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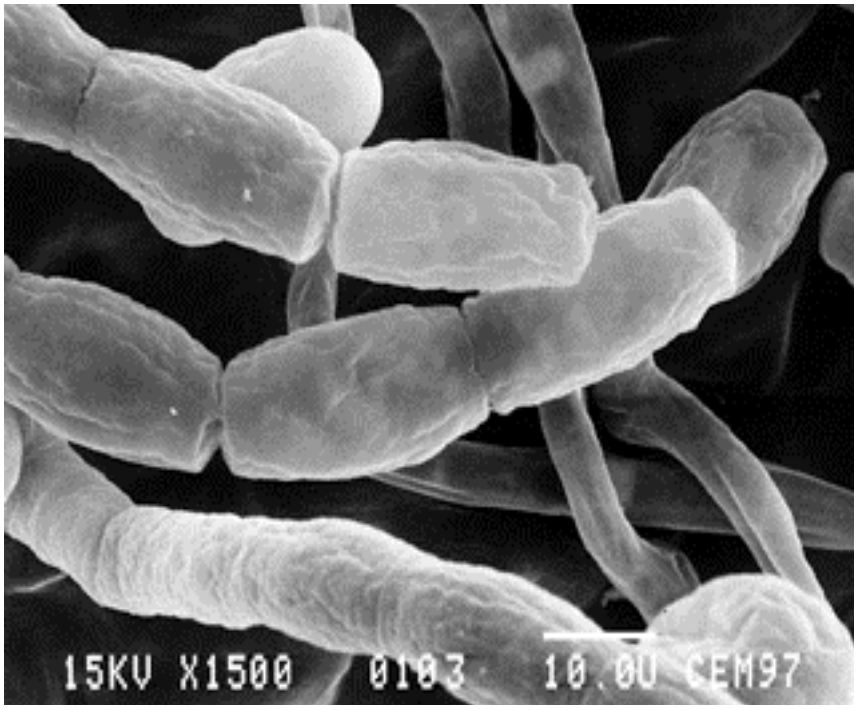
How to evaluate the environmental safety of plant protection products of natural origin
Proposals for decision trees for microbial, semio-chemical and plant-derived biopesticides
(version 1.0)

B.J.W.G. Mensink



T:OH

Electron scan image of *Pseudozyma flocculosa*, a fungal biopesticide, evaluated in 2001 by RIVM on the environmental safety. Evaluation to include the active ingredient on Annex I of the EU Directive 91/414/EEC. There is also a joint review programme with PMRA (Canada) on sporodex, a biopesticidal product with *Pseudozyma flocculosa*.

This investigation has been performed by order and for the account of the Directorate-General for Environmental Protection and the Directorate for Soil, Water, and Rural Areas, within the framework of project M/601450/01/BL, Risk Assessment Methodology.

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ABSTRACT

How to evaluate the environmental safety of plant protection products of natural origin[1]

The environmental safety of crop protection products of natural origin (biopesticides) is to be re-evaluated within a few years' time by the European Union. Also, new biopesticides will enter the EU market, due to the increasing importance of sustainable agriculture. There is, however, almost no guidance to evaluate the environmental safety of these products. Therefore, in this desk study risk decision trees have been developed for three groups of products, *i.e.* microbial organisms, signalling chemicals (including pheromones) and plant-derived substances. These trees help to discern acceptable from unacceptable potential risks. Also, *summary tables* have been developed to record and evaluate environmental tests with microbial pesticides. In this way the reliability and usefulness for the safety evaluation can be assessed per test. The decision trees and the *summary tables* are intended to improve the safety evaluation for regulatory purposes. Biopesticides are allegedly less toxic and persistent than synthetic chemicals. However, this should not exclude them from a proper environmental safety evaluation, since microbial and plant-derived biopesticidal ingredients can be infective, pathogenic or toxic. This depends on their microbiological or chemical properties, the dosage and the site and type of application. It is of primary importance to find out the identity of the micro-organism(s) or the active substance(s), the microbial or chemical characteristics, the mode of action, the origin, the role under natural conditions and the range of hosts or target organisms, if relevant. Subsequently, the scientific literature and/or the results of special environmental tests should be used to assess the safety of biopesticides to the environment.

RAPPORT IN HET KORT

Hoe zijn de milieurisico's vast te stellen van gewasbeschermingsmiddelen van natuurlijke oorsprong[2]

De milieuveiligheid van diverse gewasbeschermingsmiddelen van natuurlijke oorsprong zal de komende jaren door de EU opnieuw worden beoordeeld. Bovendien zullen nieuwe middelen op de markt komen vanwege het toenemende belang van duurzame landbouw. Er zijn echter nauwelijks richtlijnen om de milieuveiligheid van dergelijke middelen te evalueren. Daarom worden in deze bureaustudie twee hulpmiddelen aangereikt om de milieuveiligheid van natuurlijke gewasbeschermingsmiddelen beter te kunnen beoordelen. Het eerste betreft beslisbomen om voor drie groepen onacceptabele potentiële risico's te kunnen scheiden van acceptabele. De groepen zijn microbiële gewasbeschermings-middelen, signaalstoffen als feromonen en middelen van plantaardige oorsprong. Het tweede hulpmiddel bestaat uit "samenvattings"tabellen als format om de uitkomsten van experimenten met microbiële gewasbeschermingsmiddelen en de betrouwbaarheid en bruikbaarheid daarvan voor veiligheidsevaluaties vast te leggen. Van nature voorkomende gewasbeschermingsmiddelen zijn doorgaans minder persistent en toxisch dan synthetische gewasbeschermingsmiddelen. Het is echter niet juist om middelen van natuurlijke oorsprong derhalve te vrijwaren van milieuveiligheidsevaluaties. Ze kunnen infecties of vergiftigingen veroorzaken afhankelijk van de microbiële of chemische eigenschappen, de dosering, het type en de plaats van toepassing. Het is van het eerste belang de identiteit van het micro-organisme, de stof of het mengsel van stoffen, de microbiële of (bio)chemische eigenschappen, het werkingsmechanisme, de oorsprong, de eventuele rol onder natuurlijke omstandigheden en het gastheerbereik vast te stellen, indien van toepassing. Vervolgens kan, mede op grond van relevante wetenschappelijke literatuur en/of speciaal daartoe uitgevoerde experimenten, worden vastgesteld in hoeverre het gebruik van deze middelen veilig voor het milieu is.

[1] Keywords: pesticide, biopesticide, evaluation, environment, risk, safety.
[2] Trefwoorden: gewasbeschermingsmiddel van natuurlijke oorsprong (GNO), evaluatie, milieu, risico, veiligheid.

PREFACE

Biopesticides are predominantly naturally occurring micro-organisms, substances or extracts to be used as plant protection products. In the context of this report, synthetic pesticides resembling natural compounds are not included. Biopesticides are used for both biological and chemical control of (non-) agricultural pests. This report focuses on the agricultural use of biopesticides. Biological control is not necessarily restricted to biological or integrated crop protection. It may be useful for conventional crop protection as well. As a substitute for synthetic pesticides, the generally less hazardous — though not necessarily! — biopesticides may contribute to sustainable agriculture. The focus of this report is on three major groups of biopesticides: microbial biopesticides, signalling chemicals (*e.g.* insect pheromones) and plant-derived biopesticides (extracted, isolated or purified). This enormous variety in structure and function hinders the drawing up of a uniform safety evaluation scheme to assess the potential environmental impact. The challenge is, of course, to provide transparent and consistent guidance that on one hand improves the uniformity and the mutual acceptance of risk assessments and on the other hand meets this variety in structure and function. A more uniform approach, as far as possible and desired, may save labour, funds and test animals. This report offers tools for data and test evaluations and for environmental risk assessments.

The investigation was by order and for the account of the Directorate-General for Environmental Protection and the Directorate for Soil, Water, and Rural Areas, within the framework of project M/601450/01/BL, Risk Assessment Methodology.

The author thanks dr. J. Scheepmaker (RIVM/SEC) and drs. M. Rikken (RIVM/SEC) for critically reviewing this report.

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SUMMARY

The environmental safety of crop protection products of natural origin (biopesticides) is to be re-evaluated within a few years' time by the EU. They are used for both chemical and biological pest control (in the Netherlands in glasshouse horticulture in particular), and comprise circa 290 existing products with circa 130 active ingredients. Also, new biopesticides are expected to enter the market in view of EU policies towards a more sustainable agriculture. There is, however, almost no guidance to evaluate the environmental safety of these products. Therefore, in this desk study for risk assessors and scientists, guidance is presented to evaluate the environmental safety of biopesticides. The study has been based on scientific literature and various documents of the EU.
Biopesticides are allegedly less toxic and persistent than synthetic chemicals. However, this should not exclude them from a proper environmental safety evaluation, since they can be infective, pathogenic or toxic. This depends on their microbiological or (bio)chemical properties, the dosage and the site and type of application. The study proposes risk decision trees for three groups of products, *i.e.* microbial organisms, signalling chemicals (including pheromones) and plant-derived substances. These are the biopesticides with the highest use potentials. The trees help to discern acceptable from unacceptable potential environmental risks. Also, *summary tables* have been developed to record environmental tests with microbial pesticides. In this way the reliability and usefulness for the safety evaluation can be assessed per test. The decision trees and the *summary tables* are intended to improve the safety evaluation for regulatory purposes. They show that it is of primary importance to find out the identity of the micro-organism(s) or the active substance(s), the microbial or (bio)chemical properties, their mode of action, their origin, their role under natural conditions and the range of hosts or target organisms, if relevant. Screen and efficacy tests can be useful in this respect. Subsequently, the scientific literature and/or the results of special environmental tests should be used to assess the safety of biopesticides to the environment. In this way, the safety evaluation will promote the safe use of biopesticides.

SAMENVATTING

De milieuveiligheid van diverse gewasbeschermingsmiddelen van natuurlijke oorsprong zal de komende jaren door de EU opnieuw worden beoordeeld. Deze middelen worden voor zowel chemische als biologische gewasbescherming gebruikt (in Nederland vooral in de glastuinbouw). Het gaat om circa 290 bestaande middelen met circa 130 actieve ingrediënten. Bovendien zullen nieuwe middelen op de markt komen vanwege het toenemende belang van duurzame landbouw. Er zijn echter nauwelijks richtlijnen om de milieuveiligheid van dergelijke middelen te evalueren. Daarom worden in deze bureaustudie voor risicobeoordelaars en wetenschappers hulpmiddelen aangereikt om de milieuveiligheid van natuurlijke gewasbeschermingsmiddelen beter te kunnen beoordelen. De studie is gebaseerd op wetenschappelijke literatuur en documenten van de Europese Unie. Van nature voorkomende gewasbeschermingsmiddelen zijn doorgaans minder persistent en toxisch dan synthetische gewasbeschermingsmiddelen. Het is echter niet juist om middelen van natuurlijke oorsprong derhalve te vrijwaren van milieuveiligheidsevaluaties. Ze kunnen immers infecties of vergifigingen veroorzaken afhankelijk van de microbiële of (bio)chemische eigenschappen, de dosering, het type en de plaats van toepassing. Als eerste hulpmiddel stelt de studie voor drie groepen van middelen risicobeslisbomen voor. Het zijn de microbiële gewasbeschermingsmiddelen, signaalstoffen als feromonen en middelen van plantaardige oorsprong. Het zijn de groepen die naar verwachting het meest worden gebruikt. De risicobeslisbomen helpen om potentiële onacceptabele effecten te onderscheiden van de acceptabele. Het tweede hulpmiddel bestaat uit “samenvattings”tabellen als format om de uitkomsten van experimenten met microbiële gewasbeschermingsmiddelen en de betrouwbaarheid en bruikbaarheid daarvan voor veiligheidsevaluaties vast te leggen. Beide hulpmiddelen, beslisbomen en tabellen, zijn bedoeld om de veiligheidsevaluatie van gewasbeschermingsmiddelen van natuurlijke oorsprong te verbeteren. Ze geven aan dat het van het eerste belang is de identiteit van het micro-organisme, de stof of het mengsel van stoffen, de microbiële of (bio)chemische eigenschappen, de oorsprong, de eventuele rol onder natuurlijke omstandigheden en het gastheerbereik vast te stellen, voor zover van toepassing. Vervolgens kan, mede op grond van relevante wetenschappelijke literatuur en/of speciaal daartoe uitgevoerde experimenten, worden vastgesteld in hoeverre de potentiële risico’s onacceptabel zijn. Op deze wijze kan de veiligheidsevaluatie voor deze middelen van natuurlijke oorsprong bijdragen aan het veilige gebruik ervan.

ABBREVIATIONS

ai	active ingredient (as a collective term for micro-organisms and substances)
CFU	colony forming units
DT_{50}	median dissipation or degradation time (the time in which 50% of the biopesticide degrades or dissipates)
EC	European Commission
EC_{50}	median effective concentration (the amount of a substance affecting 50% of the test organisms or affecting 50% of an endpoint, *e.g.* growth)
EPPO	European and Mediterraneum Plant Protection Organisation
ET_{50}	median effective titer (the amount of a micro-organism affecting 50% of the test organisms or affecting 50% of an endpoint *e.g.* growth)
EU	European Union
GAP	good agricultural practice
GLP	good laboratory practice
ID_{50}	median infective dose or inoculum size (the amount of micro-organisms infecting 50% of the test organisms)
incl	including
MID	minimal infective dose
MOPA	micro-organism with pesticidal action
NOEC	no-observed-effect concentration (*i.e.* the highest amount of a (mixture of) substance(s) or toxin(s) not causing any significant adverse effect)
NOET	no-observed-effect titer (*i.e.* the highest amounts of a micro-organism not causing any significant adverse effect)
NTO	non-target organism
om	organic matter
P-DP	plant-derived plant protection product
PEC	predicted environmental concentration (referring to the environmental occurrence of a substance or toxin)
PET	predicted environmental titer (referring to the environmental occurrence of a micro-organism)
RIVM	National Institute for Public Health and the Environment
SCLP	straight-chained lepidopteran pheromones
TER	toxicity exposure ratio (*e.g.* NOEC/PEC, NOET/PET or ET_{50}/PET)
TO	target organism
QA	quality assurance

1. Introduction

The environmental safety of crop protection products of natural origin (biopesticides) is to be re-evaluated within a few years' time by the EU. They are used for both chemical and biological pest control (in the Netherlands in glasshouse horticulture in particular), and comprise circa 290 existing products with circa 130 active ingredients. Also, new biopesticides are expected to enter the market in view of EU policies towards a more sustainable agriculture. There is, however, almost no guidance to evaluate the environmental safety of these products. Therefore, in this desk study for risk assessors and scientists, guidance is presented to improve the evaluation of the environmental safety of biopesticides.

