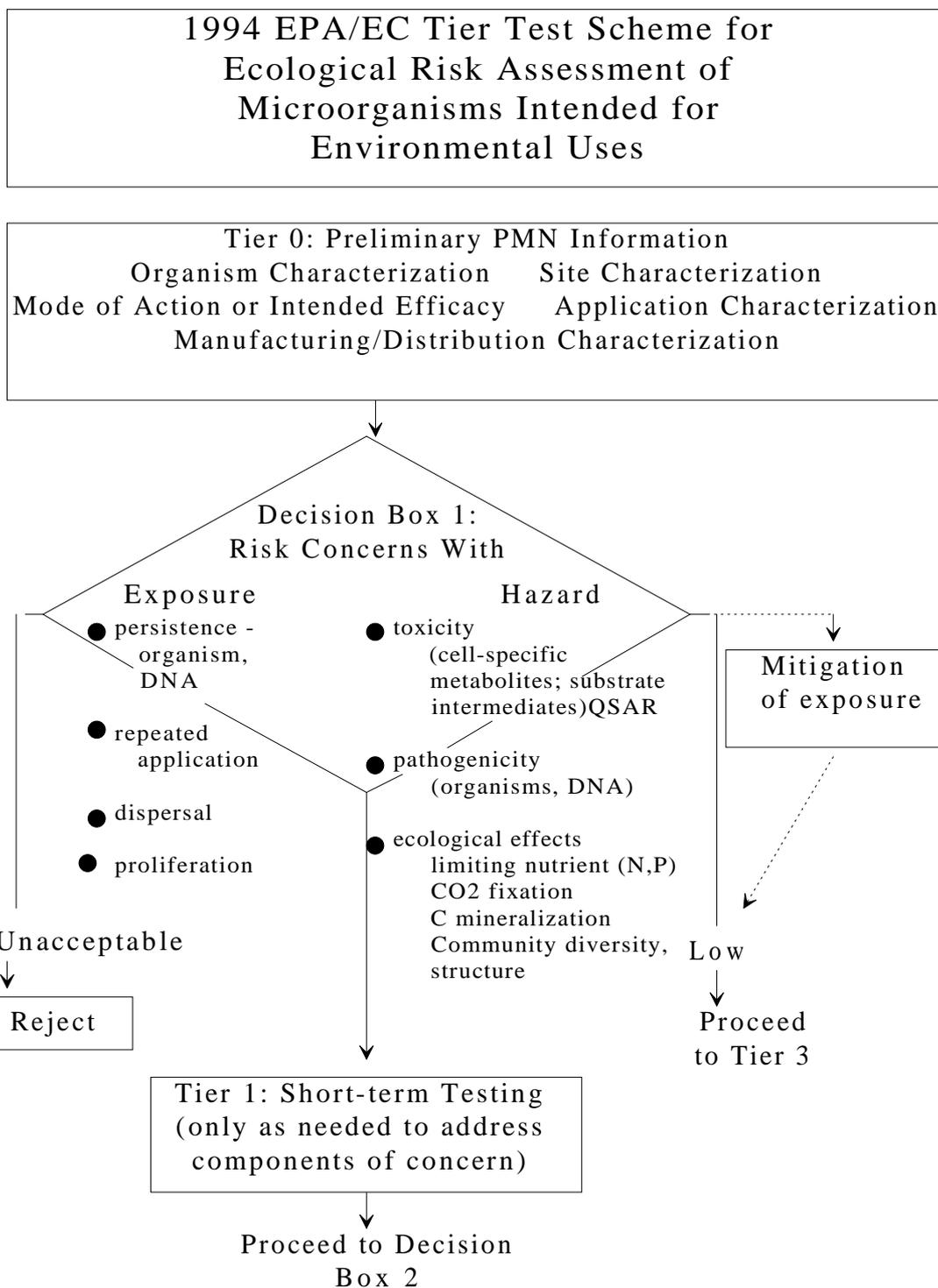


Figure 3B

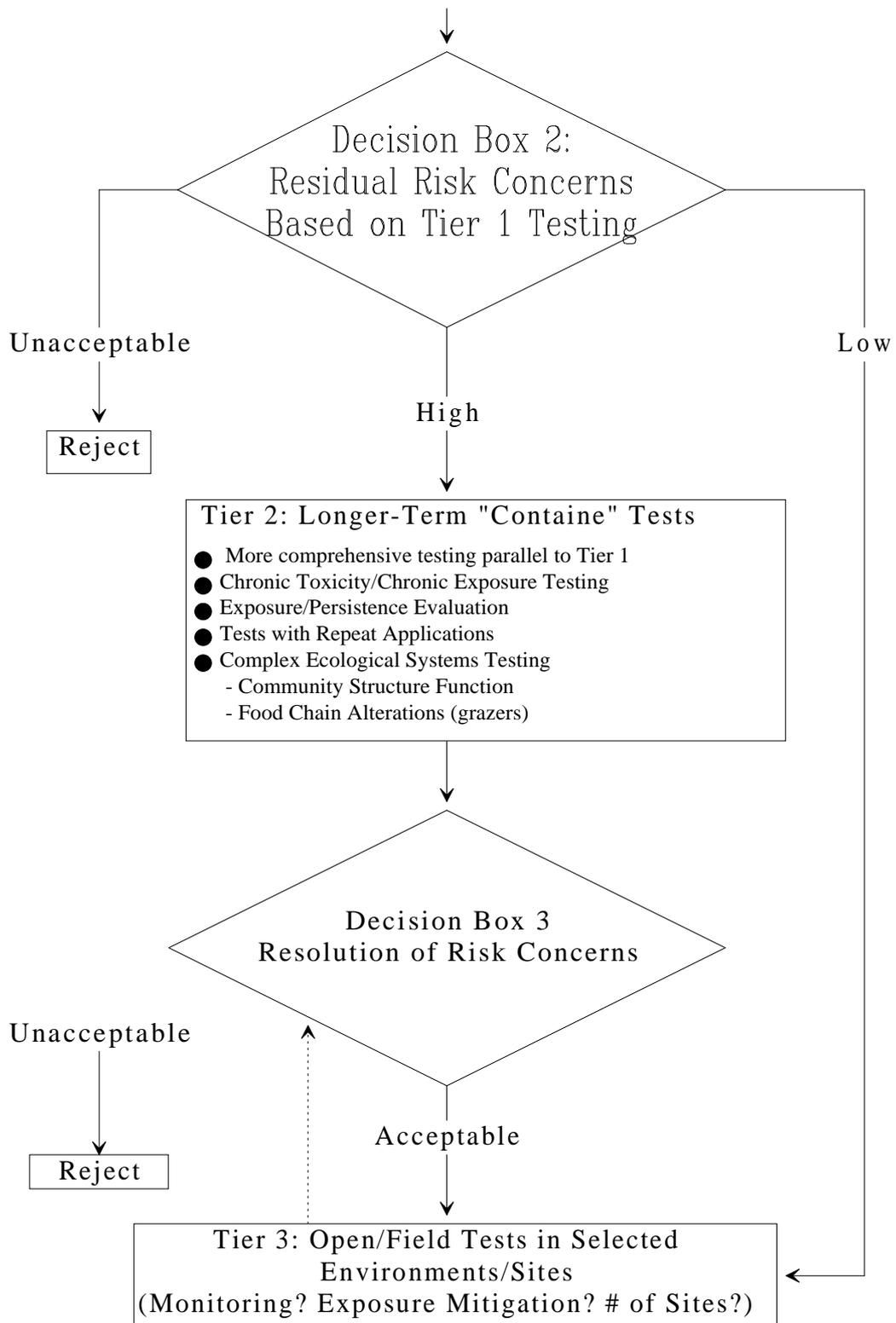


Figure 4A
Mammalian Health Effects Test Scheme

- Microbial Toxicity/Pathogenicity for a Single Microbe
 - Tier I Tests:
 - Taxonomic Identification
 - If pathogen, then only use in enclosed system (bioreactor)
 - If avirulent, then oral or pulmonary tox/path test
 - If unknown, then oral, pulmonary and systemic tox/path test
 - Allergenicity (case notification)
 - Tier II Tests:
 - Immunotoxicity

Figure 4B
Mammalian Health Effects Test Scheme

- Metabolite(s) Toxicity
 - Tier I Tests:
 - Chemical analysis
 - *In vitro* genotox
 - (Ames or Prophage induction, chromosome aberration)
 - FETAX?
 - Other rapid bioassays
 - Tier II or III Tests:
 - *In vivo* genotox
 - Teratogenicity
 - Carcinogenicity
 - Behavioural
 - Neurotoxicity
 - Immunotoxicity

Figure 4C
Mammalian Health Effects Test Scheme

- Microbial Toxicity/Pathogenicity for a Consortium
 - Tier I Tests:
 - Taxonomic Identification
 - If pathogen, then only use in enclosed system (bioreactor)
 - If avirulent, then oral, pulmonary and systemic tox/path test
 - If unknown, then oral, pulmonary and systemic tox/path test
 - Allergenicity (case notification)
 - Tier II Tests:
 - Immunotoxicity

Figure 5
Effect of Different Engineering Techniques
on Microbial and Chemical Exposures

***In situ* remediation**

Engineering tech.	Microorg. expo.	Chemical expo.
Saturated zone – Ground water reinjection	Hydrogeology? Above-ground treatment tech.?	Hydrogeology? Above-ground treatment tech?
Saturated zone – Air sparging	Limited (hydrogeology)	Vapour extraction? Water table mounding?
Vadose zone – bioventing	Limited (hydrogeology)	Vapour extraction?

***Ex situ* remediation**

Engineering tech.	Microorg. expo.	Chemical expo.
Slurry phase	Mixing; aeration	Mixing; aeration
Land farming	Mixing; moisture	Non-volatile
Soil bed reactor	Depth; leachate collection system?	Depth; leachate collection system?

Figure 6
NIOSH Standards for Chemical Hazards

Chemical	Exposure Limit~	Personal protection	Respirator
Aroclor 1242	1 µg/m ³	Avoid possible skin contact	Self-contained breathing apparatus; air-supplied positive-pressure respirator; full-face respirator with organic vapour canister & HEPA filter (at any concentration)

Partial Risk Assessment for a PCB-degrading *Pseudomonad*

Dr Philip G. Sayre

United States Environmental Protection Agency

Preliminary information on two recombinant pseudomonads, with either plasmid- or transposon-borne genes, for aerobic degradation of polychlorinated biphenyls (PCBs) was reviewed to assess the safety of their use in a small-scale field test (**Figure 1**). The rationale for designing the pseudomonads and the aerobic degradation process for PCBs (**Figure 2**) and the rationale for designing the recombinant pseudomonads (**Figure 3**) were discussed. These recombinant pseudomonads can degrade congeners up to heptachlorobiphenyls aerobically in the absence of toxic chemical inducers. Further, these pseudomonads are able to utilize a unique carbon substrate which most bacteria cannot metabolize: a surfactant which solubilizes PCBs, making them more bioavailable for degradation.

A partial risk assessment for this microorganism was presented, based on the risk assessment scheme provided from the 1993 EPA-Environment Canada Bioremediation Risk Assessment Workshop (see previous paper, "Findings of the 1993 US EPA-Environment Canada Bioremediation Risk Assessment Workshop," Figures 1A and 1B). Items examined included partial information on organism characterization, site characterization, microbe-contaminant interaction, and engineering/application characterization.

Organism characterization information available led to fairly complete descriptions of the two recombinants (**Figure 4**). In both cases, the recipient microorganism was characterized as a *Pseudomonas putida*, although some outstanding questions still need to be examined to confirm this identity. In both constructs, *bph* or biphenyl-degrading genes have been added from *Pseudomonas sp strain ENV307*. These are the genes that allow aerobic degradation of higher congener PCBs in the absence of toxic inducers. Although there are taxonomic uncertainties in identifying the species of the *bph* donor microorganism, it may only be necessary to examine the *bph* sequences to determine if any risks are posed by this introduced DNA. The substrate range of the *bph* genes was also examined to see if hazard issues would arise due to degradation of biologically important materials: the substrate range appears to be confined to PCBs and other aromatic substrates such as toluene. Differences in the two recombinants arise from the different vector DNA used to introduce the *bph* genes into the recipient microorganisms: one construct involves carriage of these biphenyl-degrading genes on a plasmid derived from plasmid pRK2; the second construct involves the use of a disarmed Tn5 transposon to stably insert the *bph* genes into the chromosome of the recipient pseudomonad.

Site characterization and the microbe-contaminant interactions were examined next. For this small-scale field test, the site chosen (**Figure 5**) was an isolated one owned by the Tennessee Valley Authority (an electrical power generation company). PCBs there have leaked from an electrical capacitor bank, where they were used, primarily into the gravel-soil interface where the estimated concentration is 5 to 140 ppm. Although the PCBs are bioavailable to some degree, there are few bacteria present in this material. Tests run with

bacteria at the site showed that they were incapable of aerobically degrading the PCBs, even in the presence of added nutrients, pH adjustments and surfactants. Microbe-contaminant interactions could result in the formation of 1) PCB metabolites such as chlorobenzoates (toxic to terrestrial vascular plants) and 2) surfactant metabolites such as nonylphenols (toxic to aquatic organisms). These metabolites are likely not to pose risks due to low concentrations and limited exposures. Mobility of PCBs and metabolites is also unknown at this time, but added surfactants are intended to increase aqueous solubility of PCBs and therefore enhance mobility of the PCBs and metabolites. There were no predicted problems due to the interaction of co-occurring contaminants at the site; additional contaminants could be toxic to introduced bacteria and/or lead to a greater variety of metabolites. However, there were no known contaminants with the possible exception of low levels of herbicides applied to the site several years earlier.

Application characterization, the last category of pretest information (Step 1 of Figure 1A in the previous paper), was not yet available for consideration.

