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MICROBIAL PESTICIDES II

[*data evaluation and environmental risk assessment: a desk study*]

B.J.W.G. Mensink, B.P. Loos & J.B.H.J. Linders

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National Institute of Public Health and the
Environment / RIVM
P.O. Box 1, 3720 BA Bilthoven,
The Netherlands
Tel.: (31) 30 2749111, Fax: (31) 30 2742971

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PREFACE AND ACKNOWLEDGEMENTS

This study should be seen as a discussion paper: what are the (im)possibilities of a structured and transparent system to assess the environmental risks of microbial pesticides? An increase in the use of microbial pesticides may be expected in the Netherlands in the near future, due to governmental policies to reduce chemical pesticide emissions and the dependence of the Dutch agriculture on chemical pesticides. However, contrary to chemical pesticides and transgenic (micro-)organisms — covered by the Dutch Pesticide Act and the Decree on Genetically Modified Organisms, respectively — , there is no such system for the environmental risk assessment of microbial pesticides. The examples in this study referring to transgenic microorganisms — with or without pesticidal action — or chemical pesticides are meant for comparative reasons: what can we learn from the expertise in the Netherlands respecting the registration and environmental risk assessment of these. In summary, the focus of this study is on the non-transgenic microbial pesticides.

This study presents a concise overview of key aspects of data evaluation (*i.e.* the interpretation of test data submitted for registration procedures) and environmental risk assessment (*i.e.* qualifying or quantifying the potential risks, prior to introduction and based on the submitted test data) for microbial pesticides. The major subdivision in microbial pesticides, as distinguished in the present study, is non-viral *versus* viral. The study should be seen as a pilot in developing a more consistent and adequate framework for the data evaluation and the environmental risk assessment for microbial pesticide registration purposes. When genetically modified microbial pesticides will be registered, they have to comply with the Pesticide Act. As the registration procedures concerning genetically modified (micro)organisms have already been developed into more detail, these may serve as examples. Also, some basic aspects of environmental behaviour, fate, and effects of genetically modified microorganisms may be the same as for non-modified microorganisms. Therefore RIVM experts have been consulted on the environmental impact of the introduction of genetically modified microorganisms — with or without pesticidal action — into the natural environment. For this the authors express their gratitude to dr. ir. K. Wernars and dr. E. Smit.

The completion of a consistent framework for evaluating submitted test data and, subsequently, assessing the environmental impact of microbial pesticides will be a highly time-consuming effort, as there is yet not much *integral* expertise and experience that comprise the scientific fields of molecular biology, microbiology, microbial ecology and ecotoxicology on this issue. It is therefore not surprising that in many countries, incl. the Netherlands, such data evaluations and environmental risk assessments for new microbial pesticides occur on a case by case basis. In the near future this will remain inevitable to some

extent. There is, however, the challenge to find "the common elements" to structure and simplify — if possible — such a framework. This study pretends to make a start, anticipating the developments in *e.g.* microbial ecology and molecular microbiology that may result in new concepts for — integrated? — pest management in the near future (*e.g.* more environmentally friendly and more pest specific). The authorities should be able to cope with these developments in a proper way. It is expected that a "structured and transparent framework", as stated above, may facilitate registration procedures, if its rationale is clear to all participants in the registration process. This should always be kept in mind, prior to further development.

As this study reflects a state-of-the-art, it is not surprising that the key aspects of environmental risk assessment are primarily dealt with in a qualitative way. A probabilistic approach is even more ahead of us. Experts of the US EPA and the Canadian PMRA — who are now combining forces in developing some more general ideas on this issue — do not expect such a quantitative approach before 2010.

ABSTRACT

A 1998 study discusses the (im)possibilities of a more structured and transparent system for assessing the environmental risks of microbial pesticides. As current environmental risk assessments by registration authorities are on a case-by-case basis, it is a challenge to transform the existing experience — most of it gained in Canada and the US — into a more systematic approach. This will probably result in a more structured, transparent and reproducible environmental risk assessment. It is to be expected that such an approach may enable governments to cope with the increasing number of registrations of (micro-)biological pesticides. A concurrent advantage of such a framework for risk assessment — with the prerequisite of a transparent rationale — may be the acceleration of registration procedures for such pesticides. Major impediments for the development of such a framework are:-I- the diversity and complexity of microbial organisms and their behaviour and interactions in the environment; -II- the lack of experience with *in situ* behaviour, fate, and adverse effects of pesticidal microorganisms to biota (apart from some work with *Bacillus thuringiensis* and several Baculoviridae, which are two groups without almost any adverse environmental effect); -III- small market shares to be expected in the near future for new microbial pesticides. Nevertheless, the authors feel that in spite of these impediments progression can be made towards a more systematic, transparent and harmonised approach. Therefore, the present study successively stipulates for microbial pesticides general aspects of identification of hazards, exposure and effects assessment, and risk characterisation. As the identification of hazards includes the evaluation of individual tests, key items are listed and annotated for tests on distribution and fate. This is also done for effects on organisms in the environment. These lists can be used to screen for the purpose of summarising and evaluating experimental tests for pesticide registration.

CONTENTS

ABBREVIATIONS

AI	Active ingredient
APHIS	Animal and Plant Health Inspection Service (section of US Department of Agriculture)
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft (Germany)
BPPD	Biopesticides and Pollution Prevention Division (section of OPPTS/EPA) (US)
CA	Competent authorities
COGEM	Committee for Genetically Modification (in Dutch: COmmissie GEnetische Modificatie) (Netherlands)
CSR	Centre for Substances and Risk Assessment (section of RIVM; in Dutch: Centrum voor Stoffen en Risicobeoordeling) (Netherlands)
CTB	Board for Authorisation of Pesticides (in Dutch: College voor de Toelating van Bestrijdingsmiddelen) (Netherlands)
EDR	Environmental data requirement
EPA	Environmental Protection Agency (US)
ERA	Environmental risk assessment
GMM	Genetically modified microorganism
GMO	Genetically modified organism
ICP	Insecticidal crystalline protein
IPO-DLO	Research Institute for Plant Protection (Netherlands)
IU	International unit
KEMI	National Chemicals Inspectorate (Sweden)
MGB	Microbiological Laboratory for Health Protection (section of RIVM; in Dutch: Microbiologisch Laboratorium voor Gezondheidsbescherming) (Netherlands)
MO	Microorganism
MOPA	Microorganism with pesticidal action
NBCI	National Biological Control Institute (section of APHIS) (US)
NPV	Nucleopolyhedrosis virus
NTO	Non-target organism
OPP	Office of Pesticides Programs (section of EPA) (US)
OPPTS	Office of Prevention, Pesticides and Toxic Substances (section of EPA) (US)
PMRA	Pest Management Regulatory Agency (Canada)
RI	Reliability index
TO	Target organism
UBA	Umweltbundesamt (Germany)

SUMMARY

Microbial pesticides are different from chemical pesticides, since their main active constituent — a microorganism with pesticidal action — may proliferate in the environment. Contrarily, the active ingredient of chemical pesticides generally decrease in due course. However, such an increase of *e.g.* a non-indigenous microorganism, has, up till now, not been observed *in situ*. Another essential difference is that a pesticidal microorganism — whether indigenous or not — can be assumed to transfer genetic material to related species. Such genetic transfer — mainly via conjugation — has been observed in the laboratory. However, there are some indications that laboratory tests may overestimate the probabilities of genetic transfer. Therefore the ecological relevance of the genetic transfer in the laboratory is unclear.

The present report is the second in a series concerning the environmental evaluation of microbial pesticides with the following triplet: data requirements, data evaluation, and risk assessment. The first report dealt in particular with the kind of data that could be required for (re)registration purposes. It discussed, amongst others, the differences in data requirements between the EC, the US, Canada, the Netherlands, and Denmark. The present report is a discussion paper: what are the (im)possibilities of a more structured and transparent system for assessing the environmental risks of microbial pesticides? Environmental risk assessments by registration authorities are generally performed on a case-by-case basis, probably due to the substantial variety between microorganisms, their complex biology, or their similarity with microorganisms with little or without adverse environmental effects. Nevertheless, the current market on microbial pesticides is dominated by only two groups: *Bacillus thuringiensis* and some Baculoviridae, both with almost no adverse environmental effects (as documented in the scientific literature). The application areas for other registered microbial pesticides are generally small. It is however expected that in the near future more microbial pesticides will enter the market as the use of chemical pesticides will be regulated more strictly. In view of this expected increase, it is a challenge to transform the existing experience with the triplet of data requirement, data evaluation, and risk assessment — especially in Canada and the US — into a more systematic approach, whenever this is possible. If the rationale of this approach is transparent for all participants in the registration process, we believe that this will facilitate that process.

A more systematic way of environmental risk assessment appears to be impeded primarily by to the complexity of microbial ecosystems. These are difficult to simulate under laboratory conditions, and the results of these laboratory experiments will always raise the question whether the same results can be observed in the field. Experience on this subject has already been gained in the Netherlands by the registration authorities for genetically modified organisms. The registration authorities for pesticides should benefit from this experience.

They should also benefit from the experience in Canada and the US, as these are joining forces on this issue.

The present study successively stipulates for microbial pesticides general aspects of identification of hazards, exposure and effects assessment, and risk characterisation. As the identification of hazards includes the evaluation of individual tests, key items are listed and annotated for tests on distribution and fate. This is also done for the effects on organisms in the environment. These lists can be used to screen for the purpose of summarising and evaluating experimental tests for pesticide registration.

The present study also stipulates that there are serious data gaps in the scientific fields impeding the further development of a proper triplet on data requirements, data evaluation, and environmental risk assessment. These gaps are especially in the field of microbial ecology, *e.g.* which factors will determine the extent of propagation and spatial distribution in the field. Important research topics should also comprise *e.g.* the extent of genetic exchange of those parts responsible for toxin production in the field and the extent of fitness differences between indigenous and non-indigenous populations of microbial microorganisms with pesticidal action. The ecological relevance of significant differences in the laboratory should be investigated more properly. Special attention should be paid to the role of competition between populations and to the suitability of the "no-effect level" and the "dose-effect" concept. These concepts have shown to be very useful for chemical pesticides. For microbial pesticides this is not necessarily the same.

SAMENVATTING

Microbiële bestrijdingsmiddelen kunnen verschillen van chemische bestrijdingsmiddelen in die zin dat het werkzame bestanddeel — *i.e.* het pesticidale microorganisme — in het milieu kan groeien en in aantal toenemen. De hoeveelheid werkzaam bestanddeel van een chemisch bestrijdingsmiddel daarentegen zal in de meeste gevallen na verloop van tijd afnemen. Een dergelijke toename van een niet-indigeen microorganisme is echter tot op heden *in situ* niet waargenomen. Microbiële bestrijdingsmiddelen zijn — zowel inheems als niet inheems — in staat tot het uitwisselen van genetische informatie met verwante soorten. Dergelijke genetische uitwisseling via conjugatie is waargenomen voor diverse microorganismen in het laboratorium. Er zijn echter aanwijzingen dat de in het laboratorium waargenomen frequenties van genetische uitwisseling worden overschat. De ecologische relevantie van de in het laboratorium waargenomen genetische transfer is derhalve onduidelijk.

Deze studie is een tweede in een reeks over de milieuevaluatie van microbiële bestrijdingsmiddelen aan de hand van het volgende triplet: gegevensvereisten, gegevensevaluatie en tenslotte risicobeoordeling. De eerste studie ging vooral in op het soort van gegevens dat gevraagd wordt voor (her)registratiedoeleinden. Het besprak o.a. de verschillen hierin tussen de EU, de VS, Canada, Nederland en Denemarken. De nu voor u liggende studie is vooral een discussiestuk: welke zijn de (on)mogelijkheden om een meer gestructureerd en transparent systeem op te zetten voor het beoordelen van de milieurisico's van microbiële bestrijdingsmiddelen? De competente autoriteiten voeren tot op heden dergelijke beoordelingen uit op een *ad hoc* basis, waarschijnlijk door de grote soortenrijkdom van de microorganismen, hun complexe biologie, of de verwantschap met microorganismen met weinig of geen milieuschadelijke effecten. Toch wordt de markt momenteel slechts door een beperkt aantal microbiële bestrijdingsmiddelen overheerst: *Bacillus thuringiensis* en sommige Baculoviridae, twee groepen waarvan in de wetenschappelijke literatuur — bijna — geen milieuschadelijke effecten worden gerapporteerd. De overige hebben een beperkt afzetgebied. Het ligt echter in de verwachting dat in de nabije toekomst meer microbiële middelen op de markt zullen komen omdat het gebruik van chemische middelen strikter gereguleerd zal worden. Gezien deze verwachting is het de uitdaging om de bestaande kennis met betrekking tot het bovengenoemde triplet — zoals die vooral in de V.S. en Canada bestaat — om te vormen tot een meer systematische aanpak, voorzover mogelijk.

Een dergelijke systematische benadering van de milieuevaluatie lijkt vooral te worden bemoeilijkt door de enorme complexiteit van microbiële ecosystemen. Deze zijn moeilijk te simuleren onder laboratoriumcondities en de uitkomsten van dergelijke studies roepen onvermijdelijk de vraag op of dezelfde uitkomsten in het veld verwacht mogen worden. In Nederland is met betrekking daartoe expertise opgebouwd bij de registratie van genetisch

gemodificeerde organismen. De Nederlandse registratie-autoriteiten voor bestrijdingsmiddelen kunnen hopelijk profiteren van deze expertise. Hopelijk kunnen ze ook profiteren van de expertise in met name de V.S. en Canada, zeker nu deze hun krachten bundelen op dit gebied.