1.1. Biopesticides: definitions and context

Biopesticides are products that contain naturally occurring micro-organisms, macro-organisms, substances or extracts to be used as plant protection products. They are used for both biological and chemical control of (non-) agricultural pests[3]. This study focuses on the agricultural use of biopesticides. Synonyms for biopesticides are biological pesticides and natural plant protection products. However, to avoid confusion, the term biopesticides is preferred in this report. This group comprises a wide array of substances, mixtures of substances, micro-organisms and macro-organisms. Table 1 shows the variety in structure and function of biopesticides.

Table 1. Biopesticides, their structure and function.

STRUCTURE	**FUNCTION**				
	pesticidal action s.s.[1]	**attractant**	**repellant**	**plant growth re-gulator**	**pest population regulator[2]**
1. micro-organisms living	⊗				⊗
2. micro-organisms dormant	⊗				⊗
3. microbial metabolites[3]	×				
4. metabolites/extracts of plants					
a. biochemicals	×			×	
b. extracts	⊗		⊗		
5. metabolites/extracts of animals					
a. biochemicals[4]	×	⊗			
b. extracts	×		×		
6. beneficial arthropods					×
7. genetically modified organisms	×				×

*Combinations with × actually exist (including ⊗). The combinations with a ⊗ are dealt with in this report. **1**: pesticidal action sensu stricto refers to direct toxicological or infective interaction of a natural chemical with a pest; **2**: a population regulator affects a pest indirectly via processes as predation, competition or parasitism; **3**: refer to all micro-organisms in conformity with [12]; **4**: include pheromones and other semiochemicals.*

[3] Biological and chemical control refer to pest control by living organisms and specific (bio)chemicals, respectively. Both types of control are not strictly separated as the effect of a living micro-organism may be based on a particular toxin.

Other biopesticides as synthetic, though natural products resembling pesticides, macro-organisms (*e.g.* spiders and predatory mites) and genetically modified organisms are not included in this study. The huge variety of biopesticides indicates the difficulty of formulating a uniform concept for the safety evaluation, including the risk assessment. However, the industrial, scientific and regulatory activities in the field of biopesticides are increasing (*e.g.* [2;3;15;19;21]), thus prompting for a consistent and transparent approach to evaluate the environmental safety of biopesticides.

Biopesticides are generally derived from living systems as animals, plants and micro-organisms. Various biopesticidal products as microbials contain living systems as (incl survival structures as spores and cysts). Derivation occurs:

1. *via selecting and culturing micro-organisms;*
2. *via extraction from living organisms;*
3. *via isolation and purification of biochemicals from living organisms;*
4. *via artificially synthesising biochemicals similar to naturally occurring biochemicals.*

Examples of these four types of derivation are (1) the culturing of the various strains of *Bacillus thuringiensis*, an insectide with a worldwide use, (2) the extraction of biochemicals from the neem (*Azadiracta indica*) and tea (*Melaleuca alternifolia*) shrubs, (3) the isolation and purification of spinosad from *Saccharopolyspora spinosa*, an insecticidal metabolite of a soil actinomycete, and (4) the synthesis of plant growth regulators — *e.g.* dikegulac-sodium, maleic hydrazide and piproctanyl bromide— and pyrethroids as allethrin and permethrin, respectively. Within the context of this desk study, group (4) of the synthetic biopesticides is not included.

The difference between biopesticides and chemical pesticides and the implications of these differences for data requirements, data evaluation and risk assessment is an issue. On the one hand, both pesticide types fall within the scope of the EU Directive 91/414/EEC, thus guaranteeing comparable starting-points. On the other hand, data requirements for biopesticides may diverge. In the Netherlands, since 2001, data requirements for new microbial biopesticides have to follow a separate EU Directive [12]; other biopesticides and since 2003 specific pheromones [6], have to be evaluated on a case by case basis though preferably in conformity with the Uniform Principles for synthetic pesticides [11]. In the EU, a proposal for data requirements for plant extracts is under discussion [1]. It should be noted that the data requirements do not necessarily match with the data, actually needed for risk assessment as the data requirements also represent a jurisdictional context (*e.g.* on labelling and classification).

Because of the natural character of biopesticides, it may be suggested that biopesticides are less toxic and less persistent than chemical pesticides. But though this may apply to a lot of products, particularly in respect of the persistence, it is definitely not valid for all biopesticides. The application of fungi spores in “unnaturally” high amounts in a greenhouse may *e.g.* cause respiratory adverse effects to employees and pesticide applicators, though not much data are available to underpin this assumption. Toxicological research on the effects of aerial exposure via spores is scarce. Plant extracts with botanical secondary metabolites may have been evolved evolutionary to deter herbivores from consuming the plant or tree. It is therefore not surprising that plant extracts can be very toxic.

The major domains dealing with (bio)pesticides are represented in Fig. 1. The safety evaluation refers to the scientific process of integrating data to assess the safety of a biopesticidal ingredient or product, before it enters the market. Regulation refers to legislation, policies, decision making and enforcement: these aspects determine whether a biopesticide will be registrated and under which conditions. Production, use and effects refer to the empirical chain of cause and effects. This domain represents that what actually happens with biopesticides being produced, used, and emitted to the environment, possibly causing unwanted effects. The feed-back arrow in Fig. 1 refers to new scientific or regulatory insights or developments leading to a new evaluation, taking these aspects into account. The *major domain* dealt with in this report is the *safety evaluation*. It is important to realise that EU *regulation* will be prevalent in the next decades. However, whereas decisions on active ingredients (a.i.) will be taken by the European Commission (EC), the final decisions on the products with those ingredients will be made by the individual EU member states. The EC confirms the approval of an a.i. by placing it on the Annex I of Commission Directive 91/414/EEC (conform [10]). Only then, products with this a.i. are potentially marketable in the countries of the EU. The first new biopesticide entered the Annex I in 2001 (*Paecilomyces fumosoroseus*). Apart from these *new* microbial biopesticides, circa 290 *existing* products with circa 130 active ingredients will be re-evaluated in the next years (the 4th stage of the review programme of the EC) [1][4][13][5]. The Netherlands will be involved as lead rapporteur member state for the microbial biopesticides, together with Sweden. These 4th stage microbials are listed in Table 2. The regulatory domain and the domain of the causal chain from production and use, via emission to exposure and effects are not further dealth with in this report.

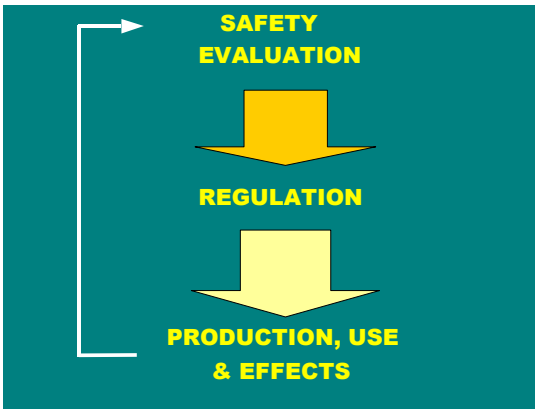


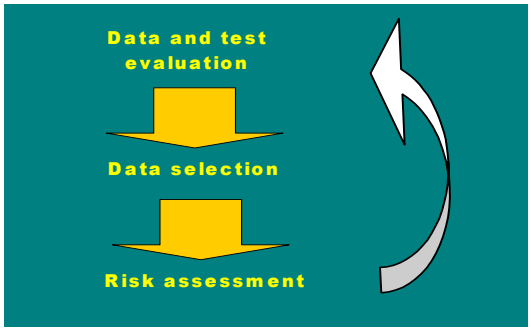
Figure 1. Major domains dealing with (bio)pesticides.

1.2. Safety evaluation

An essential aspect of evaluating the environmental safety of a biopesticide comprises the route from test and data evaluation, via data or endpoint selection to risk assessment (see Figure 2). There is consensus on the notion that microbial biopesticides generally need a safety evaluation (including risk assessment) that differs from synthetic pesticides [14-17]. Until further notice, biopesticidal products with other ingredients than micro-organisms have to comply with the Uniform Principles for pesticides in general [11]. After inclusion of a micro-organism on Annex I of Directive 91/414/EEC, the member states have to use the Uniform Principles for microbial biopesticides to decide on registration of a product per member state [15]. In this way, a common approach in biopesticide registration and evaluation should be promoted. The Uniform Principles contain the criteria and statements whether hazards or risks are acceptable or not.

[4] Existing biopesticides are those marketed before 25 July 1993. Whereas a basic notification may suffice for most of these biopesticides, full notifications are required for the micro-organisms of this 4th stage list (see Table 2). All 4th stage biopesticides are listed in Appendix 3. Their re-registration is supported by a (group of) notifier (s).

[5] The 4th stage list of (bio)pesticides comprises various biopesticides besides microbials, semiochemicals, plant-derived biopesticides and synthetic pesticides. Examples of biopesticides are substances used in human foodstuffs or animal feeding stuffs as fatty acids and urea; animal products or derived thereof by simple processing; commodity substances as *e.g.* kieselgur (diatomaceous earth) and lime sulphur; substances used on stored plants or plant products as ethanol and ethylene; repellants and attractants other than pheromones or other semiochemicals.



Although the regulatory context and the criteria of the safety evaluation of biopesticides, particularly for the microbial products, are getting more clear, common grounds for various technical and scientific aspects of the evaluation process are not yet available. Common guidance on various aspects is lacking. In this way there is a need for proper safety evaluation tools, especially on a more detailed, scientific level.

Figure 2. Main aspects of the safety evaluation route.

Table 2. Existing microbial biopesticidesin the EU [13].

Active substances (including any variants thereof) that were on the market before 25 July 1993 which:
1. are microorganisms including viruses, including the following:
Aschersonia aleyrodis
Agrotis segetum granulosis virus
Bacillus sphaericus
Bacillus thuringiensis including: (*)
— subspecies *aizawai*
— subspecies *israelensis*
— subspecies *kurstaki*
— subspecies *tenebrionis*
Beauveria bassiana
Beauveria brongniartii (syn. *B. tenella*)
Cydia pomonella granulosis virus
Mamestra brassica nuclear polyhedrosis virus
Metarhizium anisopliae
Neodiprion sertifer nuclear polyhedrosis virus
Phlebiopsis gigantea
Streptomyces griseoviridis
Tomato mosaic virus
Trichoderma harzianum
Trichoderma polysporum
Trichoderma viride
Verticillium dahliae Kleb.
Verticillium lecanii

In general, guidance and statements on the following aspects are lacking:

1. *test protocols (which to use or prefer?) (test protocols determine the endpoints[6] to be used for risk assessment);*
2. *data and test evaluation (how to evaluate the data and individual tests submitted by registrants or producers?);*
3. *selection of endpoints for risk assessment (how to chose proper endpoints of the submitted data and tests to use for environmental risk assessment?);*
4. *risk assessment (when is a potential risk acceptable and when is it not?);*
5. *waivers (how to deal with waivers or statements put forward by registrants or producers to be exempted from submitting particular data or tests, e.g. if it is argued that the terrestrial compartment will not be contaminated by a biopesticide and that terrestrial non-target organisms therefore cannot be exposed and thus need not to be tested?);*

[6] An endpoint is any reponse measure in a test, *i.e.* the measure(s) or value(s) derived from the test that constitutes the test results (*e.g.* DT_{50}, MID, NOEC, EC_{50}).

6. *criteria for higher-tier assessments (are there first-tier criteria that trigger a higher-tier test or evaluation if exceeded?).*

1.3. Study objectives

Scientific and technical guidance on the safety evaluation of biopesticides is limited or lacking, whereas the scientific, regulatory and industrial activities in the field of biopesticides evolve rapidly. These dynamics are due to the increasing role of biopesticides in a more sustainable agriculture, the policy goal of an increasing number of countries, including the Netherlands and Germany. This report offers tools for the safety evaluation of biopesticides:

1. *to improve the technical and scientific guidance on recording and evaluating environmental data and tests with microbial biopesticides (tests submitted for regulatory purposes);*
2. *to improve the environmental risk assessment for three groups of biopesticides (micro-organisms, semiochemicals and plant-derived (bio)chemicals.*

In this way, the present report focuses on the numbers 2, 3 and 4 of the list on page 18. The major groups of biopesticides dealt with in this report are: microbial biopesticides (§ 3.2.2), semiochemical biopesticides, including signalling chemicals as pheromones (§ 3.2.3) and plant-derived biopesticides (§ 3.2.4).

Some technical terms are phrased within quotation marks. These stress the relativity of these terms. Then there are no clear unambiguous definitions, although these terms are useful in the context (*e.g.* “indigenous”, “exotic” and “background” level) and may be defined more precise in the future.

2. Methodology

In this report, tools are presented to improve the environmental safety evaluation of biopesticides. The tools are primarily based on:

1. *scientific literature in the field of biopesticides, their efficacy and effects;*
2. *regulatory literature on data requirements and risk and decision criteria;*
3. *the experience of RIVM in evaluating biopesticides (including environmental risk assessment) by order of the Board for Authorising Pesticides for both national and EU registrations.*

The format of the environmental risk decision trees is freely adapted from the decision-schemes of EPPO (see also [6]).

3. Results

Fig. 3 refers to the sections per group of biopesticides. Three groups are actually dealt with:

1. *microbial biopesticides;*
2. *semiochemical biopesticides;*
3. *plant-derived biopesticides.*

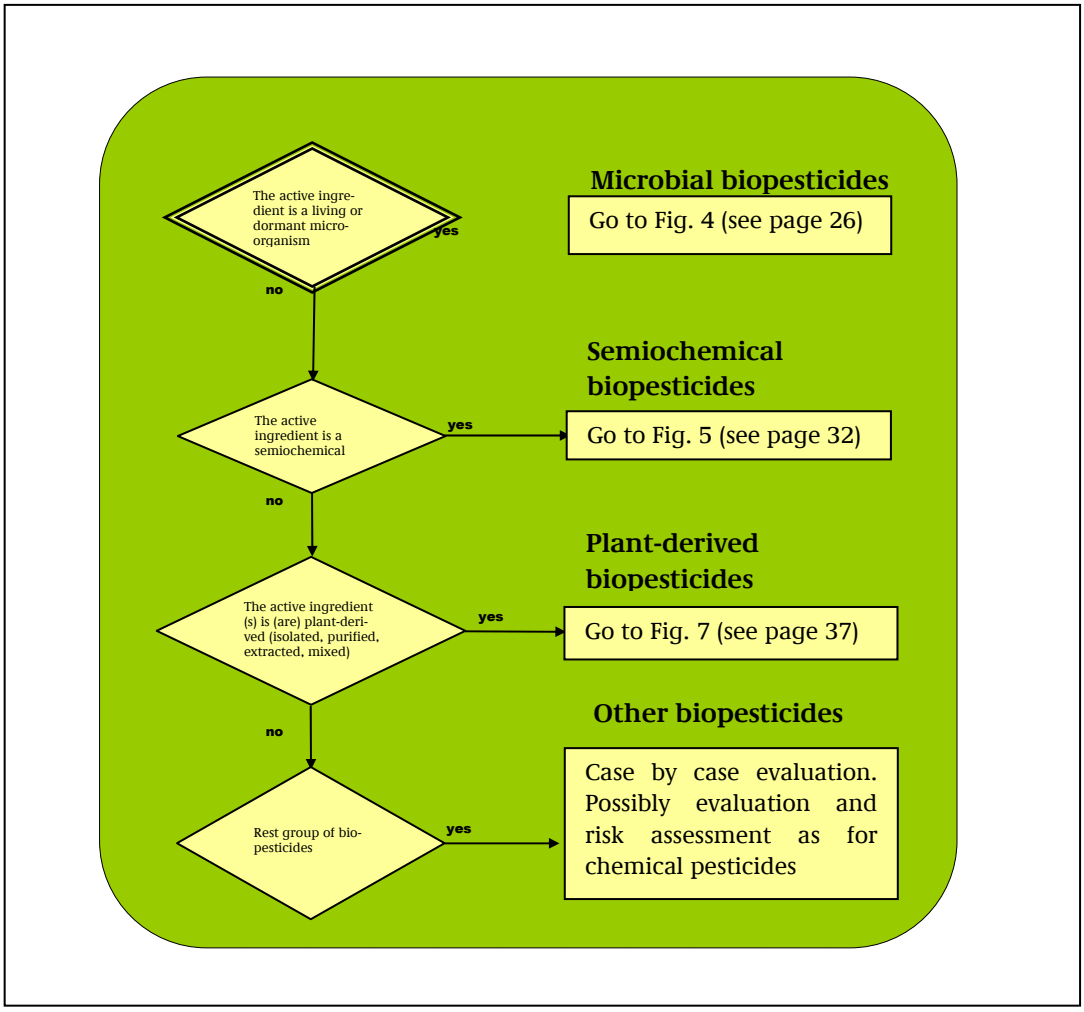


Figure 3. Determination tree for biopesticides.