Since all Step 1 information was unavailable, the exposure and hazard assessments (Steps 2 and 3) are incomplete. Limited information on application characterization included the amount of interface material to be remediated. The most likely engineering design will probably involve landfarming of the interface material. Containment may be provided if the landfarming is conducted on an impermeable liner; also, a concrete pad below the capacitor bed material may provide some amount of containment if an *in situ* engineering technique is selected. Efficacy data was provided which gave insight into the fate of the PCBs and recombinant microorganisms in site soils. Although no degradation occurred in the absence of added microorganisms (**Figure 6**), PCBs were degraded and there was measurable plasmid loss from one of the constructs after 32 days in sterile soil microcosms tests (**Figure 7**). Finally, worker exposure to both PCBs and microorganisms may be limited if workers wear the protective clothing and respirators recommended by the National Institute for Occupational Safety and Health for workers who handle PCBs.

Figure 1
Partial Risk Assessment for a PCB-degrading Pseudomonad

- 1) PCB degradation
- 2) Design of recombinant pseudomonad
- 3) Duluth workshop guidance

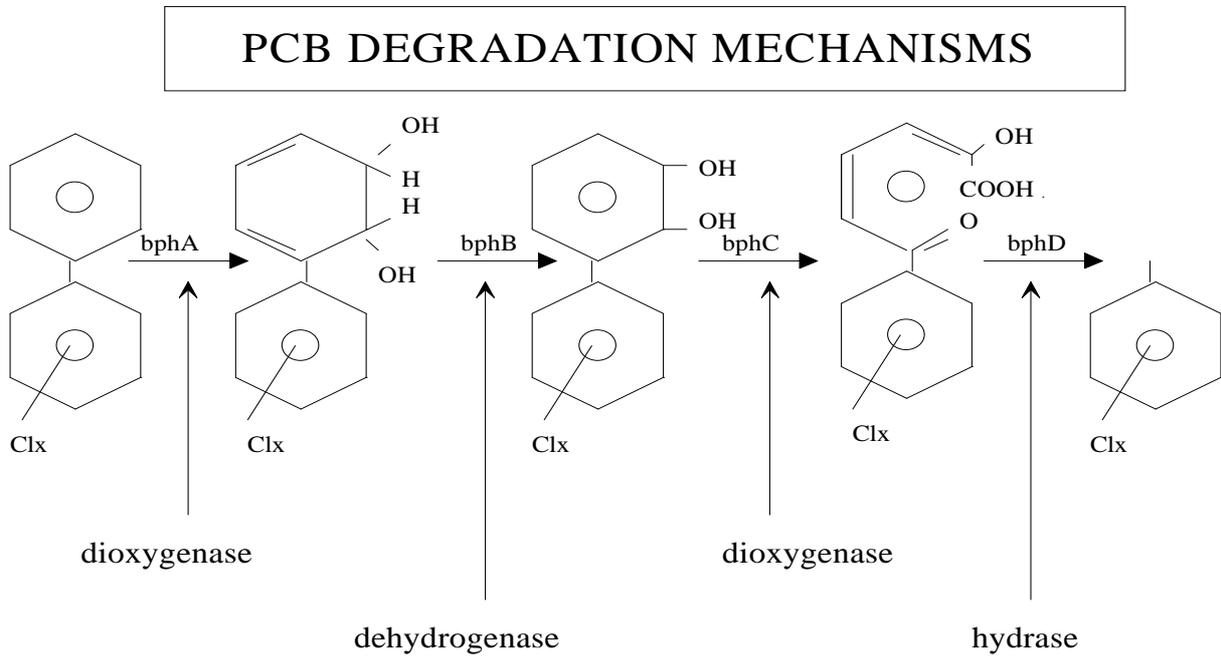
Step 1

- a) Organism characterization
- b) Site characterization
- c) Microbe-contaminant interaction
- d) Application characterization

Step 2

- a) Exposure assessment
- b) Hazard assessment

Figure 2



I. [Anaerobic Cl removal from higher congeners] → [Aerobic ring cleav. of lower congeners]

II. Selected Aerobes degrade higher congeners up to heptachlorobiphenyls (INDUCER)

Figure 3
Design of Recombinant Pseudomonad

Goal: Establish non-adaptive genes

- Aerobic ring cleavage
- Constitutive expression of *bph* genes
- Selective substrate
- Substrate solubilizes PCBs

Figure 4
Organism Characteristics

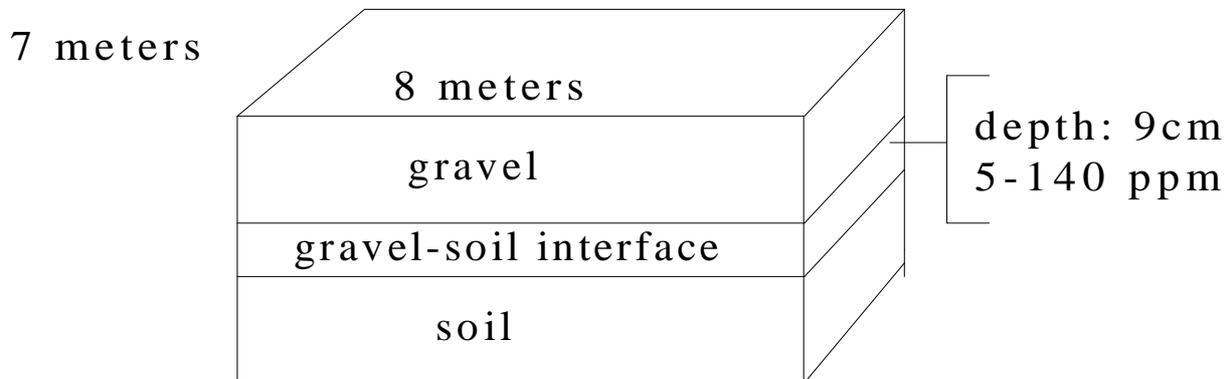
Recipient:	<i>Pseudomonas putida</i> IPL5 – ? identification techniques – Metabolizes surfactant – ? plasmids
Donor:	<i>P. sp.</i> ENV307 – ? identification techniques – Examine <i>bph</i> sequences
Vector:	Plasmid pRK290 and Tn903 Kan promoter Tc (PRK2 and pRK2501)
2nd Vector:	Disarmed Tn5 and TN903 Kan promoter; Tc (PRK2 and PRK2501)
Pathogenicity:	Soft rot?
Substrate specificity:	Other aromatic substrates (biphenyl, toluene)

Figure 5

SITE CHARACTERIZATION

- Restricted access TVA site in Tennessee
- Electrical capacitor banks shut down between 1971 & 1992
- Weathered Arochlor 1242, 1248 leakage into capacitor beds

Capacitor_Bed_3_Description:



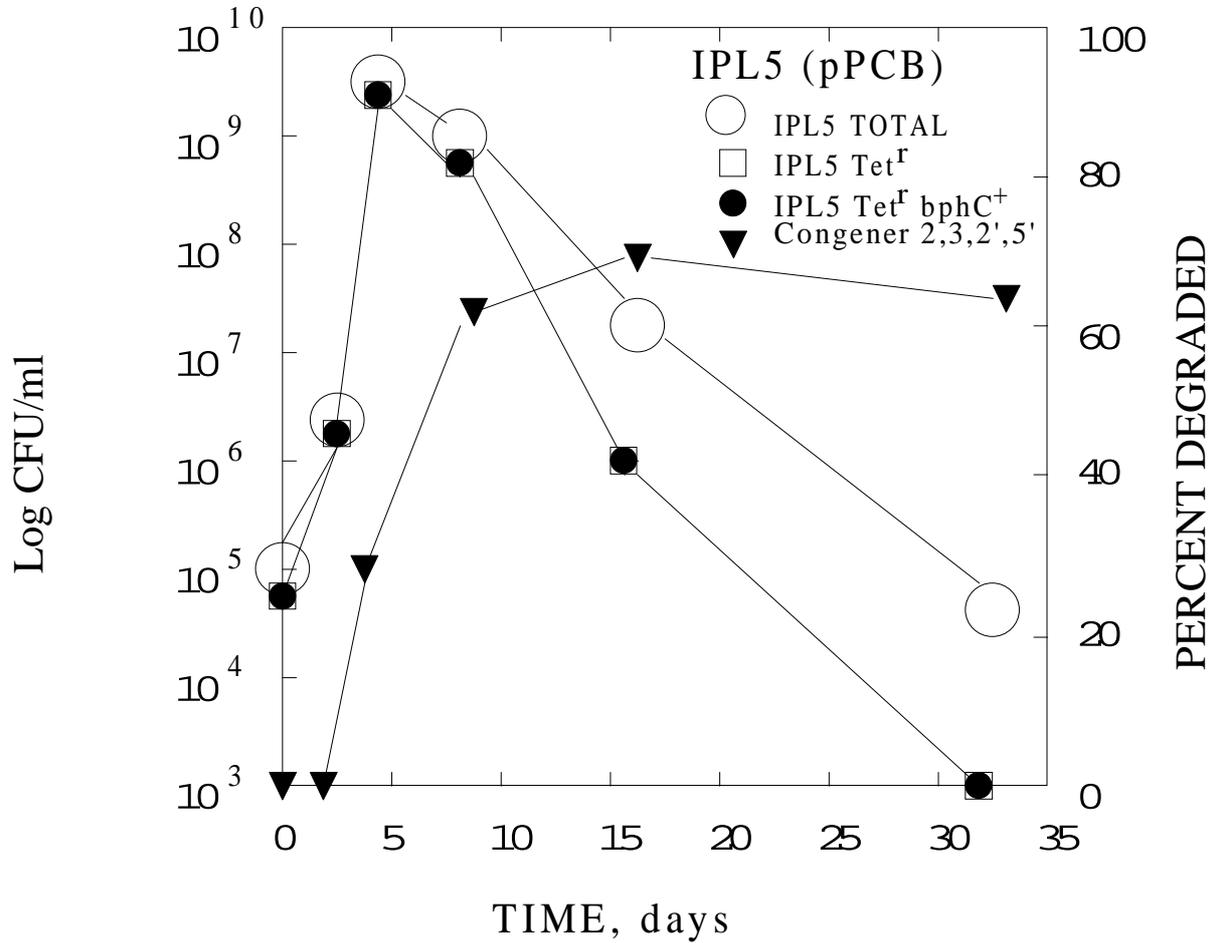
- PCBs bioavailable, but few bacteria
- Few other contaminants present
- PCB mobility limited

Figure 6
Exposure Assessments

Fate of microorganism, DNA, contaminant

1. Treatability studies with two constructs
 - Interface material with nutrients, surfactant ----> no change (100 days)
2. Worker protection for PCB exposure

Figure 7



Inoculum growth, gene stability and PCB degradation in electric power substation soil. Degradation of PCB congener 2,3,2',5' and time course concentrations of the surfactant degrading strain IPL5 (IPL5 TOTAL) and the same strain maintaining tetracycline resistance and bphC from plasmid pPCB in soil slurries (1g soil/5ml PAS medium) amended with 1.0% (wt/vol) surfactant (Igepal CO-720)

Provisional Method for Evaluating Environmental Effects of Bioremediation

Mr Hideto Yoshida and Dr Osami Yagi

Environment Agency of Japan

The Environment Agency of Japan has produced a provisional draft of a method for evaluating environmental effects of bioremediation (**Figure 1, Table 1**). This method consists of five steps: identification of the contaminated site, consideration of characteristics of utilized microorganisms, site characterization, laboratory tests, and field tests.

There is considerable soil and groundwater contamination in Japan (**Table 2**). In 1991, the Environment Agency issued Environmental Quality Standards for soil pollution. Much attention was focused at that time on the application of bioremediation to soil and groundwater clean-up.

If bioremediation technology is going to be used, environmental risk assessment should be carried out. This five-step scheme is based on the research activities at the National Institute for Environmental Studies and the Committee on Environmental Risk Assessment for Bioremediation, organized by the Environment Agency of Japan. Studies on bioremediation of soil and groundwater contaminated by volatile organic chlorines (VOCs) and heavy metals have taken place since 1986. Research activities have involved the identification of trichloroethylene-degrading bacteria, pathogenicity/infectivity, environmental effects, trichloroethylene-contaminated site characterization, laboratory tests and field tests.

Evaluation items for this method are listed in Figure 1 and Table 1. We consider these items to represent minimum data requirements. Characterization of microorganisms, toxicity of metabolites, and site characterization are particularly important (**Figures 2-5, Tables 3-6**). Moreover, laboratory tests and field tests (**Table 7**) are indispensable in environmental risk assessment for bioremediation since each site's characteristics are different.