Deze studie benadrukt een aantal algemene aspecten van achtereenvolgens de gevaarsidentificatie, de blootstellings- en de effectbeoordeling, en de risicokarakterisatie bij het gebruik van microbiële bestrijdingsmiddelen. Als onderdeel van de gevaarsidentificatie zijn ook een aantal tabellen opgenomen met hierin de belangrijkste aandachtspunten voor het samenvatten en beoordelen van individuele testen, zowel wat betreft verspreiding en lotgevallen als milieueffecten.

De studie geeft ook aan dat er substantiële leemtes zijn in de wetenschappelijke kennis die nodig is om het bovengenoemde evaluatie-triplet verder te ontwikkelen. Deze leemtes betreffen vooral de microbiële ecologie, bijvoorbeeld welke factoren de voortplanting en verspreiding bepalen van — pesticidale — microorganismen in het veld. Belangrijke onderzoeksthema's zouden kunnen zijn: het vaststellen en verklaren van eventuele verschillen in "fitness" tussen indigene en niet-indigene microbiële populaties en het vaststellen van de mate van genetische uitwisseling — bijvoorbeeld van dat deel dat zorgt voor de aanmaak van toxines. Daarnaast zou de ecologische relevantie van in het laboratorium vastgestelde significante verschillen tussen blootgestelde en niet-blootgestelde microbiële populaties beter onderzocht moeten worden. Speciale aandacht moet uitgaan naar de rol van competitie tussen populaties en de bruikbaarheid van het "no-effect" en het "dosis-effect" concept. Beide concepten zijn belangrijk bij de evaluatie van chemische bestrijdingsmiddelen. Voor microbiële bestrijdingsmiddelen zijn zij niet altijd noodzakelijkerwijs toepasbaar.

1. INTRODUCTION

This study is primarily a discussion paper: what are the (im)possibilities of a structured and transparent system to assess the environmental risks of microbial pesticides? The study also refers to the evaluation of experimental data from individual studies, prior to the environmental risk assessment (ERA[1]). As it is the follow-up of the study by Mensink and Linders (1997) on the data that could be required from the industry by the competent authorities (CA), it completes the triplet of data requirement, data evaluation, and risk assessment. Hopefully, it gives guidance on the further elaboration of this triplet to be used for the registration of microbial pesticides.

Currently, no structured and transparent framework is operational for the evaluation of submitted test data and the environmental risk assessment (ERA) for registration purposes, prior to the introduction of new microbial pesticides (Mensink & Linders, 1997). Environmental data on the distribution, fate and adverse effects, if required by the CAs at all, are evaluated in many countries on a case by case basis (person. communic. of *e.g.* EPA, PMRA, BBA, KEMI). The major criterion in this approach appears to be the (dis)similarity to indigenous species or strains. If a microbial pesticide is not genetically modified, and if it resembles a naturally occurring microorganism (MO), the European countries will generally registrate such a pesticide without much additional environmental data requirements (EDRs). Wäckers (1992) published a proposal for data requirements concerning the environmental behaviour and fate, and the human and ecotoxicological effects[2] on behalf of the Dutch Board for Authorisation of Pesticides[3]. However, this proposal has never become operational.

Why is it important to focus upon microbial pesticides? An increasing market share of microbial pesticides may be expected in view of the increasing demand for biological pest control. It is *e.g.* expected that the current *Bacillus thuringiensis* market will increase at a rate of 20% per year (Adams *et al.*, 1996). In view of the rapid developments in biotechnology, the introduction within the European Union of *e.g.* transgenic corn (*Zea mays*) with a gene from *Bacillus thuringiensis* appears to be more rapid than expected (person. communic. of Koppert Biological Systems bv to RIVM). These developments have both governmental, agronomical and public incentives: environmental safety, insect selectivity, the increasing resistance of insects against chemical insecticides and the increasing banning of chemical pesticides (Adams *et al.*, 1996).

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[1] for reasons of convenience the explanation of most abbreviations is repeated once in the beginning of the Chapters 1, 2, 3, 4, 5, and the Appendices. Beyond, the reader is kindly referred to the abbreviation list.

[2] this proposal has been discussed and summarised in Mensink & Linders (1997).

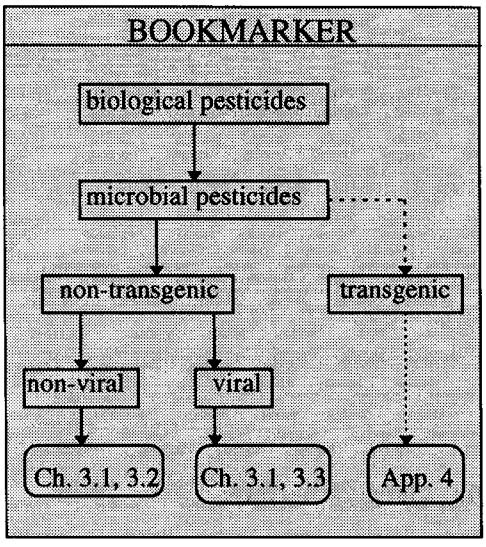
[3] in Dutch: het College voor de Toelating van Bestrijdingsmiddelen (CTB).

Increasing resistance has been reported by Smits and Vlak (1994). The beet armyworm (*Spodoptera exigua*) showed an increasing resistence against the chemical pesticides methomyl and diflubenzuron and their combinations in Dutch greenhouses growing *Chrysanthemum*. To cope with these noctuid moth caterpillars, the application frequency had to be increased up to once a week or even more. Both environmental and agronomical aspects brought IPO-DLO, a Dutch research institute on plant protection, to take the lead in commercially marketing a baculovirus against *Spodoptera*. At first industry was not interested, as they expected the fees for registration to be too high, and the field of application too small. However, in January 1994 SPOD-X^R came on the Dutch market, after renewed commercial interest.

As the biotechnological developments will be rapid — and therefore the increasing options for producing microbial pesticides — one may prognosticate that a case by case approach for assessing the environmental risks of microbial pesticides will be impractical and insufficient. Therefore, this study aims at a more consistent approach, so far as feasible. On one hand this study will lean on the current approaches for assessing environmental risks of chemical pesticides. On the other hand, it will lean on the current developments in the fields of microbiology, microbial ecology, ecotoxicology, biotechnology, and risk assessment.

The Dutch Directorate-General for Environmental Protection acknowledges the gap in the legislation on microbial pesticides: expertise on the risk assessment should be developed rapidly within a European context (de Boer, 1997). The Directorate-General also acknowledges that the legislative framework for chemical pesticides is not applicable to biological pesticides. Manufacturers are generally complaining about the slow registration procedures for microbial pesticides in the Netherlands. Besides, they often consider the registration fees too high in relation to the often small agricultural areas for application. c. DFL 40,000 had to be paid in 1994 for the evaluation of a registration (Smits & Vlak, 1994)

Having noted the reasons for focusing upon microbial pesticides, one may wonder about the number and nature of those microbial pesticides that — in spite of the difficulties, as stated above —, are actually registered in *e.g.* the Netherlands and the US. These lists of microbial pesticides — see Appendix 2 and 3 — at least tell us for which microbial pesticides experience has been gained in evaluating the test data for registration purposes. The Animal and Plant Health Inspection Service (APHIS) has gained considerable experience in regulating microbial pesticides in the US (NBCI, 1996).



Any microbial pesticide in this study is assumed to contain a living microbial microorganism with pesticidal action[4] (MOPA). A MOPA (incl. any associated metabolite) can be considered as the "active ingredient". In case the pesticidal action is due to a toxin, the MO — producing the toxin — is a MOPA, if the product contains the living toxin-producing MO. If the product contains the toxin only, the pesticide is defined as a chemical pesticide.

Non-transgenic MOPAs may be indigenous species, non-indigenous species or species that have been obtained by traditional methods for selecting and upgrading particular strains. Examples are the various insecticidal strains of *Bacillus thuringiensis*[5] (non-viral) and Baculoviridae (viral).

The registration and the environmental risk assessment of non-transgenic microbial pesticides should be seen in the context of EU Directive 91/414. Genetically modified microorganisms (GMMs) with pesticidal action should be judged on the basis of EU Directives 90/219 and 90/220. The Directive 90/220 deals in particular with the introduction of a GMM — with or without pesticidal action — into the field. After such a judgement — on the particular aspects of being transgenic — a further judgement will take place on its particular aspects of being a pesticide. In as far as the Dutch expertise on GMMs may elucidate comparable approaches for non-transgenic microorganisms, these are described in Appendix 4.

[4] *e.g.* direct efficacy via interaction with a target (*e.g.* inhibition of an enzyme), or indirect efficacy via niche competion (*e.g.* the microfungus *Trichoderma*).

[5] *Bacillus thuringiensis* may produce insecticidal crystal proteins (ICPs) that can be activated in the midgut of pest insects to a toxin killing the insect (see also sect. 3.2.2).

In summary, the aims of this study are:

I. to give guidance for evaluating and summarising[6] experimental data that are submitted for the registration of a microbial pesticide.
II. to evaluate the (im)possibilities of developing a structured, transparent, and reproducible type of environmental risk assessment (ERA)
    - II A to pinpoint the key elements that should be part of such a structured, transparent, and reproducible type of ERA.
    - II B to pinpoint the scientific drawbacks that hamper the development of such ERAs for microbial pesticides.

Three additional questions have been considered helpful to reach this aim:

a) Can the current Dutch practices on the data evaluation and environmental risk assessment of GMOs contribute to the development of such a framework — for the data evaluation and environmental risk assessment — for microbial pesticides?
b) Is the division into viral and non-viral — sufficient for an initial qualitative ERA?
c) Can the current Canadian/US practices on the environmental evaluation of microbial pesticides provide guidance for the Dutch or European practices in this field?

[6] a summary refers to a concise text with the most relevant aspects of a particular test. Summaries are made for registration procedures.

2. METHODOLOGY

This desk study is a literature and source investigation. All definitions in the field of risk assessment for chemical substances are in conformity with those in use for (E)USES, a system for risk assessment for governmental ministries and institutes (RIVM, VROM & WVC, 1994; EC, 1996; Linders & Jager, 1997). The relevant parts of environmental risk assessment (ERA) — and their definitions as used in this study — are listed in Table 1.

Table 1. Goals of environmental data requirements for microbial organisms with pesticidal action (MOPAs). Environmental risk assessment (ERA) is the process that contains some of or all these four elements (adapted from Van Leeuwen & Hermens, 1995)

ENVIRONMENTAL RISK ASSESSMENT	DEFINITIONS
HAZARD IDENTIFICATION	*identifying the inherent capacity of a MOPA or its formulation to cause adverse effects to the environment (incl. e.g. fate in soil, infectivity to birds, toxicity to mammals),*
EXPOSURE ASSESSMENT:	*predicting the distribution and fate of MOPAs in the area of MOPA application to estimate MOPA amounts to which non-target species or populations may be exposed; the exposure assessment refers to the environmentally relevant compartments in the area of MOPA application,*
EFFECTS ASSESSMENT:	*identification of effect or no-effect levels of MOPAs for various ecologically relevant non-target groups (e.g. an NOEC for aquatic organisms); the effects assessment refers to the environmentally relevant compartments in the area of MOPA application; as it focuses upon effects dependent on a particular dose, the concept of dose-effect relations is included in the effects assessment (if applicable).*
RISK CHARACTERISATION:	*predicting the incidence or probability of the adverse effects likely to occur in an environmental compartment due to the predicted exposure to a MOPA (e.g. the risk of killing fish, terrestrial predators or large vertebrates).*

This study has been limited to those ERA aspects as listed in Table 1. The valuation of the outcomes of the risk characterisation, often referred to as the risk evaluation, which is the next step in risk assessment after risk characterisation, has not been taken into account.

3. RESULTS

The following test results — to be submitted by a manufacturer — are considered as a basis for a proper environmental risk assessment (ERA), as part of registration procedures. Prior to an ERA these experimental data should be evaluated:

I. characteristics and taxonomy of the microorganism with pesticidal action (MOPA),
II. behaviour and fate in the environment (of MOPAs, but also of propagules[7], *e.g.* toxic spores),
III. (eco)toxicology incl. dose-effect relations (dependent on the mode of action),
IV. infectivity and pathogenicity (dependent on the mode of action).

All relevant aspects of an ERA are graphically summarised in Figure 1 (see also Table 1).

Assessing accurately the environmental risks of new microbial pesticides implies detailed knowledge of its distribution and fate in the various compartments, its interaction with the targets[8] in both pest and non-pest organisms, and the subsequent effects on the environment — includingits inhabitants. The occurrence and attainability of other other targets in the surrounding environment will determine the extent of the actual risk. The spatial distribution depends therefore on both biotic and abiotic factors.

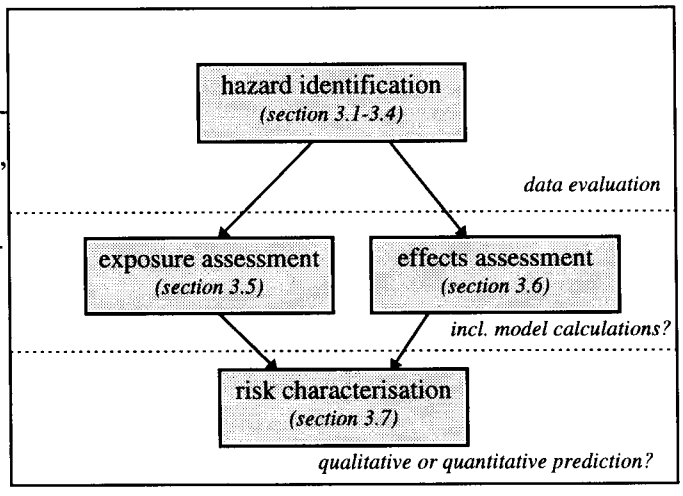


Figure 1 The systematic procedure of risk assessment for new microbial pesticides as discussed in this study (adapted from Van Leeuwen & Hermens, 1995)

As it has been shown by Burgess (1981) that modelling of a unidirectional system[9] is already complex and difficult, it should be clear that a predictive approach in quantitative terms is difficult. Therefore, this study tries to outline qualitative rather than quantitative approaches.