The rest group of biopesticides in Fig. 3 comprises biopesticides of natural origin though clearly not microbial, semiochemical or plant-derived.

3.1. Data and test evaluations of biopesticides

Generally, technical and scientific tests provide the data for risk assessment. In this way, the scientific reliability and usefulness of tests are the foundation of the risk assessment. As they may vary, a strategy must be derived to evaluate individual tests and to select the relevant elements for risk assessment. Various sources can be helpful in evaluating biopesticides. Such technical or scientific sources are *e.g.* [5] and [9]. Specific information

on the comprehensiveness and set-up of laboratory and field tests with microbial biopesticides is found in [8]. An overview of the data and tests to be submitted for EU regulation is in [12]. A format for recording and evaluating relevant scientific tests had been presented as *summary tables* in [17]. An updated version of these tables is presented in Appendix 1 and 2 of this report. The tables serve for assessing the quality and the usefulness of the data in the test reports for risk assessment. They can also function as a checklist for risk assessors, peer-reviewers and scientists. In this way such a format can be used for QA as well. Appendix 1 refers to the environmental distribution and fate of microbial biopesticides, Appendix 2 refers to their effects on living systems.

3.2. Environmental risk assessment

Biopesticides, in view of their natural character, are allegedly less persistent and hazardous than their synthetic counterparts[7]. Various biopesticides are used throughout the world, without demonstrable adverse effects to the environment and to wildlife. Safety evaluations sofar have not aroused substantial suspicion, although various areas seem underexposed: *e.g.* the potential inhalatory effects on employees in greenhouses after spraying spores of pesticidal fungi at high concentrations. *Bacillus thuringiensis* is not detrimental to human health when used as recommended. However, the genetical make-up is identical to that of the human pathogen *Bacillus cereus*. Apparently small differences may have large consequences. Therefore, biopesticides can be potentially detrimental dependent on:

1. *the mode of action and other (micro)biological properties (incl the environmental requirements for persistence, growth and replication as pH, temperature, humidity, organic matter availability, if relevant);*
2. *the dosage of toxins or (bio) chemicals in case of a dose-response relation;*
3. *the potency of infectivity and pathogenicity;*
4. *the site and type of application (determining the area and the sort of potential exposure).*

Therefore risk assessment is important. Risk assessment conform [20] entails the following actions: (1) effects assessment, comprising (1a) hazard identification and (1b) dose-response assessment, (2) exposure assessment and (3) risk characterisation (*i.e.* the estimation of the incidence and severity of the adverse effects likely to occur due to the actual or predicted exposure)[8]. The risk characterisation may include "risk estimation" *i.e.* the quantification of that likelihood, *e.g.* via the toxicity exposure ratios NOEC/PEC or NOET/PET[9]. If quantification is not possible, the risk assessment should be qualitative.

A more narrow host range or a narrow micro-habitat of a microbial organism with pesticidal action (MOPA) may increase the environmental safety, unless such a host range or micro-habitat still comprises relevant non-target organisms. In this way, proper data on the mode of action and other (micro)biological properties, the host range and natural

[7] The synthetic pesticides that can be used against the same pest as the plant protection products of natural origin.

[8] **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 to estimate amounts to which non-target species or populations may be exposed; the exposure assessment refers to the environmentally relevant compartments; **effects assessment**: identification of effect or no-effect levels of MOPAs for various ecologically relevant non-target groups (*e.g.* an NOET or NOEC for aquatic organisms); the effects assessment refers to the environmentally relevant compartments; as it focuses upon effects dependent on a particular concentration or dose, the concept of concentration- or 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 small vertebrates).

[9] See p. 13 for the abbreviations.

distribution may preclude the exposure of non-target organisms (NTOs). If *e.g.* the monitoring of fungal spores in a greenhouse indicates the rapid inactivation or dissipation of a MOPA, then risk assessments for outdoor environments may not be necessary, as spores may not dissipate to outdoor environments. As it is difficult to extrapolate or use such data for micro-organisms in general, the use of such a concept will not be easy.

The risk assessments of microbial biopesticides should be in accordance with the Uniform Principles for plant protection products with micro-organisms. These have been agreed upon in May 2004 by the Council of Ministers ([15]). In case the biopesticide contains an a.i. that is not a micro-organism, the environmental risk will have to be assessed in conformity with the Uniform Principles for chemical pesticides.

3.2.1 First-tier versus *higher-tier risk assessment*

First–tier risk assessment refers to the first, generally conservative, safety evaluation based on primarily submitted tests and data. These submitted tests are mostly relatively simple laboratory tests. If certain criteria or triggers are met, a second- or higher-tier assessment will be necessary. This follow-up is generally more realistic, though it may be based on more complex studies that are difficult to analyse. These may be varying from extended laboratory studies, via microcosms and mesocosms to semi-field or field studies. In this way, the first-tier of the risk assessment may reveal unacceptable risks (*e.g.* if the micro-organism is pathogenic to beneficial arthropods and exposure is likely, no authorisation shall be granted), *unless* higher-tier tests under generally more realistic conditions indicate the opposite (*i.e.* the lack of actual adverse effects under the intended conditions of use of the biopesticidal product) (see also footnote 13).

In the following sections (§ 3.2.2 – § 3.2.4), the environmental risk decision trees for biopesticides are central. These risk decision trees focus on microbial biopesticides (Fig. 4), semiochemical biopesticides (Fig. 5) and plant-derived biopesticides (Fig. 7), respectively. The reference to these figures with annotated risk decision trees is presented in Fig. 3. The numbers in the individual risk decision trees refer to the correspondingly numbered passages in the texts.

3.2.2. Microbial biopesticides

The environmental risk decision tree for microbial pesticides is presented in Fig. 4 (next page). The goal is to discern the acceptable from the unacceptable risks in view of the intended use of the biopesticidal product and the submitted tests and data. The decision tree reflects the final draft of the Uniform Principles for biopesticidal products with micro-organisms [15]. Conform the Directive 91/414/EEC, risks are unacceptable if certain criteria are not met, unless tests under field conditions reveal the opposite.

Explanatory notes of Fig. 4:

(1) The box *characterisation, identification* and *efficacy* is the starting block for the risk decision tree for microbial biopesticides (therefore double-lined ▭ in Fig. 4). Characterisation is an umbrella term for aspects as the mode of action, microbial properties (persistence, growth, replication as a function of environmental parameters as temperature, pH, humidity, the availability of organic matter, if relevant), the origin of the micro-organism and its role under natural conditions.

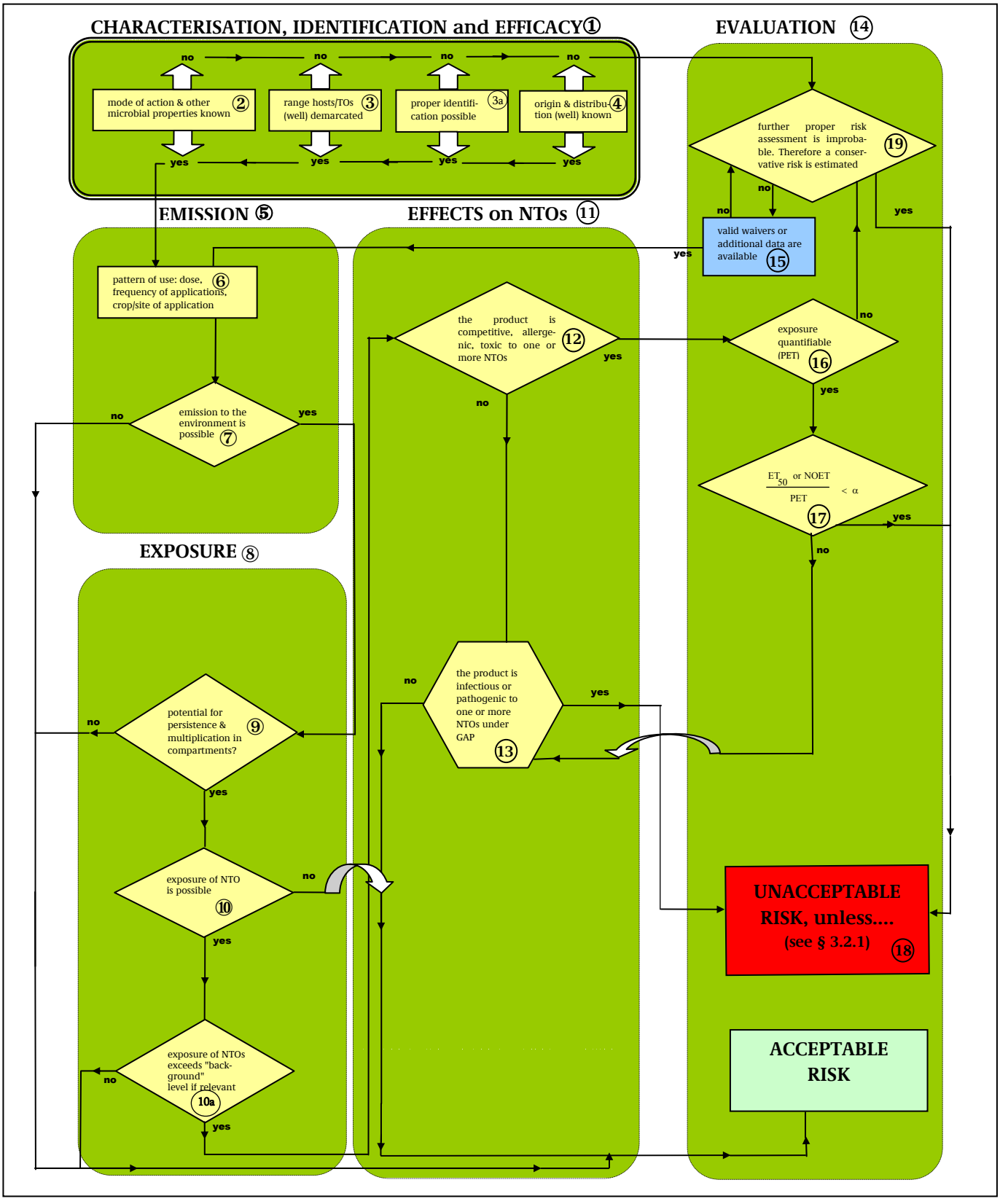


Figure 4. *Environmental risk decision tree for microbial biopesticides.*

PET: predicted environmental titer; ET_{50}: the median effective titer; NOET: no-observed-effect titer; TOs: target organisms; NTOs: non-target organisms; GAP: good agricultural practice. Note that the box *characterisation, identification and efficacy* makes a strong appeal to expert judgement, as strict yes or no answers are difficult to obtain. One should be flexible in this respect. For explanatory notes see text.

The starting block shows the issues of primary importance. They are:

1. *mode of action and other (micro)biological properties (are they known?);*
2. *the range of hosts or target organisms (what is known?);*
3. *the identification methods or analyses (how to recognise and quantify?);*
4. *the natural distribution and the role under natural conditions (what is known?).*

Note that the box *characterisation, identification and efficacy* makes a strong appeal to expert judgement, as strict yes or no answers are difficult to obtain. One should be flexible in this respect. The set, however, is considered basic for understanding the micro-ecological niche of the micro-organism with pesticidal action (MOPA). The *mode of action* may exclude risk assessment for particular non-target organisms (see also ②). Other *microbial properties* may determine the environmental expression. Therefore, these are very important for risk assessment. As an example: it was shown for a biocontrol micro-fungus that it had been difficult to maintain optimal microclimatological conditions in a greenhouse to secure the efficacy (person. communic. of a company to RIVM). Due to a narrow range of environmental requirements (a very high relative humidity in particular), repeated dosages were recommended for biocontrol with this micro-fungus in greenhouses. The difficulties in maintaining a proper efficacy indicated a poor survival under outdoor conditions. The *target* (in case of a toxic micro-organism) or *host* (in case of an infectious micro-organism) *range* can be helpful to determine whether the borderline between the target organism (TO) and the non-target-organism (NTO) is clear or not (if noctuid moths are at risk as a pest, relatively closely related non-target species may be affected as well). In this way, the submission of screening or efficacy tests may be helpful to demarcate the target organisms from the non-target organisms as clear as possible. *Identification* is necessary for various reasons. However, whereas the EC stated that micro-organisms should be identified at the strain level [12;15], submitted data and literature may not always reveal the strain. Ecotoxicologically, details on the strain may be relevant in particular when metabolites or toxins cause the effects. Then, small genotypic or phenotypic differences may have large consequences. The composition of a biopesticidal product should be evaluated and qualitatively and quantitatively (see [15]), thus including:

1. *the micro-organism, including its strain, resting or vegetative stages and the genetic stability, when relevant;*
2. *relevant metabolites or toxins;*
3. *residual growth medium;*
4. *co-formulantia;*
5. *microbial contaminants.*

Co-formulantia can be protective for micro-organisms by inhibiting the host defence mechanisms against the micro-organism. This may result in a lowered minimal infective dose (MID) [15]. Therefore, the identification of co-formulantia can be important for the risk assessment as well. The *microbial origin*, its natural *distribution* and its role role under natural conditions may be helpful to explain the action of a micro-organism as a biopesticide and the consequences.