Figure 1

The evaluation method for environmental effect of bioremediation
(Draft)

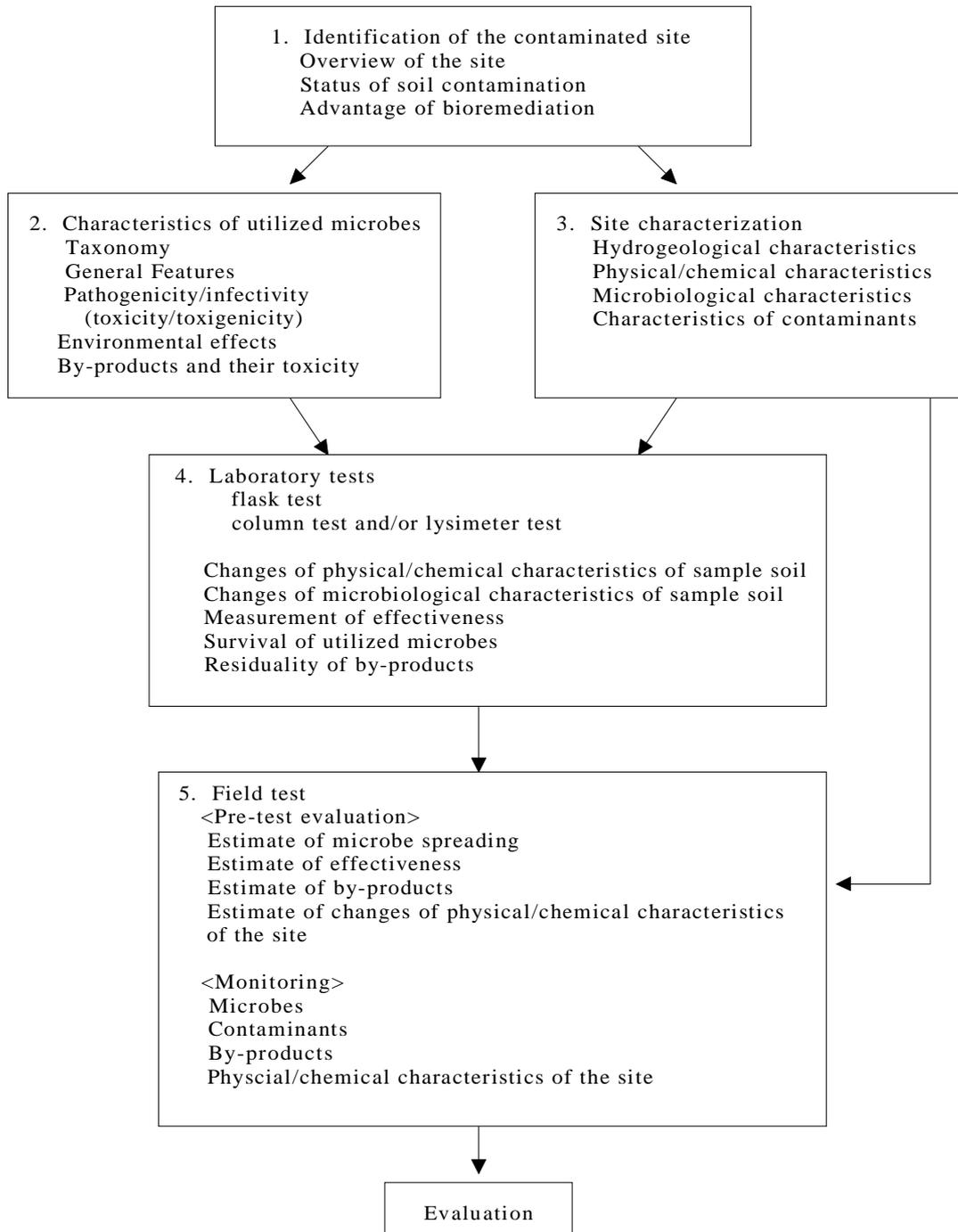


Table 1
Detailed Outline of the Evaluation Method for Environmental
Effects of Bioremediation (Draft)

1. Identification of the contaminated site

The situation of the contaminated site shall be specified in order to evaluate the necessity of bioremediation.

1) Overview of the site

- location, topography, water system, surface characteristics
- land use around the site, accessibility of the site

2) Status of soil contamination

- contaminants, concentrations, and distributions of the contaminants
- cause and history of contamination

3) Advantage of bioremediation

- potential remediation technologies
- comparison between technologies

2. Characteristics of utilized microorganisms

Among the characteristics to be clarified are taxonomy, general features, pathogenicity/infectivity (toxicity/toxigenicity), environmental effects, and by-products and their toxicity.

1) Taxonomy

- taxonomic features (Gram-positive/negative, aerobic/anaerobic, motility)
- distribution in the environment (natural background)
- known pathogenicity/infectivity (toxicity/toxigenicity)

2) General features

(in recombination, features of recombinant and its parent strain)

- growth conditions, etc.
- capability to degrade the target chemical substances (applicable concentrations, degradation rates)
- pH
- heterotrophy
- growth pattern (spore formation)
- genetic constructs (antibiotic resistance)

3) Pathogenicity/infectivity (toxicity/toxigenicity)

Effects on human health shall be evaluated

- acute toxicity
oral toxicity rat LD₅₀
inhalation toxicity
- pathogenicity/infectivity (rat)
- eye irritation (rabbit)

4) Environmental effects

Effects on ecosystem shall be evaluated

- microcosm test
- bacteria, blue-green algae, green algae, protozoa, metazoa (Rotifera, Oligochaeta)

5) By-products and their toxicity

Expected by-products and their toxicity shall be specified.

- kinds and features of by-products
- toxicity (data on oral acute toxicity and chronic toxicity)
- degradability/accumulativity (data on biodegradability and biological accumulativity)

3. Site characterization

1) Hydrogeological characteristics

The following shall be clarified in order to estimate the diffusion of utilized microorganisms and nutrients:

- strata (sand, pebble, clay, etc.)
- permeability coefficient
- level, flow direction, and velocity of ground water
- land use and water use around the site

2) Physical/chemical characteristics

The following shall be measured as the basic data to evaluate the physical/chemical effect of applying microorganisms and nutrients:

- pH
- oxidation-reduction potential
- nutrients (C, N, P)

3) Microbiological characteristics

The following shall be measured as the basic data to evaluate the proliferation of utilized microorganisms and its effect on the native microorganisms:

- number of utilized microorganisms
- number of heterotrophs (aerobic and anaerobic)
- respiration activity

4) Characteristics of contaminants

The following shall be clarified as the basic data to evaluate the remediation by the microorganisms:

- concentrations of contaminants and by-products
- distributions of contaminants and by-products

4. Laboratory tests

Laboratory tests include flask test, column test and/or lysimeter test using the site soil.

1) Changes of physical/chemical characteristics of sample soil

The following parameters have effects on the degradation of contaminants and survival of microorganisms:

<Physical characteristics>

- grain size
- water content
- adsorption by soil (for estimate of complete elimination)

<Chemical characteristics>

- pH
- oxidation-reduction potential
- nutrients (C, N, P)
- biodegradability (biodegradability of contaminants in the soil)

2) Changes of microbiological characteristics of sample soil

- heterotrophs (aerobic and anaerobic)
- respiration activity (as an indicator of degradation activity of the soil)

3) Measurement of effectiveness

- time course of contaminant concentrations

4) Survival of utilized microorganisms

- changes in distribution and number of utilized microorganisms

5) Residuality of by-products

- changes in distributions and concentrations of expected by-products

5. Field test

<Pre-test evaluation>

1) Estimate of microorganisms spreading

On the basis of the results of the lysimeter/column test and hydrogeological characteristics, the spreading area of utilized microorganisms shall be estimated.

2) Estimate of effectiveness

On the basis of the results of the lysimeter/column test and hydrogeological characteristics, changes in contaminant concentration and the area of effect shall be estimated.

3) Estimate of by-products

On the basis of the results of the lysimeter/column test and hydrogeological characteristics, changes in by-product concentrations and the area of diffusion shall be estimated.

4) Estimate of changes in physical/chemical characteristics of the site

On the basis of the results of the lysimeter/column test and the hydrogeological characteristics, changes in physical/chemical characteristics of the site and the area of effect shall be estimated.

<Monitoring>

1) Microorganisms

The number of utilized microorganisms in the soil and/or ground water within the area of effect shall be monitored.

Example: TCE bioremediation

- methanotrophs

2) Contaminants

The contaminant concentrations in the soil and/or ground water within the area of effect shall be monitored.

Example: TCE bioremediation

- trichloroethylene

3) By-products

The by-product concentrations in the soil and/or ground water within the area of effect shall be monitored.

Example: TCE bioremediation

- cis-dichloroethylene, 1,1-dichloroethylene, trans-dichloroethylene

4) Physical/chemical characteristics of the site

Physical/chemical characteristics of the soil and/or ground water within the area of effect shall be monitored.

Example: TCE bioremediation

- ground water level, temperature, pH
- dissolved oxygen, dissolved methane, methane in soil gas
- nitrate nitrogen, phosphate phosphorus

Figure 2
Construction of *Pseudomonas putida* PpY101/pSR134

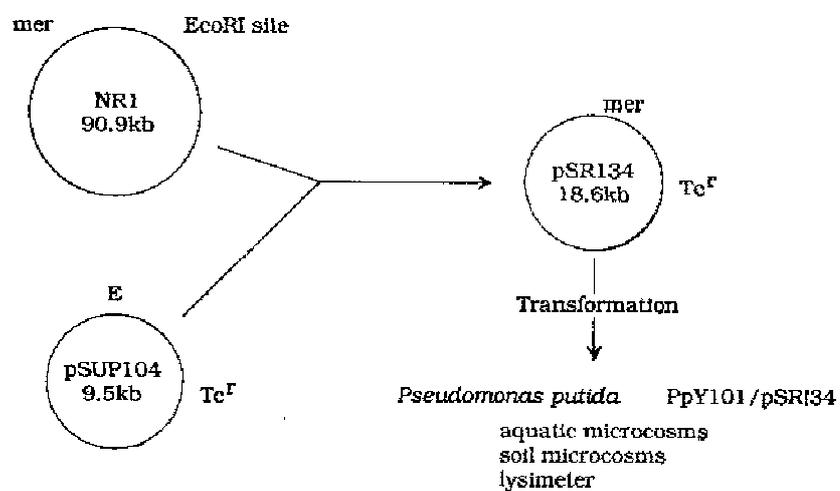


Table 2
Survey of Ground Water Polluted by VOC

Year	Chemicals	Number of well surveyed	Number of polluted wells	Ratio (%)
1984	Trichloroethylene	5720	122	2.1
	Tetrachloroethylene	5773	185	3.2
	1,1,1-trichloroethane	5476	4	0.1
1988	Trichloroethylene	19669	387	2.0
	Tetrachloroethylene	19661	835	4.2
	1,1,1-trichloroethane	19596	38	0.2
1989	Trichloroethylene	3380	30	0.9
	Tetrachloroethylene	3380	42	1.2
	1,1,1-trichloroethane	2569	2	0.1
1990	Trichloroethylene	5817	44	0.8
	Tetrachloroethylene	5817	79	1.4
	1,1,1-trichloroethane	4515	1	0.0
1991	Trichloroethylene	6458	27	0.4
	Tetrachloroethylene	6518	44	0.7
	1,1,1-trichloroethane	5135	0	0.0
1992	Trichloroethylene	4762	18	0.4
	Tetrachloroethylene	4762	35	0.7
	1,1,1-trichloroethane	3952	3	0.1

Figure 3
Survival of Various Genetically Engineered Microorganisms in Soil Microcosms

The survival patterns of GEMS were different in different soil microcosms. *E. coli* showed the highest decreasing rate and was not detected after ten days. *Krebsiella* also showed a relatively high decreasing rate. *Pseudomonas* strains showed slower decreasing rates than the other strains. The detection limit of GEMS was less than 10 CFU g⁻¹ soil.

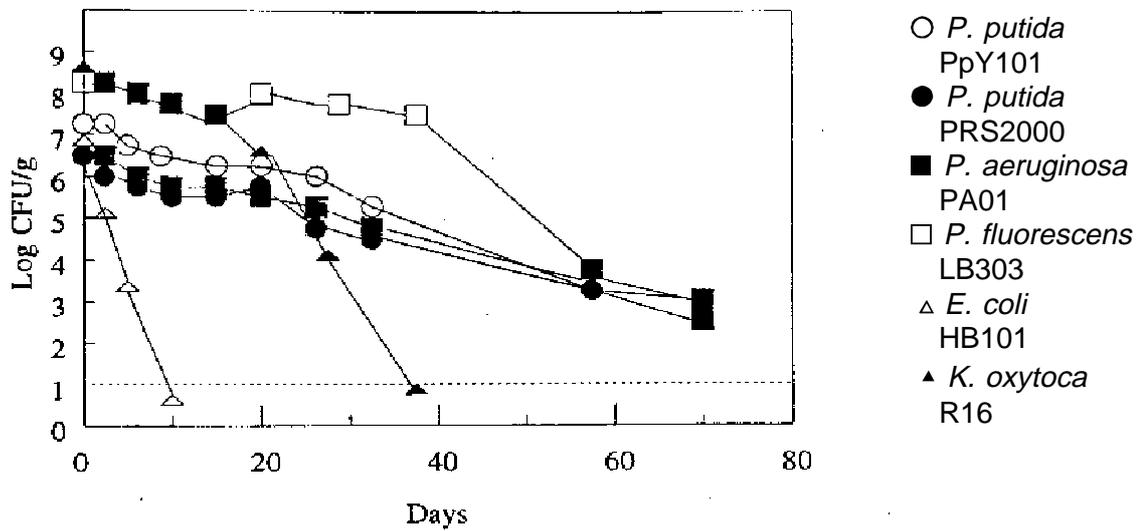


Figure 4
Effect of TCE Concentration on TCE Degradation by *Methylocystis* sp. strain M

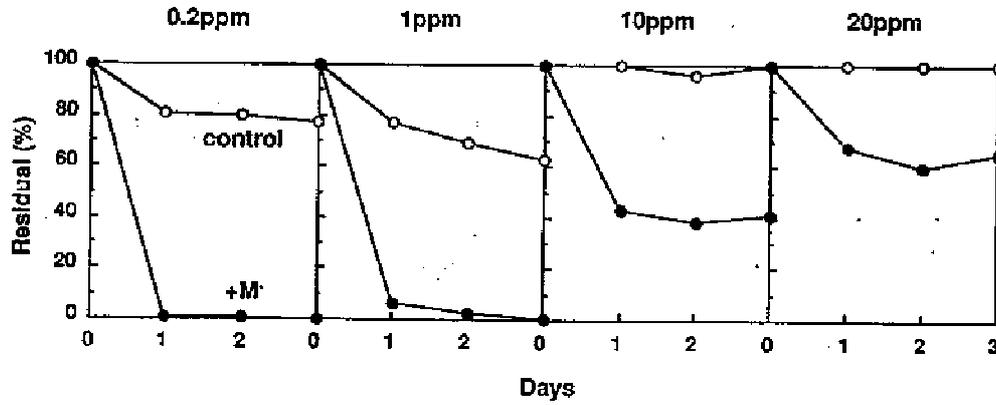


Figure 5
A Hypothetical Pathway of TCE Degradation by Stain M

MMO, methane monooxygenase; 1) chloral; 2) trichloroacetic acid; 3) 2,2,2-trichloroethanol; 4) TCE oxide; 5) carbon monoxide; 6) formic acid; 7) glyoxylic acid; 8) dichloroacetic acid