[7] a propagule is any part of an organism capable of growing into a new organism, *e.g.* an endospore.
[8] a target is the molecular site in the recipient organism where the actual interaction with the toxicant occurs.
[9] a simple microorganism/target organism system in which the microorganism population influences the host population and not the reverse.

There are two main differences between the active ingredient (AI) of a chemical pesticide and a MOPA. The first is that the concentration of an AI of a chemical pesticide generally decreases in due course, whereas MOPAs can grow due to replication under favourable environmental conditions (incl. *e.g.* the occurrence of suitable hosts for infectious MOPAs). However, such an increase of bacteria, introduced from an artificial medium into fresh soil, has never been documented in literature[10] (person. communic. of Smit, RIVM). This might have been due to stress coinciding with the translocation of the bacteria itself. It might also have been due to the fact that the microniches in the soil had already been occupied.

The second main difference is that a MOPA may transfer genetic material to other microorganisms, whether indigenous or not, and also whether this genetic material has been treated biotechnologically or not.

3.1 Hazard identification of microbial pesticides in general

Hazard identification may deal with the occurrence of unintended contaminants as *e.g.* MOs from the environment in which the MOPA has been tested, or mutated MOPAs. In the 1970s tests with particular attention for the serial passage through guts of young chickens yielded the identification of mutant *Bacillus thuringiensis* strains producing the highly toxic β-exotoxin (Burges, 1981). Subsequently, these mutant strains were banned in the US. In the 1990s these hazards can generally be avoided by using modern production facilities. As the conditions in *e.g.* large fermentors are currently well controlable, one should not expect unintended MOs or mutants to evolve. Statements of quality controls could give guarantees.

It may be necessary to verify pre-treatment procedures: *e.g.* virus production in insects may require clean room techniques, and operators may be isolated from the materials they work with as much as possible. Storage and other pre-treatments should not facilitate the intrusion of extraneous MOs. As stated above, such intrusions need not be expected using modern biotechnology.

3.1.1 Distribution and fate

The key items for the evaluation of tests on the distribution and fate of MOPAs in the environment are summarised in Table 2. The legend and the background of such a Summary Table is reported in Appendix 1.

[10] it was shown by tracing bacteria that had been marked by genetic modification.

3.1.1.1 Distribution

A MOPA with a plant as host — *i.e.* any plant infected by a microorganism — must overcome various barriers before reaching the target[11]: not only local microflora on shoot or leaf, but also unnatural barriers as pollutants or *e.g.* natural toxins as phytoalexins produced by the plant as a reaction on the MOPA may have to be tackled (Burges, 1981). Finally, the production of propagules[12] and their dispersal mechanisms are crucial for the intrusion of *e.g.* plant tissue, facilitated by the colonisation of dead or senescent tissue, or by the occurrence of natural openings or damaged surface (wounds).

Various studies have been performed on the environmental behaviour of *Bacillus thuringiensis*. A review of these will be published by IPCS in an Environmental Health Criteria (person. communic. IPCS to RIVM).

Some studies indicate that *Bacillus thuringiensis* is relatively immobile in the soil (Adams *et al.*, 1996). Leaching depths appear not to exceed c. 10 cm.

It should be noted that distribution of *e.g.* bacteria may occur via animals. It is known that spores of *e.g. Bacillus thuringiensis* may be distributed by fish or by birds and mammals feeding on infected larvae (Adams *et al.*, 1996). Earthworms may also transport MOPAs by ingestion of contaminated soil and excreting the remains within some distance from the site, where the soil has been ingested.

3.1.1.2 Persistence

Bacillus thuringiensis has been recovered in habitats all over the world: soil, water, and vegetation. Whereas rapid decline has been observed after spraying on foliage due to combinatory degradation by ultraviolet light and microbes, the spores of *Bacillus thuringiensis* may be viable in soil for several months (Adams *et al.*, 1996). The formulation of *Bacillus thuringiensis* may influence the persistence: half-lives of formulated *Bacillus thuringiensis* on foliage can be up to 10 days, whereas unformulated, half-lives were only a few hours.

The persistence of MOPAs can be assumed to be as high as technically achievable: the efficacy of MOPAs will generally be better, if a MOPA remains active (*i.e.* able to interact with the target) during a longer time. However, persistence may be highly variable, dependent *e.g.* on the (micro)biological and local abiotic conditions. According to Burges (1981)

[11] this target refers to an NTO, and not to the target for which the microbial pesticide was applied deliberately.
[12] see footnote 7.

MOPAs applied to soil are not persistent as they should be much less competitive than the indigenous soil microorganisms (Burges, 1981). However, this may not always be the case (person. communic. Wernars, RIVM).

3.1.2 Adverse effects to non-target organisms

The main aspects of tests on the effects of microbial pesticides on non-target organisms (NTOs) are summarised in Table 3. The legend and the background of such a Summary Table is reported in Appendix 1. An NTO is any organism that can be unintendedly affected by a chemical, a toxin or a microorganism, whereas target organisms are intendedly affected.

In trying to predict the incidence of adverse effects to NTOs, the mode of action should be known. This knowledge can be complicated by the occurrence of many stages of an MO: which stage may cause which effect? This is particularly important, as different stages of MOs may show very different patterns of distribution. The stages of *e.g.* viruses can be manifold: amongst others, infectious nucleic acid, virions and inclusion bodies. Parasitic MOs — *e.g.* entomophilic nematodes — may show *e.g.* preparasitic and parasitic stages.

All stages of MOs (see the paragraph above) in *e.g.* a host may be detected with current DNA techniques (person. communic. Wernars, RIVM). Therefore the incidence of false negatives — *i.e.* wrongfully indicating the absence of an MO — may be considered low.

Tier I testing in the US for potential adverse effects of MOPAs to *e.g.* aquatic NTOs, is primarily based on the site of application and the resulting potential for aquatic exposure (EPA, 1989)[13]. It is *not* based on other issues as natural geographic MO distribution, the natural MO population compared with population levels likely after application, or the MO ability to survive and replicate after application. At higher Tiers, these aspects can be taken into consideration.

Extrapolation from laboratory tests — on the adverse effects of MOPAs — to field conditions may be invalid. Do MOPAs affect NTOs in the field, as they affect NTOs in the laboratory under standardised conditions? It has been observed *e.g.* that some MOs can produce toxins on particular experimental growth media, whereas they cannot produce these in the field (Burges, 1981). Therefore the potential for causing adverse effects may be overestimated.

[13] instead of 4 days acute toxicity tests with *e.g.* fish, the EPA requires a 30 days semi-static test in which both the infectivity/pathogenicity and the toxicity must be investigated (EPA, 1989).

The following qualitative ranking of potentially adverse effects — *i.e.* from catastrophical to negligible hazard — can be made for MOPAs. This rather arbitrary list is adapted from Smit *et al.* (1992), who designated this list for genetically modified microorganisms (GMMs), therefore including GMMs with pesticidal action in particular. However, it is also applicable to microorganisms in general[14].

I. pathogenicity to man, plants or animals
II. disturbance of ecological balance
III. causing unwanted biochemical reactions
IV. mobilising toxic chemicals
V. affecting community diversity
VI. dominance over indigenous microorganisms
VII. input of extra C and N

3.1.2.1 Factors modifying infectivity and pathogenicity

An important determinant for infectivity and pathogenicity can be expected to be the type of host and its condition, and therefore the specific (micro)environmental conditions: *e.g.* the occurrence of wounds may facilitate the intrusion by viruses, and local conditions — humidity, temperature, absence of virus eliminating elements — may be optimal or less optimal for replication. As viruses may be very host specific, the taxonomical status of a potentially unintended target organism may be decisive whether an infection will actually occur or not. Viral MOPAs as *e.g.* baculoviruses can show a varying range of virulence to a wide range of hosts: *e.g.* AfNPV[15] is infectious for more than 39 agronomically important insect species from ten Lepidoptera families, whereas other Baculoviridae may have much less hosts (Leisy & Fuxa, 1996).

There appear to be complex interactions between various groups of pathogens: *e.g.* infection by bacterial and fungal pathogens may be aggravated by viral pathogens, but not *vice versa* (Burges, 1981). Therefore the extent of pathogenicity can be dependent on the occurrence of other pathogens.

EPA guidelines generally prescribe that testing for infectivity and pathogenicity should last longer than conventional testing: testing the ecotoxicological impact of *e.g.* infectious bacteria on rainbow trout *Oncorhynchus mykiss* should take 30 days, and not the conventional 96 hours for testing the acute effects of chemical pesticides.

[14] Smit *et al.* also added the dissemination of heterologous genes — between VI and VII. This hazard however does only apply to transgenic MOPAs.
[15] *Anagrapha falciphera* is a nuclear polyhedrosis virus.

3.1.2.2 Factors modifying toxicity

The toxicity of *Bacillus thuringiensis* is primarily caused by insecticidal crystal proteins (ICPs), that may differ per subspecies. Some subspecies of *Bacillus thuringiensis*, however, can produce the heat-stable β-exotoxin. This toxin can be toxic to almost all forms of life including humans, and it can contaminate formulations containing *Bacillus thuringiensis* (Adams *et al.*, 1996). The various strains of *Bacillus thuringiensis* produce a range of antibiotics, enzymes, metabolites and toxins that may differ in their effects.

3.2 Hazard identification of non-viral microbial pesticides

3.2.1 Distribution and fate

The key items, when evaluating tests on the distribution and fate of non-viral microbial pesticides in the environment are included in Table 2. The legend and the background of such a Summary Table can be found in Appendix 1.

3.2.1.1 Distribution

The main difference in the propagation and spatial distribution in the environment of non-viral *versus* viral MOPAs is the ability of the latter to be propagated and distributed by a host, whereas non-viral MOPAs — *e.g. Bacillus thuringiensis* — are not necessarily dependent on a host. It is however difficult to predict the consequences for spatial distribution. The occurrence of hosts and their frequent contact may increase the incidence of infected organisms, whereas the lack of hosts may prevent the infection from further spreading. Non-viral MOPAs can be very similar to or coincide with indigenous species or strains of MOs (*e.g. Bacillus thuringiensis*). A condition for further spatial distribution then, after being applied *in situ*, is the ability of the MOPA to compete with these indigenous species, as it can be expected that the closer the similarities, the more likely they will compete for a comparable niche. Therefore the similarities with indigenous species in *e.g.* soil or water should always be investigated.

The spatial distribution of MOPAs in the environment is expected to be different from chemical pesticides. This is primarily due to the following differences: MOPAs, showing a narrow range of target organisms, *versus* chemical substances, generally showing les specificity. Non-viral microbial pesticides can also differ from chemical pesticides by their ability to increase in a particular environmental compartment (biotic or abiotic) by multiplication.

3.2.1.2 Persistence

A laboratory test on the persistence may find out whether *e.g.* spores actually terminate or grow in soil. Spores of *e.g. Bacillus popillae* — one of the first microbial insecticides that had been registered — do not germinate in soil. Only in some scarabid beetles multiplication of the bacteria was observed (Burges, 1981). Growth was only observed *in vitro* in complex artificial media.

3.2.2 Adverse effects to non-target organisms

The main aspects of tests on the effects of non-viral microbial pesticides on NTOs are included in Table 3. The legend and the background of such a Summary Table can be found in Appendix 1.

The possibility of integration of — parts of — the genome of non-viral MOPAs, *e.g.* the parts coding for toxins, into the chromosomes of vertebrates should not be neglected. Nevertheless, it is generally accepted by experts that these risks are low, as the DNA control system — necessary for transcription of the inserted microbial DNA — in vertebrates differs too much from that in MOs (person. communic. Wernars, RIVM). In contrast, *cry*-genes on plasmids of *Bacillus thuringiensis* can be readily transferred by conjugation between *Bacillus thuringiensis* strains. They may also be transferred to other bacterial species (Adams *et al.*, 1996).

The mode of action may be decisive in predicting the potential effects to NTOs. However, this mode may be complex. *Bacillus thuringiensis*' principal pesticidal site is the crystalline protein body at one end of the bacterial cell. However, at the other side of the same cell, spores can be formed. Both spores and (pro)toxins can be pathogenic to insects (Fast, 1981). After ingestion, the protoxin is metabolised into a toxin that affects the gut epithelial cells of the targeted insect (*e.g.* a caterpillar). ATP decreases rapidly, followed by an increase of respiration and glucose uptake. Metabolic disruption is complete c. 10-15 minutes after application. Finally, death is probably due to starvation (Brock *et al.*, 1994).

3.2.2.1 Factors modifying infectivity and pathogenicity

See section 3.1.2.1.

3.2.2.2 Factors modifying toxicity

See section 3.1.2.2

3.3 Hazard identification of viral microbial pesticides

Viral microbial pesticide preparations contaminated with mutants or extraneous MOs are not likely to be produced with the current high standards for commercial biotechnological production of such preparations (see also sect. 3.1). However, production in foreign laboratories can trigger the need for verification of their production processes, if felt necessary.

3.3.1 Distribution and fate

The key items, when evaluating tests on the distribution and fate of viral MOPAs in the environment are included in Table 2. The legend and the background of such a Summary Table can be found in Appendix 1.