② If the mode of action and other microbiological properties are not known, it may be difficult to predict or confirm the potential effects on NTOs. It relies on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation. The mode of action should be evaluated as much as possible [15]. Examples of modes of action are:

1. *antibiosis;*
2. *induction of plant resistance;*
3. *interference with the virulence of a pathogenic target organism;*
4. *endophytic growth;*
5. *root colonisation;*
6. *competition of ecological niche (e.g. nutrients, habitats)*
7. *parasitisation*
8. *invertebrate pathogenicity.*

3 If the specificity of the MOPA is known, it may be more easy to explain the type of effects or risks to *e.g.* birds, fish and bees. The more clear this specificity is confined to a particular set of organisms, the more likely NTO tests with other organisms may be waived. It relies on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

3a Proper identification is required. References to (agro-)scientific publications are welcomed. A proper identification refers to a valid analysis to identify the micro-organism under registration. Only then, assessing the (dis)similarity with “indigenous” and related micro-organisms in the geographical area of application and in the relevant compartments is possible. The storage of the MOPA under registration in an internationally acknowledged culture collection is strongly recommended as it will facilitate post-registration research or monitoring in case of suspecting a MOPA to be hazardous to human health (consumers, applicators, bystanders) or to the environment, after its release. Storage will also make it possible to investigate the possibility of genetic divergence between the original micro-organism and the MOPA offspring in the field, if relevant. For various environmental data, tests with the same species but with different strains are generally useful. In case of a specific metabolite or toxin, however, one should be alert as different strains of the same species may produce different metabolites or toxins.

4 The origin and the role of the MOPA under natural conditions may be relevant as they may explain particular aspects of the MOPA. The more is known of the geographical, ecological or physical (*e.g.* in the root or soil zone, on leaves) distribution of the MOPA, the more likely the distribution and effects due to its prospective use as a pesticide can be assessed. On the other hand, extrapolation of laboratory test results with micro-organisms to ecosystems is generally tedious: the dynamics and microbial interrelations of such test systems are complex. It relies on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

5 Emissions of microbial biopesticides, *e.g.* via actual transport of spores or other life stages to nearby (agro-)ecosystems.

6 The pattern of use is a very important part of the environmental risk assessment, as it primarily determines the (potential) extent of exposure. It comprises dosage, frequency, the frequency, the site (crop, bare soil, slope) and type of applicaton. It should be noted that *e.g.* various fungi, as MOPAs, require very specific (micro-) conditions and without these requirements the efficacy is limited. In view of these limitations, biopesticides may be applied much more frequent than their chemical counterparts.

⑦ Interesting topics are *e.g.* to what extent conidiospores of a fungal biopesticide — to be used in a greenhouse — can be expected to disseminate to outdoor environments as surface water, via drainage of condense, and soil, via air and deposition. Maybe emission factors should be developed to facilitate these exposure assessments. These are fixed percentages of the indoor amounts of biopesticides likely to disseminate to outdoor areas, taking into account particular cultures as hydroponic cultivation, barriers as glass windows and the spore persistence and viability).

Ways of transport to nearby (agro-)ecosystems should be considered:
1. *via air;*
2. *via organisms (e.g. faeces, fur, feathers, berries, human shoes and cloths);*
3. *via water;*
4. *via soil particles (e.g. run-off).*

Emissions are dependent on the type of application. For instance, when the micro-organism is directly rubbed on the tree, emissions to the terrestrial compartment and the atmosphere can probably be excluded. When bulbs are treated in a bath containing micro-organisms, emissions to the air can be excluded as well.

⑧ Exposure of NTOs is primarily dependent on the pattern of biopesticide use, the dissipation patterns of the MOPA or its propagules, after application and the persistence of the MOPA. How long does a MOPA or its propagule remain intact and viable to grow and replicate in non-application areas, where it has been transported to by wind, water, soil particles or other vehicles (see also ⑦)? Because of their natural origin, it is not surprising that many micro-organisms or biochemicals can be degraded rapidly depending on the environmental conditions.

⑨ Data on growth and replication may indicate the persistence of a micro-organism or its dormant phase in (a) the agro-ecological area of application and (b) the nearby (agro-)ecological areas that may be reached by *e.g.* spores or otherwise. Proliferation of an “indigenous” MOPA should, after a short growth period, level off, and continue along the line of the background micro-organisms [15]. Relatively rapid multiplication of micro-organisms can lead to higher frequencies of mutations. Viruses can change rapidly in properties, especially virulence [15].

⑩ + ⑩A It is difficult to quantify the exposure. However, it is important to “connect” the application type and pattern with (a) the target organisms, and (b) the non-target organisms. If only leaves should be sprayed from below, it is important to know whether phylloplane non-targets as beneficial arthropods favour the upper- or underside of leaves. Their behaviour and preferences are therefore important to be known. If a natural “background” level of *e.g.* MOPA related microfungi or bacteria — already present pre-application — is not expected to be enlarged, risks may be considered “not deviating” from “normal”.

⑪ Apart from some widespread microbials as *Bacillus thuringiensis*, there is not so much known of the effects of MOPAs in a “new” environment (*i.e.* the environment of application). General knowledge, however, of the introduction of “new” (micro-) organisms on biodiversity or ecological functioning of these sites of application may be helpful.

⑫ Effects of MOPAs on NTOs can be competitive, allergenic (*e.g.* to small mammals due to high aerial concentrations of spores), toxic, infective and pathogenic. Toxicity may be caused by a range of different toxins, *e.g.* bacterial exotoxins, endotoxins or

mycotoxins. Information on the production and relevance of toxins may be deduced from (acute) toxicity studies, the mode of action and other microbiological properties, relationships with known plant, animal or human pathogens, and analytical methods [15;16]. It is recognised in [15] that colonisation, infectiveness, and toxicity comprise a complex set of interactions between micro-organisms and hosts and these endpoints may not be resolved easily as independent endpoints. Combining these endpoints, the most important aspects of a micro-organism to be assessed are :

1. *the ability to persist and multiply in a host (indicative of colonisation or infectivity);*
2. *the ability to produce (non-adverse or adverse) effects in a host (indicative of infectivity, pathogenicity and/or toxicity).*

An assessment of the infectivity and pathogenicity is considered necessary, even if the potential of exposure is deemed low [15]. The scheme in Fig. 4, however, does not indicate such an assessment if the exposure is nil or does not exceed a “background” level. Additional guidance in this respect may be necessary.

13 If in a laboratory test with *e.g.* rats, a biopesticide is shown to be infectious and/or pathogenic at a the maximum label rate (*i.e.* in accordance with GAP), the risk of affecting wild fauna is supposedly high. In such a case it seems reasonable that higher-tier (*i.e.* less conservative) tests should be performed (see also § 3.2.1). US EPA test protocols of such extended laboratory or field studies can be found in [8]. These tests are generally conducted with maximised amounts of micro-organisms (a maximum amount based on the maximum label rate times a safety factor). Therefore, these tests may not be useful for estimating infectivity and pathogenicity at more realistic scenarios, unless the results of such tests are negative. Then the results of less conservative tests will be negative as well. Infectivity and pathogenicity can be expressed in accordance with a dose-response concept. Then, a minimal infective dose (MID) and a median infective dose (ID_{50}) can be derived. It is dependent on the micro-organism whether the dose-response concept suffices. It should be noted that in case of an infective agent a single dose not necessarily causes an infection, whereas a multiple application may. Therefore, repeated applications can be required. The ID_{50} refers to the dosage or inoculum size required to infect 50% of the exposed organisms. Theoretically, an ID_1 refers to a low virulence and an ID_{99} to a high virulence. In this way, the pathogenicity is quantified in terms of virulence.

14 Risks can be assessed by the NOET/PET quotient (conform Knacker, cited in [17]). PET is the Predicted Environmental Titer, *i.e.* the amount or the number of micro-organisms in a compartment or organism. The NOET (no-observed-effect titer) is the highest amount of a micro-organism that does not cause any significant harmful effect. The NOET/PET concept assumes a dose-response relation between the number of micro-organisms and the observed adverse effects. An example of such a MOPA is *Bacillus thuringiensis* of which the mode of action is based on the protoxins of the parasporal crystal inclusion bodies. Upon ingestion by pest organisms, the protoxin is converted to four insecticidal toxins. Although this mode of action resembles the mode of action of synthetic pesticides (dose-effect relation), the elapse prior to the pest death can be much longer. Therefore, it may be important to take longer observation times into account. The evaluation of the potential risks should reveal whether these potential risks are acceptable or not. More technical and scientific aspects of assessing risks of microbial biopesticides in [16].

(15) Waivers may be granted. However, the acceptation of waivers by regulatory authorities depends on the quality and the usefulness of the registrant's rationale.

(16) Quantification of the (potential) risks by a toxicity exposure ratio (see (17)) seems only feasible respecting the toxicity of a MOPA, not due to its infectivity and/or pathogenicity. However, there may be indications that the infectivity and/or pathogenicity are concentration- or dose-related as well.

(17) The toxicity exposure ratio (TER) is compared with the α (conform the Uniform Principles for microbial pesticides [15]). The assessment factor α (5, 10 or 100) depends on the non-target group and whether the exposure is acute, short-term or chronic[10]. The NOET is the no-observed-effect titer (see also (14)).

1. terrestrial vertebrates, earthworms: $\frac{ET_{50}}{PET} < 10$ (acute exposure, high and unacceptable risk);
2. terrestrial vertebrates, earthworms: $\frac{NOET}{PET} < 5$ (chronic exposure, high and unacceptable risk);
3. fish, daphnids: $\frac{ET_{50}}{PET} < 100$ (acute exposure, high and unacceptable risk);
4. fish, daphnids: $\frac{NOET}{PET} < 10$ (chronic exposure, high and unacceptable risk);
5. algae: $\frac{ET_{50}}{PET} < 10$ (short-term exposure, high and unacceptable risk).

Definitions of unacceptable risks follow the 1st-tier evaluation. Risks should be considered unacceptable, unless the lack of effects can be demonstrated under field conditions (see also § 3.2.1). Although not mentioned in particular, "proper risk assessment" and "field condition" supposedly refer to the use of higher-tier tests (*e.g.* extended laboratory studies, mesocosms, semi-field studies). Limited guidance on higher-tier tests with microbial biopesticides is found in [8].

(18) The EC states that risks are unacceptable "unless it is clearly established through an appropriate risk assessment that under field conditions [NTO] populations are not at risk after the use of the plant protection product according to the proposed conditions of use" [15]. Criteria for some other unacceptable risks are not included in the decision trees, as they appeared in a more recent version of [16], too recent to include here. Therefore they are only briefly mentioned: (1) bees: no authorisation if hazard quotients (*i.e.* [application rate]/LD_{50}) for oral or contact exposure of honeybees exceed 50, (2) soil: no authorisation if the nitrogen or carbon mineralisation processes in laboratory studies are affected by more than 25% after 100 days.

(19) In case a proper first-tier evaluation as proposed in Fig. 4 is not possible, *e.g.* because essential data or information are lacking, it may be decided to assume an unacceptable risk, unless or until reliable and useful data are submitted.

[10] Acute toxicity involves harmful effects in an organism through a single exposure. Chronic toxicity involves harmful effects over an extended period, usually upon repeated or continuous exposure and sometimes lasting for the entire life of the exposed organism. Short-term toxicity solely refers to an exposure period of ≤ 4 days (no relation with life-cycle). Note that a 4-day toxicity test with algae is both a short-term and a chronic test referring to the test duration only, and to the various generation cycles of algae during the exposure period, respectively.

3.2.3 Semiochemical biopesticides

Semiochemicals are chemicals emitted by plants, animals and other organisms, evoking behavioural or physiological response in the individuals of the same or other species. An important group of semiochemicals are pheromones. They are (bio)chemicals, and the risk assessment criteria are the same as for other chemical pesticidal products [11]. All data should be submitted in accordance with EU Directive 91/414/EEC [10], and its subsequent amendments. Due to the availability of much technical scientific information, and the notion that semiochemicals can be effective at very low concentrations, there is consensus

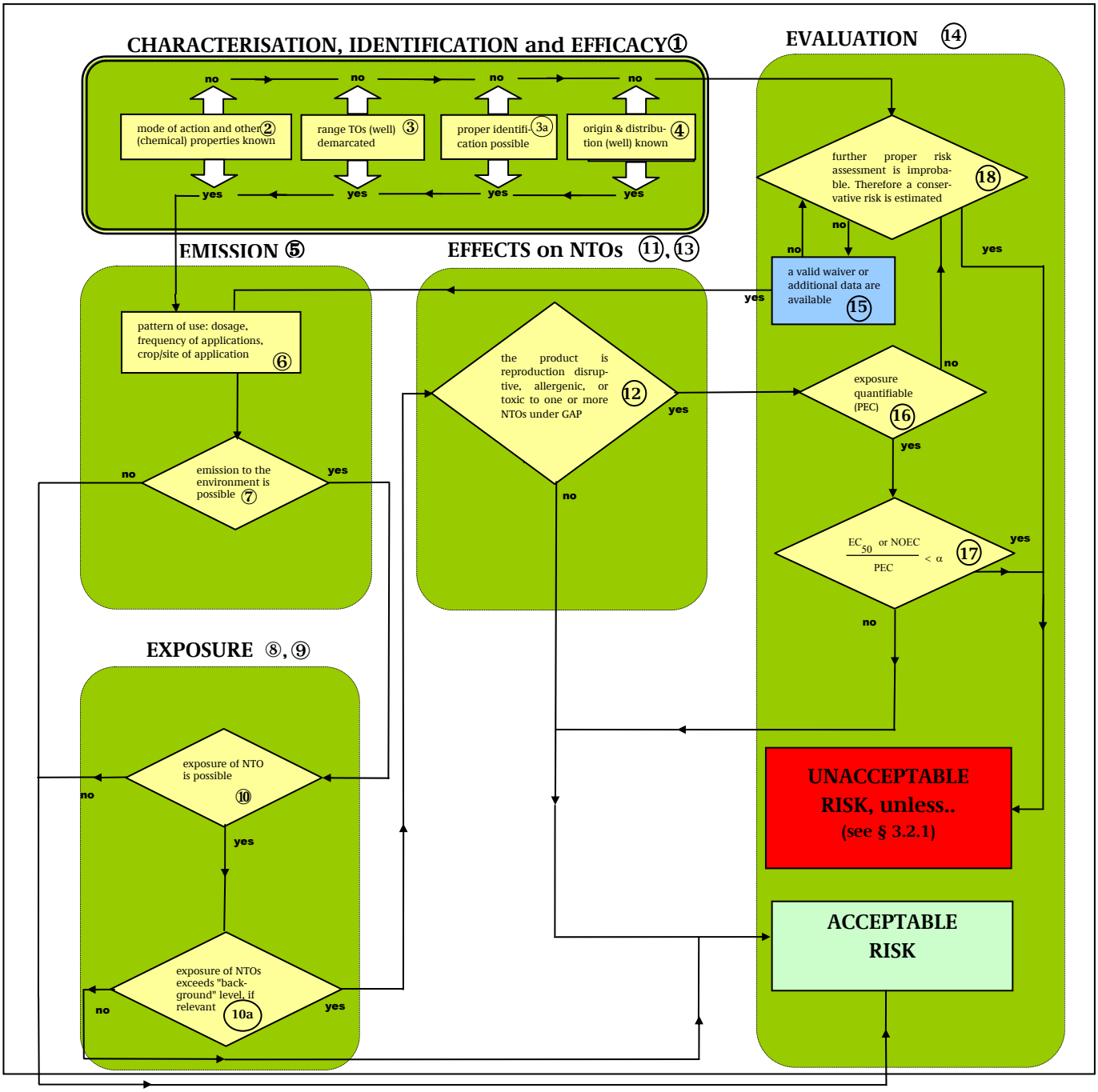


Figure 5. *Environmental risk decision tree for semiochemical biopesticides.*

TOs: target organisms; NTOs: non-target organisms; PEC: predicted environmental concentration; EC_{50}: median effective concentration; NOEC: no-observed-effect concentration. GAP: good agricultural practice. Note that the box *characterisation, identification and efficacy* makes a strong appeal to expert judgement, as strict yes or no answers are difficult to obtain. One should be flexible in this respect. For explanatory notes see text.

that common semiochemicals, as *e.g.* the straight-chained lepidopteran pheromones (SCLPs) are generally not hazardous to NTOs. SCLPs generally have a very narrow host range. In this way, waivers can be proposed that relieve industries of performing tests for regulatory purposes. Various semiochemical pesticides are listed in Appendix 3 (Part B).