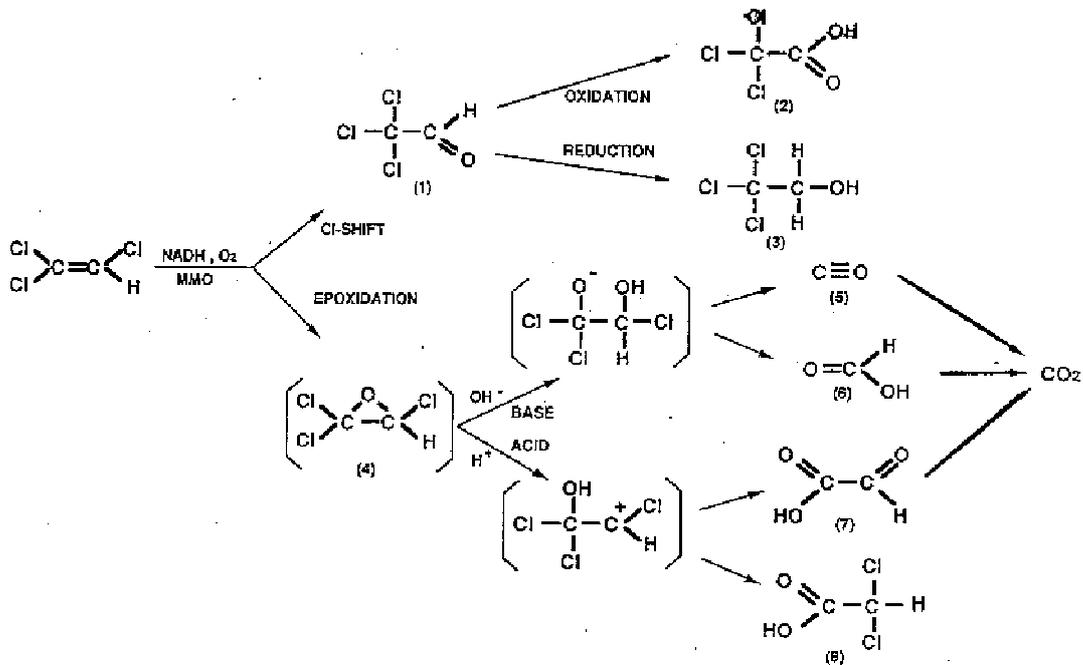


Table 3
Trichloroethylene Metabolites (Aerobic):
Drinking Water Standards

trichloroethylene	0.03 mg/l
dichloroacetic acid	0.04*
trichloroacetic acid	0.3*
1,1,1-trichloroethanol	—
glyoxylic acid	—
formic acid	—
CO	
CO ²	
TCE oxide	

* monitoring values

Table 4
Tetrachloroethylene Metabolites (Anaerobic):
Environmental Quality Standards for Soil Pollution

tetrachloroethylene	0.01 mg/l
trichloroethylene	0.03
1,1-dichloroethylene	0.02
cis-dichloroethylene	0.04
trans-dichloroethylene	— (0.04)*
vinyl chloride	—
ethylene	—
CH ₄	
CO ₂	

* for drinking

Table 5
Overview of the Contamination of Sites by TCE

	Site A	Site B	Site C
Year of discovery	1986	1990	1989
Contaminants	TCE, c-DCE, VC	TCE	TCE
Contaminated zone	first aquifer (5-14 metres deep)	upper first aquifer (14-23 metres deep)	first aquifer (6-9 metres deep)
Source of contamination	electronics parts factory	electronics parts factory	machinery factory
Status	remediation (pump and treat)	remediation (pump and treat)	site characterization

Table 6
Summary of Contaminated Site Characterization (Ground Water)

	Site A	Site B	Site C
Depth from surface (m)	5.5~14.5	14~23	6~9
Ph	7.15~7.32	6.28	5.95~6.19
Temperature (°C)	15.7~16.1	16.2	15.8~15.9
Dissolved oxygen (mg/l)	0.00	4.94	0.04~0.42
TCE (mg/l)	0.167	5.26~6.5	0.8~1.7
c-DCE (mg/l)	1.23	0.042~0.05	0.046~0.06
VC (mg/l)	0.15	0	0
Total carbon (mg/l)	5.2	–	–
Total nitrogen (mg/l)	0.08	7.5	7.3
Total phosphorus (mg/l)	2.6	0.01	0.12
Copper (mg/l)	0.007	0.1	0.008
Aerobic heterotrophs (CFU/ml)	1.1×10^3	2×10^2	$1^2 \times 10^3$
Methanotrophs (CFU/ml)	$>1 \times 10^4$	1×10^2	$0.43^3.9 \times 10^3$

Table 7
Monitoring Items for TCE Bioremediation by Methane Bacteria

1. Microbial analysis	methane-utilizing bacteria (MPN) heterotrophs (aerobic and anaerobic) soil respiration
2. Contaminants	trichloroethylene
3. By-products	1,1-, cis-, trans-dichloroethylene dichloro-, trichloroacetic acid
4. Physical/chemical characteristics	ground water level, temperature, pH, DO, methane, nitrogen, phosphorous, ORP

A Method for Predicting Environmental Impacts of Field Releases of Effective Specific Microorganisms Using Microcosm Systems

**Dr Yuhei Inamori,¹ Dr Kazuhito Murakami,¹ Dr Ryuichi Sudo²
and Dr Yasushi Kurihara³**

¹National Institute for Environmental Studies

²Tohoku University, Faculty of Engineering

³Oou University, Faculty of Dentistry

Japan

In this paper we present a very useful method for carrying out environmental assessment previous to the field release of effective specific microorganisms such as microbial pesticides and genetically engineered microorganisms (GEMs). The "step by step" method, as we have named it, consists of three model ecosystem tests: a monoxenic culture test, an aquatic flask-sized microcosm test, and a natural model ecosystem test (**Figure 1**). Several effective specific microorganisms were tested using this method. Some did not show any influence on indigenous microbiota, others showed a limited influence, and none showed a great influence. The "step by step" method can be considered a standard method for this type of environmental assessment, along with the activated sludge method which is provided in the chemical substances control law in Japan (Chemical Products Safety Division, Basic Industries Bureau, MITI, C-3/78/JAP.).

Background

With the recent advances in genetic engineering and the development of useful new microorganisms for bioremediation and environmental clean-up, microbial pesticides and other such products have been investigated for use in various fields. In the interest of environmental protection, confirmation of their safety should be made before environmental release. It is essential to understand the behaviour of effective specific microorganisms in natural ecosystems, as well as of artificial ecosystems including bio-film and activated sludge. Interactions among microorganisms (for example, between prey and predator) are especially significant. Microcosm systems which closely reflect and reproduce natural ecosystems can be used to estimate the prosperity and decay of effective specific microorganisms. These systems are comparable to natural aquatic ecosystems in regard to basic functions such as material cycle, energy flow, and the interaction of microorganisms.

Model aquatic ecosystems

Model aquatic ecosystems were used to examine prosperity and decay patterns of effective specific microorganisms such as microbial pesticides and GEMs in aquatic ecosystems, as well as the interaction between effective specific microorganisms and indigenous bacteria, protozoa, metazoa, algae, etc. These microorganisms included: *Escherichia coli* C600, *E. coli* HB101, *E. coli* HB101/pBR325, *E. coli* S17-1, *E. coli* S17-1/pSUP104, *E. coli* S17-1/pCRO1, *Pseudomonas putida* PRS2000, *P. aeruginosa* PAO1, *Bacillus thuringiensis* subsp. *aizawai* KH, *B.t. kurataki*, *B. cereus* MC, *Beauveria bassiana* B27, *Metarhizium anisopliae* M7, and *Verticillium lecanii* V5. Their characteristics are shown in **Table 1**.

Monoxenic culture testing is used to investigate prey-predator interactions between bacteria as prey, and micro animals as predators. Micro animals as predators were selected from protozoa and metazoans, taking into account their frequent appearance both in natural ecosystems, such as rivers, lakes, marshes and seas, and artificial aquatic ecosystems, such as bio-film and activated sludge. These micro animals, chosen because they were easy to culture and could be counted accurately, were *Cyclidium glaucoma*, *Tetrahymena pyriformis*, *Colpidium campylum*, *Philodina erythrophthalma* and *Aelosoma hemprichi*. They had been cultured and serially transferred in LE medium for several years. A 20 ml portion of cell suspension was poured into a 50 ml conical flask, inoculated with a micro animal, and then cultured at 20°C under dark, undisturbed conditions.

Aquatic flask-sized microcosm testing is used to investigate the prosperity and decay of effective specific microorganisms. The microcosm system consists of four species of bacteria (*Pseudomonas putida*, *Bacillus cereus*, *Acinetobacter* sp. and Coryneform bacteria), one species of protozoa (*Cyclidium glaucoma*), three species of metazoans (*Philodina* sp., *Lepadella* sp. and *Aelosoma hemprichi*), and two species of algae (*Chlorella* sp. and *Tolypothrix* sp. (**Figure 2**). Twenty-four combinations, consisting of three species of protozoans and three species of metazoans as predators, seven species of algae as producers, and four species of bacteria as decomposers, were investigated for high stability and reproductivity (**Figure 3**). It is clear that a microcosm system with this composition could be used as a standard method for environmental assessment of effective specific microorganisms, micropollutants, etc. at the ecosystem level. The aquatic flask-sized microcosm was cultured at 25°C, under 2800 lux (12L/12D), and shaken slowly by hand once a day. The vessel for cultivation was a 300 ml triangle flask. 200 ml of Taub's basal medium was added and the polypeptone concentration was adjusted to 50 mg/l, as shown in **Table 2**.

Natural model ecosystem testing is used to investigate the fate and influence of effective specific microorganisms in the natural aquatic environment, in comparison with the aquatic flask-sized microcosm system. The natural model ecosystem, which uses water from the natural environment such as that from Lake Kasumigaura, shows a very high correlation with the aquatic flask-sized microcosm system. It can therefore mediate between the aquatic flask-sized microcosm and the natural aquatic ecosystem.

Prosperity and decay patterns in the aquatic flask-sized microcosm test

The results of the aquatic flask-sized microcosm test show various patterns of prosperity and decay for the effective specific microorganisms. For example, the *B.t. aizawai* KH and *E. coli* HB101/PBR325 decreased and survived in low numbers; the *E. coli* S17-

1/PCRO1 decreased rapidly; and the *B. bassiana* B27 survived in its initial numbers (**Figure 4**). Eight patterns of prosperity and decay were recognized: 1) rapid decrease; 2) rapid decrease and survival, keeping low numbers; 3) slow decrease; 4) slow decrease and survival, keeping low numbers; 5) survival, keeping the initial numbers; 6) survival during a long period and increase in number; 7) survival in low numbers, increase with the microorganism's peculiar substrate, and continuing increase in number; 8) survival in low numbers, increase with the microorganism's peculiar substrate, and continuing increase in number (**Figure 5**). Furthermore, it was clear that prey-predator interaction between effective specific microorganisms and indigenous micro animals was quite important to the fate of these bacteria. In other words, the prosperity and decay of effective specific microorganisms were greatly affected by the predation of micro animals inhabiting the microcosm system.

Evaluation of the step by step method

The test case of the microbial pesticide *B.t. aizawai* KH, which was evaluated to determine whether it was an effective specific microorganism, has been used and is expected to be used more widely in the near future. In this case, because a microbial pesticide would be released without its peculiar substrate, the cultivation test was set up under non-growth conditions. In the monoxenic culture test (step 1), all the micro animals were able to grow in a cell suspension of *B.t. aizawai* KH; that is, *B.t. aizawai* KH was expected to decrease through predation of micro animals in the natural environment just like other bacteria. In the aquatic flask-sized microcosm test (step 2), the *B.t. aizawai* KH decreased because microbiota, especially protozoa, were greatly affected by the interaction (e.g. prey-predator interaction). In the natural lake model ecosystem test (step 3), *B.t. aizawai* KH showed the same behaviour as in the flask-sized microcosm; that is, it decreased in the microcosm through the predation of micro animals (**Figure 6**). From these outcomes, it was considered that if *B.t. aizawai* KH were released to the natural environment, it would not have a great influence on the indigenous ecosystem (**Figure 7**). The aquatic flask-sized microcosm test is a valid screening test.

Throughout this test method, the following three cases can be recognized as influencing the natural ecosystem: 1) the effective specific microorganisms supplied were not preyed on in the monoxenic culture, that is, they survived without predation by micro animals; 2) the effective specific microorganisms greatly influenced the indigenous microbiota of the aquatic flask-sized microcosm; and 3) the effective specific microorganisms did not decrease in the natural model ecosystem. These three cases, on the other hand, can be recognized as having no influence on the natural ecosystem: 1) the effective specific microorganisms were preyed on in the monoxenic culture, that is, they decreased with predation by micro animals; 2) the effective specific microorganisms almost did not influence the indigenous microbiota and decreased in the aquatic flask-sized microcosm; and 3) the effective specific microorganisms decreased in the natural model ecosystem.