3.3.1.1 Distribution

Distribution of viral MOPAs in the environment is primarily dependent on the occurrence of hosts and the attainability of such hosts. If *e.g.* a baculovirus preparation has been sprayed in a forest against particular moths, it should be investigated whether these hosts, or related species may spread the infection beyond the site of application. Whereas *e.g.* a chemical insecticide may affect a beneficial insect immediately after application, therefore causing environmental problems primarily on the site of contamination, a viral insecticide may be distributed further from the site of application as insects may live for various days after application (Leisy & Fuxa, 1996).

Baculoviruses — the main group of indigenous insecticidal viruses currently used as MOPAs — may be spread *per os* from insect larva to insect larva. Which abiotic and biotic factors determine the spread of such an infection, and therefore its spatial distribution? Although not much is really known about this, abiotic factors as rainfall, air currents, and gravity are probably important for short distance transport: *e.g.* from soil to and within an insect's host plant (Leisy & Fuxa, 1996). Biotic factors are probably more important for longer transport routes of these viruses. This was observed by adult Lepidopterae and Coleopterae. Other organisms that have been indicated vectors for entomopathogenic viruses, are predatory arthropods, saprozoites as birds and sarcophagid flies, and grazing mammals (Leisy & Fuxa, 1996). The nuclear polyhe-

Table 2. Key items for the data evaluation of tests on distribution and fate of MOPAs in the environment

	ITEMS	NOTES	RELIABILITY LOWER ?
METHODOLOGY & TEST DESCRIPTION	1. **test type**	1. **improperly reported?** *[e.g. fate and behaviour in soil, water, air? duration?]*	1. **Y**
	2. **active ingredient, purity**	2. **improper characterisation of the active ingredient? impure?** *[which MOPA — e.g. protozoan, fungus, bacteria, virus? common name? scientific name — down to strain or serotype? mutant? microbiological purity? nature and identity of impurities — e.g. mutated AIs, extraneous MOs? conditions — e.g. a crippled plant pathogen?]*	2. **Y**
	3. **formulation**	3. **(partly) unknown composition?** *[name? type? composition — e.g. quantities and function of non-active ingredients, e.g. wetting agents?]*	3. **Y**
	4. **environmental compartment**	4. **improperly reported?** *[e.g. water, soil, air? natural/artificial? sterile? temperature? light conditions? volume/weight?]*	4. **Y**
	4.1 **abiotic**		
	4.1.1 **water**	4.1.1 *[e.g. pH? sediment type? redox potential/availability of O_2?]*	
	4.1.2 **soil**	4.1.2 *[e.g. soil type? pH? % o.m.? "natural" microbes (quantities, composition)? redox potential/availability of O_2? moisture conditions?]*	
	4.1.3 **air**		
	4.2 **biotic**	4.2 *[e.g. transmission via vectors?]*	
	5. **application**	5. **improperly reported?**	5. **Y**
	5.1 **rate**		
	5.2 **type**	5.2 *[e.g. homogeneously mixed with the medium/substrate? application e.g. as a fluid inoculum, or as encapsulated spores?]*	
	6. **endpoint**	6. **improperly defined?** *[e.g. the amount of AI at the end of incubation, the extent of distribution in the compartment, the extent of interaction — competition? — with other MOs?]*	6. **Y**
	7. **analysis**	7. **invalid? inadequate?** *[e.g. extent of validation? "limit of detection"? proper bioassay?]*	7. **Y**
RESULTS	8. **endpoint**	8. **improperly reported? results non-verifiable?** *[e.g. raw data available for verification?]*	8. **Y**
	9. **statistical analysis**	9. **invalid?** *[all tests with MOs require accurate statistical analysis for proving significant differences between the control and the treatment groups]*	9. **Y**
	10. **test conditions**	10. **improperly reported?** *[are certain ranges of abiotic/biotic parameters exceeded during incubation?]*	10. **Y**
REMARKS	11. *other dissipation routes: e.g. sorption of MOs to glass, or algae?*		11. **E**
	12. *the — e.g. agricultural — history of the environmental compartment under study: does e.g. prior use of compounds may have lead to adapted MOs — e.g. in sewage sludge, or in soil that had been sprayed with chemical pesticides?*		12. **E**
	13. *the handling of the compartment under study: does e.g. the pretreatment indicate microbial populations that cannot be considered resembling "natural" conditions — e.g. soil being stored too dry?*		13. **E**
	14. *the biological meaning of statistically significant differences?*		14. **E**
	15. *microbiological properties: e.g. dispersal mechanism? occurrence of toxins? natural occurrence?* 15.1 *type of propagation: e.g. spores, mycelial fragments?* 15.2 *type of optimal culture media for propagation or growth: e.g. temperature? moisture conditions? pH? % o.m?*		
	16. *pretreatment instability of the AI or product (respecting, light, temperature, "shelf"-storage, and packaging)? instability during incubation?*		16. **Y**

drosis virus of the pest *Anticarsia gemmatalis* has been shown to be transported by predatory arthropods from a soybean field — the application site — at a rate of c. 1 meter per day during the growing season.

Besides the horizontal distribution mentioned above (*i.e.* the spreading of a virus from host to host), there may be vertical distribution (*i.e.* parent-to-offspring) as well (Leisy & Fuxa, 1996). The distribution — and therefore also the effectivity — of Baculoviridae can be modelled with input data as, amongst others, the horizontal and vertical distribution (Smits & Vlak, 1994). However, this model has not been validated yet by field experiments.

3.3.1.2 Persistence

Baculoviruses are in use in forestry and agriculture as viral insecticides in various countries. They belong to an insect virus family that is able to enclose virions — *i.e.* fully grown, mature viruses — with large proteinaceous crystals, also called occlusion bodies (Leisy & Fuxa, 1996). This natural process of encapsulation may increase the persistence of viruses in soil substantially. Baculoviruses may persist for years in soil, also in more disturbed agro-ecosystems. In forest soil, a baculovirus — effective against the douglas-fir tussock moth *Orygia* — had been shown to survive for 41 years (Thompson *et al.* 1981, cited in Leisy & Fuxa, 1996). The persistence of baculoviruses on foliage and other surfaces exposed to sunlight is generally much shorter: inactivation may occur within days or hours. This may reduce the exposure of NTOs, living on the soil surface or on foliage, substantially.

3.3.2 Adverse effects to non-target organisms

The host range of *e.g.* a baculovirus is generally thought to be limited from one single lepidopteran species to ten or more families within an insect order (Leisy & Fuxa, 1996). This high specificity was confirmed by various tests in which baculoviruses of insects did not cause adverse effects in many vertebrates, invertebrates — *e.g.* insects one order different from the targeted species), and plants (Burges, 1981). There is, however, limited evidence that insects of other orders may also be infected.

The efficacy of a baculovirus can be modelled with input data as the horizontal and vertical distribution (see section 3.3.1.1), the persistence of the virus, dose-effect relations, and the feeding behaviour of the larvae that are targeted (Smit & Vlak, 1994). The modelled virus was a multiple-enveloped nuclear polyhedrosis virus effective against the larvae of the beet armyworm *Spodoptera exigua*. The same underlying data may give a clue on the effectivity against NTOs. The model, however, has not been validated yet.

Table 3. Key items for the data evaluation of laboratory tests on the effects of MOPAs to non-target organisms

	ITEMS	NOTES	RELIABILITY LOWER ?
METHODOLOGY & TEST DESCRIPTION	1. **test type**	1. **improperly reported?** *[e.g. tests on infectivity or toxicity to honey bees or birds, or bioassays on insects to show replication of a virus in vertebrates. Duration? in vitro or in vivo?]*	1. **Y**
	2. **active ingredient, impurity**	2. **improper characterisation of the active ingredient? impure?** *[which MOPA — e.g. protozoan, fungus, bacteria, virus? common name? scientific name — down to strain or serotype? mutant? microbiological purity? nature and identity of impurities — e.g. mutated AIs, extraneous MOs? conditions — e.g. a crippled plant pathogen?]*	2. **Y**
	3. **formulation**	3. **(partly) unknown composition?** *[name? type? composition — e.g. quantities and function of non-active ingredients, e.g. wetting agents?]*	3. **Y**
	4. **application** 4.1 **rate** 4.2 **type**	4. **improperly reported?** 4.2 *[e.g. homogeneously mixed with the medium/substrate? application e.g. as a fluid inoculum, or as encapsulated spores?]*	4. **Y**
	5. **endpoint** 5.1 **virus** 5.2 **other**	5. **improperly defined?** 5.1 *[e.g. incorporation of viral DNA into the chromosomes of NTOs? induction of other viruses — viral interference?]* 5.2 *[infectivity/pathogenicity: e.g. cytopathic effects, or visible replication of a MOPA? toxicity[16]? allergenic effects — e.g. in mammalian vertebrates? mutagenicity/carcinogenicity/teratogenicity — e.g.in mammalian vertebrates?]*	5. **Y**
	6. **control**	6. **inadequate control treatment?** *[e.g. no autoclavation or UV inactivation?]*	6. **Y**
	7. **analysis**	7. **invalid? inadequate?** *[e.g. extent of validation? "limit of detection"? proper bioassay?]*	7. **Y**
RESULTS	8. **endpoint**	8. **improperly reported? results non-verifiable?** *[e.g. raw data available for verification?]*	8. **Y**
	9. **statistical analysis**	9. **invalid?** *[all tests with MOPAs require accurate statistical analysis for proving significant differences between the control and the treatment groups]*	9. **Y**
	10. **test conditions**	10. **improperly reported?** *[are certain ranges of abiotic/biotic parameters exceeded during incubation?]*	10. **Y**
REMARKS	11. *the biological meaning of statistically valid differences: e.g. does a treatment- or dose-effect relation exist?*		11. **E**
	12. *microbiological properties: e.g. dispersal mechanism? occurrence of toxins? natural occurrence?* 12.1 *type of propagation: e.g. spores, mycelial fragments?* 12.2 *type of optimal culture media for propagation or growth: e.g. temperature? moisture conditions? pH? % o.m?*		12. **Y**
	13. *pretreatment instability of the AI or product (respecting, light, temperature, "shelf"-storage, and packaging)? instability during incubation?*		13. **Y**
	14. *plating media (bioassay): e.g. type of medium? pretreatment — e.g. pasteurisation? these may influence the number of CFUs*		14. **Y**

[16] toxicity can be relevant for all groups except protozoa and virus inclusion bodies: these generally produce no toxins.

3.3.3 Factors modifying infectivity and pathogenicity

See section 3.1.2.1.

3.4 Hazard identification: examples

The Office of Pesticide Programs (OPP) of the US EPA has registered c. 50 microbial pesticides (Slutsky *et al.*, 1997; see also Appendix 3). Guidelines for data evaluation were first published in 1982, revised in 1989 (EPA, 1989) and further updated as part of a harmonisation project. Most of these guidelines are approved upon in the OECD. The OPP publishes Pesticide Fact Sheets with summarised data on *e.g.* microbial pesticides.

In the US, microbial pesticides have to be tested generally for their effects on two bird, one fish, one aquatic invertebrate, and some non-target insect and plant species (person. communic. of Kough, EPA/BPPD to RIVM).

Registration of *Bacillus thuringiensis* ssp. *aizawai* for the control of various pest insects in 1992 was supported by a dossier of the firm with various ecotoxicological tests (Anon., 1992). Tests were submitted on the toxicity to birds, fish, water fleas (*Daphnia magna*), honey bees (*Apis mellifera*), green lacewings (*Chrysoperla carnea*), predatory wasps (*Trichogramma pretiosum*) and some predacious mites. The registration was only conditionally granted: additional data were required, as three tests were not accepted. The test with the predatory wasps was not accepted due to substantial mortality in the control, the test with the water fleas due to the lack of a sterile filtrate control and an inaccurate EC_{50}, and the test with rainbow trout due to a test duration that was too short (96 hours instead of 30 days). The EPA also required additional data as some non-target invertebrates had been affected unexpectedly: rather than due to the insecticidal crystalline protein (ICP), these effects could have been due to heat-labile or heat-tolerant exotoxins contaminating the technical grade substance (Anon., 1992).

An overview of the MOPAs registered in the Netherlands is presented in Appendix 2. Most of these MOPAs are insecticides (the baculoviruses *Cydia pomonella* and *Spodoptera exiqua*; the bacteria *Bacillus thuringiensis*, and the fungi *Verticillium dahlia* and *Verticillium lecanii*). The others control pathogens: the bacteria *Streptomyces griseoviridis* is a fungicide, and a weak strain of a mosaic virus (tomato mosaic virus) is a virucide. Inactive endospores of *Bacillus thuringiensis* with toxins — sold as the wettable powder Aseptasporin CT with 7% active ingredient — are not included, because they should be considered as a chemical pesticide. It should be noted that in accordance with the Pesticide Act, the Dutch CA do not

divide microbial pesticides into non-transgenic and transgenic (see also Chapter 1). They denominate microbial pesticides as "biological preparations".

In the Netherlands all microbial pesticides have been registered by the CA without requiring additional experimental data, as all microbial pesticides were considered to consist of naturally occurring MOPAs (see Appendix 2). In contrast, the EPA frequently requires additional experimental data. Slutsky *et al.* (1997) report *e.g.* that recently reviewed test data showed toxicity of various strains of *Bacillus thuringiensis* to water fleas and honey bees. Therefore the EPA required the manufacturer to investigate the possibilities of exotoxins or metabolites being the cause of this toxicity. It was found that the toxicity was due to the fermentation media composition. As an EPA laboratory showed that various entomopathogenic fungi were toxic to fish embryo cells, fish life cycle studies were required from the manufacturer (Slutsky *et al.*, 1997).