Explanatory notes of Fig. 5:

(1) The box *characterisation*, *identification* and *efficacy* is the starting block of the risk decision tree for pheromones and other semiochemicals (double-lined ▭ in Fig. 5). Characterisation is an umbrella term for aspects as the mode of action, the (bio)chemical properties, the origin and its role under natural conditions. The starting block shows the issues of primary importance. They are:

1. *mode of action and (bio)chemical properties (are they known?);*
2. *the range of target organisms (what is known?);*
3. *the identification methods or analyses (how to recognise and quantify?);*
4. *the natural distribution and the role under natural conditions (what is known?).*

Note that the box *characterisation, identification and efficacy* makes a strong appeal to expert judgement, as strict yes or no answers are difficult to obtain. One should be flexible in this respect. In general, semiochemicals are effective at low dosages. As pheromones are generally species-specific the range of target organisms is easy to predict. The range of target organisms is expected to be much smaller than the range for microbial biopesticides and other biopesticides of natural origin. The more precise the range of target organisms is determined and demarcated, the less likely non-related non-target organisms need to be tested. It may be necessary to compare the (dis)similarity with pheromones of “indigenous” and related pheromone emitting species. It should be noted that the levels of pheromones in *e.g.* greenhouses may exceed “background” levels, dependent on the release via a dispenser or via spraying. In such cases, the risks cannot be excluded at first instance.

Together with sufficient data on the target range, a “picture” of the potential effects of semiochemicals can be “painted” that is scientifically valid and convincing for the regulatory authorities. If it is properly demonstrated that particular (micro-) environments and NTOs are not exposed, additional testing of these environments may not be necessary.

(2) If the mode of action and other (bio)chemical properties are not (exactly) known, it may be difficult to predict or confirm the potential effects on NTOs. It than relies heavily on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

(3) If the specificity of the semiochemical is known, it may be more easy to explain the type of effects or risks to *e.g.* birds, fish and bees. The more clear this specificity is confined to a particular set of organisms, the more likely NTO tests with other organisms may be waived. It relies on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

(3a) Proper identification is required. References to (agro-)scientific publications can suffice.

(4) The more is known of the geographical, ecological or physical (*e.g.* in the root or soil zone, on leaves) distribution of the organism from which the semiochemical was

derived, the more likely the (potential) effects due to its prospective use as a pesticide can be assesed. It relies on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

5 Emissions of semiochemicals to nearby (agro-)ecosystems. In view of their biological function — to facilitate and fine-tune reproduction cycles — pheromones are effective in low dosages. Therefore their emissions are much lower in comparison with microbial pesticides and P-DPs.

6 Semiochemicals may be applied in various ways. Patterns of use refer to the dosage, frequency and type of applications and crop or site of application. A common application method is via dispensers that can be hung in crops or (fruit) trees.

7 Emissions of semiochemicals will be generally low, although dependent on the type of application.

8 Exposure depends primarily on the pattern of use and the fate and behaviour of the semiochemical in the (nearby) (agro-) ecosystems. Non-dispenser applications of pheromones in greenhouses may not be affected by UV radiation, as glass prevents the passage of most UV radiation.

9 The persistence of semiochemicals is generally limited, conform their biological function (see also 5). For assessing the emission, the pattern of use must be known. If pheromones are sprayed, emission scenarios are essentially the same as for chemical pesticides. Therefore the same drift data can be used. See also 16.

10 + 10A It is difficult to quantify the exposure of semiochemicals. The persistence of pheromones is generally limited due to *e.g.* photo-oxidation in air and UV radiation. When applied in retrievable-sized dispensers, the use of a UV screen and an anti-oxidant may prevent decomposition in the dispenser. "Background" levels of pheromones probably depend on their specific role in the reproduction process of TO relatives in the field. If necessary, concentrations of semiochemicals in air can be calculated in accordance with Fig. 6. Degradation is then assumed to follow first-order kinetics, a common assumption for synthetic pesticide degradation in risk assessment.

$$\ln C_t = \ln C_{t=0} - [\frac{\ln 2}{DT_{50}} \times t]^*$$

C_t *is* concentration in air at t (mg/m^3);
$C_{t=0}$ *is* concentration in air at t is 0 (mg/m^3);
DT_{50} time in which half of the initial amount has dissipated or degraded (days);
t time in days (since application).

* $C_t = C_{t=0} \times e^{-\ln 2 / DT_{50}}$

Figure 6. *Concentrations of semiochemicals in air following natural degradation.*

11 There is not so much known on the possibility of dose-response relations of semiochemicals. It is probably more a switch knob on signalling, indicating "treshold" levels.

12 In general, pheromones are not expected to disrupt the reproduction of species other than the target species. This is due to the co-evolution of species and their features. If pheromones would attract other species as well, the reproduction and selection of the pheromone emitting species self could be at risk. As the gas-phase concentrations of pheromones are very low — *e.g.* insects have very sophisticated sensors needing very low amounts to track down the source — other organisms in the area of application are generally assumed not to be at risk. This would be particularly the case when the

pheromone under registration has a natural occurrence in the area of application. The situation might be different when a pheromone of an “allochthonous” species will be introduced or when pheromones will be used at concentrations much higher than “background” levels. Therefore, as beneficial arthropods may be used in integrated pest management in greenhouses, it is important to check for the potential risks of beneficial arthropods’ impairment due to use of pheromones. Not only the relatively high concentrations of the active substance may pose potential risks, the occurence of co-formulantia can be detrimental as well. The general expectation, however, seems justified that the environment is not at risk, unless perhaps the gas-phase concentrations become so high that breathing problems, inhalatory allergy or other effects can occur. In conclusion, risks due to the use of semiochemicals seem limited, due to their intrinsic nature and function. Only when relatively high amounts are emitted, there may be potentially ecotoxicological risks.

13 The effects of semiochemicals on NTOs probably depend on the aerial concentrations. There are no clear indications that NTOs are really jeopardised by the use of *e.g.* pheromones, other than the target species.

14 The evaluation is primarily dependent on the submitted data. In the Netherlands, these data are based on data requirements presented in a workshop of the OECD Biopesticide Steering Group in 1999 in Ottawa; it should be noted that this report has been revised and published in [19].

15 Waivers may be granted. However, the acceptation of waivers by regulatory authorities depends on the quality and the usefulness of the registrant's rationale and the available data.

16 When possible and considered necessary, toxicity exposure ratios may be calculated (see below).

17 The toxicity exposure ratio (TER) is compared with the α. The assessment factor α (5, 10 or 100) depends on the non-target group and whether the exposure is acute, short-term or chronic[11]. Note that the NOEC is the no-observed-effect concentration (*i.e.* the highest amount of a semiochemical not causing any significant adverse effect).

1. terrestrial vertebrates, earthworms: $\frac{EC_{50}}{PEC} < 10$ (acute exposure, high and unacceptable risk).
2. terrestrial vertebrates, earthworms: $\frac{NOEC}{PEC} < 5$ (chronic exposure, high and unacceptable risk).
3. fish, daphnids: $\frac{EC_{50}}{PEC} < 100$ (acute exposure, high and unacceptable risk).
4. fish, daphnids: $\frac{NOEC}{PEC} < 10$ (chronic exposure, high and unacceptable risk).
5. algae: $\frac{EC_{50}}{PEC} < 10$ (short-term exposure, high and unacceptable risk).

18 In case a proper first-tier evaluation as proposed in Fig. 5 is not possible, *e.g.* because essential data or information are lacking, it may be decided to assume a high risk, unless reliable and useful data are submitted.

[11] See footnote 10.

3.2.4 Plant-derived biopesticides

As the biopesticides of Fig. 7 are isolated, extracted or otherwise derived from plants, and as their chemical constitution is generally known (though less well-identifiable when mixtures appear with variable contents of chemicals) the risk assessment should be as for a synthetic pesticide. Therefore, all data should be submitted in accordance with [1], [10] and their subsequent follow-ups and amendments. So formally, the data to be submitted for *e.g.* azadirachtin are the same as the data to be submitted for, for instance, parathion. Plant extracts can be detrimental to fauna, possibly due to the function of some secondary plant metabolites to deter herbivores. Various existing plant-derived biopesticides (P-DPs) are listed in Appendix 3 (56-59).

Explanatory notes of Fig. 7 (see next page):

(1) The box *characterisation*, *identification* and *efficacy* is the starting block of the risk decision tree for P-DPs (double-lined ▭ in Fig. 7). Characterisation is an umbrella term for aspects as the the mode of action, the (bio)chemical properties, the origin and role under natural conditions. The starting block shows the issues of primary importance. They are:

1. *mode of action and (bio)chemical properties (are they known?);*
2. *the range of target organisms (what is known?);*
3. *the identification methods or analyses (how to recognise and quantify?);*
4. *the natural distribution and the role under natural conditions (what is known?).*

Note that the box *characterisation, identification and efficacy* makes a strong appeal to expert judgement, as strict yes or no answers are difficult to obtain. One should be flexible in this respect. In general, P-DPs may function as any other synthetic chemical pesticide. Therefore, the range of target organisms may be difficult to predict on the base of the characterisation, identification and efficacy. The range of target organisms may be much wider than for MOPAs or semiochemicals. Although contents of extracts or mixtures may differ, *e.g.* due to production or formulation techniques, the composition should be known as exact as possible. If submitted data on batches do not satifactorily reveal the composition and its ranges, it can be difficult to use scientific public literature (as one is never sure whether the products are the same). It than relies heavily on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

(2) If the mode of action and the (bio)chemical properties are not (exactly) known, it may be difficult to predict or confirm the potential effects on NTOs. It than relies heavily on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

(3a) Proper identification is required. References to (agro-)scientific publications can suffice.

(4) The more is known of the geographical or ecological distribution of the source plant, the more likely the (potential) effects due to its prospective use as a pesticide can be assessed. It relies on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

(5) P-DPs are generally applied in the same way as synthetic pesticides. Emission may therefore be assessed with the same tools as synthetic pesticides.

⑥ The pattern of use — dosage, frequency, crop or application site, type of application — may vary dependent on crop and its pests, and the specific use of a P-DP within a crop cycle.

⑦ Emissions depend on various factors. Primarily it depends on the type and site of application. If P-DPs are sprayed, emissions depend on the same conditions as for spraying synthetic pesticides.

⑧ Exposure depends primarily on the pattern of use, the location, the persistence and the fate and behaviour of the P-DP active ingredient(s). Generally, P-DPs will show a slight persistence. However, co-formulantia may be added to enhance the persistence,

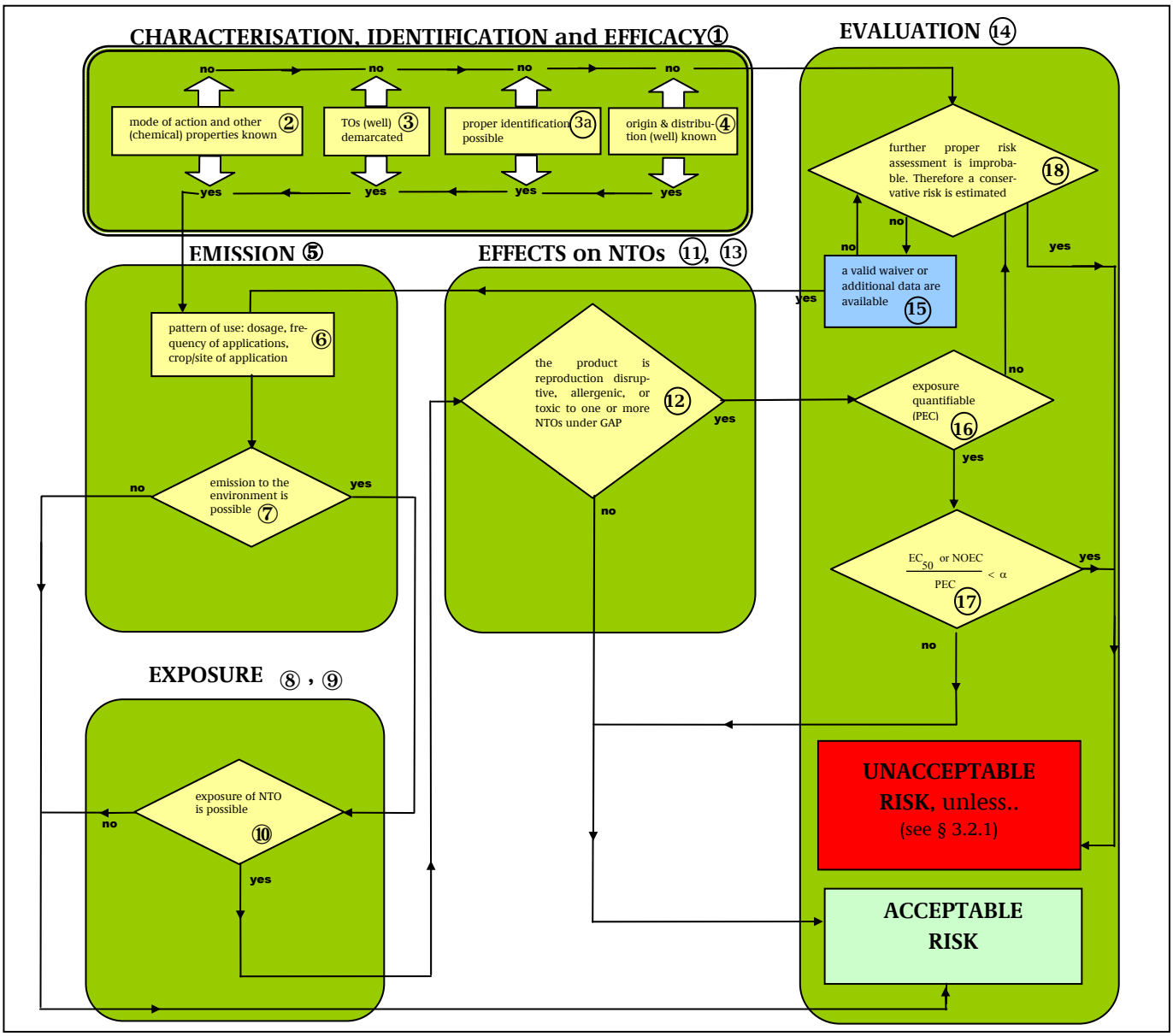


Figure 7. *Environmental risk decision tree for plant derived biopesticides, including extracts.*

TOs: target organisms; NTOs: non-target organisms; PEC: predicted environmental concentration; EC_{50}: median effective concentration; NOEC: no-observed-effect concentration. GAP: good agricultural practice. Note that the box *characterisation, identification and efficacy* makes a strong appeal to expert judgement, as strict yes or no answers are difficult to obtain. One should be flexible in this respect. For explanatory notes see text.

thus enhancing the efficacy. If it is properly demonstrated that particular (micro-) environments and NTOs are not exposed, additional testing of these may not be necessary.