Effectiveness of the aquatic flask-sized microcosm testing method

In evaluating the prosperity and decay of effective specific microorganisms in an aquatic environment, an estimation using aquatic flask-sized microcosm systems is a valid method as regards high reproductivity and reflectivity of the natural environment. In this testing method, several patterns of the prosperity and decay of effective specific microorganisms were recognized. The importance of the interaction between effective specific

microorganisms and micro animals was clear. It was established that the interaction between these microorganisms and algae cannot be ignored. From these results, it was considered that the characteristics of effective specific microorganisms and seasonal changes of microbiota should be evaluated before environmental release. Furthermore, there was a high correlation between the results of the aquatic flask-sized microcosm experiment and those of the natural lake model ecosystem experiment. Thus, a more intensive comparable evaluation of the experimental results of this aquatic flask-sized microcosm system test would lead to a more accurate estimation of the prosperity and decay of effective specific microorganisms. In addition, possible impacts of effective specific microorganisms on the environment could be evaluated more correctly.

From an ecological viewpoint, no bacterial field release can be viewed as safe. Based on the results of this study, it is impossible to say "safety" but it may be possible to say "relief". This screening test system is considered to be an essential method of environmental assessment for field release of effective specific microorganisms.

References

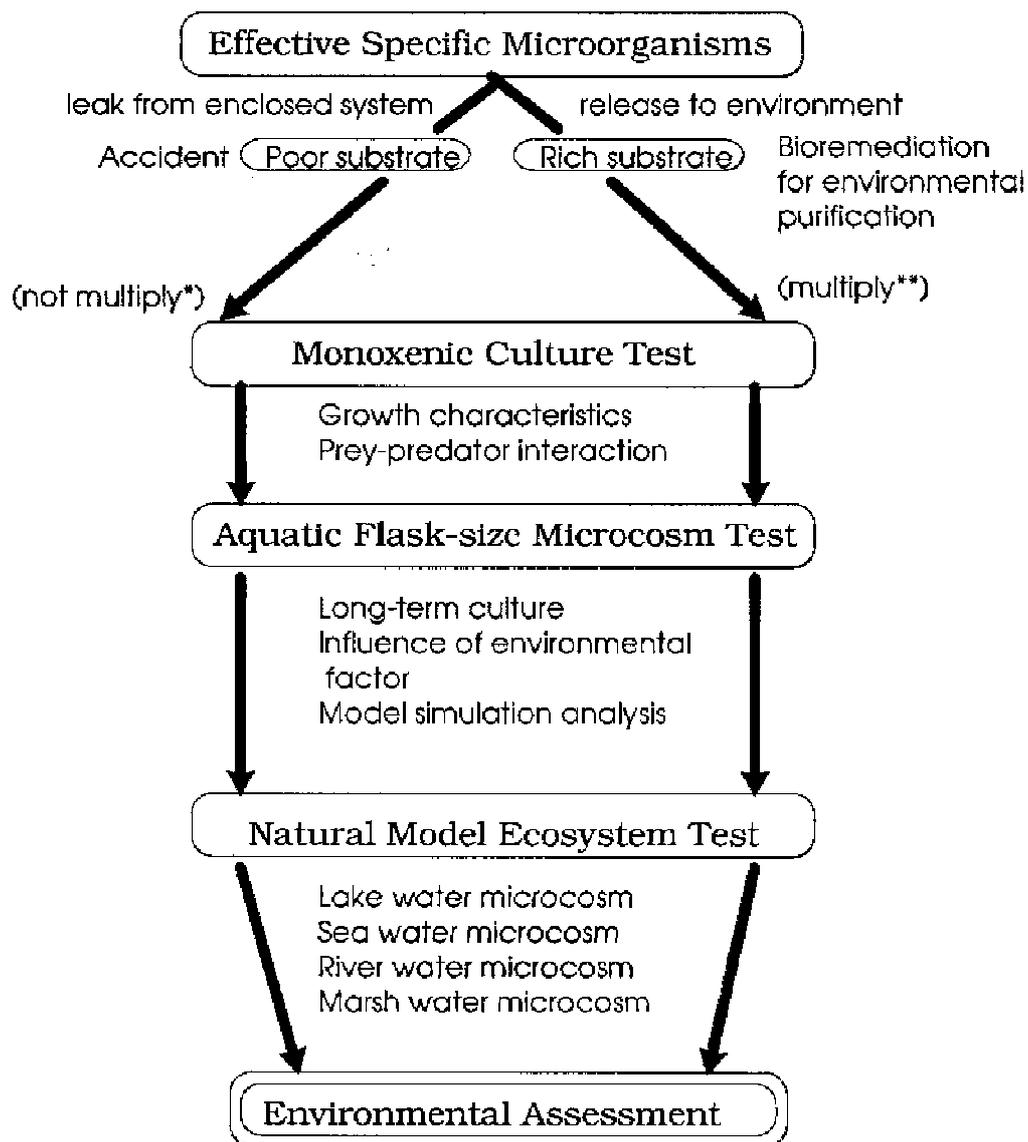
1. R. Sudo, Y. Inamori, K. Murakami, M. Okada and H. Ohtake (1990) Effect of Micro Animals on Prosperity and Decay of Plasmid DNA Transconjugant, *Proceeding of 15th IAWPRC*, pp. 331-334.
2. Y. Inamori, K. Murakami, R. Sudo, Y. Kurihara, N. Tanaka (1992) Environmental Assessment Method for Field Release of Genetically Engineered Microorganisms using Microcosm Systems, *Water Science and Technology* 26, pp. 2161-2164.
3. K. Murakami, Y. Inamori, T. Sudo and Y. Kurihara (1992) Effect of Temperature on Prosperity and Decay of Genetically Engineered Microorganisms in a Microcosm System, *Water Science and Technology* 26, pp. 2165-2169.
4. K. Murakami, Y. Inamori, N. Hayashi and R. Sudo (1993) Effect of Microbial Pesticide on Growth of Micro Animals, *Japanese Journal of Water Treatment Biology* 29, pp. 31-38.
5. Y. Inamori, K. Murakami, M. Tsunoda, T. Sato and Y. Kurihara (1993) Studies on Interaction between Genetically Engineered Microorganisms and their Parental Strains, *Japanese Journal of Water Treatment Biology* 29, pp. 39-49.
6. N. Tanaka, Y. Inamori, K. Murakami, T. Akamatsu and Y. Kurihara (1995) Effect of Species Composition on Stability and Reproductivity of Small-scale Microcosm System, *Water Science and Technology* 30, pp. 125-131.
7. K. Murakami, Y. Inamori, M. Okada, R. Sudo and Y. Kurihara (1994) Prosperity and Decay of Microbial Pesticide, *Bacillus thuringiensis* in Aquatic Model Ecosystems for Environmental Assessment Method, IAQW, Water Quality International '94.

Table 1
Characteristics of Model Effective Specific Microorganisms

Bacterial strain	
<i>Escherichia coli</i> C600	
<i>Escherichia coli</i> HB101	(Smr)
<i>Escherichia coli</i> HB101/PBR325	(Smr, Apr, Tcr, Cmr)
<i>Escherichia coli</i> S17-1	(Smr)
<i>Escherichia coli</i> S17-1/PSUP104	(Smr, Tcr, Cmr)
<i>Escherichia coli</i> S17-1/PCRO1	(Smr, Cmr, Crr)
<i>Pseudomonas putida</i> PRS2000	(Cmr, Smr, Apr)
<i>Pseudomonas aeruginosa</i> PAO1	(Cmr, Smr, Apr)
<i>Bacillus thuringiensis</i> subsp. <i>aizawai</i> KH	(microbial pesticide)
<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>	(microbial pesticide)
<i>Bacillus cereus</i> MC	
Fungal strain	
<i>Beauveria bassiana</i> B27	(microbial pesticide)
<i>Metarhizium anisopliae</i> M7	(microbial pesticide)
<i>Verticillium lecanii</i> V5	(microbial pesticide)

Table 2
Composition of Taub's Basal Medium

a) Stock solution	g/D.W. 1000 ml
A sol. MgSO ₄ • 7H ₂ O	24.65 g
B sol. KH ₂ PO ₄ NaOH	13.6 g 2.8 g
C sol. CaCl ₂ • 2H ₂ O (or CaCl ₂)	14.7 (or 11.1) g
D sol. NaCl	5.84 g
E sol. FeSO ₄ • 7H ₂ O Na ₂ EDTA	24.9 g 13.6 g
F sol. H ₃ BO ₃ ZnSO ₄ • 7H ₂ O MnCl ₂ • 4H ₂ O Na ₂ MoO ₄ • 2H ₂ O CuSO ₄ • 5H ₂ O Co(NO ₃) ₂ • 6H ₂ O	1.854 g 0.287 g 1.98 g 0.024 g 0.0499 g 0.291 g
b) Taub's basal medium	ml/D.W. 1000 ml
A sol.	1 ml
B sol.	1 ml
C sol.	20 ml
D sol.	30 ml
E sol.	0.125 ml
F sol.	0.5 ml
c) TP broth: Taub's basal medium + polypeptone	
Taub's basal medium	1000 ml
polypeptone	50 mg/l



* not multiply : release into the environmental condition without its peculiar substrate

** multiply : release into the environmental condition with its peculiar substrate

Fig.1 Environmental Assessment Using Microcosm System

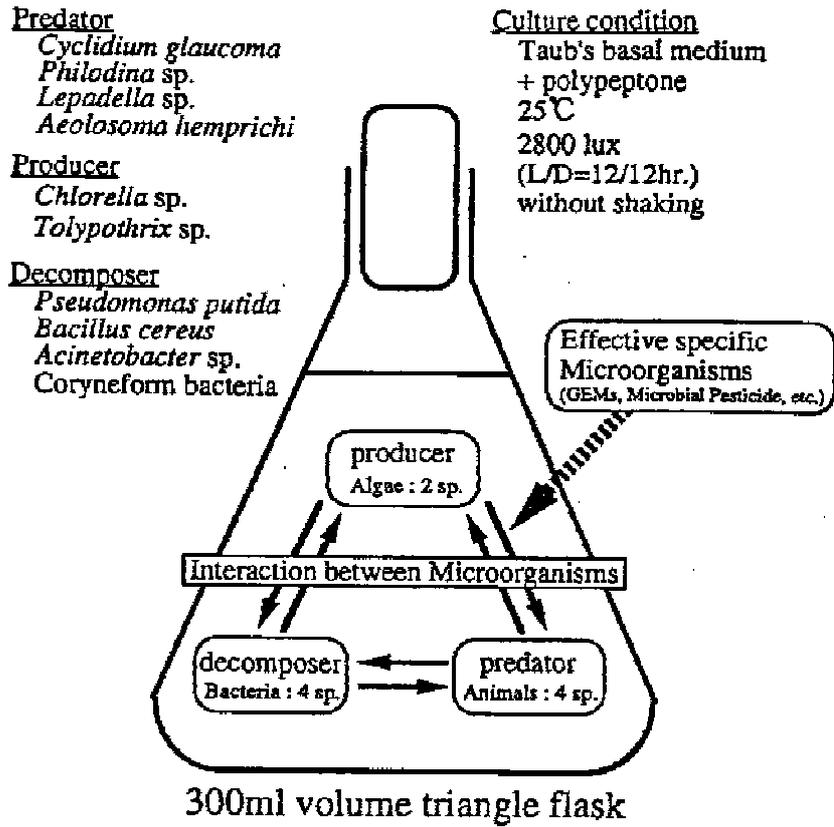
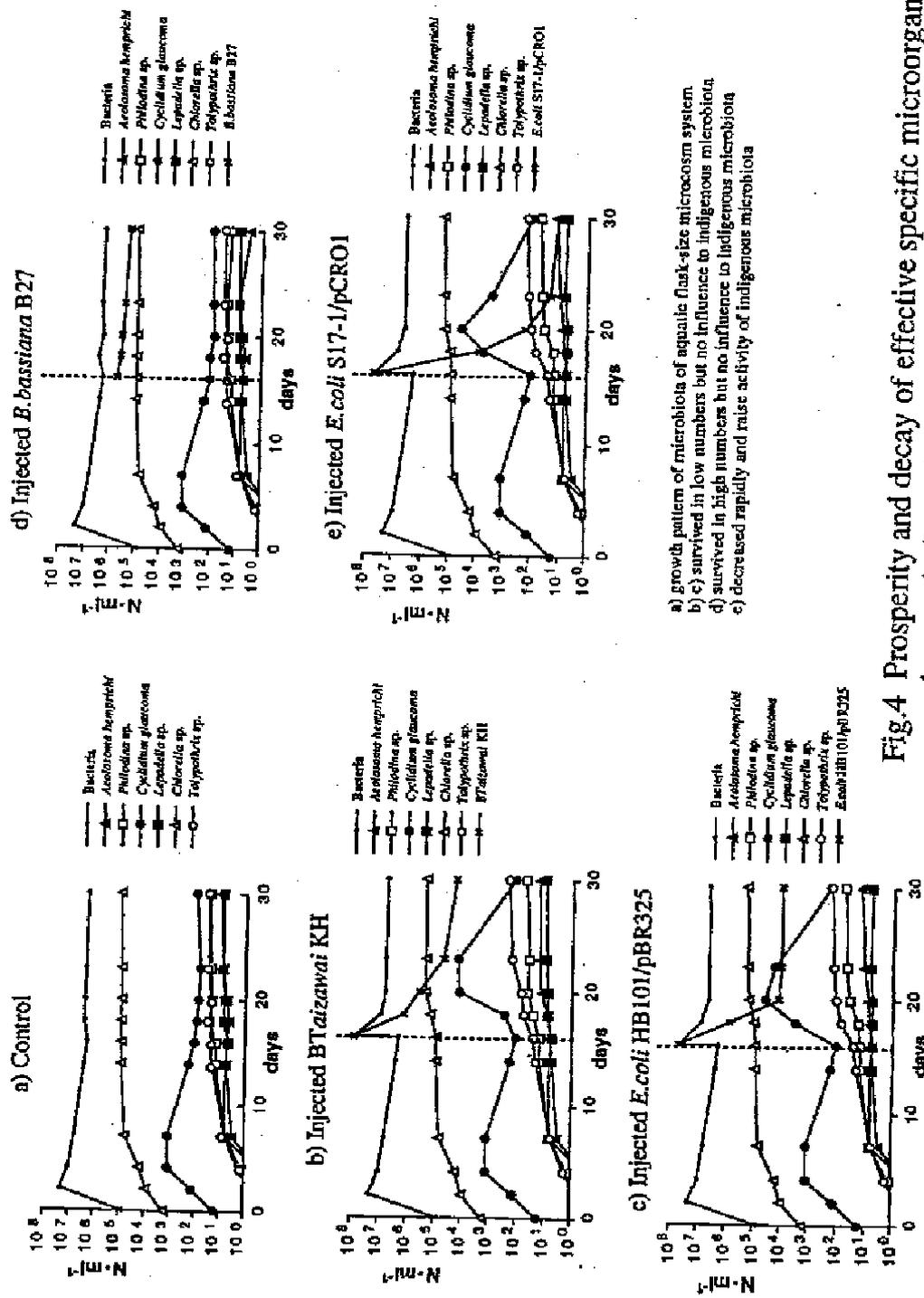