HAZARD IDENTIFICATION, SOME HISTORY

Spores of *Bacillus popilliae* — one of the first registered microbial insecticides — had been fed to starlings and chickens, causing no effects and remaining viable after passing the avian gut (Burges, 1981). No germination occurred. *Bacillus popilliae* was not allied to any vertebrate pathogen. The targets in the beetles were in the blood: *Bacillus popiliae* caused septicaemia, an infection of lymph and blood. Production of toxins was not suspected.

Bacillus thuringiensis — with a safe and currently wide-spread agricultural use — is closely related to *B. cereus*, which in its turn showed biochemical similarities with *B. anthracis*, the bacillum causing anthrax by especially cows. However, no evidence for contamination of NTOs in the field was observed (Burges, 1981). *Bacillus thuringiensis* exerts its pesticidal action by producing crystal toxins, the δ endotoxins. Therefore, when *B. thuringiensis* had to be registered in the 1960s, the authorities not only required tests that were also required for the registration of chemical pesticides (regarding the toxin as a chemical), but particular infectivity tests as well (Burges, 1981). Specific attention was given to the possibilities of the bacteria growing in human food. Finally, the probability of *B. thuringiensis* to mutate into *B. anthracis* was considered exceedingly small, because the modes of action involved two clearly different toxins, and mutations were not likely to achieve such a change.

Later, some strains of *B. thuringiensis* were found to produce another toxin: β-exotoxin or thuringiensin. This toxin showed adverse effects to some mammals (parenterally) and birds (orally), compelling the US/CA to ban these particular strains.

3.5 Exposure assessment

Exposure assessment refers to the estimation of the extent of exposure of an NTO to a potentially harmful MOPA. It includes statements or predictions on the distribution and fate of the living MOPA in the environment. The estimation of the extent of exposure may be expressed as a modelled or measured quantity of MOPAs (see also Table and Figure 1).

As MOPAs are living microorganisms, the exposure assessment may include elements that are completely different from the risk assessment for a chemical pesticide: *e.g.* survival, competition, selection, and gene transfer.

Exposure of NTOs may occur at the site of application, and the sites surrounding the application site, dependent on the ability of the living MOPAs to reach those sites. On both the application and the other sites, MOPAs may be distributed within and between the particular environmental compartments — *e.g.* from soil pore water to the soil matrix or from the subsoil to the shallow ground water.

Primarily, the — unintended — exposure of any organism in an environmental compartment to a MOPA is dependent on the characteristics of the MOPA itself: *e.g.* reproduction mechanism, and surviving mechanism under stress. Thereupon, agricultural and ecological factors can be decisive. Examples of the former are the application rate, the time and type of application — *e.g.* as seed dressing, soil or plant inoculation, tree stump or wound treatment[17], spraying in summer or winter —, its frequency, and the corresponding time-interval. Ecologically relevant factors may be the extent of fitness in comparison with related species, and those factors that will determine whether the MOPA can be distributed via abiotic — *e.g.* percolating water, via wind or soil transport — and biotic processes — *e.g.* transport via a host.

All these factors may determine the quantities of a MOPA in an environmental compartment, due to an intended application. Scientific disciplines necessary for qualifying or quantifying these processes may comprise micro- and molecular biology, population biology and microbial ecology.

Exposure assessment may comprise the following items (person. communic. of Knacker, ECT Oekotoxikologie GmbH to RIVM):

I. distribution and survival in environmental media (*e.g.* water, soil, higher organisms)
II. tolerance towards environmental conditions (*e.g.* temperature, moisure, radiation)
III. survival structures (*e.g.* spores, cysts)
IV. genetic transfer and exchange (horizontal gene transfer, plasmids)

It should be noted that item IV on genetic transfer is not necessarily applicable to genetically modified microorganisms — by DNA recombinant techniques — only. Genetic recombination of DNA may also occur due to conventional selecting and outcrossing techniques: *e.g.* various wheat varieties in Europe contain a chromosomal part of rye. Genetic recombination, *e.g.* via conjugation, is for many microorganisms a natural phenomenon.

[17] an example is a small sprayer attached to a hand-held pruner, so that after a branch had been cut off, the liquid can be sprayed onto the cutting site (Burges, 1981).

The type of application can be decisive, when interpreting the results of *e.g.* an infectivity test under semi-static conditions for 21 days with *Daphnia magna*. Exposure can be directly via the water or indirectly via contamination of the feed. However, at least for some MOPAs (*e.g.* entomopathogens) dietary exposure is probably the most important route (EPA, 1989). In view of the exposure assessment, infectivity tests with the indirect route via feed may therefore be more helpful than those with the direct route.

In recapitulation, exposure assessment should give a qualified or quantified estimate of an infectious or toxic MOPA in any relevant environmental compartment, dependent on various assumptions. Due to the complexity of microbial populations and the lack of knowledge, especially in the field of microbial ecology — *e.g.* how do new MOPAs compete with indigenous MOs in the field? —, quantification of such estimates within the next ten years should not be expected (person. communic. of Schneider, EPA/BPPD, to RIVM).

3.6 Effects assessment

Effects assessment should refer to the identification or quantitation of (no-) effect levels for non-target organisms in any environmental compartment that may be contaminated due to the intended use of a microbial pesticide (see also Table and Figure 1).

As it always focuses upon the effects of a particular dose or concentration, dose-effect relations are included into the effects assessment (see also Table 1). However, whereas the concept of dose-effect relations can be helpful for the effects assessment of chemical pesticides — as an increasing dose corresponding with an increasing adverse effect is clear evidence for a causal relationship between dose and effect —, this will not always be so for the effects assessment of infectious MOPAs. A low quantity of an infectious MOPA *e.g.* may cause an infection, whereas a higher dose may not, due to the triggering of the immune system at the high and not at the low dose. Nevertheless, in the US EPA approach for environmental data requirements (EDRs), information about these dose-effect relations can be crucial in the third Tier (Mensink & Linders, 1997). This apparently indicates that these US EDRs are particularly focused upon tests with bacteria whose mode of action is based on a toxin (*e.g.* in tests with active spores of *Bacillus thuringiensis*). There appears to be no clear point-of-view on how to assess the effects of a MOPA without a dose-effect relation. This is probably a major drawback for the development of a more systematic type of effects assessment. Therefore, the case by case approach may be inevitable for these MOPAs.

When testing for dose-effect relations of MOPAs, whose activity is based on a toxin, the applied doses can be expressed as colony forming units (CFUs). However, as the number of

CFUs always depends on the type of medium for plating and its treatment, these should be verified and included into the review.

The (no-)effect levels for chemical pesticides are generally derived from reliable sources as *e.g.* scientific journals, industrial test reports or reports from governmental institutes and international organisations. An example of such a no-effect level is the Maximum Permissible Concentration for chemical pesticides, as derived by the Dutch CA (Crommentuijn *et al.*, 1997). However, there are no such equivalents yet for MOPAs producing toxins.

The concept of (no-)effect level is probably not useful for infectious and pathogenic MOPAs, as there is no particular treshold below which adverse effects can be excluded. The probability of effects from viral microbial pesticides is possibly much more dependent on the occurrence of suitable hosts and those environmental and biological conditions favouring replications and intrusions. It is not clear as to how far the "(no-)effect level" concept is useful for non-viral microbial pesticides as *e.g. Bacillus thuringiensis* producing crystals with endotoxins (ICPs). Due to the analogy with chemical pesticides, the occurrence of such treshold levels cannot be excluded. Scientific disciplines necessary for qualifying or quantifying these (no-) effect levels — if occurring — may comprise micro- and molecular biology, microbial ecology, ecotoxicology, and risk assessment (incl. statistical procedures). It may be of particular importance to identify those effects that have microbial ecological relevance.

The host specificity determines the host range in the environmental compartment under investigation, and a narrow host range may imply a smaller probability of *potentially* adverse effects of viral microbial pesticides to NTOs. A narrow host range of *e.g.* a viral insecticide under development may prevent infection and eradication of beneficial insects. This is, however, not an unequivocal incentive for commercial exploitation, as a narrow host range — concurrently — may reduce the market share and the effectiveness of a microbial pesticide (Leisy & Fuxa, 1996).

The route via which a viral or non-viral pathogen may enter a host can also be decisive in triggering adverse effects in a host or non-target host. In accordance with Burges (1981), such a pathogen may enter a host orally (via *e.g.* feed, water), respiratory (*e.g.* propagules in the air), parenterally (via *e.g.* wounds), or dermally (via *e.g.* skin and eyes).

Nematodes affecting insects are unlikely to infect vertebrates (Burges, 1981). However, adverse effects following consumption by vertebrates of contaminated dead insects or infected nematodes cannot be excluded. Such "feed chains" may complicate the effects assessment.

Effects assessment for microorganisms may comprise the following items (person. communic. of Knacker, ECT Oekotoxikologie GmbH to RIVM):

I. interaction with other microorganisms, plants or animals

II. effects on nutrient cycles

In recapitulation, one of the major problems in the effects assessment is the extrapolation of submitted laboratory test data to (no-)effect levels that can be used in the "open field" for the actual risk characterisation. It remains to be seen whether the use of the dose-effect concept is useful for infectious and pathogenic MOPAs. Particularly due to gaps in the scientific knowledge of microbial population dynamics in an ecological context, the extrapolation problem appears to be more serious for microbial pesticides than for chemical pesticides. Solving this problem is essential for answering perhaps the most crucial question, before the introduction of a new MP: do we add something substantial to an ecosystem that might alter the relations between organisms adversely. However, it should be noted that also for the registration of chemical pesticides or new substances such an ecological context — *i.e.* more subtle than the mere ecotoxicological effects — is generally not taken into account, due to methodological difficulties (how to measure, how to interpret?).

3.7 Risk characterisation

After the hazard identification, the exposure and the effects assessment, the risk characterisation combines the exposure and the effects assessment. Ideally, the risk characterisation is the actual quantitation of the probability that adverse effects may occur due to the use of a microbial pesticide. However, it is generally a qualitative estimation of the actual risk by dividing the predicted environmental concentration by the (no-) effect level. This concept is often used by registration procedures for chemical pesticides. Such a concept can probably not be used for infectious and pathogenic MOPAs, and it is unclear whether it can be used for toxic MOPAs. A literature survey, that was not exhaustive, indicated no references on this issue.

A quantitative approach is under development by ECT Oekotoxikologie and the UBA (person. communic. Knacker, ECT Oekotoxikologie to RIVM). Instead of the PEC/PNEC[18] approach — commonly used to quantify ecotoxicological risks of chemical pesticides — they

[18] the PEC/PNEC ratio is the Predicted Environmental Concentration divided by the Predicted No-Effect Concentration.

develop the PET/PNET ratio[19] to quantify the risks of microorganisms. This ratio refers to a titer — cell number — rather than to a concentration.

[19] the PET/PNET ratio is the Predicted Environmental Titer divided by the Predicted No-Effect Titer.

4. DISCUSSION

This report is a discussion paper: what are the (im)possibilities of a structured and transparent system to assess the environmental risks of microbial pesticides? There is currently no such structured and transparent system for the risk assessment of microbial pesticides within a proper legislative context. Up till now, the environmental risks of microbial pesticides have been evaluated in the Netherlands on a case by case basis, and these pesticides have been registrated smoothly as they occur as indigenous species in a natural environment as well. However, this may change in the near future.

In view of the Dutch policies on agricultural pest management — *e.g.* to reduce the emissions and use of chemical pesticides and to be less dependent on the use of chemical pesticides —, and in view of rapid biotechnological developments, it may be expected that in the near future the use of microbial pesticides will increase (Mensink & Linders, 1997). It is difficult to prognosticate, but as the research on and development of biological pest management is still in an early phase — especially outside the greenhouses —, the implementation of integrated pest management (*i.e.* by biological *and* chemical means) could be seen as a major goal, rather than changing radically from chemical to biological pest management (if that is possible at all). This means that on a short term, risk assessment for substances to be used in agricultural pest management may comprise a microbial together with a chemical component.

The triplet of data requirement followed by consistent data evaluation and, subsequently, consistent and adequate environmental risk assessment based on these data for the registration of microorganisms with pesticidal action (MOPAs) is in an early phase in the Netherlands. As a follow-up of Mensink and Linders (1997), therefore, this study discusses aspects of this triplet to initiate the development of such an integral and systematic framework for microbial pesticides.

The use of *Bacillus thuringiensis* is "likely to increase dramatically in future", in particular due to the rapid degradation in the environment and the low incidence of reports on field tests indicating the occurrence of resistance (Meadows, 1992). However, currently it is more expensive to produce *Bacillus thuringiensis* than chemical pesticides, although it can be produced on an industrial scale by liquid fermentation.

Proposals for some parts of an integral framework have been published in the Netherlands by Wäckers (1992) and Smit *et al.* (1992). Wäckers proposed a decision tree, for the registration of microbial pesticides, to require particular endpoint data — *e.g.* a laboratory test on the degradation rate in soil — dependent on the results of other experiments — *e.g.*

does the microorganism (MO) naturally occur in the soil at the same or higher amounts compared with the amounts following the recommended dosage? Therefore this approach is a mix of data requirements, and exposure assessment. Smit *et al.* (1992) proposed a decision tree for genetically modified microorganisms (GMMs) about when to start a small scale release of GMMs in the environment. This tree included data requirements, exposure and effects assessment (see also Appendix 4). The approaches of Smit *et al.* and Wäckers show that decision trees — though both can be used for registration procedures — can be quite different. The comparison makes clear that, when making an overall framework, the purposes and the underlying concepts should be made very clear. However, the major crucial starting-point for such a framework should always be whether residues of a microbial pesticide can be expected in the various environmental compartments or not.