9 Environmental factors influencing persistence of P-DP ingredients are vital for understanding the exposure of nearby (agro-)ecosystems.

10 "Background" levels of the P-DP under registration are not likely. Exceptions may be P-DPs with local plant biochemicals that theoretically may enter the terrestrial environment via fallen leaves. When it is possible to determine the DT_{50}, than the amounts can be determined at any time in a greenhouse or field crop with the formula in Fig. 8. Then first-order decay of the a.i. can be assumed, which is a common assumption for synthetic pesticide degradation in risk assessment. Some plant products such as ascorbic acid (vitamin C) however may occur in the soil. When a formulation with ascorbic acid is sprayed on plants, a certain proportion may reach the soil. Information on the natural concentration of ascorbic acid in the soil might be retrieved from the public scientific literature.

$$\ln C_t = \ln C_{t=0} - [\frac{\ln 2}{DT_{50}} \times t]^*$$

C_t *is* concentration in soil at t (mg/kg);
$C_{t=0}$ *is* concentration in soil at t is 0 (mg/kg);
DT_{50} time in which half of the initial amount has dissipated or degraded (days);
t is time (days since application).

* $C_t = C_{t=0} \times e^{-\ln 2 / DT_{50}}$

Figure 8. Concentrations of biochemicals in soil following natural degradation

11 Unlike the micro-organisms, the P-DPs are not potentially pathogenic or infectious. Potential effects on non-target organisms are *e.g.* allergenic, toxic or sublethal effects such as reproduction disruption. The ecotoxicity of P-DPs is probably very variable. Whereas *e.g.* the insecticide azadirachtin can be toxic to aquatic wildlife, the application of garlic is not expected to be hazardous to the aquatic wildlife under particular conditions (apart perhaps of being repellant because of the odour).

12 As many beneficial insects or other arthropods may be used in integrated pest management systems in *e.g.* greenhouses, it is important to check for the potential risks of beneficial arthropods' impairment due to use of P-DPs. Not only the relatively high concentrations of the active ingredient may pose risks, the occurrence of co-formulantia may be detrimental as well.

13 Effects on NTOs may be difficult to assess, when a P-DP can consist of various ingredients, and the specific (group of) a.i. ('s) is not known. In such cases, the various components may even follow different routes of fate and behaviour.

14 A mixture of plant-derived chemicals may be difficult to evaluate due to the presence of different biochemicals, showing individual variety in persistence, fate and behaviour. The evaluation is primarily dependent on the submitted data. The data requirements for a specific group of P-DPs, *i.e.* the plant extracts, are under discussion in the EU [1].

15 Waivers may be granted. However, the acceptation of waivers by regulatory authorities depends on the quality and the usefulness of the registrant's rationale. If it is properly demonstrated that particular (micro-) environments and NTOs are not exposed, additional testing of these may not be necessary. The more clear the

specificity is confined to a particular set of organisms, the more likely NTO tests with other organisms may be waived. It relies on expert judgement whether the risk assessor judges the submitted information as sufficient for a proper evaluation.

16 When possible and considered necessary, toxicity exposure ratios (TERs) may be calculated (EC_{50}/PEC, NOEC/PEC). See also below.

17 The toxicity TER is compared with the α. The assessment factor α (5, 10 or 100) depends on the non-target group and whether the exposure is acute, short-term or chronic[12]. Note that the NOEC is the no-observed-effect concentration (*i.e.* the highest amount of a plant-derived biopesticide (or the major active substance in a mixture) not causing any significant adverse effect).

1. terrestrial vertebrates, earthworms: $\frac{EC_{50}}{PEC} < 10$ (acute exposure, high and unacceptable risk).
2. terrestrial vertebrates, earthworms: $\frac{NOEC}{PEC} < 5$ (chronic exposure, high and unacceptable risk).
3. fish, daphnids: $\frac{EC_{50}}{PEC} < 100$ (acute exposure, high and unacceptable risk).
4. fish, daphnids: $\frac{NOEC}{PEC} < 10$ (chronic exposure, high and unacceptable risk).
6. algae: $\frac{EC_{50}}{PEC} < 10$ (short-term exposure, high and unacceptable risk).

18 In case a proper first-tier evaluation as proposed in Fig. 7 is not possible, *e.g.* because essential data or information are lacking, it may be decided to assume an unacceptable risk, unless reliable and useful data are submitted, indicating a proper environmental safety.

[12] See footnote 10.

4. Conclusions, discussion and recommendations

4.1. Conclusions

The environmental safety of crop protection products of natural origin (biopesticides) is to be re-evaluated within a few years' time by the EU. They are used for both chemical and biological pest control (in the Netherlands in glasshouse horticulture in particular), and comprise circa 290 existing products with circa 130 active ingredients. Also, new biopesticides are expected to enter the market in view of EU policies towards a more sustainable agriculture. There is, however, almost no guidance to evaluate the environmental safety of these products. Therefore, in this desk study for risk assessors and scientists, guidance is presented to improve the evaluation of the environmental safety of biopesticides. The study has been based on scientific literature and various documents of the EU. Biopesticides are allegedly less toxic and persistent than synthetic chemicals. However, this should not exclude them from a proper environmental safety evaluation, since microbial and plant-derived biopesticides can be infective, pathogenic or toxic. This depends on their microbiological or (bio)chemical properties, the dosage and the site and type of application. The study proposes risk decision trees for three groups of products, *i.e.* microbial organisms, signalling chemicals (including pheromones) and plant-derived substances. These are the biopesticides with the highest use potentials. The trees help to discern acceptable from unacceptable potential environmental risks. Also, *summary tables* have been developed to record and evaluate environmental tests with microbial pesticides. In this way the reliability and usefulness for the safety evaluation can be assessed per test. Also, these tables can be used as a checklist for risk assessors and for those reviewing their reports for QA purposes. The decision trees and the *summary tables* are intended to improve the safety evaluation for regulatory purposes. They show that it is of primary importance to find out the mode of action, other microbial or (bio)chemical characteristics, the range of hosts or target organisms, the identity of the micro-organism(s) or the active substance(s), the origin, the role under natural conditions, if relevant. Screen and efficacy tests can be useful in this respect. Subsequently, the scientific literature and/or the results of special environmental tests should be used to assess the safety of biopesticides to the environment. In this way, the safety evaluation will promote the safe use of biopesticides.

4.2. Discussion and recommendations

Two tools have been presented in this report to improve the environmental safety evaluation: *risk decision trees* for three groups of biopesticides and *summary tables* for microbial biopesticides. The main advantage of these tools is that they promote harmonisation by offering a uniform framework. Also, the risk decision trees offer a platform for further discussions on the safety evaluation of biopesticides, by including the latest insights. As knowledge and regulatory insight advances and as the industrial, scientific and regulatory activities on biopesticides increase, guidance should be regularly updated or adjusted. There will be an important role for sciences as microbial ecology, microbiology and entomology, different from the more traditional role of such sciences in (synthetic) pesticide registration. The *risk decision trees* should be helpful to discern the acceptable from the unacceptable risks. They are, however, proposals, as it is up to the EU member states and/or the European Commission to decide what is acceptable and what not. Specific safety criteria have been phrased by the EC for microbial biopesticides only [15].

Various elements are not yet clear or included in the trees. The trees are primarily qualitative. Only the potential impact via toxins or metabolites can be quantified to some

extent by calculating toxicity exposure ratios. Another issue is how to deal with waivers: statements of industries to exempt them from submitting particular data or tests. When emphasising serious waiver possibilities, extra care by the registrants should be taken to really discuss whether these waivers are valid for risk assessment[13]. If the regulatory authorities do not agree with waivers, they should explain why additional data are needed (preferably when other outcomes of the safety evaluation can be expected, so no data just for the data). The trees still require expert judgement and a case by case approach. This seems particularly due to the variation in structure and function of biopesticides. This variation requires a flexible approach of the safety evaluation. In the Netherlands, this approach requires new co-operation networks. For instance, when a fungal biopesticide has to be assessed for an EU registration (on Annex I of 91/414/EEC), acknowledged mycologists and microbiologists will be invited to join the RIVM team of environmental risk assessors.

Safety criteria are available in case of a micro-organism [15] or a chemical [11]. These safety criteria refer to the acceptability of risks in view of toxicity exposure ratios (metabolites, toxins, chemical active ingredients). Safety criteria for particular microbiological effects are less clear. Particular scientific concepts and criteria in the safety evaluation should therefore be developed: *e.g.* what to do with data on "background" levels of biopesticides, if relevant. The ecological role and relations of micro-organisms are generally only partially known. How should one deal therefore, with the introduction of a microbial pesticide without knowing the micro-ecological role of the pesticidal micro-organism? What is there to say about micro-ecological biodiversity in relation to the use of microbial biopesticides? What do we know and expect *e.g.* of the introduction of "exotic" bacteria or fungi in "autochthonous" agro-ecosystems respecting the local microflora and fauna? For various biopesticides such questions will probably be difficult to answer and the urgence of such questions will have to be set off against the costs to fund research to find these answers. It is not yet clear what to do if the first-tier triggers (on pathogenicity or toxicity exposure ratios) are exceeded. What to do if a small mammal is infected in a first-tier test? As first-tier safety criteria generally refer to realistic worst case scenarios, higher-tier evaluations should refer to more average scenarios.

It will be interesting to what extent safety evaluation strategies for biopesticides will be needed in the next decade, as the market for biopesticides does not grow substantially and is expected to be small compared with the synthetic pesticides. Biopesticides represent only a small part of the (mondial) pesticide market (ca. 1%, primarily *Bacillus thuringiensis* strains) [21]. However, mondially, integrated agriculture (generally without synthetic pesticides) is expected to increase from $1 billion (5%)to $ 8 billion (40%) within 25 years [21]. This expansion is likely to give a boost in research and development of biopesticides. In the Netherlands, a pilot project has been launched to support the registration of biopesticides as sulphur, chitosan, etheric oils and algae extracts, thus identifying the bottlenecks for biopesticide registration [4;7]. The environmental safety evaluation for these biopesticides was not the limiting factor. The development of safety evaluation strategies for biopesticides in the field may be hampered by the simple fact that biopesticides generally do not offer the rapid efficacy as many farmers and horticulturists have been used to when applying synthetic pesticides [18].

The safety evaluations of countries, other the EU, should be taken into account as much as possible, although EU member states are often reluctant to do so. However, in view of test animal-, labour- and fund-saving, it is recommended to weigh the pros and cons of other

[13] Preferably, all waivers should be backed up by documents (articles, reports, data sheets, personal communications).

safety evaluations and to use them when possible[14]. The Biopesticide Steering Group of the OECD is an important platform to streamline these discussions. Also the prospective EU activities on the 4th stage review programme can be helpful in this respect.

4.3. Recommendations in a nutshell

1. *to make* summary tables *for the biopesticides other than microbial biopesticides as well;*
2. *scientific issues:*
   - 2.1. *how to deal with: dose-effect related infectivities, impact of the introduction of an "exotic" strain of a micro-organism on local micro-flora and fauna, how to define microbial "background" levels as references for "normal" conditions;*
   - 2.2. *to investigate to what extent the generally supposed lesser efficacy of biopesticides is an ecological advantage, as a lower efficacy may indicate a lesser persistence in the area of application and also outside;*
   - 2.3. *to fill other data gaps (to start with literature research) and to continue exchanging experience in the safety evaluations of biopesticides with other EU and OECD countries;*
3. *to draw up documents with (scientifically or technically) valid reasons not to perform particular tests. In such document general statements on scientific and technical issues (see (2) should be made;*
4. *(based on the preceding) to adjust and improve the risk decision trees where necessary.*

[14] The Uniform Principles for microbial pesticides [15] state that "where, relevant, other authorised uses of plant protection products can be taken into consideration in the area of envisaged use, *e.g.* containing the same active ingredient or which gives rise to the same residues".

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APPENDIX 1 SUMMARY TABLE FOR TESTS ON THE FATE AND BEHAVIOUR OF MICROBIAL BIOPESTICIDES

Introduction
Submitted or available data should be "acceptable in terms of quantity, quality, consistency and reliability and sufficient to permit a proper evaluation of the dossier"[6]. In the Netherlands, these requirements are phrased in terms of the reliability and usefulness of submitted data. Table 3 is helpful in determining this reliability and usefulness.

How to use Table 3?
The information in Table 3 is presented over three columns: **item**, **notes** and **reliability lower? Items** represent the keywords: essential aspects of scientific tests for regulatory purposes. **Notes** represent additional though essential explanatory information. Each note starts with a question in bold. If such questions can be answered with **no**, than the intrinsic scientific reliability respecting any particular **item** is assumed to be sufficient. However, if one or more of these questions are answered with **yes** (in the table: **Y**), the scientific reliability may be jeopardised. **Y** in the table column indicates that if the requirement is not fulfilled, the reliability respecting this particular item is lower. **E** in the table column indicates that it is expert judgement to decide what to do, if the requirements are not met. It is up to the evaluator of the test when it is to be decided whether a test as a whole is unreliable.

In summary, Table 3 gives guidance and serves as checklist. Expert judgement for the test as a whole may surpass conclusions following the particular guidance in the table. Unreliable tests are not used for risk assessment. This is conform the Dutch pesticide evaluation procedure. Besides the unreliable test, a less reliable and a reliable test can be distinguished. The latter two may both be used for risk assessment (the less reliable data possibly useful as "circumstantial evidence"). However, reliable tests may be preferred above less reliable tests, dependent on the availability and quality of the data.

Take notice: *e.g.* items 7.1 (rate) and 7.2 (type) fall under item 7 (application).