Fig.2 Outline of aquatic flask-size microcosm test

Fig.3 Species composition of small-scale microcosm system and stability and reproductivity of microcosm system

Strains	Run																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
<i>Pseudomonas putida</i>	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
<i>Bacillus cereus</i>	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
<i>Acinetobacter</i> sp.	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Coryneform bacteria	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
<i>Chlorella</i> sp.	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
<i>Scenedesmus quadricauda</i>																								
<i>Chlamydomonas monticola</i>																								
<i>Toitypothrix</i> sp.	○	○																						
<i>Oscillatoria agardhii</i>																								
<i>Anabaena flos-aquae</i>																								
<i>Microcystis viridis</i>																								
<i>Cyclidium glaucoma</i>	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
<i>Tetrahymena pyriformis</i>																								
<i>Colpidium campyllum</i>																								
<i>Lepadella</i> sp.	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
<i>Philodina erythroptalina</i>	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
<i>Aeolosoma hemprichi</i>	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Stability*	●	●	●	●	●	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

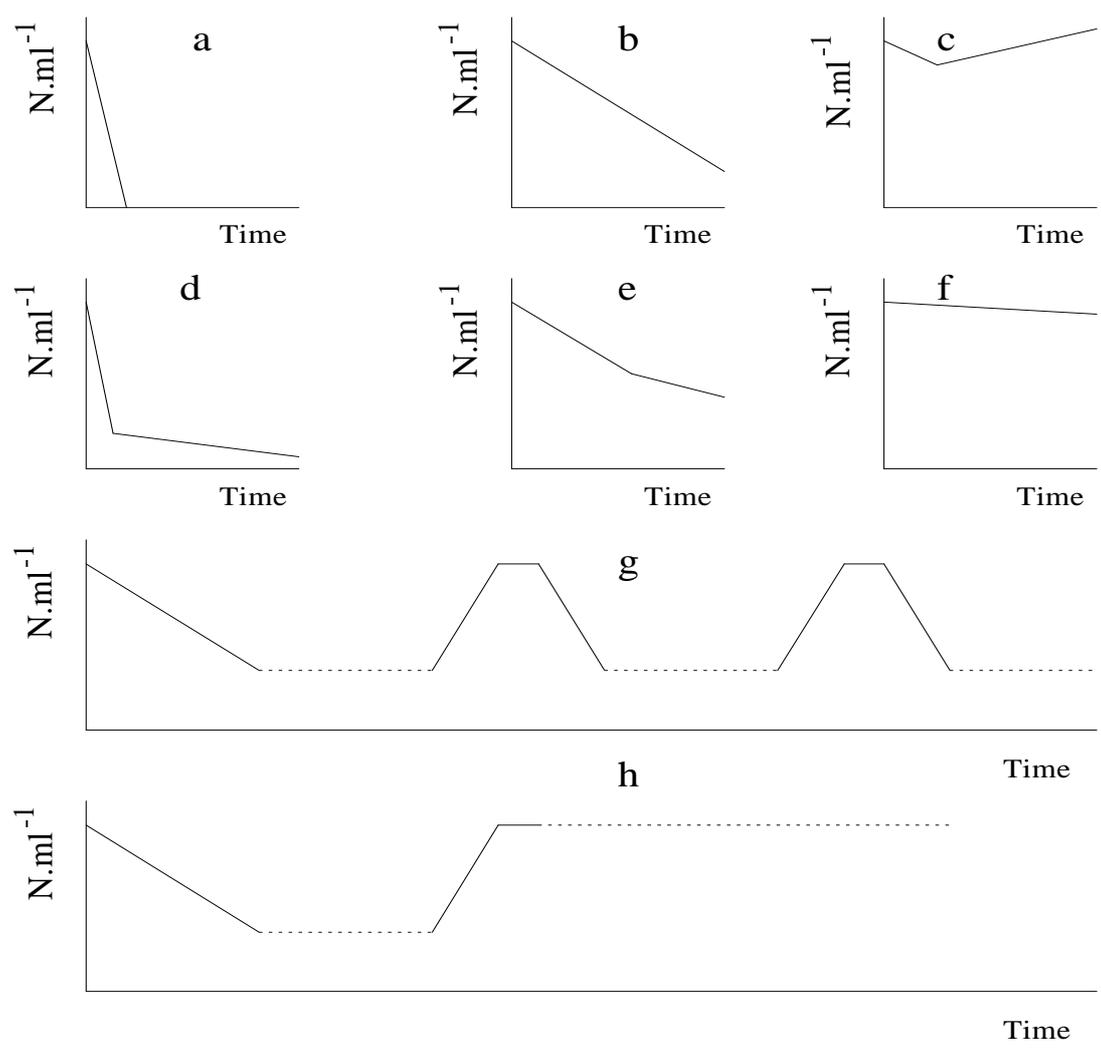
* ●: Stable, ×: Unstable



a) growth pattern of microbiota of aquatic flask-size microcosm system.
 b) c) survived in low numbers but no influence to indigenous microbiota.
 d) survived in high numbers but no influence to indigenous microbiota.
 e) decreased rapidly and raise activity of indigenous microbiota

Fig.4 Prosperity and decay of effective specific microorganisms in aquatic flask-size microcosm system in actual experiment

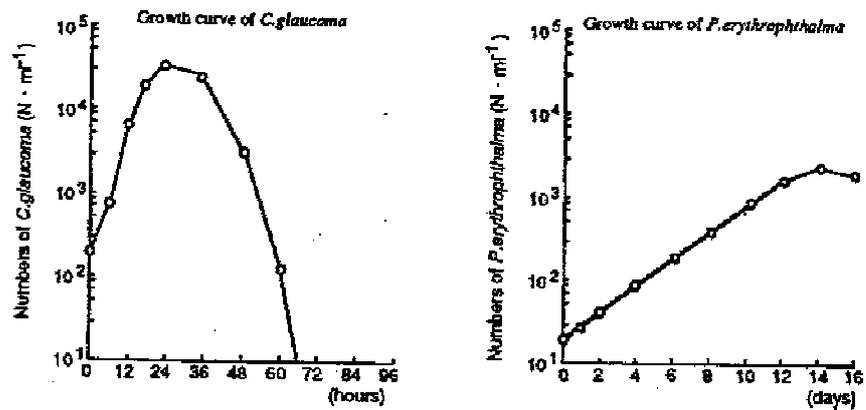
- decreased and fade out (a,b)
- increased and replaced (c,h)
- stayed and coexist (d,e,f)
- decreased after degradation of substrate (g)



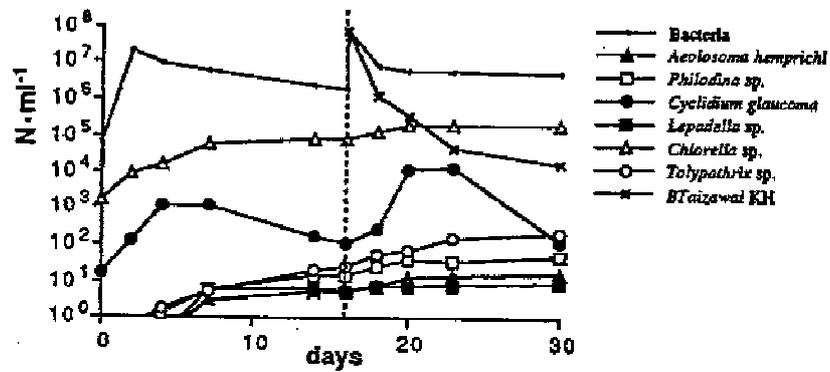
pattern c,f,h,: cannot release to environment
 pattern a,b,d,g, : can release to environment

Fig. 5 Patterns of prosperity and decay of some kinds of effective specific microorganisms in microcosm system under natural environmental conditions

step 1. Monoxenic culture test



step 2. Aquatic flask-size microcosm test



step 3. Natural model ecosystem test

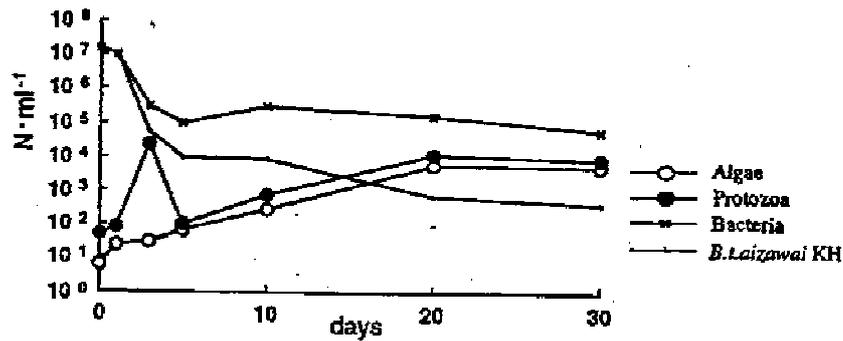
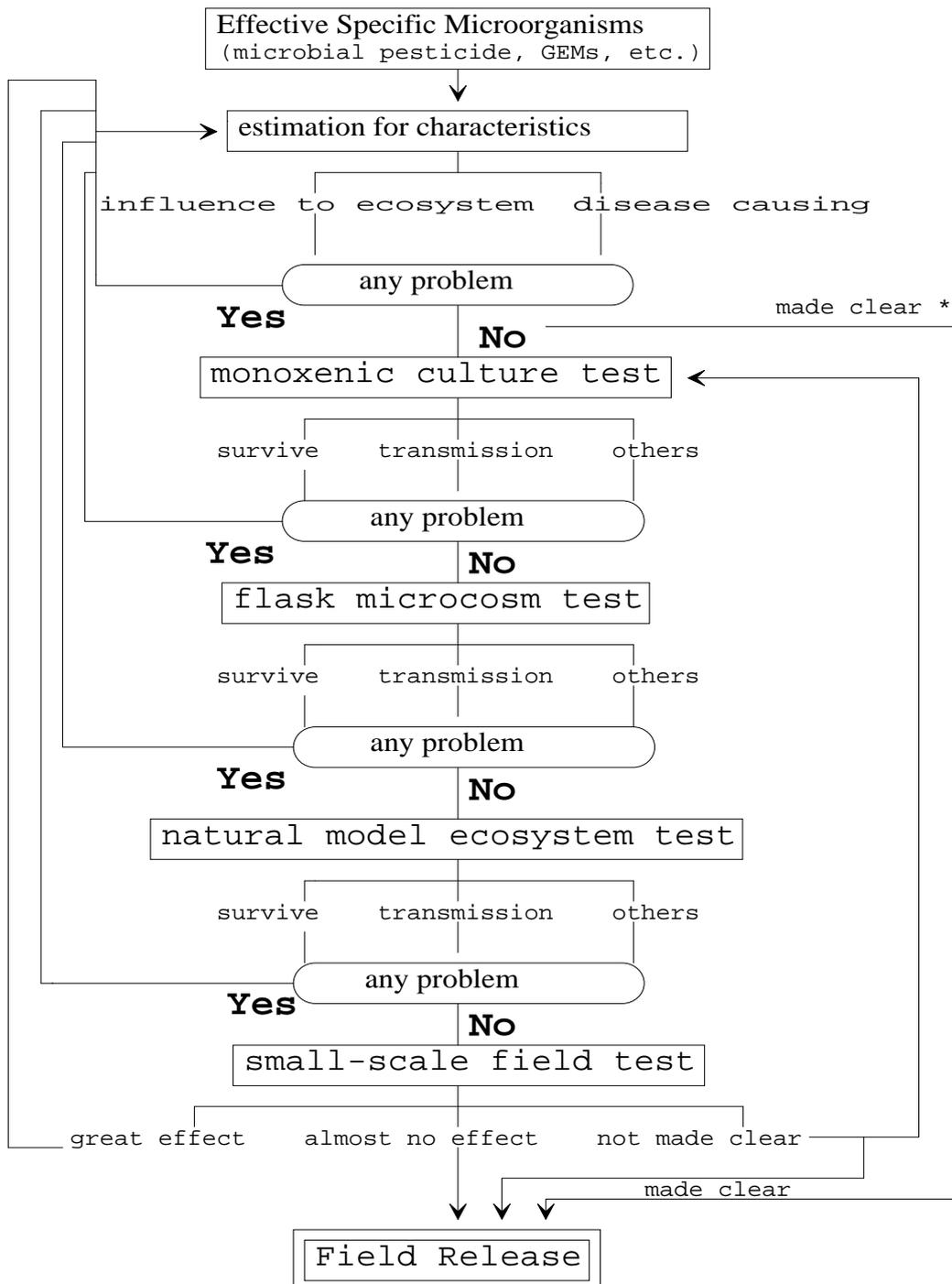


Fig.6 Environmental assessment of *Bacillus thuringiensis* subsp. *aizawai* KH using microcosm systems



* already used actually such as activated sludge bacteria, and so on.