4.1 Data requirements

The historical development of the required test battery for microbial pesticides — on which the risk assessment should be based —, is that of the conventional chemical pesticide tests, with some additions to cover infectivity followed by pathogenicity (see *e.g.* EPA, 1989). In general the study duration should be longer than conventional tests to allow for sufficient microbial growth and/or toxin production, if occurring at all (Slutsky *et al.*, 1997). The EPA compensates the higher costs — due to longer testing — by requiring fewer dose levels. Limit tests with one maximum hazard dose level may be used at Tier I. Unequivocal adverse effects will trigger exposure testing (Tier II), to find out whether maximum hazard doses may occur in the environment. As these Tier II tests depend greatly on the properties of the MOPA — *e.g.* environmental expression, population dynamics —, the EPA does not prescribe protocols for these tests. Neither do they prescribe protocols for Tier III — *e.g.* subchronic or chronic multiple dose tests including life cycle and dose-range — or Tier IV *e.g.* actual or simulated field tests (Slutsky et al, 1997).

Special attention in registration procedures should be given to aspects of a microbial pesticide that may permit waivers, and also to those aspects that require additional testing of a microbial pesticide. The environmental effects of *e.g.* Baculoviridae against Lepidopterae and sawflies are so much well-known, that various waivers in registration procedures can be granted, whereas for Baculoviridae against other insect groups complete test batteries should be considered (Burger, 1981).

4.2 Data evaluation

Burges (1981) considered making overall guidelines for testing microbial pesticides on their environmental impact as probably impossible. However, the development of scientific knowledge on the various aspects of microbial pesticides is rapidly evolving. Therefore, a format for evaluating and summarising experimental data for MP registration procedures in a more systematic and transparent way may be helpful. Such a proposal is represented as Summary Tables in Table 2 and 3, one for the distribution and fate in the environment (Table 2), and another for the effects to non-target organisms (Table 3). Such Summary Tables may be extended and refined in the near future. Such tables may be used as a checklist for environmental data evaluation that can be updated at any time in view of the expanding knowledge. They may also serve as a tool to establish the quality of these data, and to decide whether to use them for further risk assessment or not.

4.3 Environmental risk assessment

Overall risk assessment schemes that qualify the environmental risks, as *e.g.* in the EPPO decision-making risk schemes for chemical pesticides (EPPO, 1993, 1994), should not be expected within a short term. The US EPA and the Canadian PMRA — leading the way in this field and currently combining efforts — estimate that a more consistent approach than a case by case basis should not be expected to be operational within the first decade (person. communic Schneider, EPA, to RIVM). These uncertain prognoses will require an increasing flexibility of both the registration authorities and the manufacturers.

In Germany, a quantitative approach for risk characterisation is under development that compares the predicted titer of microorganisms in a particular environmental compartment with the predicted titer that is assumed not to affect this compartment. This approach is currently under investigation for six microorganisms (person. communic. Knacker, ECT Oekotoxikologie to RIVM). Two aspects are particularly important in these investigations: which effects are of microbial ecological relevance, and how can ecological effects relevant for microorganisms be detected?

4.4 A registration framework for microbial pesticides: feasible to what extent?

The following part of this subsection refers to some of the ideas of the authors: they are not necessarily the *opinio communis* within the National Institute of Public Health and the

Environment (RIVM) or within the Directorate-General for Environmental Protection. They should be regarded as the contribution of the authors to the discussions on this subject.

The authors of this study believe that the crux of the matter is the explication of the environmental criteria that are — roughly stated — decisive for distinguishing between beneficial and harmful, and therefore decisive for registration or not. Those explicit environmental criteria for registration procedures are yet lacking. As many uncertainties can be involved — *e.g.* the fitness of a MOPA in the field compared with the fitness in a microcosm — it will be difficult to develop these criteria in a scientifically sound way.

How could these environmental criteria be developed? They could be developed concurrently with the overall framework for environmental data requirement (EDR), data evaluation and environmental risk assessment (ERA). These three elements are interdependent: *e.g.* data requirements may be adjusted, if an ERA for a particular MOPA indicates particular risks that until then were never taken into account. Therefore it is recommended not to develop a rigid framework first, but to develop it in an iterative way, with the possibilities of immediate adjustment of one of the elements. The environmental criteria should be based on this development, and may be adjusted as well, *e.g.* if research reveals new aspects, and if new MOPAs are proposed for registration. It is hoped for that in this flexible approach, the registration framework can follow market developments — which some experts expect to be drastic — without unnecessarily delaying registration procedures. This approach will be highly dependent on the ability to recognise by monitoring adverse or unwanted effects in the environment. If this ability exists, CA decisions may be reconsidered after the first release. However, such a flexible approach with adjusting environmental criteria from time to time — to put it bluntly —, may cause uncertainty for the manufacturer: one month a microbial pesticide may not be granted admission to the market, whereas the next month, it might be granted, due to an adjusted environmental criterion (*e.g.* due to new scientific opinions, an upheaval of the PET/PNET ratio for triggering higher tier tests). Such uncertainties, however, could be accepted by the manufacturers, if clear agreements can be made with them to develop such a framework with trial and error during a period of *e.g.* ten years, and to consider this period as transitional.

In general, there appears to be concensus among scientists about the fact that "microbial pesticides are inherently different from chemical pesticides, with fundamentally different modes of actions and consequently lower risks of adverse effects, due to their agricultural use" (*e.g.* Burges, 1981; Adams *et al.*, 1996). However, one of the main features of microbial pesticides, discriminating them from chemical pesticides, is that the MOPAs can propagate in a suitable environmental compartment, whereas concentrations of chemical pesticides generally decrease in due course.

There are indications that propagation of MOPAs may be observed in relatively simple microcosms, whereas it may not occur in terrestrial ecosystems (see Chapter 3).

Monitoring can be considered as the last step in the risk management process (Van Leeuwen & Hermens, 1995). It requires analytical techniques to detect a specific MOPA in an NTO or an environmental compartment. If there is not such a technique, monitoring in the field is impossible. These techniques may already have been used in the seperate tests for hazard identification, *e.g.* a test on the degradability or viability in soil. It will be important to reach scientific consensus on the following issues (person. communic. of Beringer, University of Bristol to RIVM):

I. what to monitor?
II. what to do with low frequency events in microbial populations?
III. what is cause and what is an effect (*e.g.* which effects are biologically relevant)?

In most countries, the first tiered registration tests — if required at all by CAs — for microbial pesticides will be maximum challenge tests (see Mensink & Linders, 1997): NTOs are exposed to large quantities of infectious or toxic MOPAs by usual or unusual routes of administration. Therefore positive results — *i.e.* establishing the occurrence of adverse effects — do not necessarily imply adverse effects at more realistic doses (*i.e.* doses that will be recommended for practical use). No risk assessment can be based on such tests only, and therefore the consecutive step can only be to test at more realistic levels.

The current case by case test batteries — required by the CAs, if required at all — may need improvements, as there are still many gaps in the scientific knowledge of the fate, behaviour, and effects of MOPAs. Many studies focus upon bacterial survival and gene transfer, whereas *e.g.* the role of competition between MOPAs and indigenous, related species is still underexposed (Smit *et al.*, 1992). The transport mechanisms in the environment, and the extent of harmful effects on an ecosystem are also underexposed.

In conclusion, environmental risk assessment for microbial pesticides, of which the MOPAs may be distributed among indigenous MOs, can be tedious, in spite of the environmental safety for various microbial pesticides that are already on the market. This is mainly due to the fact that most experience with registrating and regulating microbial pesticides is based on only a few MOPAs, mainly *Bacillus thuringiensis* and Baculoviridae. Although this experience indicates substantial environmental safety, this need not necessarily be the case, when different MOPAs will be introduced, *e.g.* non-indigenous strains. As it has been shown in many cases that aggressive non-indigenous species may become dominant in particular

ecosystems[20], such predominances cannot be excluded, when introducing non-indigenous MOPAs. In view of the multitude of MOs in the environment, the scientific ignorance of many of their interactions, and their population dynamics in an ecological context, fundamental research on these topics with a tendency for application for registration purposes, should be considered necessary. Smit *et al.* (1992) recommend to perform more research with microcosms and biological containment systems for GMMs. This may apply to non-transgenic MOPAs with demonstrable adverse environmental effects in the laboratory as well. Relevant scientific disciplines are molecular biology and microbiology, microbial ecology, ecotoxicology and biotechnology. Important research topics may be:

I. The extent of genetic exchange of MOPA genome parts, responsible for toxin production under field conditions.
II. The extent of fitness differences between indigenous and non-indigenous MOPA populations and their explanation.

Besides more fundamental experimental research, another important feature to focus upon is the possibility of using some concepts that — up till now — have been useful in the ERA development for the registration of chemical pesticides. These are:

I. The concept of a no-effect level — is it possible to conceive such a treshold concept for MOs, and in particular, for infectious MOPAs?
II. The "dose-effect" concept — to what extent do different quantities of MOPAs in the environment result into different effects?

The concept of risk characterisation by comparing quantities of a chemical in the environment with those quantities that cause harmful effects — to what extent is this concept applicable to MOPAs? It is related with concepts I and II, but besides, it implies the quantitation of risks, which may not be feasible for microbial pesticides, in view of the current science. NBCI (1996) also points out these difficulties, and refers to a different approach based on a framework using "similarity" and "functional equivalence" standards for examining unprecedented organisms.

[20] *e.g.* the invasion in Dutch deciduous forests of *Prunus padus*, often called the "forest plague".

5. CONCLUSIONS AND RECOMMENDATIONS

Experimental research with consistent outcomes useful for registration procedures including the environmental risk assessment for the use of microbial pesticides, prior to release, is scarce, except for *Bacillus thuringiensis* and particular Baculoviridae. More basic research should be carried out in the integral field of molecular and microbiology, microbial ecology, ecotoxicology, statistics, and environmental risk assessment.

5.1 Data evaluation

Data evaluation concerning the environmental impact of microbial pesticides implies that the registration authorities have to deal with a very heterogenous data set, *e.g.* with respect to the characteristics of microorganisms with pesticidal action (MOPAs), their distribution and fate in the environment, and their effects. Therefore this tendency for heterogeneity is on bad terms with the strife for consistent guidelines for data evaluation.
The Dutch CA — dealing with the data evaluation of pesticides — should cooperate closely with experts in the fields of micro- and molecular biology, microbial ecology, applied biotechnology, ecotoxicology and environmental risk assessment to construct a solid framework for data evaluation, not only for proper evaluation, but also for requiring the appropriate data from the industries. The integration of these disciplines should be emphasised.

Evaluating and summarising test reports[21] on the environmental effects of microbial pesticides in a consistent and adequate way, is in the Netherlands yet in an early phase.
It is recommended to refine the Summary Tables as represented in this study. These tables may function as a checklist for environmental data evaluation, serving as a tool to establish the quality of these data, and to decide whether to use these data for further risk assessment or not. The division of microbial pesticides into two major groups (non-viral and viral) is sufficient for the next step of refining these Summary Tables.

5.2 Environmental risk assessment

The development of a consistent and adequate environmental risk assessment (ERA) for microbial pesticides, based on a proper data evaluation, is yet in an early phase in the Netherlands.

[21] summarising refers to a concise text including the most relevant aspects of a particular test. These summaries are made for registration procedures.

The Dutch CA — dealing with ERAs for pesticides — should cooperate closely with experts in the fields of micro- and molecular biology, microbial ecology, applied biotechnology, and risk assessment to construct a solid framework for risk assessment, based on proper data evaluation.

Risk assessment for chemical pesticides is based on three major concepts: the occurrence of (no-)effect levels, the "dose-effect" concept, and the concept that risk characterisation can be based on comparing the quantities in the environment with those quantities that cause harmful effects.
It remains to be seen, whether the development of a systematic approach for assessing the environmental risks of infectious MOPAs should be based on the same concepts as used for chemical pesticides. Due to various gaps in the scientific knowledge on the distribution, fate and effects of non-indigenous MOPAs in the environment, even a more qualitative approach is disputable. However, there is one current German attempt to quantify effects by comparing environmental titers with titers causing no effects. Therefore this "no-effect" concept may be useful for further development.

5.3 Framework for registration procedures

An overall framework for registration procedures linking data requirements by the CA, data evaluations (of the submitted test reports), and subsequently ERAs is yet in a premature stage. However, proposals for some elements of this framework have been published and can be used as a starting-point for the elaboration of an integral framework, that comprises all relevant elements. It is expected that a structured and transparent overall framework for data requirements, data evaluation, and environmental risk assessment will facilitate registration procedures, if its rationale is clear to all participants in the registration process.
Proposals for some parts of an integral framework have been published by Wäckers (1992) and Smit et al. (1992). The comparison of their decision trees makes clear that, when making an overall framework, the underlying concepts should be made very clear. Internation cooperation is essential for harmonisation purposes in this field.

Registration procedures in use for genetically modified microorganisms (GMMs) may be useful for registration purposes for microbial pesticides.

Genetically modified (micro)organisms are covered by a specific decree[22]*. Therefore transgenic MOPAs will be registrated in accordance with this decree. However, as they are also pesticides, they have to comply with the Dutch Pesticide Act. As no transgenic MOPA has been registered yet in the Netherlands, there is no jurisprudence on this issue. In the meantime, expertise should be exchanged between the CA dealing with GMOs and the CA dealing with pesticides.*

[22] in Dutch: Besluit genetisch gemodificeerde organismen.