Table 3: Key items for the data evaluation of fate and behaviour of MOPAs

	ITEMS		NOTES	RELIABILITY LOWER ?
METHODOLOGY & TEST DESCRIPTION	1	test type	**improperly reported or characterised?** [laboratory or field experiment? sampling under natural conditions without description of the test situation?]	Y
	2	active ingredient, (im)purity	**improper characterisation of the active ingredient?** [common name? scientific name — down to strain or serotype? mutant? taxonomy, identification (classification based on biochemical or molecular studies), origin? cosmopolitan? microbiological purity? nature and identity of impurities — *e.g.* mutated a.i.'s]	Y
	3	formulation	**(partly) unknown composition?** [name? type? composition — *e.g.* number of CFU's, quantities and function of non-active ingredients, *e.g.* wetting agents? components that act as endocrine disrupter?]	E
	4	mode of action	**improperly reported?** [hyperparasite? action through metabolites? reproduction? time needed to kill? host switching by mutation?]	E
	5	biological properties	**improperly reported?** [description of growth form, resting phase, life cycle, type of propagation (*i.e.* spores, mycelial fragments), possible nutrient substrates, optimum pH, temperature, relative humidity, host specificity, saprophytic proliferation possible?, natural "background" concentration, germination conditions?]	E
	6	environmental compartment	**improperly reported?** [*e.g.* water, soil, air? natural/artificial? sterile? temperature? light conditions? volume/weight?]	E
	6.1	water	**transport/sorption/shelter by water/sediment improperly reported?** [*e.g.* pH? sediment type? redox potential/availability of O_2? floating or settling at the bottem?]	E
	6.2	soil	**transport/sorption/shelter by soil improperly reported?** [*e.g.* soil type? pH? % o.m.? natural pathogen (possible quantities, composition)? redox potential/availability of O_2? moisture conditions? occurrence of MOPA in cracks and crevices of stones or bark]	E
	6.3	air	**transport by air?**	E
	6.4	biotic	**requirement of vertebrate, invertebrate host?** [*e.g.* way of transmission via vectors?] **requirement of leaf surface?** [restricted to leaf surface, host, colonisation rate, growth rate and survival in absence of host, rhizosphere competence]	E
	7	application	**improperly reported?**	Y
	7.1	rate	[*e.g.* L product/ha; start of application and interval between applications; applications inthe fiield or in the greenhouse; specific conditions required, *e.g.* relative humidity]	Y
	7.2	type	[*e.g.* homogeneously mixed with the medium/substrate? application *e.g.* as a fluid inoculum, or as incapsulated spores?]	Y
	8	analysis	**invalid? inadequate?** [*e.g.* extent of validation? "limit of detection"? proper bioassay?]	Y

Contd Table 3

	ITEMS		NOTES	RELIABILITY LOWER ?
RESULTS	**9**	**endpoint(s)**	**improperly reported? results non-verifiable?** [*e.g.* raw data available for verification?]	Y
	10	**statistical analysis**	**invalid?** [all tests with MOPAs require accurate statistical analysis for proving significant differences between the control and the treatment groups]	E
	11	**test conditions**	**improperly reported?** [are certain ranges of abiotic/biotic parameters exceeded during incubation?]	E
REMARKS	**12**	microbiological properties: *e.g.* dispersal mechanism? natural occurrence?		E
	13.1	type of propagation: *e.g.* spores, mycelial fragments?		E
	13.2	type of optimal culture media for propagation or growth: *e.g.* temperature? moisture conditions? pH? % o.m?		E
	14	pretreatment instability of the a.i. or product (respecting. light, temperature, "shelf"-storage, and packaging)? Instability during incubation?		E
	15	plating media (bioassay): *e.g.* type of medium? pretreatment — *e.g.* pasteurisation? these may influence the number of CFUs		E
	16	test performed according GLP?		E

Sub item 2.
Cosmopolitan: the a.i. of a product may have been originally isolated in another country of the EU or in another part of the world. Although a fungus, bacterium or virus may occur all over the world, this may not be confirmed in the literature.

Sub item 4.
Host switching by mutation: this has been proved once for a baculovirus. The BmNPV which has *Bombix mori* as a host switched to *Autographa californica* due to a mutation. This experiment was performed under high selection presssure which cannot be expected under natural conditions [16].

Sub 5.
If an a.i. is endemic and is applied at high rates in the field (*e.g. Cydia pomonella)*, the number of CFU may still be lower than the natural rates which may occur during outbreaks of the viruses. A natural outbreak only occurs when the host is present at very high density and is thus a regulatory factor in the population dynamics of the host. In the control treatments the virus usually builds up to equal densities later in the season. Then, the effect of the virus application is no longer visible. In general: an application of a virus does not bring about an additional risk relative to the natural background of the virus. Saprophytic proliferation by fungi that normally grow on living insects is not likely to be an important route.

Sub 6.2.
In some cultures such as cucumbers, plants are mostly grown on artificial soil, such as rockwool. In these cases data on fate and behaviour in soil may not be necessary. When soil is used, this may be steamed before cultivation. This must be checked, as this information is not always included in the dossier. Viruses such as baculoviruses are extremely persistent (they may persist as long as 40 years in sheltered places (*e.g.* in crack of tree trunks).

APPENDIX 2 SUMMARY TABLE FOR TESTS ON THE EFFECTS OF MICROBIAL BIOPESTICIDES ON NON-TARGET ORGANISMS

Introduction
Submitted or available data should be "acceptable in terms of quantity, quality, consistency and reliability and sufficient to permit a proper evaluation of the dossier" [6]. In the Netherlands, these requirements are phrased in terms of the reliability and usefulness of submitted data. Table 4 is helpful in determining this reliability and usefulness.

How to use Table 4?
The information in Table 4 is presented over three columns: **item**, **notes** and **reliability lower? Items** represent the keywords: essential aspects of scientific tests for regulatory purposes. **Notes** represent additional though essential explanatory information. Each note starts with a question in bold. If such questions can be answered with **no**, than the intrinsic scientific reliability respecting any particular **item** is assumed to be sufficient. However, if one or more of these questions are answered with **yes** (in the table: **Y**), the scientific reliability may be jeopardised. **Y** in the table column indicates that if the requirement is not fulfilled, the reliability respecting this particular item is lower. **E** in the table column indicates that it is expert judgement to decide what to do, if the requirements are not met. It is up to the evaluator of the test when it is to be decided whether a test as a whole is unreliable.

In summary, Table 4 gives guidance and serves as checklist. Expert judgement for the test as a whole may surpass conclusions following the particular guidance in the table. Unreliable tests are not used for risk assessment. This is conform the Dutch pesticide evaluation procedure. Besides the unreliable test, a less reliable and a reliable test can be distinguished. The latter two may both be used for risk assessment (the less reliable data possibly useful as "circumstantial evidence"). However, reliable tests may be preferred above less reliable tests, dependent on the availability and quality of the data.

Take notice: *e.g.* items 4.1 (rate) and 4.2 (type) fall under item 4 (application).

Table 4: Key items for the data evaluation of laboratory test on the effects of MOPAs to non-target organisms

	ITEMS		NOTES	RELIABILITY LOWER ?
METHODOLOGY & TEST DESCRIPTION	1	test type	**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*?]	Y
	2	active ingredient, (im)purity	**improper characterisation of the active ingredient? impure?** [which, virus? common name? scientific name — down to strain or serotype? mutant? microbiological purity? nature and identity of impurities — *e.g.* mutated a.i.'s, extraneous micro-organisms?]	E
	3	formulation	**(partly) unknown composition?** [name? type? composition — *e.g.* quantities and function of non-active ingredients, *e.g.* wetting agents?]	Y
	4	application	**improperly reported?**	Y
	4.1	rate	**not reported?**	Y
	4.2	type	[*e.g.* homogeneously mixed with the medium/substrate? application *e.g.* as a fluid inoculum, or as encapsulated spores?]	Y
	5	endpoint(s)	**improperly defined?**	Y
	5.1	virus	[*e.g.* incorporation of viral DNA into the chromosomes of NTOs? induction of other viruses — viral interference?]	E
	5.2	other	[infectivity/pathogenicity: *e.g.* cytopathic effects, or visible replication of a MOPA? toxicity? allergenic effects — *e.g.* in mammalian vertebrates? mutagenicity/carcinogenicity/teratogenicity — *e.g.* in mammalian vertebrates?]	E
	6	control	**inadequate control treatment?** [*e.g.* no autoclavation or UV inactivation?]	E
	7	analysis	**invalid? inadequate?** [*e.g.* extent of validation? "limit of detection"? proper bioassay?]	Y
RESULTS	8	endpoint(s)	**improperly reported?** results non-verifiable? [*e.g.* raw data available for verification?	Y
	9	statistical analysis	**invalid?** [all tests with MOPAs require accurate statistical analysis for proving significant differences between the control and the treatment groups]	E
	10	test conditions	**improperly reported?** [are certain ranges of abiotic/biotic parameters exceeded during incubation?]	E

Contd Table 4

	ITEMS	NOTES	RELIABILITY LOWER ?
REMARKS	**11**	the biological meaning of statistically valid differences: *e.g.* does a treatment- or dose-effect relation exist?	E
	12	microbiological properties: *e.g.* dispersal mechanism? occurrence of toxins? natural occurrence?	E
	13	type of propagation: *e.g.* spores, mycelial fragments?	E
	14	type of optimal culture media for propagation or growth: *e.g.* temperature? moisture conditions? pH? % o.m?	E
	15	pretreatment instability of the a.i.. or product (respecting. light, temperature, "shelf"-storage, and packaging)? instability during incubation?	E
	16	plating media (bioassay): *e.g.* type of medium? pretreatment, *e.g.* pasteurisation? these may influence the number of CFUs	E
	17	test performed according GLP?	E

APPENDIX 3 EXISTING BIOPESTICIDES OF THE 4th STAGE LIST

PART A:

Active substances for which the use is authorised in human foodstuffs or animal feeding stuffs in accordance with EU-legislation (Lead Rapporteur: Ireland):

Active substance		Notifier
Acetic acid		PAB-SE-001
		PUN-DK-001
		TEM-DE-001
Amino acids / Gamma Aminobutyric acid		AGR-ES-001
Amino acids / L-Glutamic acid		AGR-ES-002
Amino acids / L-Tryptophan		VAL-IT-012
Ammonium carbonate		ABC-GB-005
Ethoxyquin		XED-FR-003
Fatty acids / Decanoic acid		PBI-GB-005
Fatty acids / Fatty acid methyl ester (CAS 85566-26-3)		OLE-BE-001
Fatty acids / Fatty acid potassium salt		FBL-DE-003
		IAB-ES-003
		NEU-DE-003
Fatty acids / Fatty acid potassium salt (CAS 7740-09-7)		DKI-NL-002
Fatty acids / Fatty acid potassium salt (CAS 10124-65-9)		ERO-IT-199
Fatty acids / Fatty acid potassium salt (CAS 13429-27-1, 2624-31-9, 593-29-3, 143-18-0, 3414-89-9, 38660-45-6, 18080-76-7)		DXN-DK-001
Fatty acids / Fatty acid potassium salt (CAS 18175-44-5, 143-18-0, 3414-89-9)		DXN-DK-002
Fatty acids / Fatty acid potassium salt (CAS 61788-65-6)		TBE-ES-001
Fatty acids / Fatty acid potassium salt (CAS 61790-44-1)		VAL-IT-008
Fatty acids / Fatty acid potassium salt (CAS 61790-44-1, 70969-43-6)		STG-GB-002
Fatty acids / Fatty acid potassium salt (CAS 67701-09-1)		CRU-IT-004
Fatty acids / Heptanoic acid		DKI-NL-007
Fatty acids / Octanoic acid		PBI-GB-006
Fatty acids / Oleic acid		ALF-ES-014
Fatty acids / Pelargonic acid		ERO-IT-200
		NEU-DE-001
Fatty acids / potassium salt - decanoic acid (CAS 334-48-5)		NSC-GB-003
Fatty acids / potassium salt - caprylic acid (CAS 124-07-2)		ADC-DE-003
Fatty acids / potassium salt - lauric acid (CAS 143-07-7)		NSC-GB-004
Fatty acids / potassium salt - oleic acid (CAS 112-80-1)		NSC-GB-001
Fatty acids / potassium salt - oleic acid (CAS 112-80-1, 1310-58-3)		BCS-DE-002
Fatty acids / potassium salt - oleic acid (CAS 142-18-0)		SBS-IT-004
Fatty acids / potassium salt - oleic acid (CAS 143-18-0)		VIO-GR-003
		STG-GB-001
Fatty acids / potassium salt - pelargonic acid(CAS 112-05-0)		NSC-GB-002
Fatty acids / potassium salt - tall oil fatty acid (CAS 61790-12-3)		ADC-DE-002
Fatty acids / tall oil fatty acids (CAS 61790-12-3)		ACP-FR-002

Formic acid		KIR-NL-853
Maltodextrin		BCP-GB-001
Potassium hydrogen carbonate		PPP-FR-002
Sodium hydrogen carbonate		CLM-NL-002
		SLY-FR-111
Sodium metabisulphite		ESS-IT-001
		FRB-BE-100
Urea (see also Attractant/Rep.)		FOC-GB-002
Wheat gluten		OMX-GB-003 ESA-NL-001

casein (CZ)
milk albumen(CZ)

Active substances which are derived or extracted from plant (Lead Rapporteurs: France and UK):

Active substance	Notifier
Azadirachtin	AGI-IT-001
	ALF-ES-015
	CAP-FR-002
	CRU-IT-002
	FBL-DE-001
	IAB-ES-002
	MAS-BE-001
	NDC-SE-001
	PBC-ES-002
	PRO-ES-414
	SIP-IT-001
	TRF-DE-001
	VAL-IT-002
cis-Zeatin	VAL-IT-005
Citronellol (see also Attractant/Rep)	ACP-FR-003
Citrus extract Notified as Bactericide	ALF-ES-011
Citrus extract / Grapefruit seed extract Notified as Disinfectant	BOB-DK-002
Folic acid	AMI-IT-002
	CHE-DK-001
	ISA-IT-014
Garlic extract Notified as Repellant	ALF-ES-016
	CRU-IT-005
	ECY-GB-001
	IAB-ES-001
	PBC-ES-004
	SBS-IT-003
	SIP-IT-002
	TRD-FR-001
	VAL-IT-011