Fig. 7 Flowchart of environmental assessment for field release of effective specific microorganisms

Bioremediation of PAH-contaminated Soils from a Gasworks Site with the Ebiox Vacuum Heap™ System: A Swiss Case Study

**Daniel Rolf Eiermann, Project Co-ordinator,
and Reinhard Bolliger, Safety and Technology Engineer**

**Ebiox AG
Sursee, Switzerland**

A former gasworks site in the industrial city of Winterthur, Switzerland, was extremely contaminated with the typical pollutants PAHs, BTEX, phenols, ammonia and mineral oils. During the summer of 1993 three vacuum heaps with a total volume of 10,500 m³ contaminated soil were prepared on site for microbiological treatment. The clean-up project, which is still going on, is an integrated part of the whole building project for realising an administration centre for the state gas, water and civil engineering departments. A carefully carried out separation of excavated soil material into different soil fractions upon excavation was crucial for the proper recycling of the extremely heterogeneous material. The excavation and separation process took more than ten months to complete, continuously delivering contaminated soil material for bioremediation. The material was conditioned and piled up into three vacuum heaps on three different locations and conditioned with the appropriate nutrients.

Ebiox Bioremediation enhances natural decontamination processes through biostimulation of the indigenous contaminant-adapted microflora. A carefully tuned mixture of macronutrients and trace elements (micronutrients) is added to the ecosystem. Sufficient oxygen is provided by an efficient bioventing system. Because no mechanical treatment is needed, the soil structure remains unchanged during the whole bioremediation process. Therefore the cleaned soil can be reused as a high-value filling material for civil engineering purposes.

The clean-up process is controlled by a sophisticated analytical monitoring programme. Thorough sampling for extended chemical and microbiological analyses is carried out at one month intervals. Weekly controls of the biosystem, including a reduced site monitoring with field test kits, are done to assure a smooth operation. The preliminary data set clearly demonstrates that the pollution degradation is proceeding as expected from the results of the treatability studies. The PAH concentrations dropped from 1316 to 457 mg/kg for vacuum heap 1 (65 per cent decrease within 269 days) and from 500 to 83 mg/kg for vacuum heap 2 (83 per cent decrease within 270 days). The PAHs were the major source of contamination. Minor contaminants like mineral oil and BTEX were removed even more efficiently. The legal target values were reached within the first three months in vacuum heap 1 and within two months in vacuum heap 2. No intermediates accumulated, indicating that the contaminants were in fact eliminated or incorporated into the stable organic fraction of the soil ecosystem. In both heaps the enhanced toxicity of the contaminated material could be reduced to the background levels of uncontaminated reference soils from the surrounding area.

The Ebiox Vacuum Heap™ system is well suited for large-scale treatments of excavated soil (thousands of cubic metres at a time). The technique is particularly interesting for bioremediation projects requiring short completion deadlines, confined working space, and tough air emission restrictions.

In order to meet high quality standards, a monitoring programme should cover the whole range of possible pathways of organic matter breakdown as well as the potential human health hazards. Suitable safety guidelines and standards have yet to be established. Today the required know-how and experience is only available from a few specialists. It is very important that these specialists communicate their knowledge and experience in multi-disciplinary committees and work groups. A professional and environmentally safe application of new biotechnology concepts is only possible through a balanced combination of scientific know-how and fundamental experience. The case presented is an example of the most advanced technology in the field of bioremediation. The applied quality standards are highly progressive and set the trends for future regulations.

Annex I

Information Elements from Environment Monograph 100

(Tables I-III)

The information elements identified in Tables I to III of Environment Monograph No. 100 (Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology) are listed in this Annex. Working Groups I and II examined the types of information needed to satisfy these information elements (see the "Report on the Working Groups", beginning on page 20).

**Information Elements – General Scientific Considerations
(Taken from Environment Monograph No. 100, Table I)**

A. Characteristics of Donor and Recipient Organisms

1. *Taxonomy, identification, source, culture*
 - 1a Names and designations;
 - 1b The degree of relatedness between the donor and recipient organisms and evidence indicating exchange of genetic material by natural means;
 - 1c Characteristics of the organism which permit identification, and the methods used to identify the organisms;
 - 1d Techniques employed in the laboratory and/or environment for detecting the presence of, and for monitoring, numbers of the organism;
 - 1e The sources of the organisms;
 - 1f Information on the recipient organism's reproductive cycle (sexual/asexual);
 - 1g Factors which might limit the reproduction, growth and survival of the recipient organism.

2. *Genetic characteristics of donor and recipient organisms*
 - 2a History of prior genetic manipulation;
 - 2b Characterisation of the recipient and donor genomes;
 - 2c Stability of recipient organism in terms of relevant genetic traits.

3. *Pathogenic and physiological traits of donor and recipient organisms*
 - 3a Nature of pathogenicity and virulence, infectivity, or toxigenicity;
 - 3b Host range;
 - 3c Other potentially significant physiological traits;
 - 3d Stability of these traits.

B. Character of the Engineered Organism

- 4a Description of the modification;
- 4b Description of the nature, function and source of the inserted donor nucleic acid, including regulatory or other elements affecting the function of the DNA and of the vector;
- 4c Description of the method(s) by which the vector with insert(s) has been constructed;
- 4d Description of methods for introducing the vector-insert into the recipient organism and the procedure for selection of the modified organism;
- 4e Description of the structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;
- 4f Characterisation of the site of modification of the recipient genome. Stability of the inserted DNA;
- 4g Frequency of mobilisation of inserted vector and/or genetic transfer capability;
- 4h Rate and level of expression of the introduced genetic material. Method and sensitivity of measurement;
- 4i Influence of the recipient organism on the activity of the foreign protein.

**Information Elements – Human Health Considerations
(Taken from Environment Monograph No. 100, Table II)**

Characteristics of the Engineered Organism

1. Comparison of the engineered organism to the recipient organism regarding pathogenicity.
2. Capacity for colonisation.
3. If the organism is pathogenic to humans (or to animals if appropriate):
 - 3a Diseases caused and mechanism of pathogenicity including invasiveness and virulence;
 - 3b Communicability;
 - 3c Infective dose;
 - 3d Host range, possibility of alteration;
 - 3e Possibility of survival outside of human host;
 - 3f Presence of vectors or means of dissemination;
 - 3g Biological stability;
 - 3h Antibiotic-resistance patterns;
 - 3i Toxigenicity;
 - 3j Allergenicity.

Health Considerations Generally Associated with the Presence of Non-viable Organisms or with the Products of rDNA Processes

4. Toxic or allergenic effects of non-viable organisms and/or their metabolic products.
5. Product hazards.

Management of Personnel Exposure

6. Biological Measures:
 - 6a Availability of appropriate prophylaxis and therapies;
 - 6b Availability of medical surveillance.
7. Physical and organisational measures.

**Information Elements –
Environmental and Agricultural Considerations
(Taken from Environment Monograph No. 100, Table III)**

Ecological Traits Relating to the Donor and Recipient

- 1a Natural habitat and geographic distribution. Climatic characteristics of original habitats;
- 1b Significant involvement in environmental processes;
- 1c Pathogenicity – host range, infectivity, toxigenicity, virulence, vectors;
- 1d Interactions with and effects on other organisms in the environment;
- 1e Ability to form survival structure (e.g. seeds, spores, sclerotia);
- 1f Frequency of genotypic and phenotypic change;
- 1g The role of the genetic material to be donated on the ecology of the donor organism;
- 1h The predicted effect of the donated genetic material on the recipient organism.

Application of the Engineered Organism in the Environment

- 2a Geographical location site, physical and biological proximity to man and/or any other significant biota;
- 2b Description of site including size and preparation, climate, temperature, relative humidity, etc.;
- 2c Containment and decontamination;
- 2d Introduction protocols including quantity and frequency of application;
- 2e Methods of site disturbance or cultivation;
- 2f Methods for monitoring applications;
- 2g Contingency plans;
- 2h Treatment procedure of site at the completion of application.

Survival, Multiplication and Dissemination of the Engineered Organism in the Environment

Detection, identification and monitoring techniques

- 3a Description of detection, identification and monitoring techniques;
- 3b Specificity, sensitivity and reliability of detection techniques;
- 3c Techniques for detecting transfer of the donated DNA to other organisms.

Characteristics affecting survival, multiplication and dissemination

- 4a Biological features which affect survival, multiplication or dissemination;
- 4b Behaviour in simulated natural environments such as microcosms, growth rooms, greenhouses, insectaries, etc.;
- 4c Known and predicted environmental conditions which may affect survival, multiplication, dissemination.

(continued next page)

**Information Elements –
Environmental and Agricultural Considerations (continued)**

Interactions of Engineered Organism(s) with Biological Systems

Target and non-target populations

- 5a Known and predicted habitats of the engineered organism;
- 5b Description of the target ecosystems and of ecosystems to which the organism could be disseminated;
- 5c Identification and description of target organisms;
- 5d Anticipated mechanism and result of interaction between the engineered organism and the target organism(s);
- 5e Identification and description of non-target organism(s) which might be exposed.

Stability

- 6a Stability of the organism in terms of genetic traits;
- 6b Genetic transfer capability;
- 6c Likelihood of post-release selection leading to the expression of unexpected and undesirable traits by the engineered organism;
- 6d Measures employed to ensure genetic stability, if any;
- 6e Description of genetic traits which may prevent or minimise dispersal of genetic material.

Routes of dissemination

- 7a Routes of dissemination, physical or biological;
- 7b Known or potential modes of interaction, including inhalation, ingestion, surface contact, burrowing and injection.

Potential Environmental Impacts

Potential effects on target and non-target organisms

- 8a Pathogenicity, infectivity, toxigenicity, virulence, vector of pathogen, allergenicity, colonisation;
- 8b Known or predicted effects on other organisms in the environment;
- 8c Likelihood of post-release shifts in biological interactions or in host range.

Ecosystems effects

- 9a Known or predicted involvement in biogeochemical processes;
- 9b Potential for excessive population increase.

Annex II

Workshop Participants

The Fribourg Workshop on Industrial Products of Modern Biotechnology Intended for Release to the Environment (May 1994)

AUSTRIA

Dr Helmut Gaugitsch
Federal Environmental Agency
Vienna

BELGIUM

Dr Jean-Marc Collard
Institute of Hygiene and Epidemiology
Biosafety and Biotechnology
Brussels

CANADA

Dr Desmond Mahon
Environment Canada
Hull, Quebec

Mr Bart Bilmer
Agriculture and Agri-Food Canada
Nepean, Ontario

Mr John Smith
Health Canada
Head, Biotechnology Section
Environmental Health Centre
Ottawa, Ontario

Dr Nigel Skipper
Head, Biotechnology Section
Environment Canada
Ottawa, Ontario

DENMARK

Mr Holger Pedersen
Danish Environmental Protection Agency
Copenhagen

GERMANY

Prof. Dr B. Appel
Robert Koch - Institute of the Federal Health Agency
Berlin

Dr Martin Mieschendahl
Federal Environmental Agency
Berlin

ITALY

Dr Laura Nicolini
National Institute of Health
Biological Service
Rome

Ms Gabriella Levi
ENEA Dipartimento Ambiente
Rome

JAPAN

Dr Tsuguyoshi Suzuki
Director General
The National Institute for Environmental Studies
Ibaraki

Mr Hideto Yoshida
Deputy Director
Environmental Research and Technology Division
Planning and Coordination Bureau
Environment Agency
Tokyo

Dr Osami Yagi
Head, Water Quality Science Section
Water and Soil
Environment Division
The National Institute for Environmental Studies
Ibaraki

Prof. Masanori Fujita
Professor
Environmental Engineering Department
Osaka University
Osaka

Mr Minoru Nishimura
Senior Consultant
Technology Department
Integrated Research Division
The Japan Research Institute Limited
Tokyo

Dr Ryuichiro Kurane
Director
Research Planning Office
National Institute of Bioscience and Human Technology
Agency of Industrial Science and Technology
MITI
Ibaraki

Mr Haruhito Fujita
Biochemical Industry Division
Basic Industries Bureau
MITI
Tokyo

NORWAY

Ms Guri Tveito
Norwegian Pollution Control Authority
Oslo

Mr Tormod Briseid
SINTEF
Oslo

SWEDEN

Dr Britta Hedlund
Swedish Environmental Protection Agency
Environmental Supervision Department
Unit for Environmental Monitoring
Solna

SWITZERLAND

Dr Francois Pythoud
Federal Office of Environment, Forests and Landscape
Bern

Dr Georg Karlaganis
Federal Office of Environment, Forests and Landscape
Bern

Dr Hans Hosbach
Federal Office of Environment, Forests and Landscape
Bern

Dr Werner Tosch
BIAC, Ciba-Geigy Ltd.
Basel

Dr Reinhard Bolliger
Ebiox AG
Sursee

Dr Ch. Wenger
BUWAL
Bern

Dr Urs Vogeli
KCGU
Basel

Dr Heinz Reust
Federal Office of Public Health
Bern

Dr T. Egli
EAWAG
Dübendorf

Dr Karoline Dorsch-Häsler
SKBS
Zürich

Dr Paul Steffen
Federal Office of Agriculture
Bern

Mr Bruno Milani
Federal Office of Environment, Forests and Landscape
Bern

Mr Bernard Jaggi
Federal Office for Foreign Economic Affairs
Bern

UNITED KINGDOM

Dr Iain M.M. Gillespie
Department of the Environment
London

Dr Firoz Amijee
Department of the Environment
London

Dr Penny Bramwell
NERC Institute of Virology and Environmental Microbiology
Oxford

Dr Mike Griffiths
(Department of Trade and Industry)
Mike Griffiths Associates
Woking, Surrey

UNITED STATES

Dr Lawrence Zeph
Environmental Protection Agency
Washington, D.C

Dr David Giamporcaro
Environmental Protection Agency
Washington, D.C.