GLOSSARY

Definitions should be seen within the context of this study

Cry-gene	a specific gene in *Bacillus thuringiensis* encoding the insecticidal crystal protein (*Cry* is crystal)
Data evaluation	see *data requirements*
Data requirements	the scientific data required by the CA in a more broader sense, including the dossier requirements for an applicant. *Data requirements* should yield valid test reports that can be evaluated properly by the CA. This data evaluation is followed by proper *risk assessment*
Host	any organism infected by a *microorganism* (*e.g.* a caterpillar after ingestion of Bacillus thuringiensis or a baculovirus)
Infection	condition in which a host is colonised by multiplying *microorganisms*
Infectivity	the ability of a *microorganism* to colonise a *host*
Microbial pesticide	contains a living — non-transgenic or transgenic — *microorganism* with *pesticidal action*. A pesticide may also contain adjuvantes that enhance the efficacy
Microorganism	any microbial entity, cellular or non-cellular, capable of replication or of transferring genetic material. Examples are: bacteria, fungi, protozoans, viruses, and viroids
Non-target organism	any organism that can be unintendedly affected by a chemical, a toxin or a *pathogen.*
Pathogen	any microorganism able to cause *pathogenicity* in a host following infection. A host can be any living organism.
Pathogenicity	any adverse effect — generally a disease — caused by a *pathogen* in a host, following an *infection.*

Propagule	any part of an organism capable of growing into a new organism; *e.g.* spore, a mycelial fragment.
Risk assessment	the overall proces of qualifying or quantifying the risks due to the use of *e.g.* a microbial pesticide (see for more detailed definitions Table 1)
Target	molecular site in the recipient organism where the actual interaction occurs with the active ingredient of *e.g.* a microbial pesticide. The molecular interaction at this site — *e.g.* an enzyme receptor that becomes blocked — can eventually lead to an adverse effect. It should be noted that a *target* may be found in both *target* - and *non-target organisms*
Target organism	any pest organism that can be intendedly affected by a chemical, a toxin or a *pathogen*
Toxicity	any adverse effect on an organism caused by a chemical or toxin (therefore not necessarily preceeded by an *infection*)[23]
Transgenic	genetically modified by introduction of genetic material of another (micro)organism with *e.g.* DNA recombinant techniques. The species barrier may be crossed
Virulence	the degree of *pathogenicity* of a pathogen

[23] it should be noted that there can be a gradual difference only between toxicity and pathogenicity, as the latter may be caused by toxicants as well (*e.g.* viruses releasing enzymes to solve a cell).

REFERENCES

Adams L.F., Liu C.L., MacIntosh S.C. & Starnes R.L. (1996) Diversity and biological activity of *Bacillus thuringiensis*. In: Crop protection agents from nature [natural products and analogues] Copping L.G. (ed), p. 360-388. The Royal Society of Chemistry, Cambridge.

Anonymus (1992) Pesticide Data Fact Sheet of US EPA on *Bacillus thuringiensis aizawai.*

Anonymus (1997a) **Working document** on the data requirements for the authorisation of biological pesticides in the European Union (Annex **II**). 4992/VI/95 rev. 1. Commission of the European Communities.

Asselbergs D.J.M., van Nierop S., Oomen P.A. & Oostelbos P.F.J. (eds) (1996) [Guide for Crop Protection, 14th revised edition. Plant Protection Service, Wageningen] Verslagen en mededelingen nr. 182 (in Dutch).

Bishop D.H.L., Cory J.S. & Possee R.D. (1992) The use of genetically engineered virus insecticides to control insect pests. In: Release of genetically engineered and other micro-organisms. Fry J.C. & Day M.J. (eds). Plant and Microbial Biotechnology Research Series **2**: 137-146.

Brock T.D., Madigan M.T., Martinko J.M. & Parker J. (1994) Biology of microorganisms. 7th edition. Prentice-Hall International, Inc., Englewood Cliffs, New Jersey.

Burges H.D. (1981) Safety, safety testing and quality control of microbial pesticides. In: Microbial control of pests and plant diseases, 1970-1980. Burges (ed). Academic Press, London/New York, Toronto, p.737-767.

Corke A.T.K & Rishbeth J. (1981) Use of microorganisms to control plant diseases. In: Microbial control of pests and plant diseases, 1970-1980. Burges (ed). Academic Press, London/New York, Toronto, p.717-736.

Crommentuijn T., Kalf D.F., Polder M.D., Posthumus R. & van de Plassche E. (1997) Maximum Permissible Concentrations and Negligible Concentrations for pesticides. RIVM Report No. 601501002.

De Boer (1997) Letter of the Dutch Ministry of Housing, Spatial Planning and Environment to the Dutch Board for the Authorisation of Pesticides, april 1997, DWL/97090179.

EC (1996) EUSES, the European Union System for the Evaluation of Substances. National Institute of Public Health and the Environment (RIVM), the Netherlands. Available from the European Chemicals Bureau (EC/JRC), ISPRA, Italy.

EPA (1989) Microbial and Biochemical Pest Control Agents. Part A Microbial Series 150A-158A-1. Subdivision M. Report No. 540/09-89-056. Environmental Protection Agency, Washington, US.

EPPO (1993) Decision-making scheme for the environmental risk assessment of plant protection products. EPPO/Council of Europe, EPPO Bulletin **23**.

EPPO (1994) Decision-making scheme for the environmental risk assessment of plant protection products. EPPO/Council of Europe (Chapter 11. Terrestrial vertebrates), EPPO Bulletin **24**: 37-87.

Fast P.G. (1981) The crystal toxin of *Bacillus thuringiensis*. In: Microbial control of pests and plant diseases, 1970-1980. Burges H.D. (ed). Academic Press, London/New York, Toronto, p. 223-248.

Leisy D.J. & Fuxa J.R. (1996) Natural and engineered viral agents for insect control. In: Crop protection agents from nature [natural products and analogues] Copping L.G.(ed), p. 389-425. The Royal Society of Chemistry, Cambridge.

Linders J.B.H.J. & Jager D.T. (eds) (1997) USES 2.0, The Uniform System for the Evaluation of Substances, version 2.0 [supplement to EUSES]. RIVM Report No. 679102037.

Meadows M.P. (1992) Environmental release of *Bacillus thuringiensis*. In: Release of genetically engineered and other micro-organisms. Fry J.C. & Day M.J. (eds). Plant and Microbial Biotechnology Research Series **2**: 120-136.

Mensink B.J.W.G. and Linders J.B.J.H. (1997) Microbial pesticides [data requirements for environmental risk assessment]. RIVM Report No. 679102036.

Mensink B.J.W.G., Montforts M., Wijkhuizen-Maślankiewicz L., Tibosch H. & Linders J.B.J.H. (1995) Manual for summarising and evaluating the environmental aspects of pesticides. RIVM Report No. 679101022

NBCI (1996) Options for changes in biological control regulations and guidelines in the US: a strawman for comment. National Biological Control Institute.

Plimmer J.R. (1996) The registration of new natural pesticides. In: Crop protection agents from nature [natural products and analogues] Copping L.G. (ed), p. 468-489. The Royal Society of Chemistry, Cambridge.

RIVM, VROM, WVC (1994) Uniform System for the Evaluation of Substances *(USES), version 1.0.* National Institute of Public Health and Environmental Protection (RIVM), Ministery of Housing, Spatial Planning and the Environment (VROM), and Ministery of Welfare, Health and Cultural Affairs (WVC). Distribution No. 11144/150.

Scheepens P.C. & Lotz L.A.P. (1994) [Perspectives for biological weed control]. Institute for Agrobiological and Soil Fertility Research. Report No.1 (in Dutch).

Šebesta K., Farkaš J., Horská K. & Vaňková J. (1981) Thuringiensin, the beta-exotoxin of *Bacillus thuringiensis*. In: Microbial control of pests and plant diseases, 1970-1980. Burges H.D. (ed). Academic Press, London/New York, Toronto, p. 249-281.

Slutsky B., Schneider W.R. & Kough J.L. (1997) The regulation of microbial pesticides and plant-pesticides by the US Environmental Protection Agency. Unpublished report of EPA.

Smit E., van Elsas J.D. & van Veen J.A. (1992) Risks associated with the application of genetically modified microorganisms in terrestrial ecosystems. FEMS Microbiology Reviews **88**: 263-278.

Smits P.H. & Vlak J.M. (1994) Registration of the first viral insecticide in the Netherlands: the development of SPOD-X, based on *Spodoptera exigua* nuclear polyhedrosis virus. Med. Fac. Landbouww. Univ. Gent 59/2a: 385-392.

Van Leeuwen C.J. & Hermens J.L.M. (eds.) (1995) Risk assessment of chemicals: an introduction. Kluwer Academic Publishers, Dordrecht.

Wäckers F.L. (1992) [Differentiated Guidelines for the Registration of Biological Pesticides]. Dutch Board for the Authorisation of Pesticides (in Dutch).

APPENDIX 1. Summary Tables: their use and background

Table 2 and 3 — Summary Tables, in conformity with Mensink *et al.*, 1995 — help to structure the abundance of information and to tag a reliability index (RI) to a particular test or a part of it (see Table 4). In such a table the reviewer find concisely the requirements which have to be met for a test item, *e.g.* test conditions, the validity of the results, and the type of endpoints. These test items refer to the *reliability* of a test, *i.e.* the scientifically inherent reliability of a test. It doesn't say something about another important aspect of the quality of a test: its usefulness (*e.g.* for risk assessment or standard setting procedures). As yet, Tables 2 and 3 should be seen as provisional: a convenient checklist, and a tool to foster discussion for further development of data evaluation and risk assessment for microbial pesticides. They could be extended and specified in the near future.

Table 4. The inherent reliability of scientific studies (Mensink et al., 1995)

RELIABILITY INDEX	DEFINITION	NOTES
1	reliable	the methodology and the test description are in accordance with the instructions*
2	less reliable	the methodology and/or the test description are less in accordance with the instructions*
3	not reliable	the methodology and/or the test description are not in accordance with the instructions*
4	no original data	the RI cannot be attributed to a test (part), as the data needed for verification are not available

* in official (*e.g.* EC, OECD, EPPO) and/or less official guidelines (*e.g.* as handled by RIVM or CTB, often in addition to the official guidelines)

If items reported are less or not in accordance with the Summary Tables, the reliability of a study is expected to decrease. In the column with the heading "Reliability lower?" this is indicated by a Y(es) or a E(xpert judgement):

I. Y(*es*) indicates that solely based on not fulfilling this requirement for this item, the reliability of the entire test is expected to decrease. This can be reflected in tagging an RI 2 to a test, or even tagging an RI 3. It is up to expert judgement to decide how many "Y"-items are required for tagging an RI 2 or 3 to a particular test in its entirety.

II. E(*xpert judgement*), indicates that no clear guidance can be given. The reviewer can consult a specialist.

It should always be reported clearly in the review or summary[24] why a certain RI has been tagged, so that this can be verified. It should be noted that to a certain extent the tagging of an RI to a test or relevant parts of a test is always inevitably expert judgement. Therefore the Summary Tables are supposed to give guidance when tagging an RI rather than always providing reproducible and unequivocal results. Expert judgement — reflecting *e.g.* the weight of evidence — can overrule the Summary Tables.

[24] see footnote 21.

APPENDIX 2. Registered microbial pesticides in the Netherlands (June, 1997)

Registrated MOPAs in the Netherlands. No environmental data were requested by the CA, as these microbial pesticides were based on naturally occurring MOPAs (person. communic. of CTB to RIVM; Asselbergs *et al.*, 1996).

Table 5. Registered microorganisms with pesticidal action in the Netherlands

MOPA	TRADE NAME	TYPE PRODUCT	CONTENT OF MOPA
VIRUS			
Cydia pomonella granulose virus	Asepta Carpovirusine	-	-
Spodoptera exiqua nuclear polyhedrosis virus	SPOD-X GH	-	10^8 polyeders/ml
Tomato mosaic virus (weak strain)	Virus No M II	various	0.1 mg viral protein/litre suspension
FUNGI			
Verticillium dahliae Kleb	Trigger	-	10^6 conidia/ml
Verticillium lecanii	Mycotal	WP	10^6 spores/mg
BACTERIA			
Streptomycis griseoviridis	Mycostop	WP	10^8 CFU/g
Bacillus thuringiensis	Bactimos Spuitpoeder	-	-
Bacillus thuringiensis	Abbott-Biob L	-	-
Bacillus thuringiensis	Abbott-Biob WP	-	-
Bacillus thuringiensis	Bactospeine	WP	16000 IU/mg
Bacillus thuringiensis	Bactospeine XLV	WP	13000 IU/mg
Bacillus thuringiensis	Biobit Vloeibaar	SC	13000 IU/mg
Bacillus thuringiensis	Biobit WP	WP	16000 IU/mg
Bacillus thuringiensis	Delfin	WG	32000 IU/mg
Bacillus thuringiensis	Dipel	WP	16000 IU/mg
Bacillus thuringiensis	Dipel ES	-	17600 IU/mg
Bacillus thuringiensis	Kobacthur L	-	-
Bacillus thuringiensis	Kobacthur WP	WP	-
Bacillus thuringiensis	Pokon Bio-Rups	WP	16000 IU/mg
Bacillus thuringiensis	Scutello	-	100%
Bacillus thuringiensis	Scutello L	SC	100%
Bacillus thuringiensis	Turex 50 WP	WP	25000 IU/mg

- = not reported; WP = wettable powder; SC = suspension concentrate; WG = water dispersible granules

APPENDIX 3. Registered microbial pesticides in the US (January, 1997)

Personal communication of EPA to RIVM.