Gibberellic acid	AIF-IT-002
	ALF-ES-008
	ALT-FR-182
	CEQ-ES-001
	FIN-GB-005
	GLO-BE-003
	HRM-BE-009
	NLI-AT-002
	PRO-ES-415
	SUM-FR-003
	VAL-IT-004
Gibberellin	ALF-ES-007
	FIN-GB-006
	GLO-BE-004
	GOB-IT-005
	HRM-BE-008
	NLI-AT-003
	SUM-FR-002
Indolylacetic acid	ALF-ES-006
	GOB-IT-004
	RHZ-NL-001
Indolylbutyric acid	ALF-ES-002
	BCS-FR-003
	CRT-GB-001
	GOB-IT-003
	GTL-GB-001
	HOC-GB-002
	RHZ-NL-002
Lecithin	DUS-DE-001
	FBL-DE-004
	PBC-ES-006
Marigold extract	ALF-ES-010
Mimosa Tenuiflora extract	ALF-ES-012
Nicotine	JAH-GB-001
	PBC-ES-001
	UPL-GB-001
Pepper Notified as Repellant	BOO-GB-002
	PBI-GB-001
Plant oils / Black currant bud oil Notified as Repellant	IAS-SE-005
Plant oils / Citronella oil	BAR-GB-001
	PBI-GB-002
Plant oils / Clove oil Notified as Repellant	IAS-SE-001
	XED-FR-004
Plant oils / Etheric oil (Eugenol) Notified as Repellant	DEN-NL-003
	DKI-NL-008

Plant oils / Eucalyptus oil	CFP-FR-456
	SIP-IT-003
Plant oils / Gaiac Wood oil	IAS-SE-004
Plant oils / Garlic oil	DEN-NL-001
	GSO-GB-002
Plant oils / Lemongrass oil Notified as Repellant	IAS-SE-002
Plant oils / Marjoram oil Notified as Repellant	DEN-NL-004
Plant oils / Olive oil	DKI-NL-009
Plant oils / Orange oil Notified as Repellant	GSO-GB-003
Plant oils / Pinus oil	ACP-FR-001
	DKI-NL-010
	IBT-IT-002
	MIB-NL-001
	SPU-DE-002
Plant oils / Rape seed oil	CEL-DE-001
	CRU-IT-003
	DKI-NL-011
	FBL-DE-007
	NEU-DE-002
	NOV-FR-001
	PBI-GB-003
	VIT-GB-001
Plant oils / Soya oil Notified as Repellant	DEN-NL-005
	DKI-NL-012
	PBC-ES-005
Plant oils / Spear mint oil	XED-FR-005
Plant oils / Sunflower oil	DKI-NL-013
	PBI-GB-004
	TRD-FR-002
Plant oils / Thyme oil Notified as Repellant	DEN-NL-006
Plant oils / Ylang-Ylang oil Notified as Repellant	IAS-SE-003
Pyrethrins	ALF-ES-018
	BRA-GB-001
	CAP-FR-001
	FBL-DE-008
	MGK-GB-001
	ORI-GB-001
	PBC-ES-003
	PBK-AT-001
	PYC-FR-001
	SAM-FR-001
	SBS-IT-002
Quassia	AGE-IT-001
	CAP-FR-003
	FBL-DE-009

	TRF-DE-002
	ALF-ES-009
Rotenone	FBL-DE-010
	IBT-IT-003
	SAP-FR-001
	SBS-IT-001
	SFS-FR-001
Sea-algae extract	ASU-DE-005
	LGO-FR-001
	OGT-IE-002
	VAL-IT-013
Seaweed	AGC-FR-001
Seaweed	ASF-IT-001
	OGT-IE-001
	VAL-IT-003
	ALF-ES-013
	ESA-NL-002
	KAL-IE-001

Equisetum arvense (SL)

Active substances which are animal products or derived thereof by simple processing (Lead Rapporteur: Denmark):

Active substance	Notifier
Chitosan	ALF-ES-017
	CLM-NL-001
	IDB-ES-001
Gelatine	MIB-NL-002
Hydrolysed proteins (see also Attractant/Rep)	SIC-IT-002

Active substances which are commodity substances (Lead Rapporteur: UK):

Active substance	Notifier
1-Decanol	CRO-GB-010
	OLE-BE-002
	JSC-GB-001
Aluminium sulphate	FER-GB-001
	GSO-GB-001
Calcium chloride	FBL-DE-002
Calciumhydroxide	CTB-NL-001
Carbon dioxide Notified as Insecticide/Disinfectant	FBL-DE-019
EDTA and salts thereof	DKI-NL-014
Fatty alcohols / Aliphatic alcohols	JSC-GB-002
Iron sulphate	BNG-IE-001
	HTO-GB-001
	KRO-DE-001
	MEL-NL-001
Kieselgur (Diatomaceous earth)	ABP-DE-001
	AGL-GB-001
	AMU-DE-001
	DKI-NL-004
	FBL-DE-021

Lime sulphur	FBL-DE-017
	PLS-IT-001
	STI-IT-002
Paraffin oil	FBL-DE-005
Paraffin oil / (CAS 64741-88-4)	BPO-GB-003
	SUN-BE-002
Paraffin oil / (CAS 64741-89-5)	BPO-GB-002
	PET-PT-002
	SUN-BE-001
	SUN-BE-003
	XOM-FR-002
Paraffin oil / (CAS 64741-97-5)	BPO-GB-004
Paraffin oil / (CAS 64742-46-7)	TOT-FR-001
	TOT-FR-002
	TOT-FR-003
Paraffin oil / (CAS 64742-54-7)	CVX-BE-003
Paraffin oil / (CAS 64742-55-8 / 64742-54-7)	SAG-FR-001
Paraffin oil / (CAS 64742-55-8)	CPS-ES-001
	CVX-BE-002
	XOM-FR-001
Paraffin oil / (CAS 64742-65-0)	XOM-FR-003
Paraffin oil / (CAS 72623-86-0)	TOT-FR-006
Paraffin oil / (CAS 8012-95-1)	AVA-AT-002
Paraffin oil / (CAS 8042-47-5)	ASU-DE-008
	ECP-DE-007
	NEU-DE-004
Paraffin oil / (CAS 97862-82-3)	TOT-FR-004
	TOT-FR-005
Petroleum oils	FBL-DE-006
Petroleum oils / (CAS 64742-55-8 / 64742-57-7)	GER-FR-001
Petroleum oils / (CAS 74869-22-0)	CVX-BE-001
	RLE-ES-002
Petroleum oils / (CAS 92062-35-6)	RML-IT-001
Potassium permanganate	CNA-ES-001
	FBL-DE-016
	VAL-IT-009
Aluminium silicate (Kaolin)	PPP-FR-001
Sodium aluminium silicate Notified as Repellant	FLU-DE-004
Sulphur and Sulphur dioxide	ACI-BE-001
	AGN-IT-001
	BAS-DE-008
	CER-FR-001
	CPS-ES-002
	FBL-DE-012
	GOM-ES-001
	HLA-GB-001
	JCA-ES-001
	NSC-GB-005
	OSK-ES-001
	PET-PT-001
	RAG-DE-001
	RLE-ES-001

	SAA-PT-001
	SML-GB-001
	STI-IT-001
	SYN-GB-050
	UPL-GB-002
	ZOL-IT-001
Sulphuric acid	NSA-GB-001

nitrogen lime (CZ)
Calcium polysulphide
Mangan dioxide (SK)

Active substances which are used on stored plants or plant products (Lead Rapporteur: Spain):

Active substance	Notifier
2-Phenylphenol	BCH-DE-001
Ethanol	CGL-GB-001
Ethylene	BRM-GB-636
	COL-FR-002

Active substances which are repellants and attractants (other than pheromones or other semiochemicals) (Lead Rapporteurs: Belgium and Greece):

Active substance	Notifier
Aluminium ammonium sulfate	SPL-GB-001
Ammonium acetate	LLC-AT-004
Anthraquinone	TOM-FR-002
Bone oil Notified as Repellant	BRI-GB-001
	FLU-DE-007
	IOI-DE-001
	ASU-DE-002
Calcium carbide	CFW-DE-003
Citronellol Notified as Repellant (see also Plant Extract)	ASU-DE-003
	CAL-FR-004
Daphne oil	FLU-DE-006
Denathonium benzoate	ASU-DE-001
	MFS-GB-001
Dodecyl alcohol	SEI-NL-002
Farnesol / (Z,E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol	CAL-FR-002
Hydrolysed proteins Notified as Attractant (see also Animal Pr.)	BIB-ES-001
	PHY-GR-002
	SIC-IT-001
Methyl nonyl ketone	PGM-GB-001
Quartz sand	ASU-DE-004
Quartz sand	AVA-AT-003

Quartz sand	DKI-NL-003
Quartz sand	FLU-DE-002
Repellants (by smell) of animal or plant origin / Blood meal	GYL-SE-001
Repellants (by smell) of animal or plant origin / Essential oils	BAR-GB-002
Repellants (by smell) of animal or plant origin / Fatty acids, fish oil	ASU-DE-010
Repellants (by smell) of animal or plant origin / Fish oil	FLU-DE-001
Repellants (by smell) of animal or plant origin / Sheep fat	KWZ-AT-001
Repellants (by smell) of animal or plant origin / Tall oil (CAS 8016-81-7)	FLU-DE-005
Repellants (by smell) of animal or plant origin / Tall oil crude (CAS 93571-80-3)	ASU-DE-009
Trimethylamine hydrochloride	LLC-AT-005
Urea Notified as Attractant (see also Foodstuff, Feed)	PHY-GR-001

Lanolin/ repellent (SK)

PART B:

Active substances which are pheromones or other semiochemicals (Lead Rapporteur: Austria):

Active substance	Notifier
(2E,13Z)-Octadecadien-1-yl acetate	SEI-NL-004
	SEI-NL-005
	SEI-NL-020
(7E,9Z)-Dodecadienyl acetate	BAS-DE-002
	CAL-FR-017
	ISA-IT-006
	LLC-AT-009
	RUS-GB-004
	SDQ-ES-005
	SEI-NL-006
(7E,9Z)-Dodecadienyl acetate; (7E,9E)-Dodecadienyl acetate	SHC-FR-004
(7Z,11E)-Hexadecadien-1-yl acetate	SEI-NL-014
	SEI-NL-013
(7Z,11Z)-Hexadecadien-1-yl acetate ; (7Z,11E)-Hexadecadien-1-yl acetate	ABC-GB-001
	LLC-AT-008
(9Z,12E)-Tetradecadien-1-yl acetate	RUS-GB-001
(E)-11-Tetradecenyl acetate	SEI-NL-023
(E)-8-Dodecenyl acetate	CAL-FR-015
	SEI-NL-008
(E,E)-8,10-Dodecadien-1-ol	BAS-DE-003
	CAL-FR-013
	ISA-IT-004
	LLC-AT-002
	RUS-GB-002
	SDQ-ES-004
	SEI-NL-001
	SHC-FR-003
	VIO-GR-002

	MAS-BE-005
(E/Z)-8-Dodecenyl acetate	BAS-DE-005
	CAL-FR-018
(E/Z)-8-Dodecenyl acetate ; (Z)-8-Dodecenol	ISA-IT-005
	LLC-AT-003
	SDQ-ES-006
(E/Z)-9-Dodecenyl acetate ; (E/Z)-9-Dodecen-1-ol ; (Z)-11-Tetradecen-1-yl acetate	TRF-DE-003
(Z)-11-Hexadecen-1-ol	SEI-NL-022
(Z)-11-Hexadecen-1-yl acetate	SEI-NL-021
(Z)-11-Hexadecenal	SEI-NL-017
(Z)-11-Hexadecenal ; (Z)-11-Hexadecen-1-yl acetate	LLC-AT-007
(Z)-11-Tetradecen-1-yl acetate	BAS-DE-004
	SEI-NL-010
(Z)-13-Hexadecen-11-ynyl acetate	SDQ-ES-002
(Z)-13-Octadecenal	SEI-NL-019
(Z)-7-Tetradecenal	SEI-NL-024
(Z)-8-Dodecenol	SEI-NL-009
(Z)-8-Dodecenyl acetate	CAL-FR-014
	SDQ-ES-003
	SEI-NL-007
(Z)-8-Dodecenyl acetate ; Dodecan-1-yl acetate	ISA-IT-007
(Z)-9-Dodecenyl acetate	BAS-DE-001
	LLC-AT-010
	SDQ-ES-007
	SEI-NL-012
	SHC-FR-005
(Z)-9-Dodecenyl acetate ; Dodecan-1-yl acetate	ISA-IT-008
(Z)-9-Hexadecenal	SEI-NL-018
(Z)-9-Hexadecenal ; (Z)-11-Hexadecenal ; (Z)-13-Octadecenal	RUS-GB-003
	SDQ-ES-001
(Z)-9-Tetradecenyl acetate	SEI-NL-011
(Z,Z,Z,Z)-7,13,16,19-Docosatetraen-1-yl isobutyrate	SHC-FR-001
1,4-Diaminobutane (Putrescine)	LLC-AT-006
1,7-Dioxaspiro-5,5-undecan	VIO-GR-001
1-Tetradecanol	SEI-NL-003
2,6,6-Trimethylbicyclo(3.1.1)hept-2-en-4-ol	SHC-FR-006
3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol (Nerolidol)	CAL-FR-003
3,7-Dimethyl-2,6-octadien-1-ol (Geraniol)	CAL-FR-005
5-Decen-1-ol	BAS-DE-006
	SEI-NL-016
5-Decen-1-yl acetate	BAS-DE-007
	SEI-NL-015
5-Decen-1-yl acetate ; 5-Decen-1-ol	LLC-AT-001
	ISA-IT-003

PART C:

Micro-organisms including viruses (Lead Rapporteurs: Netherlands and Sweden)[15]:

Active substance	Notifier
Bacillus sphaericus	SUM-FR-008
Bacillus thuringiensis aizawai	ISA-IT-009
	MAS-BE-004
	SIP-IT-005
	SUM-FR-005
Bacillus thuringiensis israelensis	SIP-IT-006
	SUM-FR-007
Bacillus thuringiensis kurstaki	ALF-ES-019
	ASU-DE-011
	IAB-ES-004
	MAS-BE-002
	PRO-ES-421
	SIP-IT-004
	SUM-FR-004
	IBT-IT-001
	ISA-IT-011
Bacillus thuringiensis tenebrionis	SUM-FR-006
Beauveria bassiana	AGB-IT-001
	AGR-ES-003
	CAL-FR-007
	MEU-GB-001
Beauveria brongniartii	CAL-FR-006
Cydia pomonella granulosis virus	MAS-BE-003
	CAL-FR-001
	IBT-IT-004
	PKA-DE-001
	SIP-IT-007
Metarhizium anisopliae	AGF-IT-004
	IBT-IT-006
	TAE-DE-001
Neodiprion sertifer nuclear polyhedrosis virus	VRA-FI-003
Phlebiopsis gigantea	FOC-GB-001
	VRA-FI-002
Streptomyces griseoviridis	VRA-FI-001
Trichoderma harzianum	BBI-SE-002
	IAB-ES-005
	IBT-IT-005
	ISA-IT-012
	AGF-IT-002
	KBS-NL-001
	MAK-BE-001
Trichoderma polysporum	BBI-SE-001
Trichoderma viride	AGB-IT-002
	ISA-IT-013
Verticillium dahliae	ARC-NL-001
Verticillium lecanii	KBS-NL-002

[15] The Netherlands is also Rapporteur Member State for *Metarhizium anisopliae*, *Verticillium dahliae* and *Verticillium lecanii.*