Dr Philip Sayre
Environmental Protection Agency
Office of Pollution Prevention and Toxics
Washington, D.C.

EUROPEAN COMMISSION

Dr Joanna Tachmintzis
DG XI-A-2
Brussels
Belgium

Dr Manfred H.J. Schmitz
DG III E2, N9, 1/24a
Brussels
Belgium

Dr Guy Van Den Eede
Joint Research Centre
Ispra (Va)
Italy

Ursula Herranz Gomez
Joint Research Centre
Ispra (Va)
Italy

CZECH REPUBLIC¹

Professor Jaroslav Drobnik
Institute of Biotechnology
Charles University, Faculty of Science
Prague

Ing. Helena Štěpánková
Institute of Biotechnology
Charles University, Faculty of Science
Prague

HUNGARY

Mr Gabor Csernussi
Ministry for Environment and Regional Policy
Department for Public Relations
Budapest

¹ The Czech Republic became an OECD Member country in 1995.

OECD Secretariat

Dr Peter Kearns
Environmental Health and Safety Division
2 rue André Pascal
75775 Paris Cedex 16
France

Ms Lisa Zannoni
Environmental Health and Safety Division
2 rue André Pascal
75775 Paris Cedex 16
France

Dr Seizo Sumida
D.S.T.I. Biotechnology Unit
2 rue André Pascal
75775 Paris Cedex 16
France

Mr Yoshitaka Ando
D.S.T.I. Biotechnology Unit
2 rue André Pascal
75775 Paris Cedex 16
France

Ms Tiffany Larsen
Environmental Health and Safety Division
2 rue André Pascal
75775 Paris Cedex 16
France

Mme. Marie Siegenthaler
Switzerland

PUBLICATIONS LIST

As of March 1996

**OECD ENVIRONMENT DIRECTORATE,
ENVIRONMENTAL HEALTH AND SAFETY DIVISION**

**2 rue André-Pascal
75775 Paris Cedex 16
FRANCE**

Fax: (33-1) 45 24 16 75

E-mail: ehscont@oecd.org

**More information (and the full text of some publications)
are available on the OECD's World Wide Web site
(<http://www.oecd.org/ehs/>)**

Contents

The OECD Environment Monograph Series	123
OECD Priced Publications on Environmental Health and Safety	128
Some Environmental Health and Safety Publications in Preparation for 1996	129

PLEASE NOTE:

^F following a title indicates that the entire publication is available from the OECD in a separate French translation. The other publications listed are available in English only, but they often contain a French summary.

^{GLP} following a title indicates that the publication is part of the OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Translations of this series into Russian, Polish, Czech, Slovak, Hebrew, Spanish and Italian either exist or are planned. For more information, please contact the Environmental Health and Safety Division.

The OECD Environment Monograph Series:

The purpose of the Environment Monograph Series is to make technical documents prepared by the OECD Environment Directorate available to the public. The Environment Monographs on this list were prepared by the Environmental Health and Safety Division. Copies are available upon request at no charge, in limited quantities.

No. 14, *Final Report of the Expert Group on Model Forms of Agreement for the Exchange of Confidential Data on Chemicals* (1988)^F

No. 15, *Final Report of the Working Group on Mutual Recognition of Compliance with Good Laboratory Practice* (1988)^F

No. 17, *The Use of Industry Category Documents in Source Assessment of Chemicals* (1989)^F

No. 24, *Accidents Involving Hazardous Substances* (1989)^F

No. 25, *A Survey of Information Systems in OECD Member Countries Covering Accidents Involving Hazardous Substances* (1989)^F

[superseded by the *Users Guide to Information Systems Useful to Emergency Planners and Responders Available in OECD Member Countries* (1991)]

No. 26, *Report of the OECD Workshop on Ecological Effects Assessment* (1989)^F

No. 27, *Compendium of Environmental Exposure Assessment Methods for Chemicals* (1989)^F

No. 28, *Workshop on Prevention of Accidents Involving Hazardous Substances: Good Management Practice* (1990)^F

No. 29, *Workshop on the Provision of Information to the Public and on the Role of Workers in Accident Prevention and Response* (1990)^F

No. 30, *Workshop on the Role of Public Authorities in Preventing Major Accidents and in Major Accident Land-Use Planning* (1990)^F

No. 31, *Workshop on Emergency Preparedness and Response and on Research in Accident Prevention, Preparedness and Response* (1990)^F

No. 35, *A Survey of New Chemicals Notification Procedures in OECD Member Countries* (1990)^F

No. 36, *Scientific Criteria for Validation of In Vitro Toxicity Tests* (1990)^F

No. 39, *International Survey on Biotechnology Use and Regulations* (1990)^F

[no number] *Users Guide to Hazardous Substance Data Banks Available in OECD Member Countries*, OCDE/GD(91)102 (1991)^F

[Also translated into Spanish by the United Nations Environment Programme's Industry and Environment Office (UNEP IE).]

[no number] *Users Guide to Information Systems Useful to Emergency Planners and Responders Available in OECD Member Countries*, OCDE/GD(91)103 (1991)^F

[Also translated into Spanish by UNEP IE.]

No. 43, *International Directory of Emergency Response Centres* (1992)^F

[The International Directory is a co-operative project of OECD and UNEP IE. The emergency response centres listed in this Directory are located in both OECD and non-OECD countries.]

No. 44, *Workshop on Prevention of Accidents Involving Hazardous Substances: The Role of the Human Factor in Plant Operations* (1992)

No. 45, *The OECD Principles of Good Laboratory Practice* (1992)^{F, GLP}

No. 46, *Guides for Compliance Monitoring Procedures for Good Laboratory Practice* (1992)^{F, GLP}

[superseded by No. 110, *Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice* (1995)]

No. 47, *Guidance for the Conduct of Laboratory Inspections and Study Audits* (1992)^{F, GLP}

[superseded by No. 111, *Revised Guidance for the Conduct of Laboratory Inspections and Study Audits* (1995)]

No. 48, *Quality Assurance and GLP* (1992)^{F, GLP}

No. 49, *Compliance of Laboratory Suppliers with GLP Principles* (1992)^{F, GLP}

No. 50, *The Application of the GLP Principles to Field Studies* (1992)^{F, GLP}

No. 51, *Guiding Principles for Chemical Accident Prevention, Preparedness and Response: Guidance for Public Authorities, Industry, Labour and Others for the Establishment of Programmes and Policies related to Prevention of, Preparedness for, and Response to Accidents Involving Hazardous Substances* (1992)^F

[The Guiding Principles are also available in Russian. They are being translated into Spanish, and may also be translated into other languages. For more information, please contact the Environmental Health and Safety Division.]

No. 52, *Report of the OECD Workshop on Monitoring of Organisms Introduced into the Environment* (1992)

No. 58, *Report of the OECD Workshop on Quantitative Structure Activity Relationships (QSARS) in Aquatic Effects Assessment* (1992)

No. 59, *Report of the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment* (1992)

No. 60, *Report of the OECD Workshop on Effects Assessment of Chemicals in Sediment* (1992)

No. 65, *Risk Reduction Monograph No. 1: Lead* (1993)

No. 66, *Report of the OECD Workshop on Strategies for Transporting Dangerous Goods by Road: Safety and Environmental Protection* (1993)

[The OECD's Chemical Accidents Programme and Road Transport Research Programme co-operated in organising this workshop.]

No. 67, *Application of Structure-Activity Relationships to the Estimation of Properties Important in Exposure Assessment* (1993)

No. 68, *Structure-Activity Relationships for Biodegradation* (1993)

No. 69, *Report of the OECD Workshop on the Application of Simple Models for Exposure Assessment* (1993)

No. 70, *Occupational and Consumer Exposure Assessments* (1993)

No. 73, *The Application of the GLP Principles to Short-term Studies* (1993)^{F, GLP}

No. 74, *The Role and Responsibilities of the Study Director in GLP Studies* (1993)^{F, GLP}

No. 76, *OECD Series on the Test Guidelines Programme No. 1: Guidance Document for the Development of OECD Guidelines for Testing of Chemicals* (1993; reformatted 1995)^F

No. 77, *Data Requirements for Pesticide Registration in OECD Member Countries: Survey Results* (1993)

No. 81, *Health Aspects of Chemical Accidents: Guidance on Chemical Accident Awareness, Preparedness and Response for Health Professionals and Emergency Responders* (1994)^F

[Four international organisations collaborated in the preparation of this publication: the International Programme on Chemical Safety (IPCS), OECD, UNEP IE, and the World Health Organization – European Centre for Environment and Health (WHO-ECEH).]

No. 88, *US EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships* (1994)

No. 90: *Ottawa '92: The OECD Workshop on Methods for Monitoring Organisms in the Environment* (1994)*

No. 91: *Compendium of Methods for Monitoring Organisms in the Environment* (1994)*

[*Monographs No. 90 and 91 are companion documents.]

No. 92, *Guidance Document for Aquatic Effects Assessment* (1995)

No. 93, *Report of the OECD Workshop on Chemical Safety in Port Areas* (1994)

[This Workshop was co-sponsored by OECD, the International Maritime Organization (IMO) and UNEP.]

No. 94, *Report of the OECD Special Session on Chemical Accident Prevention, Preparedness and Response at Transport Interfaces* (1995)

No. 95, *Report of the OECD Workshop on Small and Medium-sized Enterprises in Relation to Chemical Accident Prevention, Preparedness and Response* (1995)

No. 98, *OECD Series on the Test Guidelines Programme No. 2: Detailed Review Paper on Biodegradability Testing* (1995)

No. 99, *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (1995)

No. 100, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology* (1995)

No. 101, *Risk Reduction Monograph No. 2: Methylene Chloride* (1994)

No. 102, *Risk Reduction Monograph No. 3: Selected Brominated Flame Retardants* (1994)

No. 103, *Risk Reduction Monograph No. 4: Mercury* (1994)

No. 104, *Risk Reduction Monograph No. 5: Cadmium* (1994)

No. 105, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment* (1995)

No. 106, *Data Requirements for Biological Pesticides* (1996)

No. 107, *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* (1995)

No. 108, *Final Report on the OECD Pilot Project to Compare Pesticide Data Reviews* (1995)

No. 110, *Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice* (1995)^{F, GLP}

No. 111, *Revised Guidance for the Conduct of Laboratory Inspections and Study Audits* (1995)^{F, GLP}

No. 115, *Guidance for the Preparation of GLP Inspection Reports* (1995)^{F, GLP}

No. 116, *The Application of the Principles of GLP to Computerised Systems* (1995)^{F, GLP}

OECD Priced Publications on Environmental Health and Safety:

OECD Guidelines for Testing of Chemicals (updated 1995)^F
(OECD No. 97 93 50 1) ISBN 92-64-14018-2 992 pages
Price in France: FF 800
Price in other countries: FF 1040 US\$ 178.00 DM 300

[Also available in CD-ROM version: for more information, contact the OECD Publications Service]

Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles (1993)^F
(OECD No. 93 04 1) ISBN 92-64-13859-5 80 pages
Price in France: FF 80
Price in other countries: FF 100 US\$ 19.00 DM 33

[Prepared in collaboration with the OECD Directorate for Science, Technology and Industry]

"OECD Documents" Series

Aquatic Biotechnology and Food Safety (1994)
(OECD No. 97 94 05 1) ISBN 92-64-14063-8 100 pages
Price in France: FF 80
Price in other countries: FF 100 US\$ 18.00 DM 30

[Prepared in collaboration with the OECD Directorate for Science, Technology and Industry]

Environmental Impacts of Aquatic Biotechnology (1995)
(OECD No. 97 95 14 1) ISBN 92-64-14666-0 171 pages
Price in France: 130 FF
Price in other countries: 170 FF US\$ 35.00 DM 49 £ 22

[Prepared in collaboration with the OECD Directorate for Science, Technology and Industry.]

The priced publications above may be ordered directly from: OECD Publications Service, 2 rue André-Pascal, 75775 Paris Cedex 16, France. Telex: 640 048. Telefax: (33-1) 49 10 42 76.

Some Environmental Health and Safety Publications in Preparation for 1996:

Activities to Reduce Pesticide Risks in OECD and FAO Member Countries

Guidance Document for the Conduct of Field Studies of Exposure of Pesticides in Use

Comparison of Ecological Hazard/Risk Assessment Schemes

Report of the SETAC/OECD Workshop on Avian Toxicology

The OECD Guidelines for the Testing of Chemicals (7th addendum)

Guidance Document on Dose Level Selection in Carcinogenicity Studies

Detailed Review Paper on Aquatic Toxicity Testing Methods

Report of the Final Ring Test of the Daphnia magna Reproduction Study

Report of the OECD Workshop on Risk Assessment and Risk Communication in the Context of Accident Prevention, Preparedness and Response

Report of the OECD/UN-ECE Workshop on Chemical Accidents

Guidance Concerning Chemical Safety in Port Areas (prepared as a joint effort with the International Maritime Organization)

Guidance Concerning Health Aspects of Chemical Accidents

Report of the OECD Cadmium Workshop^{*}

^{*} Proposed for publication in the "OECD Documents" series.

*Report the OECD Food Safety Workshop at Oxford**

Consensus Document on Information Used in the Assessment of Environmental Applications Involving Pseudomonas

Consensus Document on Information Used in the Assessment of Environmental Applications Involving Rhizobiacea

Consensus Document on Information Used in the Assessment of Environmental Applications Involving Bacillus

Consensus Document on Virus Resistance through Coat Protein–mediated Protection

Consensus Document on the Biology of Brassica Napus L (Oilseed Rape)