Table 6. Registered microorganisms with pesticidal action in the US (Jan. 1997)

MOPA
VIRUS
Heliothis nucleopolyhedrosis virus (NPV)
Douglas fir tussock moth NPV
Gypsy moth NPV
Beet armyworm NPV
Autographa californica NPV
Autographa falcifera NPV
Cydia pomonella granulose virus
BACTERIA
Bacillus popilliae & B. lentimorbus
Bacillus thuringiensis kurstaki
Agrobacterium radiobacter K84
Bacillus thuringiensis israelensis
Bacillus thuringiensis san diego
Bacillus thuringiensis tenebrionis
Pseudomonas fluorescens EG1053
Pseudomonas fluorescens A506
Pseudomonas fluorescens 1629RS
Pseudomonas syringae 742RS
Bacillus thuringiensis kurstaki EG2348
Bacillus thuringiensis kurstaki EG2424
Bacillus thuringiensis kurstaki EG2371
Bacillus sphaericus
Bacillus subtilis GBO3
Bacillus thuringiensis aizawai GC-91
Bacillus thuringiensis aizawai
Burkholderia cepacia type Wisconsin
Streptomyces griseoviridis K61
Bacillus thuringiensis kurstaki BMP123
Bacillus subtilis MBI 600
Pseudomonas fluorescens NCIB 12089
Bacillus thuringiensis kurstaki EG7673
Pseudomonas syringae ESC 10
Pseudomonas syringae ESC 11
Bacillus thuringiensis kurstaki M-200
Bacillus thuringiensis kurstaki EG7841
Burkholderia cepacia type Wisc. isol.J82
YEAST
Candida oleophila I-182
FUNGI
Phytophthora palmivora MWV
Colletotrichum gloeosporioides aeschynomene ATCC 20358
Trichoderma harzianum ATCC 20476
Trichoderma polysporum ATCC 20475
Gliocladium virens G-21
Trichoderma harzianum rifai KRL-AG2
Lagenidium giganteum
Metarhizium anisopliae ESF1
Puccinia canaliculate (Schweinitz) Langerheim ATCC 40199
Ampelomyces quisqualis M10
Beauvaria bassiana GHA
Beauvaria bassiana ATCC 74040
PROTOZOA
Nosema locustae

APPENDIX 4. Genetically modified microbial pesticides

Certain aspects of genetically modified microbial pesticides are already regulated under the Directive 90/220/EC. The definition of a genetically modified organism given in this Directive is: "genetically modified organism (GMO) means an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination". To be able to judge whether or not a organism is a GMO it is important to know which techniques are considered to result in a GMO. The Directive gives three categories of techniques.

First the techniques which lead in any case to the construction of a GMO are:

- recombinant DNA techniques using vector systems as previously covered by Council Recommendation 82/472/EEC (1);
- techniques involving the direct introduction into an organism of heritable material prepared outside;
- the organism including micro-injection, macro-injection and micro-encapsulation;
- cell fusion (including protoplast fusion) or hybridization techniques where living cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

Secondly, techniques which are not considered to result in a GMO, on condition that they do not involve the use of recombinant DNA molecules or GMOs, are:

- in vitro fertilization;
- conjugation, transduction, transformation or any other natural process;
- polyploidy induction.

Thirdly, techniques which lead to the construction of a GMO, but these GMOs are to be excluded from the Directive, on condition that they do not involve the use of GMOs as recipient or parental organisms, are:

- mutagenesis;
- cell fusion (including protoplast fusion) of plant cells where the resulting organisms can also be produced by traditional breeding methods.

A consent to placing on the market of a product which contains or consists of genetically modified organisms or a consent for the performance of a field trial with a GMO, can — on the basis of the Directive — only be withheld with regard to the protection of human health and the environment.

For this purpose, a risk assessment is carried out. This risk assessment is based on the characteristics of the genetically modified organism and of its intended use. In this respect, the following general questions are important:

1. Are there reasons to assume that the genetically modified organisms or its progeny, due to the genetic modification, will become hazardous to human health or the environment?
2. Could the genetic material inserted into the genetically modified organisms be transferred to other organisms, and are there reasons to assume that those organisms, as a result, will become hazardous to human health or the environment?

Until today no genetically modified microbial pesticide has been placed on the market in the European Union. But maize expressing the *Cry IA(b)* protein with an insecticidal function of *Bacillus thurigiensis*, has been placed on the market. There are some field trials with genetically modified micro-organisms, which are supposed to have a pesticidal effect. These experiments are still in a early phase. Table 5 gives the registered field trials with genetically modified microorganisms with pesticidal action up till 21 November 1997.

Table 5. List of notifications for field releases with micro-organisms with a pesticidal function up till 21 November 1997[25]

ORGANISM	MAIN TRAIT	COMPANY	NUMBER
Bacillus sp.	Bioinsecticide	Procida	B/FR/93/12/01
Pseudomonas sp.	Biocontrol of tomato bacterial wilt	INRA Trigalet A	B/FR/92/12/11 B/FR/94/03/15
Pseudomonas sp.	Chitinase synthesis phenazine-1-carboxylic acid synthesis	Utrecht University - Dep. of Plant Ecology and Evolutionary Biology	B/NL/96/06
AcNPV	Efficacy	NERC Instute of Virology	B/GB/93/R3/2 B/GB/94/R3/4

An example of such a field trial is with the use of *Pseudomonas putida* which contains genes for phenazine, a anti-microbial agent. Seeds of wheat are coated with these bacteria and the bacteria are supposed to spread along with the root growth of the wheat plant. The phenazine gene product is supposed to decrease the effect of pathogenic fungi on the wheat germ plant. In the risk assessment a.o. a great deal of attention is paid to the effect on non-target organisms and the spread and persistence of the modified strain in the soil. Therefore a limited introduction was consented and extensive monitoring and reporting is required to gain knowledge on the actual effect of this introduction and to verify assumptions in the risk assessment.

[25] Source: list of SNIFS circulated under article 9 of Directive 90/220/EEC, European Commission December 1997, XI/559/94-Rev 8.

Another example of an organism that is likely to be used as a genetically modified microbial pesticide is AcNPV, a baculovirus. As this virus is very host-specific, the risk-assessment should amongst others focus on the effects of the genetic modification on the host-specificity.

In the analysis of a notification the following specific aspects have to be taken into account. Not all the points included will apply to every case. It is to be expected, therefore, that individual notifications will address only the particular subset of considerations that are appropriate to individual situations. The level of detail required in response to each subset of considerations is also likely to vary according to the nature and the scale of the proposed release.

4.1 Information relating to the GMO

A *Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s):*

1. scientific name;
2. taxonomy;
3. other names (usual name, strain name, cultivar name, etc.);
4. phenotypic and genetic markers;
5. degree of relatedness between donor and recipient or between parental organisms;
6. description of identification and detection techniques;
7. sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;
8. description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts;
9. potential for genetic transfer and exchange with other organisms;
10. verification of the genetic stability of the organisms and factors affecting it;
11. pathological, ecological and physiological traits:
    (a) classification of hazard according to existing Community rules concerning the protection of human health and/or the environment;
    (b) generation time in natural ecosystems, sexual and asexual reproductive cycle;
    (c) information on survival, including seasonability and the ability to form survival structures *e.g.*: seeds, spores or sclerotia;
    (d) pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms;
    (e) antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylactics and therapy;

(f) involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.

12. Nature of indigenous vectors:
    (a) sequence;
    (b) frequency of mobilization;
    (c) specificity;
    (d) presence of genes which confer resistance.
13. History of previous genetic modifications.

B *Characteristics of the vector:*

1. nature and source of the vector;
2. sequence of transposons, vectors and other non-coding genetic sequences used to construct the GMO and to make the introduced vector and insert function in the GMO;
3. frequency of mobilisation of inserted vector and/or genetic transfer capabilities and methods of determination;
4. information on the degree to which the vector is limited to the DNA required to perform the intended function.

C *Characteristics of the modified organism:*

1. information relating to the genetic modification:
    (a) methods used for the modification;
    (b) methods used to construct and introduce the insert(s) into the recipient or to delete a sequence;
    (c) description of the insert and/or vector construction;
    (d) purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function;
    (e) sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question with particular reference to any known harmful sequence.

2. Information on the final GMO:
    (a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;
    (b) structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;
    (c) stability of the organism in terms of genetic traits;
    (d) rate and level of expression of the new genetic material. Method and sensitivity of measurement;
    (e) activity of the expressed protein(s);
    (f) description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector;

(g) sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;
(h) history of previous releases or uses of the GMO;
(i) health considerations:
  (i) toxic or allergenic effects of the non-viable GMOs and/or their metabolic products;
  (ii) product hazards;
  (iii) comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity;
  (iv) capacity for colonisation;
  (v) whether the organism is pathogenic to humans who are immunocompetent
    - diseases caused and mechanism of pathogenicity including
    - invasiveness and virulence,
    - communicability,
    - infectious dose,
    - host range, possibility of alteration,
    - possibility of survival outside of human host,
    - presence of vectors or means of dissemination,
    - biological stability,
    - antibiotic resistance patterns,
    - allergenicity,
    - availability of appropriate therapies.

4.2 Information relating to the conditions of release and the receiving environment

A *Information on the release:*

1. description of the proposed deliberate release, including the purpose(s) and foreseen products;
2. foreseen dates of the release and time planning of the experiment including frequency and duration of releases;
3. preparation of the site previous to the release;
4. size of the site;
5. method(s) to be used for the release;
6. quantities of GMOs to be released;
7. disturbance on the site (type and method of cultivation, mining, irrigation, or other activities);
8. worker protection measures taken during the release;
9. post-release treatment of the site;
10. techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment;

11. information on, and results of, previous releases of the GMOs, especially at different scales and in different ecosystems.

B *Information on the environment (both on the site and the wider environment):*

1. geographical location and grid reference of the site(s) (in case of notifications under Part C the site(s) of release will be the foreseen areas of use of the product);
2. physical or biological proximity to humans and other significant biota;
3. proximity to significant biotopes or protected areas;
4. size of local population;
5. economic activities of local populations which are based on the natural resources of the area;
6. distance to closest areas protected for drinking water and/or environmental purpose;
7. climatic characteristics of the region(s) likely to be affected;
8. geographical, geological and pedological characteristics;
9. flora and fauna, including crops, livestock and migratory species;
10. description of target and non-target ecosystems likely to be affected;
11. a comparison of the natural habitat of the recipient organism with the proposed site(s) of release;
12. any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

4.3 Information relating to the interactions between the GMOs and environment

A *Characteristics affecting survival, multiplication and dissemination:*

1. biological features which affect survival, multiplication and dispersal;
2. known or predicted environmental conditions which may affect survival, multiplication and dissemination (*e.g.* wind, water, soil, temperature, pH);
3. sensitivity to specific agents.

B *Interactions with the environment:*

1. predicted habitat of the GMOs;
2. studies of the behavior and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses;
3. genetic transfer capability:
   a) post-release transfer of genetic material from GMOs into organisms in affected ecosystems;
   (b) post-release transfer of genetic material from indigenous organisms to the GMOs;
4. likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism;

5. measures employed to ensure and to verify genetic stability. Description of genetic traits which may prevent or minimise dispersal of genetic material. Methods to verify genetic stability;
6. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including *e.g.* inhalation, ingestion, surface contact and burrowing;
7. description of ecosystems to which the GMOs could be disseminated.

C *Potential environment impact:*

1. potential for excessive population increase in the environment;
2. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s);
3. identification and description of the target organisms;
4. anticipated mechanism and result of interaction between the released GMOs and the target organism;
5. identification and description of non-target organisms which may be affected unwittingly;
6. likelihood of post-release shifts in biological interactions or in host range;
7. known or predicted effects on non-target organisms in the environment, impact on population levels of competitors: preys, hosts, symbionts, predators, parasites and pathogens;
8. known or predicted involvement in biogeochemical processes;
9. other potentially significant interactions with the environment.

4.4 Information on monitoring, control, waste treatment and emergency response plans

A *Monitoring techniques:*

1. methods for tracing the GMOs, and for monitoring their effects;
2 specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques;
3. techniques for detecting transfer of the donated genetic material to other organisms;
4. duration and frequency of the monitoring.

B *Control of the release:*

1. methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of release or the designated area for use;
2. methods and procedures to protect the site from intrusion by unauthorised individuals;
3. methods and procedures to prevent other organisms from entering the site.

C *Waste treatment*

1. type of waste generated;
2. expected amount of waste;
3. possible risks;
4. description of treatment envisaged.

D *Emergency response plans:*

1. methods and procedures for controlling the GMOs in case of unexpected spread;
2. methods for decontamination of the treats affected, *e.g.* eradication of the GMOs;
3. methods for disposal or sanitation of plants, animals, soils, etc. that were exposed during or after the spread;
4. methods for the isolation of the area affected by the spread;
5. plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

4.5 Additional information required in the case of notification for placing on the market

A *General information:*

1. name of the product and names of GMOs contained therein;
2. name of the manufacturer or distributor and his address in the Community;
3. specificity of the product, exact conditions of use including, when appropriate, the type of environment and/or the geographical area(s) of the Community for which the product is suited;
4. type of expected use: industry, agriculture and skilled trades, consumer use by public at large.

B *The following information shall be provided, when relevant:*

1. measures to take in case of unintended release or misuse;
2. specific instructions or recommendations for storage and handling;
3. estimated production in and/or imports to the Community;
4. proposed packaging. This must be appropriate so as to avoid unintended release of the GMO's during storage, or at a later stage;
5. proposed labeling. This must include, at least in summarised form, the information referred to in points A.1, 2 A., A. 3, B. 1 and B. 2.